

# Appendix G

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Green and Golden Bell Frog management  
plan

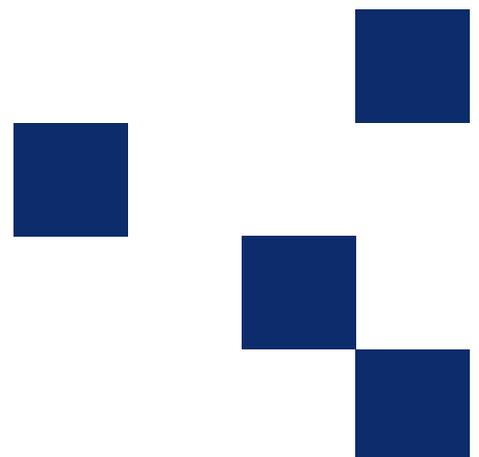


**Transport**  
Roads & Maritime  
Services

# **DUPLICATION OF TOURLE STREET AND CORMORANT ROAD, KOORAGANG**

Management Plan for the Green and  
Golden Bell Frog

JUNE 2014



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# Roads and Maritime Services

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## Duplication of Tourle Street and Cormorant Road, Kooragang

Management Plan for Green and Golden Bell Frog

June 2014

Prepared by  
Parsons Brinckerhoff Australia Pty Limited  
Level 3  
51 – 55 Bolton Street  
NEWCASTLE NSW 2300  
PO Box 1162  
NEWCASTLE NSW 2300  
Australia  
Telephone +61 2 4929 8300  
Facsimile +61 2 4929 8382  
Email [newcastle@pb.com.au](mailto:newcastle@pb.com.au)

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Authors: K Lentz, A Richardson .....

Signed:  .....

Reviewer: Toby Lambert .....

Signed:  .....

Approved by: Alex Cockerill .....

Signed:  .....

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# 1 Introduction

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## 1.1 Purpose

The Green and Golden Bell Frog (GGBF) is listed as Vulnerable under the *Commonwealth Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) and Endangered under the *NSW Threatened Species Conservation Act 1995* (TSC Act). The GGBF is known to occur within study area of the proposed 3.8 km duplication of Tourle Street, including Tourle Street Bridge, and Cormorant Road (the proposal). The GGBF is also known to occur on lands to the north, north-east and north-west of the study area, with breeding habitat also known to occur therein. The Roads and Maritime Services of NSW (Roads and Maritime) and Parsons Brinckerhoff developed this GGBF Management Plan to minimise impacts to any GGBFs encountered during construction and operation. This GGBF Management Plan must be included in the Construction Environmental Management Plan (CEMP).

## 1.2 Background

New South Wales (NSW) Roads and Maritime Services (Roads and Maritime) propose to duplicate a 3.8 kilometre (km) section of Tourle Street and Cormorant Road between Industrial Drive, Mayfield West and Egret Street, Kooragang, NSW. The proposal will improve this route from a single lane in each direction to two lanes in each direction. The route forms part of a regional transport corridor (MR108) and is the key link between Newcastle and areas to the north, including industrial operations on Kooragang Island, Williamtown Airport and residential areas such as Stockton and Medowie. The proposal would facilitate an increase in commercial, industrial, and domestic traffic to and from the identified growth areas of Newcastle airport, Medowie, and Nelson Bay which is consistent with the Lower Hunter Regional Strategy.

The proposal is being assessed under Part 5 of the *Environmental Planning and Assessment Act* (EP&A Act) in the form of a Review of Environmental Factors (REF) and prepared in accordance with clause 228 of the *Environmental Planning and Assessment Regulation 2000*, the *Threatened Species Conservation Act 1995* (TSC Act), the *Fisheries Management Act 1994* (FM Act), and the Australian Government's *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act). To inform the REF a Biodiversity Impact Assessment (Parsons Brinckerhoff 2014) was completed to examine the terrestrial and aquatic flora and fauna assemblages and their habitats in the proposal area and quantify potential biological impacts associated with the construction and operation of the proposal.

The GGBF was surveyed by Parsons Brinckerhoff ecologists during optimal weather conditions in January 2013 and included call detection, call playback, spotlighting, and targeted diurnal and nocturnal searching of potential habitat. These targeted surveys were undertaken in accordance with Threatened Species survey and assessment guidelines: field survey and methods for fauna - Amphibians (NSW Department of Environment 2009), the Survey Guidelines for Australia's threatened frogs (Department of the Environment Water Heritage and the Arts 2010b) and the Significant impact guidelines for the vulnerable Green and Golden Bell Frog (*Litoria aurea*) (Department of the Environment 2009).

