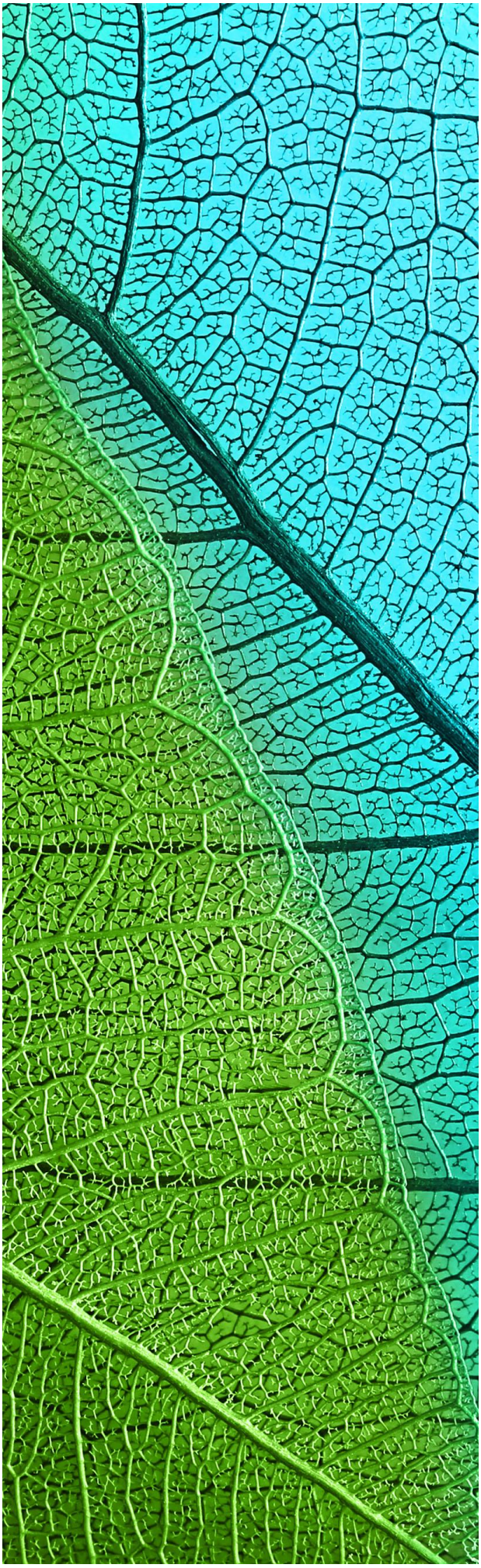

Environmental Management Plan

Project: Rossiter Park
EPBC No. 2023/09694

Prepared for Rossiter Road Investments Pty Ltd
C/- KLM SPATIAL
Suite 1, Building 2, 3 Ordish Rd
Dandenong South VIC 3175

May 2025
Report No. 22058.02 (2.7)

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Cover Page

EPBC Number: 2023/09694

Project Name: Rossiter Park

Proponent organisation: Rossiter Road Investments Pty Ltd

Proponent ABN/ACN: 651095377

Proposed Action: Multi-lot subdivision into residential lots, lifestyle village, and associated works at 145 Rossiter Road, Koo Wee Rup.

Date of preparation of the Environmental Management Plan (EMP): April 2024 – updated July 2024 September 2024 and February 2025.

Person accepting responsibility for the EMP: Rossiter Road Investments Pty Ltd

Declaration of Accuracy:

In making this declaration, I am aware that section 491 of the *Environment Protection and Biodiversity Conservation Act 1999* (Cth) (EPBC Act) makes it an offence in certain circumstances to knowingly provide false or misleading information or documents to specified persons who are known to be performing a duty or carrying out a function under the EPBC Act or the Environment Protection and Biodiversity Conservation Regulations 2000 (Cth). The offence is punishable on conviction by imprisonment or a fine, or both. I am authorised to bind the approval holder to this declaration and that I have no knowledge of that authorisation being revoked at the time of making this declaration.

Signed:

Full name: Caroline Tan, Senior Botanist and Project Manager

Organisation: Nature Advisory Pty Ltd Pty Ltd

Date: 1 May 2025

The following table outlines the revisions of this EMP.

Table 1. Document Version Control

Document Version	Date	Name	Changes made
2.0	April 2024	Ezra Janetzki & Caroline Tan	Draft prepared for feedback. Only sent to the client in April 2024 so an internal EMP.
2.1	April 2024	Caroline Tan	Updated based on further information provided by the client and issued to DCCEEW via email on 26th April 2024.
2.2	July 2024	Kate Thurkle & Caroline Tan	Updated based on DCCEEW's comments/further information provided by KLMS Spatial. Only sent to the client in July 2024 so an internal EMP.
2.3	July 2024	Caroline Tan	Amendments such as the staging of works and management objectives. Only sent to the client in July 2024 so an internal EMP.
2.4	July 2024	Caroline Tan	Updated to reflect date of issue of DCCEEW and issued to DCCEEW via email on 23 July 2024.
2.5	September 2024	Arend Kwak & Caroline Tan	Updated to address comments from DCCEEW via email on 30th August 2024 and update date of issue on the cover page.
2.6	March 2025	Ezra Janetzki & Caroline Tan	Updated to reflect changes to the development layout, which were made in response to discussions with DCCEEW and the Cardinia Shire Council in Victoria.
2.7	May 2025	Caroline Tan	Updated to reflect change in document name on the front cover and removed references to permanent fencing as requested by DCCEEW.

Executive Summary

The proposed action is for a residential development at 145 Rossiter Road, Koo Wee Rup, in Victoria (Figure 1). The proposed action will include clearance and redevelopment across most of the site.

Based on the proposed action, three farm dams providing aquatic habitat for Growling Grass Frog, and the surrounding areas of terrestrial habitat (introduced grassland using as grazing land) will be lost.

The existing swamp scrub habitat for Southern Brown Bandicoot that is present along the northern boundary of the site will be retained.

Existing habitat for Growling Grass Frog

- Aquatic habitat – occurs in three farm dams on site, which have a total combined area of **0.17 hectares**. The targeted surveys detected the species at one dam. The targeted survey considered that these dams may be used for breeding, however, the dams are likely to be ephemeral and so not permanent breeding habitat. Dams 1 and 2 were visited in May 2024 and found to be dry.
- Breeding habitat – unlikely that the farm dams have water permanence, and the species is dependent on areas of permanent water for breeding, such as shallow parts of freshwater lagoons.
- Terrestrial habitat for dispersal between waterbodies, foraging, shelter and overwintering – limited to the drainage lines running through the site, as discussed in further detail in Appendix 2. These drainage lines are approx. 337 metres and 155 metres at the northwestern section and 962 metres along the southwestern boundary. This is a combined length of approx. 1,454 metres. At generally 2 metres wide, this covers a total area of **0.2908 hectares**.

Detailed discussion regarding habitat for the Growling Grass Frog is provided in Appendix 2

Reasoning for the existing Growling Grass Frog habitat being replaced

Apart from the lower quality of the habitat for Growling Grass Frog on site (ephemeral farm dams and shallow drainage lines within a site that are used for hay cutting and grazing), it was also considered that the southwestern dams are located close to Rossiter Road with residential area across the road and powerlines cross overhead nearby. As the road presents a dispersal barrier, frog dispersal would be occurring between the Bunyip River north of the site and the dams on site. As such, habitat quality is decreased due to the existing degree of development in the surrounds (roads and buildings).

The existing dispersal habitat in the site is in association with frog access to the dams only. The proposed action is not impacting on a dispersal corridor between habitat areas that will continue to exist after the proposed action. Also, dispersal within and to/from any existing habitats in the western properties (paddocks and farm dams) are not expected to be impacted by the proposed action.

It was considered unfeasible to retain the existing dams on site for the following reasons:

- The dams are not structured for permanence. They are old farm dams which are only around 1.2 metres deep and had spoil pushed up around them to shape the walls.
- Dam 1 and Dam 2 in particular are not well placed in terms of rain fall or flows to feed the dams. Only water that falls directly on that area goes into the dams. As shown with the spot level on survey plans, there is minimal flow directly to the dams. The spot levels and fill mound surround the dams to make them the form of a 'Turkey nest', and therefore they are only fed by rainfall. This may be why the dams have low or absent water levels.

- The small embankment around Dam 1 and 2 is eroding and collapsing, showing the earth forming the dams is generally not stable and will continue to erode into the floor and become a muddy swell over time. It was noted that a number of the surrounding trees here are dead.

Detailed discussion regarding the need to remove the farm dams on site are provided in Appendix 2.

Proposed replacement habitat for Growling Grass Frog

The proposed action will include construction of replacement Growling Grass Frog habitat at the northwestern section of the site. The new Growling Grass Frog habitat will include a dedicated wetland for aquatic habitat, with a 50-metre terrestrial buffer of open grassland. The key parameters for the new Growling Grass Frog habitat will include the requirements under the *Growling Grass Frog Habitat Design Standards – Melbourne Strategic Assessment* (DELWP 2017).

- The proposed new wetland suitable for breeding will be **0.2467 hectares,** with an additional littoral zone that is **0.0647 hectares**.
- There will also be a **50-metre-wide buffer** of open grassland around the wetland within the site which covers **1.4878 hectares**, which will provide terrestrial habitat including dispersal, foraging, shelter and overwintering.
- In total, the new Growling Grass Frog habitat will be **1.845 hectares**.

Ultimately, the new Growling Grass Frog habitat will contain superior quality habitat compared to what is offered by the existing dams, which are located in the context of a cow paddock with relatively narrow seasonal dispersal opportunities. The constructed habitat will be situated closer to the Bunyip River north of the site than the dams, with permanent dispersal ability to and from the Bunyip River as well as the neighbouring property to the west (which exists as introduced grassland for grazing, similar to the site).

Furthermore, the drainage reserve containing a treatment wetland and sediment basin for stormwater management on site has been situated at the northwestern section of the site, between the site's northern boundary and the Growling Grass Frog habitat. This drainage reserve can provide additional aquatic and terrestrial habitat for dispersal, foraging and shelter for Growling Grass Frog.

The Growling Grass Frog may utilise the Southern Brown Bandicoot habitat buffer as terrestrial habitat along the site's northern section, which also abuts existing swamp scrub north of the site.

- The drainage reserve is **1.418 hectares** in size.
- The Southern Brown Bandicoot habitat buffer is **2.276 hectares** in size.
- These two areas are **3.694 hectares** combined.

New habitat buffer for Southern Brown Bandicoot

The proposed action will include an approximately 30-metre-wide habitat buffer for Southern Brown Bandicoot (also called the 'Bandicoot Corridor' on the development plan), which will adjoin the existing swamp scrub habitat present along the northern boundary of the site and will be revegetated to reflect swamp scrub and native grassland. This habitat buffer will provide separation between the proposed residential development (roads and houses) and the existing habitat.

Management actions

The new replacement habitat and habitat buffer will be secured and protected from the proposed residential land uses on the site and be managed for the purposes of conservation of Growling Grass Frog and Southern Brown Bandicoot (see Section 3). Regular monitoring will be implemented for the suitability of management actions, including an adaptive management approach (refer Section 3.10 and 3.11).

Contents

Tables

Figures

Appendices

1. Introduction

1.1. Project description

The proposed action is for the subdivision of the land into residential lots, a lifestyle village and associated works. The proposed development site (herein called 'the site) is located in Victoria at Lot 2 PS 321029K, which is also known as 145 Rossiter Road, Koo Wee Rup. The site is currently used for agriculture including grazing and hay cutting and is approx. 24.4 hectares in size.

The proposed development layout is provided in Appendix 1.

Two Matters of National Environmental Significance (MNES), Growling Grass Frog and Southern Brown Bandicoot, have the potential to be impacted by the proposed action. These potential impacts have been assessed in the MNES Report (Nature Advisory 2024).

The proposed action was referred to the Commonwealth Department of Climate Change, Energy, the Environment and Water (DCCEEW) and determined to be a controlled action, which required further assessment by Preliminary Documentation (EPBC ref 2023/09694).

1.2. Purpose of the EMP

This EMP for MNES forms the Preliminary Documentation for assessment under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act).

This EMP has been developed following the *Environmental Management Plan Guidelines* (Commonwealth of Australia, 2014) and addresses several key aspects of the proposed works:

- Environmental management roles and responsibilities (Section 1.6);
- Environmental training (Section 3.1);
- Environmental emergencies (Section 3.12);
- Potential environmental impacts and responding strategy (Section 1.4);
- Environmental management measures (Section 3);
- Adaptive management (Section 3.11); and
- Review of the plan (Section 3.13).

1.3. Limitations or uncertainties in this EMP

The proposed action is currently in the early stages of planning and design. The proposed development layout is provided in Appendix 1 of this EMP, however, there are not yet detailed design plans such as a detailed landscaping plan, detailed wetland design and detailed stormwater plan. We recognise this lack of detailed information is a limitation to the current EMP. However, this EMP will outline the commitments that will be documented and met in the detailed design plans and the Construction Contractor's Construction Environmental Management Plan (CEMP), in relation to avoiding and mitigating impacts on Growling Grass Frog and Southern Brown Bandicoot.

Review and update of this EMP for final approval will be required once additional project details become available. Approval conditions could also include requirements to prepare the detailed design plans and CEMP in accordance with this EMP.

1.4. Main Potential Impacts to MNES

Growling Grass Frog

The *Significant impact guidelines for the vulnerable growling grass frog (Litoria raniformis)* (DEWHA 2009) does not define core habitat or key habitat for the Growling Grass Frog. Targeted surveys for Growling Grass Frog were undertaken and an assessment of habitat provided by Practical Ecology (2022). The targeted surveys for Growling Grass Frog on site detected the species at only one of the existing dams in the southwestern part of the site.

Existing habitat for the species includes:

- Aquatic habitat – occurs in three farm dams on site, which have a total combined area of 0.17 hectares. The targeted surveys detected the species at one dam. The targeted survey considered that these dams may be used for breeding, however, the dams are likely to be ephemeral and so not permanent breeding habitat. Dams 1 and 2 were visited in May 2024 and found to be dry.
- Breeding habitat – unlikely that the farm dams have water permanence and the species in dependent on areas of permanent water for breeding, such as the shallow part of freshwater lagoons.
- Terrestrial habitat for dispersal between waterbodies, foraging, shelter and overwintering – likely limited to the drainage lines running through the site as shown in the Targeted Frog Survey Report by Practical Ecology, considering the site's land uses as discussed in Appendix 2. These drainage lines are approx. 337 metres and 155 metres long at the northwestern section and 962 metres along the southwestern boundary. This is a combined length of approx. 1,454 metres. For 2 metres width, this covers a combined total area of 0.2908 hectares of terrestrial dispersal habitat.

Further detailed discussion regarding habitat for the Growling Grass Frog and need for removal of the existing farm dams on site are provided in Appendix 2.

The table below outlines the main potential impacts to Growling Grass Frog and the responding strategy to avoid or mitigate the risk in this EMP. The 10-year management objectives are provided in Appendix 3.

Table 2. Impacts to Growling Grass Frog and responses

Impacts	Response
Direct loss of a total of **0.17 hectares** of aquatic habitat for Growling Grass Frog, due to the removal of three farm dams in the site. All three farm dams are conservatively assumed to be habitat, although the species was identified at only one of the dams during targeted surveys. Unlikely that the farm dams have water permanence, and the species is dependent on areas of permanent water for breeding, such as the shallow part of freshwater lagoons.	Unavoidable loss of habitat as discussed in Appendix 2. Mitigation measure: The proposed action will provide Growling Grass Frog replacement habitat that will be managed and protected in perpetuity. It will include a constructed wetland that can provide breeding habitat for Growling Grass Frog (aquatic habitat) with a surrounding 50 metre buffer of open grassland (terrestrial habitat), while considering dispersal for frogs between this new habitat and the Bunyip River north of the site (refer to Section 2.1 and Section 2.2).
Direct loss of terrestrial habitat for dispersal between waterbodies, foraging, shelter and overwintering – however limited to the drainage lines running through the site. This is a combined	The proposed new wetland suitable for breeding will be **0.2467 hectares**. The **50-metre-wide buffer** of open grassland around the wetland will cover **1.5525 hectares**, which will provide terrestrial

length of approx. 1,454 metres. At generally 2 metres wide, this covers a combined total area of **0.2908 hectares** of terrestrial dispersal habitat. The existing dispersal habitat in the site is in association with frog access to the dams only. There is no habitat corridor in the site that links between habitats in the landscape outside the site, such as a drainage line that links the Bunyip River with another waterway or wetland outside the site.	habitat including dispersal, foraging, shelter and overwintering. In total, the new Growling Grass Frog habitat will be **1.845 hectares** including the aquatic component and terrestrial component
Direct impacts through habitat disturbance and accidental encroachment while new constructed habitat is being established.	Avoidance measure: Existing habitat will be protected by temporary fencing and sediment fencing until the new habitat is constructed delivers Growling Grass Frog standards (refer Section 3.3)
Direct harm to frogs on site during construction works – injury and mortality.	Avoidance measure: Refer to the construction management protocols in Section 3.1
Potential spread of Chytrid fungus during construction works.	Avoidance measure: Refer to the construction management protocols in Section 3.1
Long-term degradation of constructed habitat by weed invasion.	Avoidance measure: Refer to weed management in Section 3.6.
Long-term degradation of constructed habitat by polluted run-off and sedimentation from lack of inappropriate stormwater management.	Avoidance measure: Refer to hydrology and stormwater management in Section 2.4.
Long-term impact of predation by pest animals present on site.	Avoidance measure: Refer to pest animal and predatory fish management in Section 3.8 and Section 3.9.

DCCEEW has confirmed the impacts of the action are residually significant and the implementation of an artificial wetland for the Growling Grass Frog within the site is the offset for that impact. It is noted that an offset can be provided within the boundaries of the proposed action area and be included within the scope of the proposed action.

Southern Brown Bandicoot

The existing swamp scrub habitat for Southern Brown Bandicoot that is present along the northern boundary of the site (outside the site) must be retained.

The table below outlines the main potential impacts to Southern Brown Bandicoot and the responding strategy to avoid or mitigate the risk in this EMP. The 10-year management objectives are provided in Appendix 3.

Table 3. Impacts to Southern Brown Bandicoot and responses

Impacts	Response
The site does not provide suitable habitat for Southern Brown Bandicoot, which inhabits native vegetation with a dense understorey, although Southern Brown Bandicoot in the swamp scrub located north of the site may occasionally wander into the dense pasture at the northern part of the site (hence the dense pasture on site was considered 'sub-optimal introduced pasture habitat' as stated in the MNES Report).	Impact avoided. Further avoidance measure: This sub-optimal habitat will not be impacted; it will be retained and revegetated into a 30-metre-wide habitat buffer (also called the 'Bandicoot Corridor' on the development plan) that will be managed and protected in perpetuity. It will be revegetated to contain swamp scrub and native grassland (refer Section 3.5).
Direct impacts through habitat disturbance and accidental encroachment while new constructed habitat is being established.	Avoidance measure: Refer to temporary fencing and sediment fencing along the northern site boundary in Section 3.3.
Direct harm to bandicoots in the adjacent swamp scrub during construction works – injury and mortality.	Avoidance measure: Refer to the construction management protocols in Section 3.1 – 'General construction work protocols'.
Long-term degradation of constructed habitat by weed invasion.	Avoidance measure: Refer to weed management in Section 3.6.
Long-term degradation of constructed habitat by polluted run-off and sedimentation from lack of inappropriate stormwater management.	Avoidance measure: Refer to stormwater management in Section 2.4.
Long-term impact of predation by pest animals present on site.	Avoidance measure: Refer to pest animal management in Section 3.8.