A reference site known to support a large GGBF population on nearby Ash Island was used throughout the survey period to determine GGBF detectability. Numerous GGBF were found to be easily observed at the Ash Island sites during the survey period.

Five individual GGBFs were observed in the study area (Long Pond) with a further two individuals recorded from call detection (refer to Figure 1 and Photos 1 and 2).



**Photo 1 GGBF habitat in Long Pond**



**Photo 2 GGBF observed in Long Pond**

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Source: Google (2013)

- |   |                                  |  |
|---|----------------------------------|--|
| Proposed temporary and permanent frog exclusion fence | GGBF Habitat Rehabilitation Area | Exotic Grassland                                   |
| Concept Design  | Core bell frog habitat           | Juncus/Phragmites Rushland (Coastal Saltmarsh EEC) |
| <b>Mapped Vegetation Communities</b>                  |                                  |  |
| Coastal Freshwater Wetlands (EEC)                     | Mangrove - Estuarine Complex     | Planted Vegetation                                 |
| Coastal Saltmarsh (EEC)                               |                                  |  |



**Figure 1-a**  
Green and Golden Bell Frog Management Plan

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Source: Google (2013)

- |  |   |                                      |                                   |  |  |
|--|---|--------------------------------------|-----------------------------------|--|--|
|  | Green and Golden Bell Frog Location (Parsons Brinckerhoff 2013) |                                      | GGBF Habitat Rehabilitation Area  |  | Exotic Grassland                                   |
|  | Green and Golden Bell Frog Location (Post 1995) (OEH Bionet)    |                                      | Core bell frog habitat            |  | Juncus/Phragmites Rushland (Coastal Saltmarsh EEC) |
|  | Proposed temporary and permanent frog exclusion fence           | <b>Mapped Vegetation Communities</b> |                                   |  | Mangrove - Estuarine Complex                       |
|  | Concept Design  |                                      | Coastal Freshwater Wetlands (EEC) |  | Planted Vegetation                                 |
|  |   |                                      | Coastal Saltmarsh (EEC)           |  |  |

0 50 100 150 m

**Figure 1-b**  
Green and Golden Bell Frog Management Plan

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Source: Google (2013)

- |   |                                  |  |
|---|----------------------------------|--|
| Proposed temporary and permanent frog exclusion fence | GGBF Habitat Rehabilitation Area | Exotic Grassland                                   |
| Concept Design  | Core bell frog habitat           | Juncus/Phragmites Rushland (Coastal Saltmarsh EEC) |
| <b>Mapped Vegetation Communities</b>                  |                                  |  |
| Coastal Freshwater Wetlands (EEC)                     | Mangrove - Estuarine Complex     | Planted Vegetation                                 |
| Coastal Saltmarsh (EEC)                               |                                  |  |



**Figure 1-c**  
Green and Golden Bell Frog Management Plan



Important populations of the GGBF are considered to be any individual sighted post-1995, even if individuals have not recently been recorded (Department of the Environment Water Heritage and the Arts 2009c). Those individuals recorded in the study area occur as part of the Lower Hunter important population (Kooragang Island sub-population).

A key impact of the proposal to Matters of National Environmental Significance (under the EPBC Act) is the removal of known habitat for the GGBF.

Based on available information and likely impacts of the proposal, the assessment of significance for the GGBF under the EPBC Act concluded that whilst the proposal would trigger listed thresholds for impacts upon this species, such impacts are not likely to be significant in nature.

This GGBFMP has been developed in consultation with Roads and Maritime to ensure effective management and mitigation of potential impacts to the GGBF population during construction and operation of the proposal.

### 1.3 GGBF Management Plan Objectives

The objectives of this GGBFMP are to:

- Identify potential threats to GGBFs during construction and operation
- Provide mitigation measures and procedures to minimise impact to any GGBFs found during construction
- Provide additional mitigation measures to minimise long-term impacts to the GGBF population during operation.

### 1.4 Relevant legislation and guidelines

The main legislation relevant to GGBF management for the proposal is:

- *Environmental Planning and Assessment Act 1979* (EP&A Act)
- *National Parks and Wildlife Act 1974* (NPW Act)
- *Threatened Species Conservation Act 1995* (TSC Act) – GGBF is listed as an endangered species under schedule 1 of the TSC Act
- *Environmental Protection and Biodiversity Conservation Act 1999* (Commonwealth) (EPBC Act) – GGBF listed as Vulnerable under the EPBC Act
- *Pesticides Act 1999* (Pesticides Act).