Figure 1: MNES habitats in or adjacent to the site

Project No: 22058_02
Project: 145 Rossiter Rd Koo Wee Rup
Date: 23/07/2024

- Study area
- Bunyip River
- Drainage lines identified by Practical Ecology
- Known sub-optimal Growling Grass Frog habitat
- Known sub-optimal Southern Brown Bandicoot habitat

N

Metres

0 25 50 75 100 125

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1.5. Summary of commitments and timeframes

The long-term management objectives over a 10-year period are provided in this EMP.

A 'Conditions of Approval' Reference Table outlining the approval condition requirements that the CEMP will address is in Appendix 4 of this EMP.

The strategy for staging of works is described in Section 3.2 of this EMP. The CEMP will detail the timeframe for construction works once a Construction Contractor has been appointed.

1.6. Environmental management roles and responsibilities

Responsible party

The proponent of the development (Responsible Party) is responsible for ensuring that the construction and management of the development is implemented as outlined in the approved CEMP and in accordance with any approval conditions for the project under the EPBC Act, until such management is handed to the responsibility of another authority.

The designated construction contractor will be responsible to the designated proponent for implementing the CEMP in accordance with the guidance in this EMP and in any conditions of approval of the project under the EPBC Act. Where necessary, the services of a qualified ecologist will be retained to advise on impacts and mitigation measures during construction for MNES.

Proposed designated proponent organisation details.

ABN/ACN 651095377

Organisation name Rossiter Road Investments Pty Ltd

Organisation address Suite 1, Building 2, 3 Ordish Rd, Dandenong South, VIC

Long-term management and responsible party

The CEMP which will incorporate this EMP will be implemented over a 10-year period but the requirement to manage the new Growling Grass Frog habitat and Southern Brown bandicoot habitat buffer remains in perpetuity. The 10-year management objectives are provided in Appendix 3.

It is anticipated that upon completion of a 2-year period after construction has commenced (or a period otherwise agreed upon with Cardinia Shire Council), the new Growling Grass Frog habitat and Southern Brown Bandicoot buffer will be managed by Cardinia Shire Council, following handover of these areas to the council. Cardinia Shire would be obliged to maintain the reserve in accordance with the purpose of the reserve and any EPBC approval requirements.

The habitat areas will be secured through transfer of land or an 'on title' agreement (e.g. Section 69 agreement under the Victorian *Conservation, Forests and Lands Act 1987*).

With particular regard to Growling Grass Frog habitat, Cardinia Shire Council maintains a number of constructed Growling Grass Frog wetlands within the municipality and their suitably qualified staff have been able to undertake an appropriate maintenance regime to protect and enhance the habitat and conservation values of the new habitats.

2. Proposed Habitat Creation

2.1. Layout Design

The layout design has been through multiple iterations to feasibly balance development objectives, which included meeting habitat requirements of both Growling Grass Frog and Southern Brown Bandicoot. The current design has been reached following discussions with DCCEEW and Cardinia Shire Council. The current design includes an approximately 30-metre-wide bandicoot corridor in the north of the site, which is 2.276 ha. This will be planted with indigenous vegetation that would provide suitable habitat for Southern Brown Bandicoot.

In the northwest of the site, a Growling Grass Frog habitat wetland will be constructed. This is mostly surrounded by a 50-metre buffer zone of maintained grassland (consistent with suitable terrestrial habitat conditions), only except for to the north where the buffer abuts existing swamp scrub north of the study area (which is connected to the Bunyip River). To the west of the Growling Grass Frog wetland is a drainage reserve, which is likely to also be used by the species even though the drainage reserve was not specifically designed as Growling Grass Frog habitat (given it serves the purpose of managing water hydrology and water quality). This drainage reserve, which is 1.418 ha in size, will serve as a buffer between the Growling Grass Frog habitat and the agricultural land to the west of the study area.

Previous iterations of the development layout located the drainage reserve in the north of the study area and the Growling Grass Frog habitat against the western boundary. The layout has been altered so that Growling Grass Frog habitat is only bordered by development in one direction, and is surrounded by natural and semi-natural areas in all other directions.

The current development layout ensures all natural and semi-natural areas in the northern part of the development (which serve as Growling Grass and Southern Brown Bandicoot habitat) are continuously connected, as well as the naturally occurring swamp scrub to the north of the site and open agricultural grassland to the west. Furthermore, the current development layout has incorporated more habitat for both Growling Grass Frog and Southern Brown Bandicoot than previous iterations of the development plan.

2.2. Growling Grass Frog Habitat

The proposed action will include construction of replacement Growling Grass Frog habitat at the northwestern section of the site, as shown in Appendix 1.

The new Growling Grass Frog habitat will also include a dedicated wetland, with a 50-metre buffer of open grassland with no development surrounding the wetland.

The Growling Grass Frog habitat, when established, will contain superior quality habitat compared to what is offered by the existing dams currently located in the context of agricultural land. The constructed habitat will be vegetated with native plants and be managed in perpetuity, as well as situated closer to the Bunyip River than the existing dams in the site in terms of frog dispersal to/from the river. The replacement habitat (including revegetation works with native plants and semi-permeable fencing) is expected to significantly ameliorate impacts and improve longer term outcomes related to the removal of the existing farm dams in the long-term.

- The proposed new wetland suitable for breeding will be **0.2467 hectares,** with an extra littoral zone that is **0.0647 hectares**.

- There will also be a **50-metre-wide buffer** of open grassland around the wetland within the site which covers **1.4878 hectares**, which will provide terrestrial habitat including dispersal, foraging, shelter and overwintering.
- In total, the new Growling Grass Frog habitat will be **1.845 hectares**.

In addition, the drainage reserve containing a treatment wetland and sediment basin for stormwater management on site has been situated at the northwestern section of the site, between the site's northern boundary and the Growling Grass Frog habitat. It is considered that this drainage reserve can provide dispersal habitat between the Bunyip River/swamp scrub to the north of the site and the constructed Growling Grass Frog habitat in the site. Considering that Growling Grass Frog are well-known to occupy a variety of habitats including farm dams and stormwater assets (like treatment wetlands and sediment basins), there is potential for this drainage reserve to also provide additional foraging and shelter for Growling Grass Frog on site.

It is also considered that the Growling Grass Frog may utilise the Southern Brown Bandicoot habitat buffer as terrestrial habitat along the site's northern section, which also abuts existing swamp scrub along a drainage channel north of the site. The habitat buffer will contain swamp scrub and native grassland to support habitat for dispersal, foraging, shelter and overwintering.

- The drainage reserve is **1.418 hectares** in size.
- The Southern Brown Bandicoot habitat buffer is **2.276 hectares** in size.
- These two areas are **3.694 hectares** combined.

Wetland habitat design standards

The wetland habitat and 50 metre buffer of open grassland will be created in accordance with the *Growling Grass Frog Habitat Design Standards – Melbourne Strategic Assessment* (DELWP 2017) (herein referred to as the 'Growling Grass Frog Habitat Design Standards').

Table 4 addresses and responds to the current wetland design standards.

Table 4. Wetland design standards and responses under Growling Grass Frog Habitat Design Standards 2017

Wetland Design Standard		Response	Response met? Yes/ No
Size standards	The surface area of most new wetlands in a cluster must be at least 0.3ha where space allows. Where space is limited, the surface area of wetlands can be reduced, but not below 0.15ha. In all cases the submergent zone must be at least 0.1ha in area.	The Growling Grass Frog wetland size is 0.3 hectares. A wetland cluster is not proposed. However, it should be noted that the drainage reserve for stormwater management on site has been situated at the site's northwestern corner, buffering the new frog wetland from the adjacent agricultural land. It is considered that this drainage reserve can provide potential wetland-type habitat for dispersal, foraging and shelter, although it is not designed to meet Growling Grass Frog Habitat Design Standards so not included in the 0.3 hectares of Growling Grass Frog wetland. The drainage reserve is 1.418 hectares in size.	**Yes**
	If possible, at least one wetland in a cluster should be large (greater than 0.7ha); in some case this could be achieved by merging two standard (medium) sized wetlands.		
Shape standards	Wetlands must be wide enough the efficiently provide the required area of deep water for submerge vegetation.	The shape of the proposed Growling Grass Frog wetland is considered acceptable. The submergent zone is 0.1804 hectares in area.	**Yes**
	Islands within the wetland are not permitted, as they are likely to encourage excessive numbers of waterbirds which may increase predation pressure and contaminate the shallows with concentrated droppings.	No islands.	
Water depth and gradient standards	All wetlands must incorporate a deep water submergent vegetation zone, constituting a minimum of 50 per cent and preferably 60-70 per cent of the total wetland surface area at normal water level.	The submergent zone is 0.1804 hectares in area which is 54% of the wetland area.	**Yes**
	The water depth in the submergent zone must be maintained at greater than 1.5m. Wetlands with greater maximum depths are desirable and should be constructed if feasible.	This will be a required parameter for the detailed wetland design.	

Wetland Design Standard		Response	Response met? Yes/ No
	The emergent vegetation zone should occupy approximately 30-40 per cent of the wetland area and should include a littoral zone with fluctuating water levels (e.g. between normal water level and the summer drawdown level).	The emergent zone is 0.1310 hectares, which is 42% of the wetland area. There is also a littoral zone, which is 0.0647 hectares. The total emergent area is 0.1310 square metres.	
	A variety of slopes must be incorporated into the design of the banks, including steep drop-offs wherever this can be accommodated within land managers' safety standards.	This will be a required parameter for the detailed wetland design.	
Hydroperiod standards	At least three-quarters of the wetlands in a cluster should have a permanent hydroperiod, and as many as possible should hold water over the breeding season (Sept-Feb). Ideally water levels should draw down naturally over late summer and autumn.	This will be a required parameter for the detailed wetland design.	**Yes**
	Semi-permanent and ephemeral wetlands may be acceptable where there is limited capacity to provide a permanent wetland or if there is a specific requirement (e.g. to maintain a natural Seasonal Herbaceous Wetland).	This will be a required parameter for the detailed wetland design.	
	New wetlands should be designed to allow them to be periodically dried out for management and maintenance purposes (e.g. the control of predatory fish).	This will be a required parameter for the detailed wetland design.	
Lining and substrate standards	The wetland must be lined (usually with clay rather than synthetic material) to prevent leakages.	This will be a required parameter for the detailed wetland design.	**Yes**
	A layer of soil must be placed over the liner. It must be suitable for establishment and long-term persistence of aquatic plants and must not result in high turbidity after wetland establishment. Clay soil is acceptable for use as substrate.	This will be a required parameter for the detailed wetland design.	
	Wetlands must be large and deep to provide thermal inertia.	This will be a required parameter for the detailed wetland design.	**Yes**

Wetland Design Standard		Response	Response met? Yes/ No
Thermal properties standards		The wetland is 0.3 hectares in size and will meet the required minimum 1.5 metre depth in the submergent zone.	
	Wetlands must incorporate an extensive, shallow, permanently inundated emergent zone where water temperatures will be elevated due to the heat of the sun.	This will be a required parameter for the detailed wetland design. The emergent zone is 0.1310 hectares	
	All wetlands should incorporate jumbled piles of rocks around at least 20 per cent of the margin, extending into the wetland at least one metre from normal water level.	This will be required as part of the revegetation works.	
	"Anti-chytrid" wetlands in the basalt region (where excavated material can be used on site rather than paying for disposal offsite) should incorporate rocks around 50 per cent of the wetland margin if within budget.	This will be a required parameter for the detailed wetland design.	
	Embankments to cut prevailing winds may be useful and can be constructed from excavated material.	This will be a required parameter for the detailed wetland design.	
Water source standards	Groundwater is generally preferred, where feasible.	Not applicable – it is not feasible for the wetland to be groundwater-fed because it will become highly saline as a result (which may rise above the tolerance range of Growling Grass Frog for salinity over time).	**Yes**
	Use the best possible source of water in response to each set of circumstances, using Table 1 in the *Growling Grass Frog Habitat Design Standards 2017*.	The source of water for the wetland will be rainwater/surface runoff and the proposed drainage reserve. The water in the drainage reserve will be treated to best practice standards (Melbourne Water quality standards) prior to entering the dedicated frog wetland. As such, this would achieve Table 1 of the design standards. The advantage is a more reliable source of water	

Wetland Design Standard		Response	Response met? Yes/ No
		in the dedicated frog wetland than rainfall, considering the Growling Grass Frog relies on permanent freshwater bodies for breeding.	
Water quality standards	The water quality standards in Table 2 of the *Growling Grass Frog Habitat Design Standards 2017* should be applied.	This will be a required parameter for the detailed wetland design.	**Yes**
Aquatic vegetation standards	It is assumed that initial plantings will spread quickly if wetland conditions are suitable, so the whole wetland does no need to be planted out. E.g. submergent species would normally be planted on the slopes of the wetland, rather than at the maximum depth. The proportion of a wetland to be planted will be determined during the project planning phase.	This will be a requirement for the project landscaping/revegetation plan.	**Yes**
	Planting density must be such that it results in the establishment of a dense (target 50 per cent) cover of submergent/ floating vegetation in the deep-water zone and patches of emergent vegetation within several years. As a guide, planting densities to create patches of emergent vegetation are generally 4-6 plants per square metre. Planting densities for patches or submergent vegetation can be lower.		
	A diversity of vegetation is highly desirable.		
	Species to be planted in Growling Grass Frog wetlands must be selected from those show in Appendix 1 of the *Growling Grass Frog Habitat Design Standards 2017*, taking account of local water quality conditions (brackish wetlands should be planted out with species adapted to growing moderately salty conditions).		
	In the deep-water zone, submergent/ floating species must include Water Ribbons (*Cycnogeton procerum*) and species from the genus *Potamogeton* – or if the water Is brackish, Fennel Pondweed (*Stuckenia pectinate*).		
	Exotic species must not be used.		

Wetland Design Standard		Response	Response met? Yes/ No
	Common Reed (*Phragmites australis*) and bulrushes (*Typha* spp.) do not need to be planted as they are likely to establish naturally over time.		
Predator control standards	All newly constructed wetlands must be offline. Wetlands constructed within a floodplain should incorporate bund walls to reduce the frequency of fish incursion.	The dedicated frog wetland will be connected to the proposed drainage reserve. The dedicated frog wetland and the drainage reserve are not further connected to other water bodies or streams. Ability to address incursion of introduced fish will be a required parameter for the detailed wetland design. Fish exclusion devices (for example gravel and sand filters) can be used if the main water source is from stormwater treatment wetlands. The dedicated frog wetland will be designed to allow it to be periodically dried out if needed for management and maintenance purposes (such as the control of predatory fish). However, as noted in the design standards, Growling Grass Frogs are known to inhabit and breed in a number of wetlands around Melbourne containing populations of Eastern Gambusia, so the predators' presence does not necessarily preclude the persistence of Growling Grass Frogs.	**Yes**
	Incorporate a fish exclusion filter in the hydraulic connect system between the source of treated stormwater or creek/ river water and the Growling Grass Frog Wetland.	This will be a required parameter for the detailed wetland design.	
	Also see standards for: ▪ Depth – deep water may assist with predator avoidance (especially predatory birds) and some predatory dish species such as Eastern Gambusia which	This will be a required parameter for the detailed wetland design.	

Wetland Design Standard		Response	Response met? Yes/ No
	prefer to aggregate in shallow parts of the wetland where temperatures are higher; ▪ Hydroperiod – dry wetlands out as required to remove or control populations of fish, yabbies and other predators; and ▪ Aquatic vegetation – provides refuge for tadpoles.		
Terrestrial habitat standards	A minimum 50m buffer from development must surround each wetland, in which major infrastructure such as roads, car parks, and buildings should be avoided (unless the wetland is constructed closer than 50m to the conservation area boundary because of space constraints).	The development layout includes a 50-metre buffer of open grassland around the wetland. Shared use paths, other minor infrastructure for passive recreation and stormwater assets are not 30m from the normal water level.	**Yes**
	Shared use paths, other minor infrastructure for passive recreation and stormwater assets must not be constructed 30m from the normal water level of a breeding wetland.		
	Approximately 50 per cent of the area within 10m of the wetland's normal water level must be designed to be maintained as low, grassy vegetation up to 10cm in height.	This will be a requirement for the project landscaping/revegetation plan.	
	Where tussock-forming grasses and sedges are used in the zone that is within 10m of normal water level, planting density should allow for no greater than 20 per cent cover when mature.	This will be a requirement for the project landscaping/revegetation plan.	
	Mulch must not be used within 50m of a wetland.	This will be a requirement for the project landscaping/revegetation plan.	
	Shrubs must not be planted within 10m of the wetland's normal water level.	This will be a requirement for the project landscaping/revegetation plan.	
	Rock piles at least one metre deep must be constructed adjacent to the wetland margin using a variety of rock sizes between 10cm and on metre in diameter.	This will be a required parameter for the detailed wetland design.	

Wetland Design Standard		Response	Response met? Yes/ No
		This will be a requirement for the project landscaping/revegetation plan.	
	Where possible, the area between 10m and up to 100m (where space is available) from the wetland should be designed to be maintained primarily as short, mown grass with an open structure (e.g. 20 per cent cover).	This will be a requirement for the project landscaping/revegetation plan.	
	Tree cover within 100m of a wetland should not exceed 10 per cent.		
	Shrub cover within 100m of a wetland should not exceed 10 per cent.		
	A patchy arrangement of denser plantings of tussock-forming species is encouraged to maintain some potential terrestrial shelter sites.		
	Low, grassy vegetation areas do not need to be native vegetation (mown pasture and even lawn is acceptable).	This will be a requirement for the project landscaping/revegetation plan.	
	Invasive plant species must not be used anywhere within the terrestrial habitat zone.	This will be a requirement for the project landscaping/revegetation plan.	

Measures under the significant impact guidelines

The following measures are from the *Significant impact guidelines for the vulnerable growling grass frog (Litoria raniformis)* (DEWHA 2009).

It is important to note that at the time of preparation of these significant impact guidelines in 2009, 'habitat creation' for Growling Grass Frog was considered 'experimental'. However, in current times, many developments around Victoria have successfully established purpose-built wetlands for Growling Grass Frog habitat wherein Growling Grass Frog populations were later recorded. This includes wetlands following the habitat design standards addressed in Table 2 above, which was published in 2017 and guided the creation of over 80 new wetlands in the Melbourne Strategic Assessment (MSA) area.

Table 5. Response to measures under the significant impact guidelines

Significant Impact Guidelines Measure		Response
Avoiding impacts	▪ Retain habitat known or likely to contain the growling grass frog and manage for the species. ▪ Retain terrestrial habitat and dispersal corridors: ▫ Maintain dedicated terrestrial habitat corridors, of a minimum of 100 m in width. ▫ Maintain existing hydrological regimes.	Impacts to habitat within three farm dams on site is unavoidable, as discussed in Appendix 2. To mitigate this impact, a dedicated Growling Grass Frog wetland of 0.3 hectares that will meet the Growling Grass Frog Habitat Design Standards will be designed as part of the proposed action. A dedicated terrestrial buffer zone of 50 metres that meets the Growling Grass Frog Habitat Design Standards will also be designed as part of the proposed action. A terrestrial habitat corridor through the site is not required in the design. Growling Grass Frog will be able to disperse from Bunyip River and associated scrub vegetation to the constructed habitat. The hydrological regime of the proposed action and water quality is described in Section 2.4 below.
Minimising impacts	▪ Maintain existing management regime if the site currently supports a breeding population (for example current grazing intensity). ▪ Maintain existing water quality.	See response above.
Managing habitat	▪ Enhance habitat quality: ▫ Carefully remove weeds and replace with Indigenous submergent, floating and emergent vegetation in and around water bodies. In weedy areas that support Growling Grass Frogs, weeds need to be gradually removed and replaced by natives. Any drastic and sudden removal of weeds in areas supporting Growling Grass Frogs is likely to	Weed removal in existing habitat is not applicable, as there will be a new constructed wetland. Weed invasion will be managed as discussed in Section 3.6 below. The remaining issues are addressed through complying with the Growling Grass Frog Habitat Design Standards for the constructed Growling Grass Frog habitat, as discussed in Table 2 above.

	have a negative effect on the species. ▫ Maintain open (unvegetated) areas within water bodies, potentially by increasing water depth in some sections. ▫ Remove or manage exotic fish (for example mosquitofish, carp and redfin). If required, drainage of water bodies to eliminate fish should occur during times of the year when there are few or no tadpoles present. ▫ Improve terrestrial habitat through provision of logs, rocks and riparian vegetation etc., to provide a diversity of overwintering habitat. ▫ Manage terrestrial weeds (manually, and without chemicals).	

2.3. Southern Brown Bandicoot Habitat Buffer

Purpose of the habitat buffer

The proposed action will include a **30-metre-wide** habitat buffer for Southern Brown Bandicoot (also called the 'Bandicoot Corridor' on the development plan), which will adjoin the existing swamp scrub habitat present along the northern boundary of the site. This habitat buffer will be **2.276 hectares** in size and provide for separation between the proposed residential development (roads and houses) and the existing swamp scrub habitat.

The habitat buffer will comprise a native vegetation buffer and help to:

- Provide a natural buffer to water quality impacts to the swamp scrub from runoff inside the site, e.g. through increased infiltration
- Reduce edge effects on the swamp scrub by intercepting wind, light and noise, thereby reducing disturbance to wildlife including bandicoot inside the swamp scrub
- Provide some additional habitat for wildlife while increasing the visual amenity of the site.

The development plan has undergone multiple iterations to maximise outcomes for the local population of Southern Brown Bandicoot, following discussions with DCCEEW and the Council. The current development plan has taken into account not only the swamp scrub habitat directly north of the site but also habitats within the adjacent Koo Wee Rup Regional Health land to the southeast and Cochrane Park vegetation to the east. The current development plan supports connectivity between the swamp scrub habitat and these other known habitats, as discussed with Council.

Bushfire requirements

The proposed habitat buffer for Southern Brown Bandicoot must also be consistent with the bushfire requirements for the site. The habitat buffer will be directly adjacent to swamp scrub to the north, a public park (Cochranes Park) to the east, the drainage reserve to the southwest and a 14.5-metre-wide road with the residential lots beyond.

As such, revegetation for Southern Brown Bandicoot habitat will be restricted to planting of scrub and grassland vegetation. In accordance with the *Australian Standards AS3959:2018*, the defendable space width for scrub vegetation on a flat landscape is a minimum of 27 metres.

Therefore, given 27 metres of defendable space for the residential lots, including the road adjacent to the habitat buffer, the following is recommended:

- The southern part of the long area of habitat buffer (12.5 metres wide) is to have revegetation works for native grassland. Limited shrub planting can be included if desired, i.e. 5 metre-square clumps spaced 5 metres apart according to bushfire requirements.
- The northern part of the long area of habitat buffer (17.5 metres wide) is to have revegetation of swamp scrub.

There is also a section of the habitat buffer that wraps around the east side of the proposed public open space in the northeastern corner, then along the rear of some proposed residential lots.

- This part of the habitat buffer is to have revegetation works for native grassland. Limited shrub planting can be included if desired, i.e. 5 metre-square clumps spaced 5 metres apart according to bushfire requirements.

Note that Nature Advisory is not providing advice about bushfire requirements. The recommendations above are based on our understanding of applying *AS3959:2018* and should be confirmed with a bushfire consultant prior to commencement of the revegetation works.

Measures under the draft referral guidelines for Southern Brown Bandicoot

The following threats considered and measures by the proposed action are informed by Table 2 of the *Environment Protection and Biodiversity Conservation Act 1999 draft referral guidelines for the endangered southern brown bandicoot (eastern), Isoodon obesulus obesulus* (DSEWPC 2011).

Table 6. Threats in the draft referral guidelines and response measures

Consecutive Timeline	Works
Predation	Pest animal management and monitoring is addressed in Section 3.8 below, including domestic pet control and pest animal monitoring.
Habitat loss, fragmentation and isolation	There is no suitable vegetation type in the site that provides core habitat for Southern Brown Bandicoot (i.e. heathland, shrubland, sedgeland, heathy open forest and woodland). The proposed action would not isolate or fragment existing Southern Brown Bandicoot habitat. The proposed action will establish a habitat buffer that will contain new suitable habitat and extend the existing swamp scrub habitat that is north of the site, while at the same time providing separation between the proposed residential area and the habitat north of the site. Furthermore, the proposed action supports the retention of secondary habitat inside the site and support connectivity between the known habitats for Southern Brown Bandicoot that surround the site, particularly to the north, east and southeast. (See Section 2.3).
Inappropriate fire regime	Appropriate fire management measures for the site will be implemented as part of planning permit requirements (see Section 3.7)

Habitat degradation (e.g. grazing, changes in urban or agricultural run-off and rubbish dumping)	No existing Southern Brown Bandicoot habitat in the site or to the north of the site will be degraded by the proposed action. The creation and maintenance of the dedicated habitat buffer will improve habitat quality in the site as well as reduce edge effects in the existing swamp scrub habitat north of the site.
Broad scale removal of important exotic habitat	Addressed through staged weed management and revegetation in the Southern Brown Bandicoot habitat buffer (see Section 3.6 and Section 3.5).
Roadside mortality	Installation of road signage to encourage driver awareness of Bandicoot presence and an active Bandicoot wildlife crossing.
Fencing	The draft referral guidelines provide for use of fencing on a 'case by case basis' including predator exclusion fencing. The possibility of using predator exclusion fencing was discussed with DCCEEW and Council, particularly as Council does not support permanent perimeter fencing around the new Growling Grass Frog habitat and Southern Brown Bandicoot habitat buffer (due to traffic and bushfire mitigation scenarios). As a result, permanent perimeter fencing around new habitats is not included in the plans. Section 3.3 below addressed construction fencing for the project. It should also be noted that fences for lots fronting the Southern Brown Bandicoot habitat buffer will have 90mm holes for bandicoot egress.

2.4. Hydrology and stormwater management

As discussed in Section 1.3 above, the project is not yet at the detailed design stage, however the parameters of the detailed designs, stormwater plan and CEMP will be consistent with the Growling Grass Frog Habitat Design Standards. The drainage reserve must be maintained to the Melbourne Water standards.

The proposed action will include the following:

- The new wetland for Growling Grass Frog will be fed by rainfall and surface runoff inside the Growling Grass Frog habitat. The relevant requirements in the Growling Grass Frog Habitat Design Standards will form part of the parameters for the detailed wetland design, as per Table 2 above.
- We understand the drainage reserve will be designed to be hydrologically independent of the Growling Grass Frog habitat; the subdivision will have underground pipes to direct stormwater and surface runoff to the drainage reserve for treatment.

The proposed action will need to satisfy the responsible authority (Cardinia Shire Council and Melbourne Water) regarding:

- Stormwater treatment; and
- Long-term maintenance of the dedicated frog wetland and the drainage reserve.

3. Environmental Management Measures for Constructed Habitats

Environmental management measures for the constructed Growling Grass Frog habitat and Southern Brown Bandicoot habitat buffer must be incorporated into the detailed design plans and the CEMP.

3.1. Construction management protocols

The Construction Contractor must include these protocols in the CEMP.

Site Inductions and environmental training

Induction and training will be an important element of the CEMP and must be tailored to the role of the contractor and personnel to ensure they understand their responsibilities.

All construction and site personnel will be inducted regarding the requirements of this EMP.

Records of all training conducted must be maintained and include the person's name, date the training was received, the trainer's name and a summary of the training provided.

General construction work protocols

- The site currently comprises pasture for grazing land uses. Upon commencement of the works, no livestock access into the site will be permitted.
- The following will be prohibited within the Growling Grass Frog habitat and the Southern Brown bandicoot habitat buffer:
    - Storage or dumping of any soil and other materials, equipment, vehicles, machinery or waste products.
- Any Growling Grass Frog observed in construction areas during construction works must not be handled by unauthorised or unlicensed personnel. If any Growling Grass Frogs are observed in construction areas, the project zoologist or ecologist must be immediately contacted so that appropriate salvage and relocation measures can be undertaken. All construction activities must cease until a large bucket or plastic box (or similar) has been placed securely over the frog (place with care to avoid injury to the frog). The location of the secured frog must be fenced temporarily with para-webbing or a similar material.

Hygiene controls to prevent the spread of Chytrid fungus

There is a risk of spreading Chytrid fungus into and around the site, which can significantly impact on frogs including Growling Grass Frog. Hygiene protocols to avoid this are to be included in the CEMP.

The *Hygiene Protocol for the Control of Diseases in Australian Frogs* (Murray et.al. 2011) is provided in Appendix 5 to provide guidance on best practice measures to manage Chytrid fungus.

- All footwear, equipment and vehicles must be cleaned and disinfected prior to entering or exiting the site.
- All people entering and exiting the site must use a boot wash station to disinfect their footwear.
- Tyres of all vehicles must be cleaned and disinfected before entering and exiting the new wetland habitat.

Note the above must be carried out at a safe distance from water bodies, so that the disinfecting solution can infiltrate soil rather than runoff into a nearby water body. Spraying with 'toilet duck' (active ingredient benzalkonium chloride) is recommended to disinfect car wheels and tyres. Cleaning of footwear before getting back into the car must be conducted to prevent the transfer of pathogens from/to vehicle floor and control pedals.

Frog salvage and relocation protocol

Preclearance survey prior to draining and removal of the existing farm dams must be undertaken by a qualified zoologist.

Salvage/translocation must be undertaken in accordance with specifications contained within a Management Authorisation under the Victorian *Wildlife Act 1975,* which must be obtained from DEECA prior to commencement of the salvage operation.

The salvage and translocation operation is to include:

- Any Growling Grass Frog identified during the survey will be immediately salvaged and relocated to a suitable receiving site nearby, including suitable micro-habitats such as areas containing rocks or dense vegetation.
- Latex surgical gloves must be worn when handling frogs.
- Captured frogs will be transported in disinfected plastic containers, with one frog located in each container to minimise potential disease transmission.
- Any visibly sick or injured frogs will not be relocated and will be transported to a registered veterinarian.
- Footwear will be washed in disinfectant at the beginning and end of each salvage period to prevent the introduction or spread of disease, particularly Chytrid fungus.
- A report to DEECA will be prepared under the terms of the permit for relocation which will include information on the body size and sex of relocated frogs.
- During salvage/translocation works, any incidentally captured fauna such as other frog species, reptiles or small mammals must also be removed from harm. Any other person assisting in relocation works must work under the close supervision of the individual(s) listed on the permit.

Draining or dewatering any aquatic habitats

During draining or dewatering of any aquatic habitats on site (i.e. the existing farm dams or the new frog wetland and new drainage reserve), appropriate filter systems will be required to prevent fauna (e.g. frogs, tadpoles and fish) from being sucked into the pump.

3.2. Staging of works

There is a need for a staged approach to development of the site given:

- Any frogs in the dam near the centre of the site can be salvaged and translocated to the southwestern dams prior to removal of the central dam. Due to its location, the protection of this dam and dispersal access for the frogs during construction of the new habitat areas is unlikely to be feasible. The central dam was considered to have the lowest quality of aquatic habitat for Growling Grass Frog of the existing dams and the species was not detected in this dam during targeted surveys.

- The existing habitat for Growling Grass Frog in the southwestern dams must be protected until the new habitat is established, as well as dispersal ability for frogs to the dams.
- The new habitat for Growling Grass Frog must be established to the satisfaction of and endorsed by the regulatory authority (Melbourne Water), prior to the removal of the southwestern dams.

The details for sequencing of works will be included in the CEMP, which must be consistent with the following strategy:

1. The two southwestern dams on site will be left *in situ*, with temporary construction fencing with frog-proof sediment fencing installed around them, until the new habitat for Growling Grass Frog is constructed. The dam near the centre of the site is to be removed, including pre-clearance survey and salvage translocation of native animals to the new constructed habitat by an experienced zoologist or ecologist.
2. Revegetation works for the Growling Grass Frog habitats and Southern Brown Bandicoot under the detailed landscaping/revegetation plan will be consistent with this EMP. Temporary construction fencing with frog-proof sediment fencing will be installed around the Growling Grass Frog habitats and Southern Brown Bandicoot.
3. When the new Growling Grass Frog habitats including the wetland and terrestrial 50 metre buffer sufficiently meet the Growling Grass Frog Habitat Design Standards, then works to remove the southwestern dams can commence (and the temporary fencing around them can be removed). The new Growling Grass Frog habitat will be endorsed by an ecological consultant and/or the regulatory authority to be considered established. Pre-clearance survey and salvage translocation of native animals by an experienced zoologist or ecologist must be undertaken (including translocation of Growling Grass Frogs to the new constructed habitat).
4. Frog-proof sediment fencing will be removed from the new habitats after the development of the site is completed.

3.3. Temporary construction fencing (including frog-proof sediment fencing)

Fencing is to be installed to prevent potential construction-related impacts (e.g. accidental damage by vehicles and unauthorised dumping) and to clearly define the boundaries of the constructed habitats for management purposes.

- Council does not support permanent fencing around the habitat areas for Growling Grass Frog and Southern Brown Bandicoot. This is primarily due to concerns regarding cost of ongoing maintenance of fencing, traffic, and bushfire mitigation scenarios. Therefore, such fencing is not part of the plans.
- The temporary construction fencing must be installed around the two southwestern dams prior to commencement of works on the site, to protect them until they can be removed.
    - In conjunction with this, frog-proof sediment fencing will be installed to help avoid and minimise run-off and sedimentation into the existing frog habitats.
    - The temporary fencing with frog-proof sediment fencing cannot prevent access of frogs to the two southwestern dams. It is proposed that the fencing will extend to the site's western boundary and there will not be fencing along the site's western boundary, so that frogs in the western property can move to the dams. The exact

placement of the fencing is to be confirmed in the CEMP, in consultation with the relevant authority.

- The temporary construction fencing must also be installed around the perimeter of the new Growling Grass Frog habitat and the perimeter of the Southern Brown bandicoot habitat buffer.
    - In conjunction with this, frog-proof sediment fencing will be installed to exclude frogs from entering the new habitat areas while they are under construction.
    - Frog-proof sediment fencing is to remain in place until construction of the development is complete to control run-off and sedimentation in the constructed habitats.

Temporary construction fencing

Temporary construction fencing details are presented below, as per the Victorian *DELWP requirements for Construction Environmental Management Plans under the Melbourne Strategic Assessment* (DELWP 2020):

- Posts are vertical steel pipes to a height of 1.8 metres, driven 0.7 metres into the ground at 3 metre intervals.
- Chain link or welded mesh fencing affixed to posts.

The fencing will have 'Conservation Area – NO GO ZONE' signs affixed at 15-metre intervals and at a height of 1.5 metres.

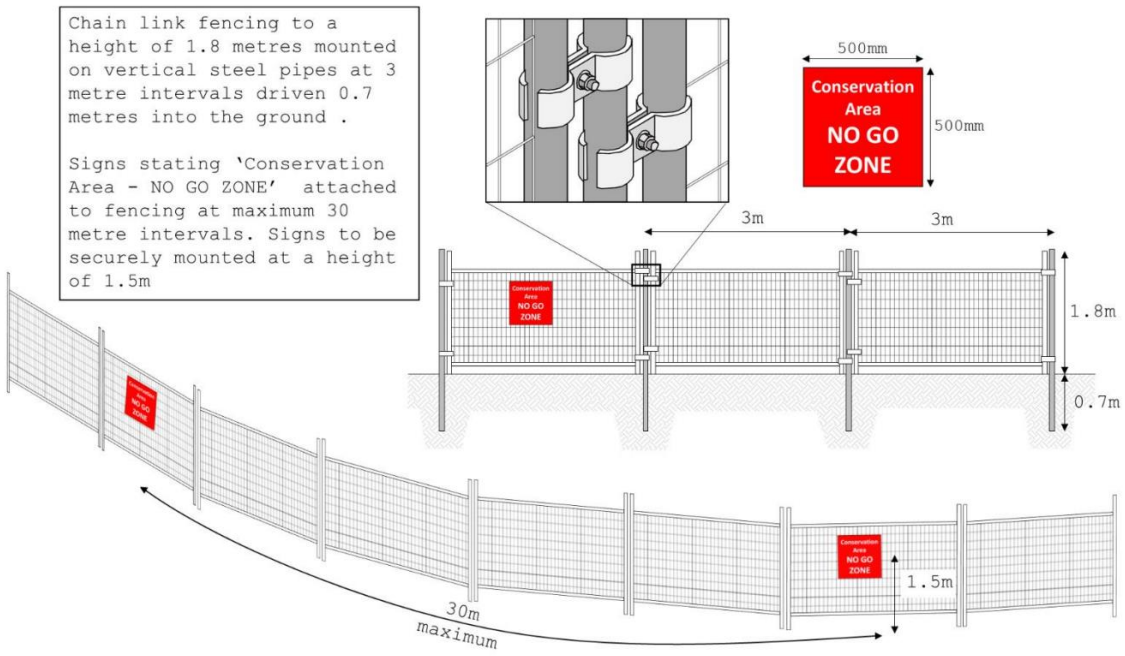


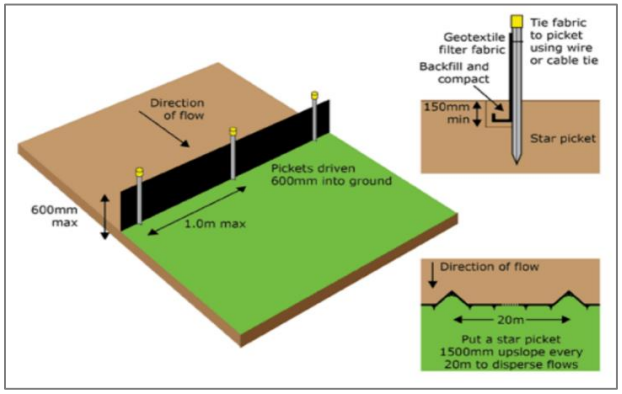
Figure 2. Temporary exclusion fencing

Sediment fencing that is frog-proof

Sediment fencing is required to be installed around the constructed Growling Grass Frog habitat and Southern Brown bandicoot habitat buffer, in conjunction with the temporary fencing for the duration of construction of the entire site.

The following measures must be undertaken to ensure that erosion is limited and indirect impacts to these aquatic environments are avoided:

- All earthworks on site must be undertaken in a manner that will minimise soil erosion and adhere to *Construction Techniques for Sediment Pollution Control* (EPA 1991).
- Water runoff from the construction site must be diverted to avoid the runoff from entering the drainage line. Sediment fencing must be installed to minimise the potential impact of water runoff into the wetlands. EPA construction guidelines are provided in the figure below (EPA Victoria 2004).



Source: EPA Victoria 2004

Source: https://www.advancedns.com.au/blog/why-compliant-silt-fence-is-important

Figure 3. EPA guidelines for sediment fencing construction

In order to be frog-proof and stop frogs from entering the site while construction works are ongoing, the sediment fencing must be:

- At least 1m high and made of silt fence material;
- Dug or pegged in so that frogs cannot move under the fence;
- Kept tight to avoid sagging; and
- Tall vegetation within 1m either side of the fence must be trimmed to prevent frogs using the vegetation to jump over the top of the fence.

3.4. Signage

The new habitat for Growling Grass Frog and the Southern Brown Bandicoot habitat buffer must be appropriately signed to prevent unauthorised access and activities in those areas. This signage must explain the purpose and importance of the new habitats to the general public, as well as identify activities which may pose a risk to Growling Grass Frog and Southern Brown Bandicoot (e.g. illegal rubbish dumping and free-roaming cats and dogs).

The Cardinia Shire Council is to approve all signage prior to installation.

3.5. Vegetation management

Revegetation of Growling Grass Frog habitat

After the wetland basin has been constructed and the site is prepared for planting, the revegetation works for the Growling Grass Frog habitat must be in accordance with Growling Grass Frog Habitat

Design Standards; the standards that are specific to the revegetation works are highlighted in green in Table 2 above.

This includes the standard that the constructed wetlands must incorporate the emergent, submergent and floating species found in Appendix 1 of Growling Grass Frog Habitat Design Standards.

A 50-metre-wide buffer of open grassland around the wetland will be established as part of the constructed Growling Grass Frog habitat. Limited tree and shrub cover is permitted under the standards regarding terrestrial habitat. These standards states that the grassy vegetation need not be native vegetation but at the same time, invasive species must not be used. Therefore, revegetation of terrestrial habitat can utilise a mix of native grass species and exotic grass species with low invasive potential, given that this vegetation can be established more rapidly and maintained more effectively than native grassland. It will also contribute to the exclusion of high threat weed species, given less bare ground will be available for their germination.

In addition, Growling Grass Frog Habitat Design Standards relating to thermal properties identify that the wetland should incorporate jumbled piles of rocks around at least 20 percent of the margin of the wetland, extending into the wetland at least one metre from normal water level. This must be undertaken as part of the revegetation works.

Revegetation of Southern Brown Bandicoot habitat buffer

Revegetation works in the Southern Brown Bandicoot habitat buffer will comprise indigenous species. Southern Brown Bandicoot typically inhabit areas with a dense understorey.

As discussed in Section 2.3, the northern part of the long length of habitat buffer (17.5 metres wide) will be revegetated to reflect swamp scrub. The remainder will be revegetated to reflect native grassland, where limited shrub planting (maximum 5 metre-square clumps spaced 5 metres apart) according to bushfire requirements may be included at the Contractor's discretion.

- The swamp scrub component will reflect the existing dense swamp scrub habitat currently present to the north of the site. The swamp scrub to the north of the site was mainly composed of a thick bush of Swamp Paperbark and intercepted with few mature eucalypts, scattered wattles and large pine trees. The understorey was also densely covered by younger Swamp Paperbark and other shrubs (with exotic grasses and dense growth of Blackberry).
- The native grassland component will comprise plantings of native grasses.

Supplementary planting of native tubestock will be undertaken in a staged manner to maintain the overall vegetation cover while replacing weeds. Weeds including pasture grasses are not to be significantly reduced within the habitat buffer, as this will leave an absence of vegetation cover and increase risk of erosion/sedimentation.

- Planting will commence early for suitable habitat structure to develop prior to significant weed removal. The locations of the tubestock planting may require initial weed removal, to prevent existing weeds from outcompeting the plantings.
- Weed control will occur after a suitable cover of native plants has become established.

3.6. Weed management

Victoria's *Catchment and Land Protection Act 1994* (CaLP Act) requires that landowners (or a third party to whom responsibilities have been legally transferred) must manage noxious weeds, i.e. eradicate regionally prohibited weeds and prevent the growth and spread of regionally controlled

weeds. Property owners who do not eradicate regionally prohibited weeds or prevent the growth and spread of regionally controlled weeds for which they are responsible, may be issued with a Land Management Notice or Directions Notice that requires specific control work to be undertaken.

The noxious weed species previously recorded on site included the following:

- Spear Thistle (Regionally Controlled);
- Hawthorn (Regionally Controlled);
- African Box-thorn (Regionally Controlled);
- Blackberry (Regionally Controlled);
- Soursob (Regionally Restricted);
- Sweet Briar (Regionally Controlled); and
- Wild Watsonia (Regionally Controlled).

The proposed action will mean that most weeds on site will be removed initially with physical removal during vegetation clearing and site preparation for development, with the exception of the Southern Brown Bandicoot habitat buffer which will remain in situ.

A suitably qualified contractor with experience in controlling weeds in the region will be engaged by the Construction Contractor to manage weeds across the whole site and more sensitively within the new Growling Grass Frog habitat and Southern Brown Bandicoot buffer:

- In the replacement habitat for Growling Grass Frog, weed control will be limited to manual removal and hand spot-spraying to avoid off-target impacts to plantings. Herbicide use in the new frog habitat must be minimised as much as possible to avoid adverse impacts on the species. Where herbicide use is necessary, waterway sensitive products (e.g. Roundup Bioactive Herbicide) is to be used.
- In the habitat buffer for Southern Brown Bandicoot, weed control will occur in conjunction with revegetation works to establish indigenous species.
- Throughout the remainder of the property where native vegetation will not be retained, weed control methods may include boom-spraying and other methods that are more time-efficient.

Prior to the commencement of works, the sub-contractor responsible for weed management must undertake baseline surveys to identify the high threat weed species and each of their covers (%) for the purpose of monitoring.

Regular monitoring for weeds in the new habitat areas will help to assess whether the current weed management regime will achieve the weed-related management objectives and inform further weed management actions.

3.7. Bushfire and biomass control in terrestrial habitats

The planning permit for the proposed action will require the development to implement the appropriate bushfire risk mitigation measures in order to ensure that the development does not materially increase the bushfire risk to the community.

A Bushfire Assessment Report (Nexus Planning 2022) has been submitted to the Cardinia Shire Council for their consideration. This report contained the bushfire hazard assessment for the site, with a response to the bushfire planning policy framework and provisions of the Cardinia Planning Scheme.

The development has been designed to incorporate the required setbacks from surrounding vegetation. As discussed in Section 2.3 above, the types of vegetation that will be established in the Southern Brown Bandicoot habitat buffer will be consistent with the site requirements for defendable space. Regular slashing in the habitat buffer will be used to manage biomass in the grassland.

Because Growling Grass Frog favours low, grassy vegetation in its immediate terrestrial habitat, the 50-metre terrestrial buffer of open grassland for Growling Grass Frog must be maintained to keep the grass short (up to 10cm in height), consistent with the Growling Grass Frog Habitat Design Standards.

3.8. Feral and domestic animal control

Permanent avoidance and mitigation measures include:

- Bans on cats as pets in the residences (or else limited to indoors and outdoor cat runs). Alternatively, a cat curfew will be implemented for the neighbourhood, to ensure that pet cats are kept indoors after dark, from dusk to dawn. These measures are aimed to avoid or minimise predation on Growling Grass Frog and Southern Brown Bandicoot by domestic cats. (This will need to be facilitated during the planning application process and discussions with Cardinia Shire Council. Cat bans or curfews may form a restriction on title.)
- The drainage reserve will be designated as 'dog on leash only' areas with signage to be installed at appropriate locations. Dogs will not be allowed in the constructed habitats. The Cardinia Shire Council will need to enforce this.
- Revegetation works in the Southern Brown Bandicoot habitat buffer will include revegetation for dense swamp scrub. Shrubs provide habitat to hide from predators and continuous scrub or shrublands provide relatively safe environments for Southern Brown Bandicoot to forage and avoid predation.

Feral predator control measures are required to be undertaken in the constructed habitats for Growling Grass Frog and Southern Brown Bandicoot:

- Regular monitoring for pest animals in the new habitat areas, to determine the need for pest animal control, including any signs of pest animals (such as scats and diggings).
- All pest animals listed under the CaLP Act that are identified in the new habitat areas must be controlled, with abundance, activity, and disturbance reduced to negligible levels (including no active rabbit warrens and fox dens).
- If rabbit or fox activity is detected on site, control must be undertaken. An integrated approach involving fumigation, hand collapsing of burrows and baiting (using safe bait for native animals and remove any carcasses to prevent poisoning of native predators/scavengers) may be necessary.

3.9. Predatory fish control

The dedicated Growling Grass Frog wetland is required to be kept fish-free, especially during the breeding season. However, it is possible that introduction of pest fish during flood events or even human introduction of fish into the wetland by members of the public could occur.

As discussed in Table 4 above, ability to address incursion of introduced fish is a required parameter for the detailed wetland design for the dedicated frog wetland. Fish exclusion devices (for example gravel and sand filters) must be used if the main water source is from stormwater

treatment wetlands, as is the case here. The dedicated frog wetland must be designed to allow it to be periodically dried out if needed for management and maintenance purposes (such as the control of predatory fish).

It is important to note that, as mentioned design standards, Growling Grass Frogs are known to inhabit and breed in a number of wetlands around Melbourne containing populations of Eastern Gambusia, so the predators' presence does not necessarily preclude the persistence of Growling Grass Frogs.

Regular monitoring will include monitoring for predatory fish in the Growling Grass Frog wetland, in particular Carp and Eastern Gambusia which feed on the frog eggs and tadpoles. If identified during monitoring, adaptive management action will be taken to address and remove this threat to Growling Grass Frog in the wetland.

3.10. Monitoring and reporting

Monitoring Commitments

Quarterly monitoring by a qualified ecologist and/or aquatic ecologist is required in Year 1 and Year 2, starting 3 months after commencement of works, and annually from Year 3.

At a minimum, the following variables will recorded during the monitoring program:

- Cover of native vegetation and listed of planted species in the new Growling Grass Frog wetland and in the terrestrial vegetation in the 50-metre terrestrial buffer;
- Cover of weeds in the new Growling Grass Frog wetland and in the terrestrial vegetation in the 50-metre terrestrial buffer, and the high-threat weed species present;
- Cover of rock piles around the edges of the new Growling Grass Frog wetland;
- Presence of predatory fish in the new Growling Grass Frog wetland;
- Water quality parameters for the new Growling Grass Frog wetland (including water level, turbidity, pH, temperature, salinity and dissolved oxygen);
- Cover of native vegetation and list of planted species in the Southern Brown Bandicoot habitat buffer;
- Cover of weeds in the Southern Brown Bandicoot habitat buffer, and the high-threat weed species present;
- Integrity of temporary fencing;
- Presence of rubbish in the new habitat areas;
- Sightings or signs of feral pest animals in the site;
- Any additional comments about the condition of the new habitat areas if appropriate; and
- Photos taken at photo monitoring points (at least one showing the Growling Grass Frog wetland, one for the 50-metre terrestrial buffer and one for the Southern Brown Bandicoot habitat buffer).

Reporting Commitments

The monitoring report will include the findings for the variables listed in Section 3.10, as well as:

- A summary of works completed;

- Progress of the revegetation works;
- Progress against the objectives in this EMP; and
- Recommendations for future management works in the site.

The monitoring report must be provided to the responsible authority within 2 months of the monitoring survey.

3.11. Adaptive management

An adaptive management approach will mean addressing new challenges that arise during management of MNES on site, guided by regular monitoring and sometimes necessitated by stochastic events.

This section does not aim to provide an exhaustive list of potential issues that may arise and their solutions, however the key issues and contingency measures are outlined below.

Monitoring of Growling Grass Frog and Southern Brown Bandicoot on site shows decline or absence at the constructed habitat for the species - adaptive response measures will include:

- Identification of potential reasons why, e.g. presence of predatory fish or unsuitable water quality.
- Response to the main potential reasons, e.g. draining the wetland to remove predatory fish or remediation of the water quality issue.

New weed invasion in the new habitats for Growling Grass Frog and Southern Brown Bandicoot – adaptive response measures will include:

- Identification of the weed during monitoring surveys and immediate adjustment to routine weed management works to include the new weed as a target species, e.g. targeted spraying or manual removal

Flooding event causes predatory fish invasion into the Growling Grass Frog – adaptive response measures will include:

- Draining the wetland.
- Provision of dense submerged and floating native plants in the wetland to provide refugia and increase survival rates by tadpoles.

Rubbish dumping in the new habitat areas – adaptive response measures will include:

- Identification of rubbish present during monitoring surveys (or routine maintenance works) and removal of the rubbish as soon as possible.

3.12. Procedures for managing environmental emergencies

In the unlikely event of an environmental emergency, there must be a procedure to follow to reduce impacts to the environment. The procedure will depend on the type of emergency and its impacts and extent.

Possible emergencies may include flooding, severe storm damage, bushfire, pollution from a spill or leak, spread of disease or pathogens.

The environmental emergency procedure must include activities to reduce the impact of the emergency such as engagement of emergency organisations, replacement of fences, rehabilitation

of vegetation, a review of this EMP, monitoring and reporting of the incident and its impacts and notification to the Minister's office.

Key personnel are required to be identified to monitor and manage the environmental emergency. These key personnel have the power to stop work on the site to manage any environmental emergencies effectively. The personal will include the proponents of the development and a lead from the construction contractor, as well as relevant public bodies. The CEMP must include all relevant emergency contact details for public bodies.

3.13. Review of this plan

This EMP must be reviewed annually, after the 4th quarterly monitoring in Year 1 and Year 2, then after the annual monitoring from Year 3 onward.

This EMP must be amended if the review finds that components of the plan require updating.

4. References

Commonwealth of Australia 2014, *Environmental Management Plan Guidelines*, Australian Government, Department of the Environment.

Department of the Environment (DoE) 2013, *Matters of National Environmental Significance*, Significant impact guidelines 1.1 *Environment Protection and Biodiversity Conservation Act 1999*, Commonwealth of Australia.

Department of Environment, Land, Water and Planning (DELWP) 2020, *DELWP requirements for Construction Environmental Management Plans under the Melbourne Strategic Assessment*, DELWP Victoria.

Environment Protection Authority (EPA) 1991, *Construction Techniques for Sediment Pollution Control*, EPA Victoria.

Murray et.al. 2011, *Hygiene Protocol for the Control of Diseases in Australian* Frogs, Prepared for the Australian Government Department of Sustainability, Environment, Water, Population and Communities.

Nature Advisory 2024, *145 Rossiter Road, Koo-Wee-Rup Matters of National Environmental Significance Assessment Report*, Prepared for Rossiter Road Investments Pty Ltd.

Nexus Planning 2022, *Bushfire Assessment Report*, Prepared for Rossiter Road Investments Pty Ltd.

Practical Ecology 2022, *145 Rossiter Road, Koo-Wee-Rup, Targeted Growling Grass Frog Survey*, Prepared for KLM Spatial.

Appendix 1. Proposed development layout plans

CONCEPT SKETCH 21

145 Rossiter Road Koo Wee Rup

LEGEND:

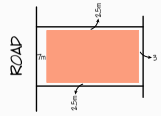
- 17 (20%) LOTS 600m² - 699m²
- 55 (64%) LOTS 700m² - 999m²
- 14 (16%) LOTS 1000m² & ABOVE
- WATER RETENTION
- VEGETATION PROTECTION RESERVE
- PRECINCT BOUNDARY
- LOT BOUNDARY
- 50m GAF WORKS BUFFER
- PEDESTRIAN ACCESS
- 6.1m PAVED ROAD (LIFESTYLE VILLAGE)
- 4.5m PAVED ACCESS (LIFESTYLE VILLAGE)
- COMMUNITY CENTRE
- BANDICOOT CORRIDOR
- PUBLIC OPEN SPACE
- LANDSCAPE BUFFER
- GROWLING GRASS FROG HABITAT
- DEFENDABLE SPACE
- 500m OPEN SPACE WALKABILITY
- SHARED PATH
- 1.8m FOOTPATH
- 1.5m LIFESTYLE VILLAGE PATH
- EXISTING DRAINAGE ALIGNMENT

PARCEL DETAILS

TOTAL SITE AREA:	24.37ha	SALEABLE LAND:	6.634ha
ROAD RESERVES:	3.098ha	N.D.A.:	9.732ha
VEG. PROTECTION RESERVE:	312.9m²	LOT YIELD:	86
LANDSCAPE BUFFER:	566m²	AVERAGE LOT SIZE:	771m²
BANDICOOT CORRIDOR:	2.276ha	VILLAGE SITE AREA:	7.419ha
GROWLING GRASS FROG HABITAT:	1.845ha	VILLAGE YIELD:	165
DRAINAGE RESERVE:	1.418ha		
PUBLIC / OPEN SPACE:	8000m²		

RESIDENTIAL NOTES:

ONLY ONE CROSSOVER PER LOT.
FRONT FENCES TO BE NO MORE THAN 1.2m IN HEIGHT.
FENCES ON SECONDARY ROAD FRONTAGE OR WITH SIDE BOUNDARY ADJACENT TO OPEN SPACE TO BE 1.8m TALL FOR NO MORE THAN 50% OF BOUNDARY LENGTH, BALANCE OF FENCE TO BE LESS THAN 1.2m, AS PER TYPICAL DESIGN GUIDELINES APPLIED BY MCP.
ACCESS FOR LOT 2 ON PS821029K AND ADJACENT PUBLIC OPEN SPACE TO BE DELIVERED AS PART OF DEVELOPMENT.



INDICATIVE BUILDING ENVELOPE APPLIES TO LOTS 1 TO 86.

VILLAGE NOTES:

WHERE THE VILLAGE ABUTS ROADS AND RESIDENTIAL LOTS SETBACKS ARE AS FOLLOWS: FRONT SETBACK TO BE 3m, SIDE SETBACK TO BE 1.2m AND REAR SETBACKS RANGE FROM 3m TO 5m, GARAGES TO BE SETBACK 5m.
TYPICAL 1.8m COLOURBOND FENCING BETWEEN DWELLINGS.
UNITS ADJACENT BANDICOOT CORRIDOR WILL BE DESIGNED TO PROVIDE PASSIVE SURVEILLANCE OF ADJACENT OPEN SPACE, ALSO INCLUDING LOW PERMEABLE BOUNDARY FENCING.
TOTAL BUILDING SITE COVERAGE FOR THE VILLAGE IS 32.8% (INDICATIVE).
PRIVATE OPEN SPACE 20m² (INDICATIVE) SUBJECT TO DWELLING DESIGN.
★ - OPPORTUNITY FOR OPEN SPACE WITHIN THE DEVELOPMENT.

- 58 TYPE A UNITS (12m X 19m) 62% SITE COVERAGE (INDICATIVE)
- 60 TYPE B UNITS (12m X 19m) 68% SITE COVERAGE (INDICATIVE)
- 47 TYPE C UNITS (12m X 19m) 64% SITE COVERAGE (INDICATIVE)

*TYPICAL DESIGN ONLY - ARCHITECTURAL PLANS TO BE PROVIDED IN DUE COURSE

TITLE INFORMATION SHOWN HEREON IS BASED ON KLM SPATIALS RE-ESTABLISHMENT SEE DRAWING 10323 REG VI DATED 17-05-2021.

PROPOSED DEVELOPMENT LAYOUT SUBJECT TO FURTHER INVESTIGATION ON SITE CONSTRAINTS RELATING TO ENVIRONMENTAL AND SERVICEABILITY. KLM SPATIAL CAN THEREFORE ACCEPT NO RESPONSIBILITY FOR RELIANCE ON THIS PLAN FOR ANY FINANCIAL DEALINGS INVOLVING THE LAND.

Nearmap PHOTOMAP FLOWN: 05/05/2024

30 0 30 60 90 120 150

1:3000 @ A3 Sheet 1 of 1

REF: 10323 CS21 Version: 1 Date: 06-11-2024

KLM SPATIAL Land Development Intelligence®

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Appendix 2. Additional information regarding Growling Grass Frog impacts

Need to remove and replace Growling Grass Frog habitat

The *Significant impact guidelines for the vulnerable growling grass frog (Litoria raniformis)* (DEWHA 2009) does not define core habitat or key habitat for the Growling Grass Frog.

Targeted surveys for Growling Grass Frog were undertaken and an assessment of habitat provided by Practical Ecology (2022). The targeted surveys for Growling Grass Frog on site detected the species at only one of the existing dams, being Dam 2. Dam 1 and Dam 2 were deemed to have the most suitable habitat for this species, while Dam 3 was deemed to lack adequate sheltering/basking structures while having less variegated vegetation structure.

Habitat for the species includes:

- Aquatic habitat – occurs in three farm dams on site, which have a total combined area of 0.17 hectares. The targeted surveys detected the species at one dam. The targeted survey considered that these dams may be used for breeding, however, the dams are likely to be ephemeral and so not permanent breeding habitat. Dam 1 and 2 were visited in May 2024 and found to be dry.
- Breeding habitat – unlikely that the farm dams have water permanence and the species is dependent on areas of permanent water for breeding, such as the shallow part of freshwater lagoons.
- Terrestrial habitat for dispersal between waterbodies, foraging, shelter and overwintering – limited to the drainage lines running through the site, considering the site's land uses as discussed in further detail in Appendix 2. These drainage lines are approx. 337 metres and 155 metres at the northwestern section and 962 metres along the southwestern boundary. This is a combined length of approx. 1,454 metres. At generally 2 metres wide, this covers a combined total area of 0.2908 hectares of terrestrial dispersal habitat.

The dams are likely ephemeral and so not permanent breeding habitat. The targeted surveys for Growling Grass Frog by Practical Ecology were undertaken in December 2021 and January 2022, after several wet years. However, the two dams in the southern corner of the site (which is where Growling Grass Frog were detected during the surveys) were found to be dry during a site visit in May 2024. The existing dams and drainage lines are not considered to be high quality nor permanent Growling Grass Frog habitat. The project will replace these habitats with high quality wetland habitat that is permanently connected to the Bunyip River.

It was also considered unfeasible to retain the existing dams on site, especially Dam 1 and Dam 2, for the following reasons:

- The dams are not structured for permanence. They are old farm dams which are only around 1.2 metres deep and had spoil pushed up around them to shape the walls.
- Dam 1 and Dam 2 in particular are not well placed in terms of rain fall or flows to feed the dams. Only water that falls directly on that area goes into the dams. The spot level on survey plans indicates that there is minimal flow directly to the dams, as the spot levels and fill mound surround the dams to form a 'Turkey nest'. Therefore, as these dams are fed by rainfall, this may explain why the dam water levels are low to absent.
- The small embankment around Dam 1 and 2 is eroding and collapsing, showing the earth forming the dams is generally not stable and will continue to erode into the floor and become a muddy swell over time. It was noted that a number of the surrounding trees here are dead.

Photo 1 and 2. Dam 1 and 2 photographed in May 2024, dried out.

Growling Grass Frog dispersal

As previously mentioned, the Growling Grass Frog Habitat Assessment Report (Practical Ecology 2022) stated that the 'areas of the site between the dams' and shallow drainage line on site could form dispersal habitat and 'terrestrial refuge'.

The existing dispersal habitat in the site is in association with frog access to the dams only. There is no habitat corridor in the site that links between habitats in the landscape outside the site, such as a drainage line that links the Bunyip River with another waterway or wetland outside the site.

Aerial imagery from Nearmap provides evidence of cutting for hay over the past several years. Hay cutting delivers tall grasses, which are not suitable for Growling Grass Frog dispersal, and then slashed open habitat over the hotter part of summer, which again is not suitable terrestrial habitat. There will be times after slashing that low grasses provide suitable foraging habitat for Growling Grass Frog, however these will be limited to a few months per year and areas cut for hay will not provide effective dispersal habitat for Growling Grass Frog. Dispersal habitat is limited to the shallow drainage lines that are not cut for hay and connect the three existing farm dams.

It was also considered that Dams 1 and 2 are located close to Rossiter Road (with residential area across the road and powerlines cross overhead nearby) and there is existing residential area beyond the road. As the road presents a dispersal barrier, frog dispersal would be occurring between the Bunyip River north of the site and the dams on site. As such, habitat quality is decreased due to the existing degree of development in the surrounds (roads and buildings).

Furthermore, the property to the west of the site is in the Green Wedge Zone and therefore it is our understanding that future urban expansion is not planned for this area by council. The Green Wedge Zone in Victoria is intended to protect non-urban lands including areas for agriculture, biodiversity conservation, parks and other scenic landscapes. There is potential Growling Grass Frog habitat in this western land adjacent to the site, including paddocks and a large farm dam similar to the current site. The proposed action will not impact on the species' ability to occur in this western land.

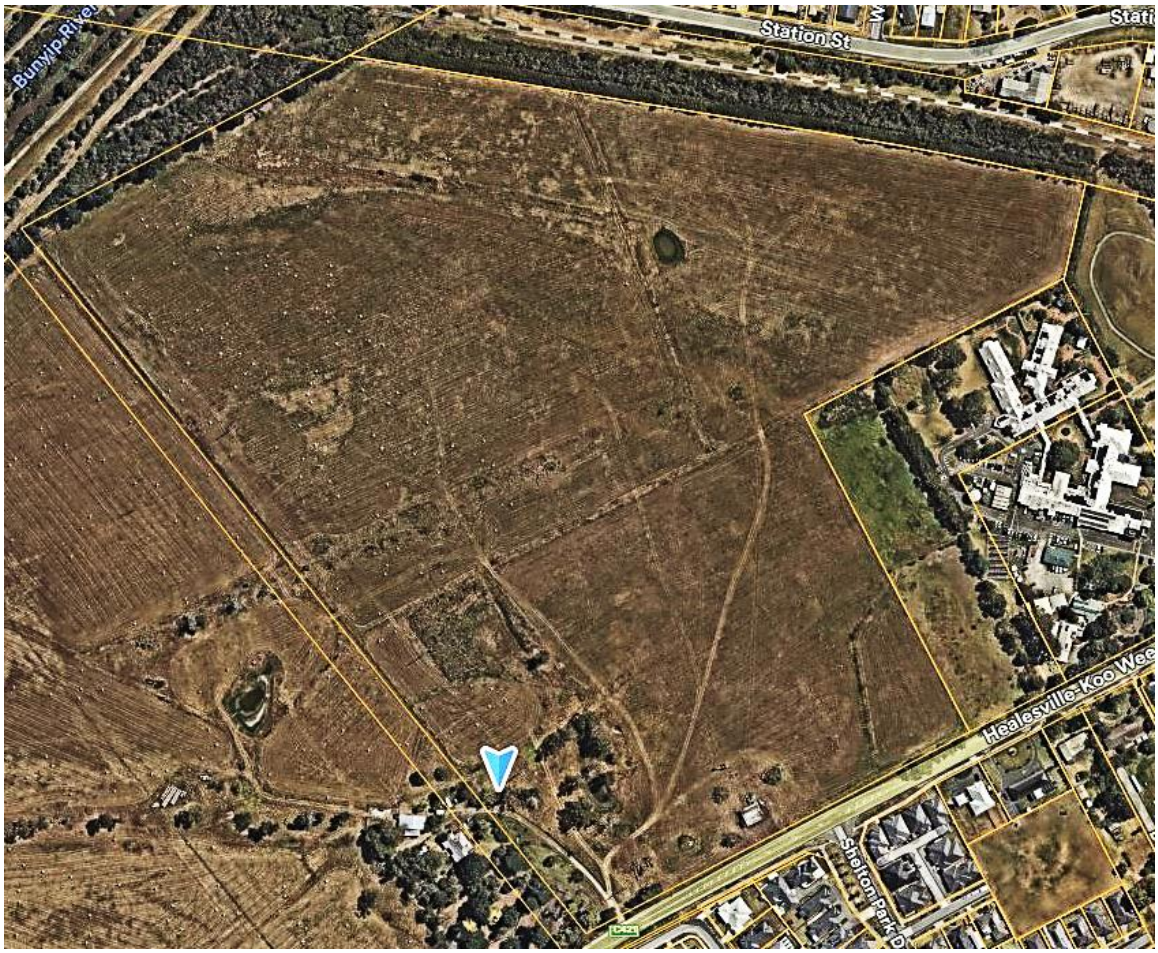

Photo 3. Aerial imagery from Nearmap showing hay cutting across the whole site, 15 February 2024.

Photo 4. Aerial imagery from Nearmap showing hay cutting at the northern section, 13 January 2022.

Photo 5. Aerial imagery from Nearmap showing hay cutting at the northern section, 17 December 2019.

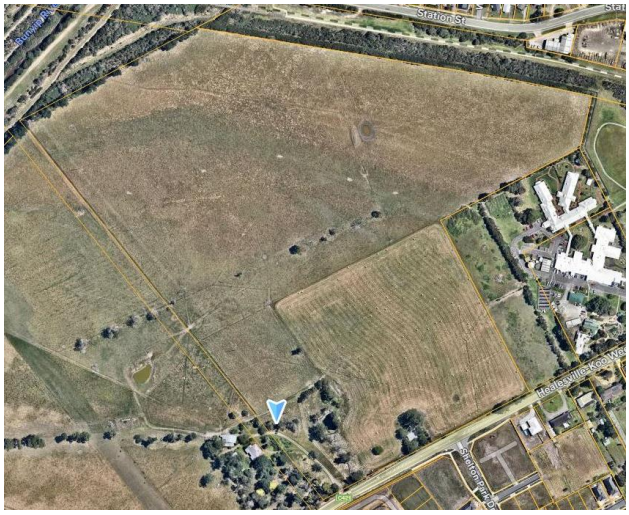

Photo 6. Aerial imagery from Nearmap showing hay cutting at the southern section, 27 December 2017.

Photo 7. Property to the west of the site – introduced grassland, photo taken in May 2024.

In addition to construction of Growling Grass Frog habitat closer to the Bunyip River, the area between the constructed habitat and the northern boundary of the site is proposed to contain an open space area with a drainage reserve and sediment basin for stormwater management. These features, though not constructed to meet breeding wetland habitat standards, are anticipated to function as dispersal habitat for Growling Grass Frog.

Overall, it is considered that although current Growling Grass Frog dispersal habitat to the existing dams will be removed by the proposed action, replacement habitat for the three dams will be provided as a single constructed wetland habitat and there will be better dispersal ability for Growling Grass Frog between the Bunyip River and the constructed habitat. The proposed action is not impacting on a dispersal corridor between habitat areas that will continue to exist after the proposed action. Also, dispersal within and to/from any existing habitats in the western property's (paddocks and farm dams) are not expected to be impacted by the proposed action.

Appendix 3. 10 Year management actions for MNES

Table of the 10-year management objectives

Main Issue	Management Actions	Timing	Measurable Management Objective	Responsible Person	EMP Reference	Completed
Pre-construction						
CEMP	The CEMP must address the matters in Section 3 of this EMP and incorporate the strategies and measures in Section 3 of this EMP.	Design and development planning during preconstruction phase	Consistency between the CEMP and this EMP.	Proponent and nominated contractor	Refer to management strategies and measures in Section 3	
Construction Phase						
Construction Management	Implementation by all relevant staff of the construction management protocols in this EMP, which are to be incorporated into the CEMP, including site inductions and environmental training, hygiene protocols to prevent spread of Chytrid fungus, frog salvage and relocation protocol, and other general protocols provided.	Construction phase	Consistency between the CEMP and this EMP.	Nominated contractor	Refer to construction management protocols in Section 3.1	
Habitat Protection and Maintenance	Install temporary construction fencing around existing Growling Grass Frog habitat and Southern Brown Bandicoot habitat. Install temporary construction fencing with sediment fencing around the new	Temporary fencing to be established prior to any construction works.	Temporary construction fencing to be erected.	Nominated contractor	Refer to staging to works in Section 3.2 and fencing in Section 3.3	

Main Issue	Management Actions	Timing	Measurable Management Objective	Responsible Person	EMP Reference	Completed
	Growling Grass Frog habitat and Southern Brown Bandicoot habitat buffer.					
Vegetation Management	Revegetation in the new Growling Grass Frog habitat and Southern Brown Bandicoot habitat buffer, in accordance with the detailed landscaping plan, which must be consistent with this EMP. Supplementary planting of native tubestock in the Southern Brown Bandicoot habitat buffer will be undertaken in a staged manner to maintain the overall vegetation cover while replacing weeds. Revegetation in the new drainage reserve as per the detailed landscaping plan.	Construction phase	Achieve at least 80% survival rate of planted species. Revegetation works in the new Growling Grass Frog habitat to be endorsed as established by the relevant authority. First stage of planting in the Southern Brown Bandicoot habitat buffer complete.	Nominated contractor	Refer to revegetation strategies in Section 3.5	
Year 1 (upon establishment of the Growling Grass Frog habitat)						
Weed Management	Baseline weed survey in the new habitats. Removal or control of invasive weeds in the new Growling Grass Frog habitat and Southern Brown Bandicoot habitat buffer.	Baseline weed survey post-construction Removal/control works as per optimal time for high-threat weed species	Invasive weeds controlled as required. Weed targets: ▪ No woody weeds. ▪ <20% cover of high-threat weeds (including	Nominated contractor	Refer to weed management in Section 3.6	

Main Issue	Management Actions	Timing	Measurable Management Objective	Responsible Person	EMP Reference	Completed
			noxious weeds) in the Growling Grass Frog habitat.			
Vegetation Management	Supplemental plantings as required especially around areas where weeds have been removed. Supplementary planting of native tubestock in the Southern Brown Bandicoot habitat buffer will be undertaken in a staged manner to maintain the overall vegetation cover while replacing weeds. Regular slashing or mowing of grass in the 50-metre terrestrial buffer for Growling Grass Frog, around the drainage reserve basins and the native grassland in the Southern Brown Bandicoot habitat buffer.	Supplementary plantings as required Routine slashing of grass	Achieve at least 80% survival rate of planted species. Routine slashing or mowing of grass. Grass to be maintained at short (<15 cm) height in the 50-metre terrestrial buffer for Growling Grass Frog.	Nominated contractor	Section 3.5	
Monitoring	Monitoring and reporting on the habitat variables in Section 3.10.	Quarterly (post-construction, 3 months, 6 months and 12 months)	Quarterly monitoring completed and reporting submitted to the relevant authority.	Ecological consultant	Section 3.10	

Main Issue	Management Actions	Timing	Measurable Management Objective	Responsible Person	EMP Reference	Completed
Years 2-10						
Weed Management	Removal or control of invasive weeds in the new Growling Grass Frog habitat and Southern Brown Bandicoot habitat buffer.	Removal/control works as per optimal time for high-threat weed species	Invasive weeds controlled as required. Weed targets to be achieved by Year 10: ▪ No woody weeds. ▪ <20% cover of high-threat weeds (including noxious weeds) in the Growling Grass Frog habitat. ▪ <50% cover of non-native understorey in the Southern Brown Bandicoot habitat buffer.	Nominated contractor	Refer to weed management in Section 3.6	
Vegetation Management	Supplemental plantings as required especially around areas where weeds have been removed. Supplementary planting of native tubestock in the Southern Brown Bandicoot habitat buffer will be undertaken in a staged manner to maintain the overall vegetation cover while replacing weeds. Regular slashing or mowing of grass in the 50-metre terrestrial buffer for	As required	Achieve at least 80% survival rate of planted species in Year 2. Routine slashing or mowing of grass. Grass to be maintained at short (<15 cm) height in the 50-metre terrestrial buffer for Growling Grass Frog.	Nominated contractor	Section 3.5	

Main Issue	Management Actions	Timing	Measurable Management Objective	Responsible Person	EMP Reference	Completed
	Growling Grass Frog, around the drainage reserve basins and the native grassland in the Southern Brown Bandicoot habitat buffer.		Year 10 targets: ▪ Minimum 50% cover of native understorey in the Southern Brown Bandicoot habitat buffer. ▪ Minimum 75% cover of native vegetation in the dedicated wetland for Growling Grass Frog.			
Pest Animal Management	Monitor for signs of feral and domestic animals, mainly rabbits and foxes. Monitor the dedicated wetland for Growling Grass Frog for predatory fish. Implement control if required.	During the quarterly monitoring Control as required	Pest animals controlled.	Nominated contractor	Section 3.8, 3.9 and 3.10	
Fencing	Monitor condition of fencing for the habitat areas. Implement repairs if required.	During the quarterly monitoring Repair as required	Fences and signage maintained in good condition. Any damaged fencing or signs to be rectified immediately.	Nominated contractor	Section 3.10	
Monitoring	Monitoring and reporting on the habitat variables in Section 3.10.	Annually	Annual monitoring completed and reporting submitted to the relevant authority.	Ecological consultant	Section 3.10	

Main Issue	Management Actions	Timing	Measurable Management Objective	Responsible Person	EMP Reference	Completed
Review of Plan	Review of this EMP	Annually	Plan reviewed after last monitoring event for the year. Required amendments to be identified and discussed with the relevant authority.	Ecological consultant	Section 3.13	

Appendix 4. Conditions of Approval under the EPBC Act reference table to be included in the CEMP

Ref	Cond.	Condition Requirement	CEMP reference	Demonstration of how the plan addresses condition requirements and commitments made in the plan to address condition requirements
1				
2				
3				
4				
5				

Appendix 5. Hygiene Protocol to guide Chytrid fungus management during construction

Australian Government
Department of Sustainability, Environment, Water, Population and Communities

JCU JAMES COOK UNIVERSITY

A REPORT FOR THE AUSTRALIAN GOVERNMENT DEPARTMENT OF SUSTAINABILITY, ENVIRONMENT, WATER, POPULATION AND COMMUNITIES

Hygiene protocols for the control of diseases in Australian frogs

June 2011

Prepared by:

[1]Kris Murray, [1]Lee Skerratt, [2]Gerry Marantelli, [1]Lee Berger, [3]David Hunter, [4]Michael Mahony and [5]Harry Hines

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The authors gratefully acknowledge:

Chris Banks, Dr John Clulow, Dr Graeme Gillespie, Professor Rick Speare and Russel Traher,for their contributions to components of the original project resulting in the production of this final report.

Cover photo: *Taudactylus eungellensis* – Eungella day frog. K. Murray

Funding for this project (Procurement Reference Number: 1011-1151) was provided by the Australian Government Department for Sustainability, Environment, Water, Population and Communities. This project progresses the implementation of the *Threat abatement plan for infection of amphibians with chytrid fungus resulting in chytridiomycosis* (Commonwealth of Australia, 2006).

This report should be cited as:

> Murray, K., Skerratt, L., Marantelli, G., Berger, L., Hunter, D., Mahony, M. and Hines, H. 2011. Hygiene protocols for the control of diseases in Australian frogs. A report for the Australian Government Department of Sustainability, Environment, Water, Population and Communities.

Table of Contents

Hygiene protocols for the control of diseases in Australian frogs

1. Who should use this document?

- This protocol is intended for use nationally by conservation agencies, zoos, scientific research staff, industry organisations (e.g., the pet industry), wildlife consultants, fauna surveyors, students, frog keepers, wildlife rescue and carer groups, frog interest groups/societies and other key interest groups who regularly deal with or are likely to encounter frogs.

- This protocol outlines the expectations of the Department of Sustainability, Environment, Water, Population and Communities (DSEWPaC) regarding precautionary procedures to be employed when working with frogs in Australia. The protocols were developed in collaboration with recognised experts in the fields of wildlife health, husbandry, research and conservation. The intention is to promote implementation of hygiene procedures by all individuals working with Australian amphibians.

- DSEWPaC recognises that some variation from the protocol may be appropriate for particular research and frog handling activities. Such variation should accompany any licence applications or renewals submitted to the relevant regulatory bodies for independent consideration. Variations should follow a risk analysis process which broadly involves hazard identification, risk assessment, risk management and risk communication.

Where *ex-situ* activities are proposed, these guidelines should be used in conjunction with the "**Guidelines for captive breeding, raising and restocking programs for Australian frogs**", which can be found here: http://www.environment.gov.au/biodiversity/invasive/projects/index.html#threat-10-11**.**

2. Objectives

The objectives of the hygiene protocols are to:

- Improve the control of diseases in Australian frogs
- **Improve preparedness for an emergency response** to new amphibian disease incursions in Australia
- **Recommend best-practice procedures** for personnel, researchers, consultants and other frog enthusiasts or individuals who handle frogs
- **Suggest workable strategies** for those regularly working or considering working in the field with frogs or where frogs may exist
- **Provide background information** and guidance to people who provide advice or supervise frog related activities
- **Inform regulatory bodies and animal care and ethics committees** for their consideration when granting permit approvals

3. Introduction

Amphibians have declined globally. In the first global amphibian assessment, at least 43% of amphibian species with sufficient data were found to have declined in recent decades, 34 species were extinct and a further 88 were possibly extinct (Stuart et al. 2004). In 2010, approximately 30% of amphibians were threatened globally (http://www.iucnredlist.org/documents/summarystatistics/2010_4RL_Stats_Table_1.pdf).

Diseases are responsible for many amphibian declines and extinctions and their risk needs to be addressed. Laurance et al. (1996) first proposed the 'epidemic disease hypothesis' to account for Australian amphibian declines. Shortly after, an unknown chytridiomycete fungus was seen infecting the skin of sick and dying frogs collected from montane rain-forests in Queensland and Panama during mass mortality events associated with significant population declines (Berger et al. 1998; Longcore et al. 1999). The fungus was subsequently found to be highly pathogenic to amphibians in laboratory trials by inducing development of skin pathology, morbidity and mortality similar to that seen in the wild frogs. The disease was called chytridiomycosis and the fungus described as a new species *Batrachochytrium dendrobatidis* (Bd), also known as the amphibian chytrid fungus.

Bd has been found infecting over 350 species in two amphibian orders (Anura and Caudata) from all continents where amphibians occur (http://www.bd-maps.net/). Sixty-three (~28%) of Australia's 223 (as listed by IUCN 2008) amphibian species are now known to be wild hosts for Bd (Murray et al. 2010a; Murray et al. 2010b), and over half of Australia's species may be naturally susceptible to Bd in the wild (Murray et al. 2011; Murray and Skerratt in press).

While the discovery of chytridiomycosis has sparked renewed appreciation for the role that diseases can play in threatening wildlife populations and species, it is not the only disease currently affecting amphibians, nor is it likely to be the last. Ranavirus, for example, has been observed to induce mass mortality events in frog and salamander populations in the UK and North America. In response to these global threats, the World Organisation for Animal Health (OIE) has listed both chytridiomycosis and ranavirus as "notifiable" diseases to help control their spread. Similarly, numerous conferences and reports have been assembled to produce standards in managing diseases in wild and captive amphibian populations. Together, these measures highlight the importance of developing **agreed hygiene protocols for the control of diseases in Australian frogs**. This document fulfils this role.

4. Key disease issues in amphibian populations

Here we review the most significant diseases of amphibians, including some that have zoonotic potential and some that have not been detected in Australia. There are many described diseases of amphibians but only a few are known to be an important threat to wild amphibians or other taxa including humans. Some become an issue in captive amphibian populations where management is inadequate. As research on this topic is limited, there are also likely to be many unknown diseases of amphibians which may pose a risk. Disinfection methods have not been validated for all pathogens. Any risk management strategy to minimise the impact of diseases of amphibians should take into account this uncertainty. For detailed reviews see Hemingway et al. (2009) and Berger et al (2009) for diseases in wild populations and Wright and Whitaker (2001) that also includes diseases in captivity.

4.1. Fungi

4.1.1. *Batrachochytrium dendrobatidis*

Batrachochytrium dendrobatidis (Bd) is a fungal pathogen capable of driving amphibian species to perilously low numbers or extinction. In Australia, the oldest record of Bd is from a museum frog specimen collected in south-east Queensland near Brisbane in 1978 (Department of the Environment and Heritage 2006a), which coincides with sudden frog declines in a number of species and two species extinctions in the region (Berger et al. 1998; Hines et al. 1999). Subsequent amphibian declines in central coastal Queensland (1985-86) and the Wet Tropics (1990-95) suggest that *B. dendrobatidis* spread north to its current northern limit at Big Tableland near Cooktown (Laurance et al. 1996; Berger et al. 1999; Skerratt et al. 2010). In southern Australia, the spread of *B. dendrobatidis* is poorly documented but its distribution extends down the entire east coast to Tasmania (first detected in 2004) (Obendorf and Dalton 2006; Pauza and Driessen 2008). Two separate foci occur in other states, one in southwest Western Australia, where the earliest record dates to 1985, and another around Adelaide in South Australia (earliest record 1995) (Murray et al. 2010a). The Northern Territory is currently considered amphibian chytrid free (Skerratt et al. 2008; Skerratt et al. 2010; Murray et al. 2011).

In the majority of infected animals for most of the time, clinical signs of chytridiomycosis are absent. The period of showing signs is typically short and mostly limited to those amphibians that die. Central nervous system signs predominate, including behavioural change, slow and uncoordinated movement, abnormal sitting posture, tetanic spasms, loss of righting reflex and paralysis. Skin changes associated with chytridiomycosis are typically microscopic and not detectable at the clinical level with any degree of confidence, although abnormal skin shedding occurs (skin shed more frequently and in smaller amounts) and erythema (tissue reddening) of ventral surfaces and digits may be seen. For what to do if you encounter a sick or dead amphibian in Australia, see section 6.7. below. For a detailed factsheet about chytridiomycosis, see the Australian Wildlife Health Network website (http://www.wildlifehealth.org.au/AWHN/FactSheets/Fact_All.aspx).

4.1.2. *Mucor amphiborium*

This fungus is an important cause of morbidity and mortality in platypus in Tasmania and amphibians are a potential reservoir host (Gust et al. 2009). Amphibian mucormycosis is a systemic disease caused by the fungus, *Mucor amphibiorum*. Severely infected amphibians have fungi disseminated through their internal organs and skin. The fungi incite formation of granulomas that consist of inflammatory cells and fibrous tissue. At postmortem, the liver contains small pale nodules up to about 5 mm in diameter and usually in massive numbers. These nodules can also be seen in other organs such as the kidney, lung, mesentery, urinary bladder, subcutaneous sinuses and skin. The microscopic fungi are found inside these nodules. *M. amphibiorum* is a primary pathogen and can infect normal amphibians, but in the wild it appears to cause only sporadic infections. Possibly the usual inoculating dose in the wild is not high enough to cause epidemic disease. In captivity it can cause fatal outbreaks in collections. For more information on mucormycosis, see http://www.jcu.edu.au/school/phtm/PHTM/frogs/mucor/mucoramphibiorum.htm.

4.1.3. Oomycetes

Water moulds (family Saprolegniaceae, phylum Oomycota) are ubiquitous in surface water. High levels of infection with *Saprolegnia ferax* caused mortality of Western toad (*Bufo boreas*) egg masses in northwestern United States and were sufficient to affect local populations (Kiesecker et al. 2001). Epidemics may be associated with fish stocking or environmental cofactors.

4.2. Viruses

There are a number of viruses that are known to cause disease and mortality in amphibians, including ranaviruses, frog erythrocytic virus, Lucké tumor herpesvirus, herpes-like virus of skin, calicivirus and leucocyte viruses (Hemingway et al. 2009). In Europe and America the most important of these for their ability to cause mass mortalities and potentially population declines are the ranaviruses (Hyatt et al. 2000). Ranaviruses have been identified in a range of ectothermic vertebrates, including fish, amphibians (frogs, toads, salamanders) and reptiles (lizards, turtles, snakes). Some species can infect a broad host range across all these taxa.

Ranaviral disease is an emerging infectious disease overseas as it is being detected over an increasing geographic range and in more species (Hemingway et al. 2009). While ranaviral disease in wild amphibians has not been frequently observed in Australia, antibodies to ranaviruses have been detected widely (NSW, Qld, NT) in cane toads (*Bufo marinus*) (Zupanovic et al. 1998). Bohle iridoviris (BIV) was first found causing death in wild caught metamorphs of *Limnodynastes ornatus* and has since been detected in wild, moribund adult *Litoria caerulea* from Townsville and captive juvenile *Pseudophryne coriacea* from Sydney (Speare et al. 2001; Cullen and Owens 2002). Laboratory studies in Australia have also shown that cane toads (*Bufo marinus*) and a range of native frogs are susceptible to BIV (Speare et al. 2001). Tadpoles appear the most susceptible, while juvenile frogs were more susceptible than adults. Data on the geographical origin and time of emergence or introduction of ranaviruses in Australia is not known. Ranaviruses not currently found in Australia can cause disease in native Australian amphibians in experimental challenges; for example, Venezuelan Guatopo virus was able to kill *Litoria caerulea* in experimental trials (http://www.jcu.edu.au/school/phtm/PHTM/frogs/otherdiseases-viruses.htm). We need to prevent the introduction of pathogenic ranaviruses into Australia.

Clinical signs of acute ranaviral disease may be seen in tadpoles, metamorphs, juveniles and adults. In general, amphibians infected with ranavirus may show decreased activity, ascites (accumulation of fluid in the peritoneal cavity), anasarca (accumulation of serous fluid in various tissues and cavities of the body), skin ulceration, focal and systemic haemorrhages and death. For what to do if you encounter a sick or dead amphibian in Australia, see section 6.7. below. For a detailed factsheet about ranaviral disease, see the Australian Wildlife Health Network website (http://www.wildlifehealth.org.au/AWHN/FactSheets/Fact_All.aspx).

4.3. Bacteria

The range of bacteria reported as causing disease in amphibians is small. Bacterial septicaemia can cause significant disease in captivity. Infection with Aeromonas spp., non-haemolytic group B Streptococcus, Flavobacteria and chlamydia have caused outbreaks in captive amphibians and Mycobacteria can cause chronic problems. Another group of bacteria can be carried by amphibians with minimal effect and are potentially capable of causing

infections in humans (zoonotic diseases). Salmonella and Leptospira are in this category and are a potential risk to humans, livestock and domestic pets, see below.

4.4. Myxozoa

Myxosporean parasites (*Myxidium* spp.) in the brain and liver of declining Australian frogs, the Green and Golden Bell frog (*Litoria aurea*) and the Southern Bell frog (*Litoria raniformis*), have recently been reported to be associated with disease and may have a significant impact on wild frogs (Hartigan et al. 2011).

4.5. Mesomycetozoa

Ichthyophonus sp. occurs the USA where it is often an incidental finding in tadpoles, frogs and salamanders but may cause morbidity and mortality. It infects muscles and adult frogs with massive infections become lethargic and emaciated. Massive acute lethal infections with numerous mortalities occur infrequently in ranid larvae (D. Green, unpubl., Mikaelian et al. 2000)

4.6. Alveolates

A *Perkinsus*-like organism is a major cause of mortality events in tadpoles in the US. Occurs predominantly in tadpoles of *Rana* spp. and may cause mortality rates of 80-99% in a pond over the course of 2-6 weeks (Davis et al. 2007). Weakly swimming, bloated and floating tadpoles are found.

4.7. Zoonotic Diseases

Guidelines for preventing human exposure to amphibian disease are available at the Centre for Disease Control website- http://www.cdc.gov/healthypets/animals/reptiles.htm

4.7.1. *Salmonella*

Amphibians may carry pathogenic *Salmonella* species, but rarely show signs of disease (Anver and Pond 1984). Prevalence of salmonellas isolated in clinically normal amphibians is generally greater than 10% and bacterial levels can be high (Sharma et al. 1974). In Australia, *Salmonella* were isolated from 12.7% (19/150) of *B. marinus* collected from the wild and 9 serotypes were identified. All nine had previously been isolated in Australia from humans and livestock (O'Shea et al. 1990). An outbreak of gastroenteritis in humans near Rockhampton possibly originated from green tree frogs (*Litoria caerulea*) contaminating drinking water in rainwater tanks (Taylor et al. 2000). Some strains of salmonellae are cosmopolitan while others are not found in Australia, but could be imported.

4.7.2. *Leptospira*

Leptospira are spirochaetal bacteria that usually invade the kidney of vertebrates and are excreted in the urine. Humans and domestic animals are susceptible to various strains of *Leptospira* usually from the species *Leptospira interrogans*. Serious acute and chronic disease occasionally with death can result. Little is known about the occurrence of *Leptospira* in amphibians, and on their significance as reservoir hosts for leptospirosis in humans. No studies appear to have been done on leptospires in amphibians in Australia. However in

Barbados, toads (*Bufo marinus*) and frogs (*Eleutherodactylus johnstonei*) were found to be reservoirs for serovars of *Leptospira* pathogenic to humans (Gravekamp 1991).

4.7.3. *Spirometra erinacei*

The adult stage of the tape worm *Spirometra erinacei* inhabits the small intestine of carnivores such as the cat, dog, fox and dingo. The first larval stage occurs in copepods and the second larval stage (spargana) are long, flat white worms that can infect amphibians and other vertebrates in muscles and under the skin. Sparganosis occurs in around 5% of Australian frogs and heavy burdens are associated with severe disease (Berger et al. 2009). Sparganosis is a public health problem in Asia, usually occurring as subcutaneous or intramuscular infections. Humans become infected by drinking water with infected copepods, eating undercooked frogs, and the worms can also migrate from frog flesh into skin wounds

5. National and border biosecurity

Unregulated trade in animals, as well as unintentional shipment, is suspected to have been a major contributor to the spread of emerging infectious diseases such as chytridiomycosis (Skerratt et al. 2007). There are numerous bodies and regulatory levels that attempt to provide guidance about how to minimise the risk of pathogen spread and transmission in amphibians.

5.1. World Organisation for Animal Health (OIE)

The World Organisation for Animal Health (OIE) lists key diseases as "notifiable" to promote the reporting and management of diseases among member countries. Preventing the spread of amphibian diseases across international borders is important, and both chytridiomycosis (Article 8.1.1) and ranavirus (Article 8.2.1:) are now listed as notifiable diseases in the OIE Aquatic Animal Health Code (http://web.oie.int/eng/normes/fcode/). To access these codes, follow these links:

- **Chytridiomycosis**: http://web.oie.int/eng/normes/fcode/en_chapitre_1.8.1.pdf
- **Ranavirus**: http://web.oie.int/eng/normes/fcode/en_chapitre_1.8.2.pdf

The codes outline recommendations for the "**Importation or transit of *aquatic animals and aquatic animal products* for any purpose from a country, zone or compartment"**:

- **Provided commodities are treated in a manner that inactivates the disease agent (Bd or ranaviruses),** Competent Authorities should not require any disease conditions when authorising the above activities, regardless of the disease status of the exporting country
- However, in cases where it could otherwise reasonably be expected that commodities pose a risk of Bd or ranavirus transmission, a risk assessment should be carried out in accordance with the recommendations in the Aquatic Code. The exporting country would then be notified of the outcome of the risk assessment before trade commences.

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Where commodities do not meet this condition and/or a reasonable risk remains, there are additional requirements that depend on the disease status of the country, zone or compartment.

Freedom from disease:

Importation of live aquatic animals from a country, zone or compartment declared free from disease (Bd or ranavirus) requires an **international aquatic animal health certificate** issued by the Competent Authority confirming disease free status.

- A country may make a **self declaration of freedom from disease** (Bd or ranaviruses) if one of the following conditions is met:

    1. It has no amphibians or other susceptible species AND basic biosecurity conditions have been continuously met for a period of 2 years
    2. There has been no observed occurrence of the disease for at least the past 10 years despite conditions that are conducive to its clinical expression AND basic biosecurity conditions have been continuously met for a period of 10 years
    3. Targeted surveillance has been in place for at least the past 2 years without detection of disease (Bd or ranaviruses) AND basic biosecurity conditions have been continuously met for a period of 2 years
    4. For a country that previously made a self declaration of freedom from disease, it may regain that status after detection of the disease if the affected area was declared an infected zone and a protection zone was established AND infected populations have been destroyed or removed from the infected zone by means that minimise the risk of further spread of the disease AND the appropriate disinfection procedures have been completed AND if the conditions of 3.) above are met.

- A zone or compartment may also be declared free from disease by the Competent Authority if it meets similar conditions to the above. Where a zone or compartment extends over more than one country, declarations must be made by all the Competent Authorities involved.

- A disease free status can be maintained if basic biosecurity conditions are continuously maintained. Targeted surveillance may be discontinued provided conditions that are conducive to clinical expression of disease exist. However, in infected countries and in all other cases where conditions are not conducive to clinical expression of disease, zones or compartments can only maintain a disease free status if targeted surveillance is maintained.

Unknown or known infected country, zone or compartment:

For the importation of live aquatic animals and aquatic animal products for any purpose (e.g., aquaculture, processing for human consumption, use in animal feed, agricultural, laboratory, zoo, pet trade, industrial or pharmaceutical use):

In general, the Competent Authority of the importing country should

- require an **international aquatic animal health certificate** stating the commodities have been appropriately treated to inactivate disease agents
- OR undertake a risk assessment and apply appropriate risk mitigation measures

The risk assessment and risk mitigation measures will vary with purpose of the importation or transit of commodities. Please see the Aquatic Code at the links provided above for more details.

5.2. AUSVETPLAN and AQUAVETPLAN

In Australia, management of animal disease emergencies normally defaults to protocols outlined in the Australian Veterinary Emergency Plan (AUSVETPLAN - http://www.animalhealthaustralia.com.au/programs/eadp/ausvetplan/ausvetplan_home.cfm) or the Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN - http://www.daff.gov.au/animal-plant-health/aquatic/aquavetplan). However, few of the diseases for which specific plans have been developed concern diseases of free-ranging wildlife. No amphibian diseases are currently included in AUSVETPLAN or AQUAVETPLAN.

5.3. Key Threatening Process and Threat Abatement Plan (TAP)

Chytridiomycosis was listed as a Key Threatening Process in Australia in 2002. A Threat Abatement Plan (TAP) for infection of amphibians with chytrid fungus resulting in chytridiomycosis was subsequently prepared by representatives of the Commonwealth Government. These documents can be accessed here:

- **Key Threatening Process:** http://www.environment.gov.au/biodiversity/threatened/ktp/frog-fungus.html
- **TAP:** http://www.environment.gov.au/biodiversity/threatened/publications/tap/chytrid.html
- **TAP Background document:** http://www.environment.gov.au/biodiversity/threatened/publications/tap/pubs/chytrid-background.pdf

Recommendation 1.1.3 of the TAP proposes that a risk-based approach be used for chytridiomycosis using AUSVETPLAN as a model (Department of the Environment and Heritage 2006b). However, this has not progressed. Nation-wide mapping protocols and disease risk models have been developed as suggested in the TAP and should serve as the basis for cost-sharing arrangements between states and for setting research and management priorities (Skerratt et al. 2008; Murray et al. 2010a; Murray et al. 2010b; Skerratt et al. 2010; Murray et al. 2011). Implementing this step remains a priority.

5.4. Biosecurity Australia

Risk analysis performed by Biosecurity Australia in **"Quarantine requirements for the importation of amphibians or their eggs into zoological facilities"** and **"Quarantine requirements for the importation of amphibians or their eggs for laboratory purposes"** (Animal Biosecurity Policy Memorandum 2003/26) does not list chytridiomycosis as a risk since it is endemic in Australia. However, this disregards the risk of importation into chytrid free areas or the introduction of novel strains. Although chytridiomycosis is not specifically mentioned, the general hygiene strategies recommended should still prevent the release of imported strains of *B. dendrobatidis* during the initial two years. After two years the amphibians can be released without testing for *B. dendrobatidis*. However, if being released into a chytrid free area, the same requirements imposed on Australian bred amphibians under the Threat Abatement Plan would apply.

Risk analysis performed by Biosecurity Australia in **"Quarantine requirements for the importation of amphibians or their eggs into zoological facilities"** and **"Quarantine requirements for the importation of amphibians or their eggs for laboratory purposes"** (Animal Biosecurity Policy Memorandum 2003/26) mentions ranaviruses:

- "The veterinary certificate must… certify that… for both live amphibians or amphibian eggs…, as far as can be determined, no case of ranavirus infection (including frog virus 3, Redwood Park virus, Regina ranavirus), or ranid herpesviruses has been diagnosed at the premises of origin during the 12 months prior to certification."

Importation of amphibians must meet the requirements of two Commonwealth departments, 1) Department of Agriculture, Fisheries and Forestry (DAFF) and 2) the DSEWPaC. The relevant documents can be accessed here:

- **DAFF:**
  Zoological facilities - http://www.jcu.edu.au/school/phtm/PHTM/frogs/aqis/2003-26a.pdf
  Laboratory purposes - http://www.jcu.edu.au/school/phtm/PHTM/frogs/aqis/2003-26b.pdf
- **DSEWPaC:** http://www.environment.gov.au/biodiversity/wildlife-trade/index.html. This site also has the requirements for export of amphibians from Australia.

6. Hygiene management

Hygiene management issues can be broadly classed into *in-situ* (field based) and *ex-situ* (facility based) categories. While general **isolation and disinfection** hygiene management principles apply to both, greater detail on '**Guidelines for captive breeding, raising and restocking programs for Australian frogs'** can be found here: http://www.environment.gov.au/biodiversity/invasive/projects/index.html#threat-10-11.

6.1. In-situ (site) hygiene management

Individuals studying frogs often travel and collect samples of frogs from multiple sites. Numerous hygiene guidelines for handling wild frogs exist, including Daszak et al. (2001), NSW NPWS (2008), NWHC (2001), Speare et al. (2004) and CCADC (2008). Most recently, Phillott et al. (2010) provide a detailed review and synthesis of hygiene considerations that aim to minimise the risk of exposure of amphibians to pathogens in field studies.

It is important to recognise that humans may aid in the:

- **transmission** (passing of disease from an infected to an uninfected individual), and
- **spread** (movement of disease geographically)

of diseases, within and among amphibian populations For researchers working with amphibians or within areas where amphibians may occur, the risk of disease transmission within these habitats and the spread of disease among populations may be increased due to:

- **movement** of frogs or personnel between isolated areas of habitat or between captive husbandry and laboratory facilities and the field
- **handling** of amphibians

It is therefore essential that personnel working with amphibians or within amphibian habitats take care to minimise disease transmission and spread. In order to do this, it is important that frog workers recognise the boundaries between sites/populations.

This is especially important where **rare, geographically restricted or threatened amphibian species** are concerned and when the spread of diseases can have serious consequences for species survival.

Phillott et al. (2010) recommend that field researchers evaluate their activities to determine the relative risk of pathogen transmission and spread compared with background levels (i.e., the risk posed by other mechanisms of disease transmission or pathogen dispersal) and implement appropriate strategies to minimise this risk during field studies. For a **hygiene protocol checklist and suggested field kit** see section 7. The risk of transmission and spread should also be evaluated by researchers, animal ethics committees and government agencies issuing permits.

6.1.1. Defining a site

Defining the boundary of a site may not be straightforward. In some places, the boundary between sites will be obvious but in others it may not. Undertaking work at a number of sites or conducting routine monitoring at a series of sites within walking distance creates obvious difficulties with boundary definitions. It is likely that defining the boundary between sites will differ among localities.

In general:

- watershed and geographical barriers should be used to designate separate sites
- river/stream tributaries should be considered separate sites
- wetlands, ponds, lakes etc. separated by dry land should be considered separate sites
- upstream locations separated by considerable distance (e.g., 500 m) should be considered separate sites
- any obvious break, barrier or change in habitats should be treated as separate sites, particularly if there is no known interchange of frogs between sites

6.1.2. Determining the order of visitation of multiple field sites

When a field trip encompasses several field sites, or a number of locations are being visited in succession, the order of visitation should be determined according to the presence of known pathogens and diseases.

- **Areas known to be absent of disease should be visited first, followed by areas of unknown status, followed by known infected areas**

6.1.3. On-site hygiene

When travelling from site to site it is recommended that the following hygiene precautions be taken to minimise the possibility of transfer of disease from personnel, footwear, equipment and/or vehicles. A list of suitable disinfectants, their required concentrations and exposure times for various purposes is summarised by Phillott et al. (2010) and is reproduced in Table 1 below.

Personnel

- **Hands, arms, knees etc. should be cleaned to remove debris and washed** or wiped with a suitable disinfectant. It is preferable to do this before entering the vehicle or moving to another site.

Footwear and clothing

- **Footwear must be thoroughly cleaned and disinfected** at the commencement of fieldwork and between each sampling site. This can be achieved by initially scraping boots clear of mud and standing the soles in a disinfecting solution. The remainder of the boot should be rinsed or sprayed with a disinfecting solution. Clothing that has significant contact with frogs and the environment should also be subjected to changing or cleaning

Disinfecting solutions should be prevented from entering any water bodies. Several changes of footwear/clothing bagged between sites might be a practical alternative to on-site cleaning. In high value sites, dedicated equipment and clothing stored at the entry to the site may be desirable. (e.g., in a lockbox)

Equipment

- Equipment such as nets, balances, callipers, bags, scalpels, headlamps, torches, wetsuits and waders etc. that are used at one site must be **cleaned and disinfected** before re-use at another site
- Disposable items should be used where practical/possible

Non-disposable equipment should be used only once during a particular field exercise and disinfected later or disinfected at the site between uses using procedures outlined below in Table 1.

Vehicles

Transmission of disease from vehicles is generally unlikely to be a problem. However, if a vehicle is used to traverse a known frog site and could result in mud and water being transferred to other bodies of water or frog sites, then wheels and tyres should be cleaned and disinfected. This is particularly important where vehicles are used in areas not normally frequented by other vehicles. Disinfection should be carried out at a safe distance from water bodies to minimise the risk of chemical contamination.

6.1.4. Principles of cleaning and disinfection

Designing an effective disinfection protocol requires understanding of the properties of disinfectants and target pathogens, and practical consideration of the equipment or processes requiring disinfection. As well as understanding the efficacy of various disinfecting processes, it is important to consider the safety of any disinfection protocol to the environment and the animals on which they will be used. Key distinctions include:

- **Cleaning**: The physical removal of all visible organic and inorganic debris from items
- **Disinfection:** A physical (e.g., UV light) or chemical (e.g., bleach) process to reduce the numbers and/or viability of microorganisms (e.g., bacteria, fungi or viruses) on an object, surface or material
- **Sterilization:** A physical or chemical process that removes all microorganisms from an object, surface or material

Thorough cleaning and disinfection reduces most of the risk of transferring amphibian pathogens. Sterilization of objects is labour intensive and less practical for most routine applications.

Cleaning alone does not render an object free of pathogens. However, it is important to thoroughly clean objects prior to disinfection or sterilization.

- Thorough cleaning physically removes many or most pathogens that are trapped in organic debris
- Thorough cleaning makes successful disinfection more likely
- Cleaning allows disinfectants to directly contact the surfaces of an object
- Warm or hot water improves the ability to remove organic materials from objects
- Regular cleaning of all items used should be performed
- Use of detergents aid cleaning by loosening organic material from the surface of objects and help to break apart biofilms of microorganisms that can resist disinfection
- Thorough rinsing of detergents from objects is essential after cleaning

Disinfection of an item by application of an appropriate chemical agent after cleaning reduces pathogen numbers and viability and minimises potential for disease transmission. Things to consider include:

- **Efficacy of the disinfectant and the type of pathogens that must be eliminated.** For example, some microorganisms such as Mycobacterium spp. or Cryptosporidium spp. are very resistant to most common disinfectants
- **The potential for toxicity to amphibians that are exposed to the disinfectant.** Amphibians are very sensitive to some disinfectant residues and thorough rinsing of all disinfectants is required after use
- **Concerns about human exposure to disinfectants and about discharge of disinfectants into the environment**
- **Safety for use on different materials.** Some disinfectants may be corrosive to materials or tools used in amphibian facilities
- **Ease of use and disposal**
- **Cost**

Table 1. Disinfection strategies suitable for killing *Batrachochytrium dendrobatidis, Mucor amphibiorum* and ranaviruses in field studies. From Phillott et al. (2010) and Webb et al. (submitted).

Application	Disinfectant	Strength	Time	Target pathogen
Surgical equipment and other instruments (e.g. scales, callipers)	Benzalkonium chloride	1 mg ml–1	1 min	*B. dendrobatidis*
	Ethanol	70%	1 min	*B. dendrobatidis* Ranaviruses
Collection equipment and containers	Sodium hypochlorite (bleach contains 4% sodium hypochlorite)	1%	1 min	*B. dendrobatidis*
		3%	1 min	Ranaviruses
	Path X or quaternary ammonium compound 128	1 in 500 dilution	0.5 min	*B. dendrobatidis*
		1 in 100 dilution	10 min	*M. amphibiorum*
	Trigene	1 in 5000 dilution	1 min	*B. dendrobatidis*
	F10	1 in 1500 dilution	1 min	*B. dendrobatidis*
	Virkon	2 mg ml–1	1 min	*B. dendrobatidis*
		1%	1 min	Ranaviruses
	Nolvasan	0.75%	1 min	Ranaviruses
	Potassium permanganate	1%	10 min	*B. dendrobatidis*
	Complete drying		>3 h	*B. dendrobatidis*
	Heat 60°C		30 min	*B. dendrobatidis* Ranaviruses
	Heat 37°C		8 h	*B. dendrobatidis*
	Sterilising UV light		1 min	Ranaviruses only
Footwear	Sodium hypochlorite (bleach contains 4% sodium hypochlorite)	1%	1 min	*B. dendrobatidis*
		3%	1 min	Ranaviruses
	Path X or quaternary ammonium compound 128	1 in 500 dilution	0.5 min	*B. dendrobatidis*
		1 in 100 dilution	10 min	*M. amphibiorum*
	Trigene	1 in 5000 dilution	1 min	*B. dendrobatidis*
	F10	1 in 1500 dilution	1 min	*B. dendrobatidis*
	Phytoclean (30% benzalkonium chloride)	0.075%	1 min	*B. dendrobatidis*
		5%	1 min	*M. amphibiorum*
	Complete drying		>3 h	*B. dendrobatidis*
Cloth (e.g. carry bags, clothes)	Hot wash 60°C or greater		30 min	*B. dendrobatidis* Ranaviruses

6.2. Handling of frogs in the field

The spread of pathogens may occur as a result of handling frogs. In addition to spreading disease among captured frogs, handling may stress animals making them more susceptible to infection from other sources or more likely to succumb to infection.

- **Capture, handling and housing of wild amphibians should be minimised or avoided where possible**
- Where handling is necessary, care must be taken to ensure individuals do not have their exposure to pathogens elevated over their background exposure levels.

Direct transfer of pathogens during capture and handling of successive adult amphibians can be reduced by using:

- **single-use gloves** (latex, nitrile or vinyl), and/or
- **single-use lightweight plastic bags**
- **adequate cleaning of hands and handling equipment**

Many researchers use disposable plastic bags to catch and/or restrain frogs followed by handling/processing with disposable gloves. As some tadpoles may suffer lethal effects when exposed to latex, nitrile or vinyl gloves (Cashins et al. 2008), researchers should only use gloves that have been proven or rendered safe (e.g., by rinsing with water) for the study species.

In situations **where gloves are not available or suitable:**

- hand washing with 70% ethanol (allowing hands to dry) between handling individual frogs is acceptable (note, repeated use on human skin is not recommended). Alcohol is toxic to frogs so hands must be washed thoroughly in water after treatment with alcohol
    - If 70% ethanol is not available or suitable, the minimum treatment is hand-washing in the water to which the amphibian is normally exposed.

In situations **where amphibians must be held temporarily:**

- Individuals should be housed in **single-use containers (e.g. plastic bags) or in containers disinfected** between each animal
- Adults should not be held in groups
- Tadpoles from the same water body may be housed for short periods in a common container, although overcrowding should be avoided

Longer holding times (>60 min) will require changes to water and the provision of appropriate food (>24 h). Tadpoles should always be treated with care to prevent damage on capture and with movement of water within holding containers. If animals must be removed from the field for greater periods and later returned, it should always be to the same site.

6.3. Housing frogs and tadpoles

- **Frogs and tadpoles should only be removed from a site when absolutely necessary.**

Detailed **'**Guidelines for captive breeding, raising and restocking programs for Australian frogs' can be found at: http://www.environment.gov.au/biodiversity/invasive/projects/index.html#threat-10-11. See also 'A Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs**'** (Pessier and Mendelson 2010) at: http://www.cbsg.org/cbsg/workshopreports/26/amphibian_disease_manual.pdf#search=%22amphibian%22

When frogs or tadpoles are to be collected and held for a period of time, the following measures are recommended:

- Isolate animals obtained at different sites
- Aquaria set up to hold frogs should not share water, equipment or any filtration system. Splashes of water from adjacent enclosures or drops of water on nets may transfer pathogens between enclosures
- Ensure that tanks, aquaria and any associated equipment are disinfected prior to housing frogs or tadpoles
- Tanks and equipment should be cleaned, disinfected and dried after frogs/tadpoles are removed

6.4. Marking, invasive and surgical procedures

Strict hygiene standards must be maintained during amphibian marking procedures including implanting internal radio transmitters, passive integrated transponder (PIT) tags, visible implant alphanumeric (VIA) tags, visible implant elastomer (VIE) tags and toe tipping or clipping.

Due to the high permeability of amphibian skin, special disinfectants are required.
The **only suitable, commercially available preparation for disinfecting wounds** is:

- **Bactine®** spray (active ingredient 0.14% w/w benzalkonium chloride and 2.6% w/w lidocaine hydrochloride in a non-alcohol base)
- **Chlorhexidine** (0.75% diluted from 2% Nolvasan®) is also suitable for surgical disinfection
- Alcohol, phenol and iodine based disinfectants **should not be used** because they are potentially toxic and can destroy mucus and wax that prevent dehydration and microbial infection of amphibian skin. Contrary to the recommendations of previous hygiene protocols, Betadine® or other povidone-iodine products are not recommended for use as disinfectants for amphibians until species-specific toxicity has been determined (Phillott et al. 2010).

Toe tipping (removal of most distal phalange) or toe clipping (amputation of a greater proportion of the digit):

- should occur through the **interphalangeal joints**

- Scissors should be **sterilised in 70% ethanol** and dried before use on frogs in the field
- For studies in which diagnostic testing of disease is important, the diagnostic test step (e.g., swabbing for Bd) should be undertaken before any other processing step to minimise the potential for false-positives due to cross contamination

PIT, VIE and VIA tags should be inserted with a **sterile, single-use applicator**.

6.4.1. Sealing wounds

- A c**ryanoacrylate** compound such as Vetbond® (active ingredient n-butyl cryanoacrylate) as a tissue adhesive after toe tipping or clipping is recommended. Vetbond® can also be used to seal incisions made during subdermal injection of VIA, VIE and PIT tags
- A disinfectant such as **Bactine**® should be applied before the adhesive to avoid trapping microbes
- Less expensive industrial adhesives ('superglues') should not be used as a replacement for surgical tissue glues

However, this procedure may only be possible in larger amphibians. In smaller animals, it can be difficult to isolate toes for application and internal marking devices such as PIT tags may be unsuitable. Moisture can interfere with setting times and adhesion so care must be taken to ensure setting has occurred before release. Problems may be experienced in their application to stream- or pond-dwelling amphibians, but can be avoided by using a small piece of sterile absorbent dressing to draw surplus water from the wound before application of the adhesive (Phillott et al. 2010).

6.4.2. Equipment

- Equipment used in marking or surgery should be appropriately **disinfected**
- Disposable sterile instruments should be used where practical/possible
- Instruments should be disinfected or changed in between each frog
- All used **disinfecting solutions, gloves and other disposable items should be stored in a sharps or other waste container and disposed of or sterilised appropriately** at the completion of fieldwork
- Disinfecting solutions must not come into contact with frogs or be permitted to contaminate any water bodies

6.5. Return of captive animals to the wild

- In general, if wild frogs or tadpoles are housed for any period of time in a captive situation (e.g. laboratory, zoo or captive breeding facility), **they should not be returned to the wild**

Exceptions to this can occur if they have been kept in isolation, their captive history is free of undiagnosed morbidity or mortality and they have had rigorous pathogen screening before release. This is usually beyond the means of most studies.

Detailed **'**Guidelines for captive breeding, raising and restocking programs for Australian frogs' can be found at:

http://www.environment.gov.au/biodiversity/invasive/projects/index.html#threat-10-11. See also 'A Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs' (Pessier and Mendelson 2010) at: http://www.cbsg.org/cbsg/workshopreports/26/amphibian_disease_manual.pdf#search=%22amphibian%22

6.6. Displaced frogs

- **Displaced frogs should be treated as if they are infected and should not be transported anywhere for release to the wild**

Displaced frogs are native frog species and introduced cane toads (*Bufo marinus*) that have been unintentionally transported from one place to another. This may typically occur with the transport of fresh produce and landscaping supplies. 'Banana Box' frog is the term used to describe several native frog species (usually *Litoria gracilenta, L. fallax, L. caerulea, L. rubella, L. infrafrenata* and *L. bicolor*) commonly transported in fruit and vegetable shipments and landscaping supplies. There is risk of spread of disease if these frogs are transferred from place to place.

When encountering a displaced frog:

- Contact a **licensed wildlife carer** organisation to collect the animal. The frog may then undergo a quarantine period along with an approved disinfection treatment
- Post-quarantine, and dependant on local state legislation and policies, the frog may be transferred to a **licensed frog keeper** once permission from the relevant regulatory body has been received. Licensed carer groups are to record and receipt frogs obtained and disposed of in this way.
- Frogs held by licensed frog keepers are **not to be released to the wild** except with relevant regulatory body approval

Displaced frogs may also be made available to recognised institutions for research projects, display purposes or offered to a museum as scientific specimens once approval has been provided by the relevant regulatory body.

- **Frogs encountered on roads, around dwellings and gardens or in swimming pools should not be considered as displaced frogs unless they are of a species not local to the area**

Local frogs encountered in these situations should be assisted off roads, away from dwellings, or out of swimming pools preferably to the nearest area of vegetation or suitable habitat.

6.6.1. Cane toads

Cane toads are known amphibian disease carriers and should not be knowingly transported or released to the wild.

If a cane toad is discovered it should be humanely euthanized in accordance with the recommended Animal Welfare procedures. Care should be taken to avoid euthanasia of native species due to mistaken identity.

6.7. Sick and dead animals

Dead amphibians or live animals showing clinical signs of disease must be regarded as having a high infection risk to healthy animals and rigorous hygiene measures are required.

- **Sick and dead frogs should be collected and sent for disease diagnosis**

No effective and practical field treatment for chytridiomycosis has been demonstrated. Similarly, no treatment regimes for ranaviral infection of frogs have been described. The collection of sick and dead frogs for expert diagnosis may improve disease surveillance activities, which can help detect disease introduction and enable emergency responses. It is also useful to assess the risk of pathogen transmission to other individuals or spread to other populations. A procedure for the preparation and transport of a sick or dead frog is given below. Adherence to this procedure will ensure the animal is maintained in a suitable condition for pathological examination and assist determining the extent of the disease and the number of species affected. For more information about sick and dead amphibians, see http://www.jcu.edu.au/school/phtm/PHTM/frogs/pmfrog.htm.

Collection:

- Do not use bare hands to handle sick or dead frogs
- Disposable gloves should be worn when handling sick or dead frogs
- New gloves and a clean plastic bag should be used for each frog specimen to prevent cross-contamination
- If the frog is dead, keep the specimen cool and preserve as soon as possible to avoid decomposition

Preserving specimens:

- Specimens can be **preserved/fixed in 70% ethanol or 10% buffered formalin**
- Cut open the belly and place the frog in about 10 times its own volume of preservative
- Where no preservative is available, **specimens can also be frozen**. If numerous frogs are collected, some should be preserved and some should be frozen. Portions of a dead frog can also be sent for analysis (e.g., a preserved foot, leg or a portion of abdominal skin)

Transportation:

- **If the frog is alive and likely to survive transportation**, place the frog into either a moistened cloth bag with some damp leaf litter or into a plastic bag with damp leaf litter and partially inflated before sealing
- Remember to **keep all frogs separated** during transportation
- **If the frog is alive but unlikely to survive transportation** (death appears imminent), euthanize the frog and place the specimen in a freezer or preservative. Once frozen/preserved the specimen is ready for shipment
- **All containers should be labelled** showing at least the species (if known), date and collection location
- Preserved samples can be sent in jars or wrapped in wet cloth, sealed in bags and placed inside a padded box
- Send frozen samples in an esky with dry ice

- Place live or frozen specimens into a small Styrofoam esky. Seal esky with packaging tape before sending
- Send the package by courier and declare any hazardous or flammable contents (e.g., 70% ethanol)

7. Hygiene protocol checklist and field kit

The following checklist and field kit are designed to assist with minimising the risk of transferring pathogens between frogs and sites in field studies (follows NSW 2008)

Have you considered the following questions before handling frogs in the field:

- Has your proposed field trip been sufficiently well planned to consider hygiene issues?
- Have you considered the boundaries between sites (particularly where endangered species or populations at risk are known to occur)?
- Have footwear disinfection procedures been considered and a strategy adopted?
- Have you planned the equipment you will be using and developed a disinfection strategy?
- Are you are planning to visit sites where vehicle disinfection will be needed? If so, do you have a plan to deal with vehicle disinfection?
- Have handling procedures been planned to minimise the risk of frog to frog pathogen transmission?
- Do you have a planned disinfection procedure to deal with equipment, apparel and direct contact with frogs?

If you answered NO to any of these questions please re-read the relevant section of the *Hygiene Protocols for the Control of Disease in Australian Frogs* and apply a suitable strategy.

Field hygiene kit

When planning to survey frogs in the field a portable field hygiene kit should be assembled to assist with implementing the hygiene protocols. Recommended contents of a field hygiene kit would include:

- Plastic box to store field equipment
- Small Styrofoam esky
- Disposable gloves
- Disinfectant spray bottle (atomiser spray) and/or wash bottle for disinfectants
- Disinfecting solutions
- Scraper or scrubbing brush for cleaning mud off footwear, vehicles etc.
- Bucket for mixing disinfecting solutions and soaking
- Plastic bags, large and small for hygienic temporary animal handling/holding
- Sharps or other container for safe waste disposal
- Materials for dealing with sick and dead frogs (see section 6.7.)

Detailed 'Guidelines for captive breeding, raising and restocking programs for Australian frogs' can be found at: http://www.environment.gov.au/biodiversity/invasive/projects/index.html#threat-10-11. See also 'A Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs' (Pessier and Mendelson 2010) at: http://www.cbsg.org/cbsg/workshopreports/26/amphibian_disease_manual.pdf#search=%22amphibian%22

8. Important Australian contacts

8.1. Sick and dead frogs

To arrange receipt and analyse sick and dead frogs, make contact with experts at any of the organisations below prior to dispatching package:

Australian Registry of Wildlife Health
Taronga Conservation Society,
Australia
PO Box 20
MOSMAN NSW 2088
Phone: 02 9978 4749

School of Public Health, Tropical Medicine and Rehabilitation Sciences
James Cook University
Douglas Campus
TOWNSVILLE QLD 4811
Phone: 07 4796 1735

School of Biological Sciences
University of Newcastle
CALLAGHAN NSW 2308
Phone: 02 4921 6014

9. References

Anver, M. R. and C. L. Pond (1984). Biology and diseases of amphibians. Laboratory Animal Medicine. B. J. C. J. G. Fox, F. M. Loew. New York, Academic Press**:** 427-447.

Berger, L., J. E. Longcore, R. Speare, A. Hyatt and L. F. Skerratt (2009). Fungal Diseases in Amphibians. Amphibian Biology, Volume 8 Amphibian Decline: Disease, Parasites, Maladies, and Pollution. H. Heatwole and J. W. Wilkinson, Surrey Beatty & Sons. NSW.

Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Slocombe, M. A. Ragan, A. D. Hyatt, K. R. McDonald, H. B. Hines, K. R. Lips, G. Marantelli and H. Parkes (1998). "Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America." Proceedings of the National Academy of Sciences of the United States of America 95(15): 9031-9036.

Berger, L., R. Speare and A. Hyatt (1999). Chytrid fungi and amphibian declines: Overview, implications and future directions. Declines and Disappearances of Australian Frogs. A. Campbell. Canberra, Environment Australia**:** 23-33.

Cashins, S., R. Alford and L. F. Skerratt (2008). "Lethal effect of latex, nitrile, and vinyl gloves on tadpoles." Herpetological Review 39: 298-301.

CCADC (2008). "Decontamination protocol to reduce the risk of spreading infectious amphibian diseases in freshwater systems. (California Centre for Amphibian Disease Control). www.ccadc.us/docs/DeconForProfessionals.pdf."

Cullen, B. R. and L. Owens (2002). "Experimental challenge and clinical cases of Bohle iridovirus (BIV) in native Australian anurans." Diseases of Aquatic Organisms 49(2): 83-92.

Daszak, P., A. A. Cunningham and H. A.D. (2001). Draft guidelines for international translocation of amphibians with respect to infectious diseases. Attachment 6. In: Speare R and Steering Committee of Getting the Jump on Amphibian Disease. Developing management strategies to control amphibian diseases: Decreasing the risks due to communicable diseases. School of Public Health and Tropical Medicine, James Cook University: Townsville. 2001: 150-156.

Davis, A. K., M. J. Yabsley, M. K. Keel and J. C. Maerz (2007). "Discovery of a novel alveolate pathogen affecting southern leopard frogs in Georgia: Description of the disease and host effects." Ecohealth 4(3): 310-317.

Department of the Environment and Heritage. (2006a). "Background document for the Threat Abatement Plan: Infection of amphibians with chytrid fungus resulting in chytridiomycosis." from http://www.environment.gov.au/biodiversity/threatened/publications/tap/chytrid/pubs/chytrid-background.pdf.

Department of the Environment and Heritage. (2006b). "Threat Abatement Plan: Infection of amphibians with chytrid fungus resulting in chytridiomycosis." 2007, from http://www.environment.gov.au/biodiversity/threatened/publications/tap/chytrid.html.

Gravekamp, C., Korver, H., Montgomery, J., Everard, C.O., Carrington, D., Ellis, W.A., Terpstra, W.J. (1991). "Leptospires isolated from toads and frogs on the Island of Barbados." Zentralblatt fur Bakteriologie 275: 403-411.

Gust, N., J. Griffiths, M. Driessen, A. Philips, N. Stewart and D. Geraghty (2009). "Distribution, prevalence and persistence of mucormycosis in Tasmanian platypuses (Ornithorhynchus anatinus)." Australian Journal of Zoology 57(4): 245-254.

Hartigan, A., I. Fiala, D. I, M. Jirků, B. Okimoto, K. Rose, D. N. Phalen and Š. J. (2011). "A suspected parasite spill-back of two novel Myxidium spp. (Myxosporea) causing

disease in Australian endemic frogs found in the invasive cane toad." PLoS One 6: e18871.
Hemingway, V., J. Brunner, R. Speare and L. Berger (2009). Viral and bacterial diseases of amphibians. Amphibian Biology, Volume 8 Amphibian Decline: Disease, Parasites, Maladies, and Pollution. H. Heatwole and J. W. Wilkinson, Surrey Beatty & Sons. NSW.
Hines, H., M. Mahony and K. McDonald (1999). An assessment of frog declines in wet subtropical Australia Declines and Disappearances of Australian Frogs. A. Campbell. Canberra, Environment Australia**:** 44-63.
Hyatt, A. D., A. R. Gould, Z. Zupanovic, A. A. Cunningham, S. Hengstberger, R. J. Whittington, J. Kattenbelt and B. E. H. Coupar (2000). "Comparative studies of piscine and amphibian iridoviruses." Archives of Virology 145(2): 301-331.
Kiesecker, J., A. R. Blaustein and C. L. Miller (2001). "Transfer of a pathogen from fish to amphibians." Conservation Biology 15: 1064-1070.
Laurance, W., K. McDonald and R. Speare (1996). "Catastrophic declines of Australian rain forest frogs: support for the epidemic disease hypothesis." Conservation Biology 10: 406-413.
Longcore, J. E., A. P. Pessier and D. K. Nichols (1999). "*Batrachochytrium dendrobatidis gen et sp nov*, a chytrid pathogenic to amphibians." Mycologia 91(2): 219-227.
Mikaelian, I., M. Ouellet, B. Pauli, J. Rodrigue, J. C. Harshbarger and D. M. Green (2000). "Ichthyophonus – like infection in wild amphibians from Québec, Canada." Diseases of Aquatic Organisms 40: 195-201.
Murray, K. A., R. Retallick, K. McDonald, D. Mendez, K. Aplin, P. Kirkpatrick, L. Berger, D. Hunter, H. B. Hines, R. Campbell, M. Pauza, M. Driessen, R. Speare, S. J. Richards, M. Mahony, A. Freeman, A. D. Phillott, J.-M. Hero, K. Kriger, D. Driscoll, A. Felton, R. Puschendorf and L. F. Skerratt (2010a). "The distribution and host range of the pandemic disease chytridiomycosis in Australia spanning surveys from 1956 to 2007." Ecology 91(5): 1557.
Murray, K. A., R. W. R. Retallick, R. Puschendorf, L. F. Skerratt, D. Rosauer, H. McCallum, L. Berger, R. Speare and J. VanDerWal (2011). "Assessing spatial patterns of disease risk to biodiversity: implications for the management of the amphibian pathogen, *Batrachochytrium dendrobatidis*." Journal of Applied Ecology 48(1): 163-173.
Murray, K. A., D. Rosauer, H. McCallum and L. F. Skerratt (2010b). "Integrating species traits with extrinsic threats: closing the gap between predicting and preventing species declines." Proceedings of the Royal Society B-Biological Sciences Published online October 27 2010(doi:10.1098/rspb.2010.1872).
Murray, K. A. and L. F. Skerratt (in press). "Predicting wild hosts for amphibian chytridiomycosis: integrating host life-history traits with pathogen environmental requirements." Human and Ecological Risk Assessment.
NSW, N. P. a. W. S. (2008). Hygiene protocol for the control of disease in frogs. Threatened Species Management**:** Information Circular No. 6. 200: p 218.
NWHC (2001). "Toe-clipping of frogs and toads standard operating procedure no. 110. NWHC (National Wildlife Health Center), Madison, WI www.nwhc.usgs.gov/publications/amphibian_research_procedures/toe_clipping.jsp.".
O'Shea, P., R. Speare and A. D. Thomas (1990). "Salmonellas from the cane toad, Bufo marinus." Australian Veterinary Journal 67: 310.
Obendorf, D. and A. Dalton (2006). "A survey for the presence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) in Tasmania." Papers and Proceedings of the Royal Society of Tasmania 140: 25-29.
Pauza, M. and M. Driessen (2008). Distribution and potential spread of amphibian chytrid fungus *Batrachochytrium dendrobatidis* in the Tasmanian Wilderness World Heritage

Area, Biodiversity Conservation Branch, Department of Primary Industries and Water, Tasmania.
Pessier, A. and J. R. Mendelson (2010). A manual for control of infectious diseases in amphibian survival assurance colonies and reintroduction programs. Apple Valley, MN, IUCN/SSC Conservation Breeding Specialist Group: .
Phillott, A. D., R. Speare, H. B. Hines, E. Meyer, L. F. Skerratt, K. R. McDonald, S. D. Cashins, D. Mendez and L. Berger (2010). "Minimising exposure of amphibians to pathogens during field studies." Diseases of Aquatic Organisms.
Sharma, V. K., Y. K. Kaura and I. P. Singh (1974). "Frogs as carriers of Salmonella and Edwardsiella." Antonie von Leeuwenhoek 40: 171-175.
Skerratt, L. F., L. Berger, H. B. Hines, K. R. McDonald, D. Mendez and R. Speare (2008). "Survey protocol for detecting chytridiomycosis in all Australian frog populations." Diseases of Aquatic Organisms 80(2): 85-94.
Skerratt, L. F., L. Berger, R. Speare, S. Cashins, K. R. McDonald, A. D. Phillott, H. B. Hines and N. Kenyon (2007). "Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs." Ecohealth 4(2): 125-134.
Skerratt, L. F., K. R. McDonald, H. B. Hines, L. Berger, D. Mendez, A. Phillott, S. D. Cashins, K. A. Murray and R. Speare (2010). "Validation of the mapping protocol for *Batrachochytrium dendrobatidis* in Queensland, Australia " Diseases of Aquatic Organisms 92: 117-129.
Speare, R., L. Berger, L. F. Skerratt, R. Alford, D. Mendez, S. Cashins, N. Kenyon, K. Hauselberger and J. Rowley (2004). Hygiene Protocol for handling amphibians in field studies. A. D. Group. Townsville, James Cook University**:** 4.
Speare, R., o. G. t. J. o. A. D. Core Working Group and o. G. t. J. o. A. Steering Committee (2001). Developing Management Strategies to Control Amphibian Diseases: Decreasing the Risks Due to communicable Diseases. S. C. o. G. t. J. o. A. D. (eds Speare R. Townsville, School of Public Health and Tropical Medicine, James Cook University.
Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman and R. W. Waller (2004). "Status and trends of amphibian declines and extinctions worldwide." Science 306(5702): 1783-1786.
Taylor, R., D. Sloan, T. Cooper, B. Morton and I. Hunter (2000). "A waterborne outbreak of Salmonella Saintpaul." Communicable Diseases Intelligence 24: 336-340.
Webb, R., A. Philips, L. Berger, J. Connolly and R. Speare (submitted). "Controlling the spread of wildlife diseases: In vitro efficacy of disinfectants against the pathogenic fungi Batrachochytrium dendrobatidis and Mucor amphibiorum." Diseases of Aquatic Organisms.
Wright, K. M. and B. R. Whitaker (2001). Amphibian Medicine and Captive Husbandry. Malabar, Florida, Krieger Publishing.
Zupanovic, Z., G. Lopez, A. D. Hyatt, B. Green, G. Bartran, H. Parkes, R. J. Whittington and R. Speare (1998). "Giant toads Bufo marinus in Australia and Venezuela have antibodies against 'ranaviruses'." Diseases of Aquatic Organisms 32(1): 1-8.