The main guidelines, specifications and policy documents relevant to this GGBFMP include:

- *Policy for translocation of Threatened Fauna in NSW: Policy and Procedure Statement No. 9 Threatened Species Unit* (NSW National Parks & Wildlife Service, 2001)
- *Threatened Species Management Information Circular No. 6 – hygiene protocol for the control of disease in frogs* (NPWS, 2001).

### 1.5 GGBF populations within the locality

Kooragang Island is located adjacent to the southern arm of the Hunter River, north-west of the Newcastle CBD and is within the Sydney Basin Bioregion and Hunter subregion. The GGBF is known to occur within the study area and surrounds, with breeding habitat also known to occur in the area.

Kooragang Island provides habitat for a regionally significant and relatively large population of the GGBF. The GGBF has been recorded in the vicinity of the study area (including Long Pond)

historically (ERM 1996, Hamer et al. 2002, ERM 2003, ERM 2004, Resource Strategies 2006a and 2006b) with the first report of this species on Kooragang Island in the 1970s (Gosper 1997 in Hamer et al. 2002). In recent years, the species has been recorded in and around the study area by Umwelt (2012), GHD (2010), Leu (2011) and Stockwell (2010). On Kooragang Island the important population of GGBF utilises a range of habitats, encompassing the Long Pond in the study area and numerous better quality habitats to the north, north-west and north-east, of the proposal. Furthermore, this species' areas of occupancy include habitats in the Hunter Wetlands National Park.

In the Lower Hunter region GGBFs are known from Kooragang and Ash Island in the estuary; from locations within Hexham Swamp; from near Medowie to the north of the estuary; and to the south near Catherine Hill Bay. The Hexham Swamp population is considered to have been large in recent historic times, and there are numerous anecdotal records of the frog occurring in urban areas adjacent to the swamp prior to the 1990s (Markwell 1984). A population occurred on the periphery of the former Hexham Swamp Nature Reserve up until 2007 (Umwelt 2012), directly adjacent to the Kooragang / Ash Island population. This population was monitored during 2004-2007 and again in 2010-2012 showing the presence of up to 100 adult individuals, but recent monitoring showed the population has since disappeared (Umwelt 2012). It is thought that the most likely explanation for the decline of the GGBF from such large swamp habitats is the detrimental role of the amphibian disease Chytridiomycosis (Chytrid Fungus (*Batrachochytrium dendrobatidis*)) (Mahony, 1996).

As a result of the disappearance of the population of GGBFs from the Hexham Swamp areas and the isolated and unconfirmed status of other populations within the Hunter region, the Kooragang population of GGBF is of high significance at a regional level (Umwelt 2012).

Given the restricted nature of known populations of the GGBF, all current populations are regarded as an 'important population' (Department of the Environment Water Heritage and the Arts 2009b). Therefore, individuals recorded in Long Pond form part of the Lower Hunter important population (Kooragang Island sub-population) (Department of the Environment Water Heritage and the Arts 2009c).

The species was formally distributed from the NSW north coast near Brunswick Heads along the NSW coast to Victoria to East Gippsland, west to Bathurst, Tumut and the ACT. Decline was noticed in the late 1970s and became severe in the 1980s, such that today the species exists as a series of isolated coastal populations within its former known range.

In NSW, breeding habitat for the Green and Golden Bell Frog includes water bodies that are still, shallow, ephemeral, unshaded, with aquatic plants and free of the introduced Eastern Gambusia (*Gambusia holbrooki*) and other predatory fish (Department of the Environment Water Heritage and the Arts 2009c; Pyke & White 1996).

Within the study area Long Pond occurs as an artificial, non-tidal freshwater wetland created using fill material from various industrial activities on Kooragang Island. Long Pond is influenced by rainfall with water levels gauged by varying wetting and drying cycles and maintains significant aquatic vegetation. For example, during initial field surveys Long Pond was observed as several large and shallow pools of standing stagnant water (significant fish/eel kill observed therein), to relatively high water levels following significant rainfall (>90 mm in seven days). Photographs 3 and 4 provide visual demonstration of this wetting and drying cycle.

Although Eastern Gambusia were observed to be abundant in Long Pond, this habitat cannot be discounted as potential breeding habitat for this species. The observation of five individual GGBFs, with a further two GGBFs recorded via call recognition may indicate that breeding is occurring, although this is not known at this stage for certain.



**Photo 3 Long Pond during a drying cycle (8 January 2013)**



**Photo 4 Long Pond following significant rainfall (19 October 2012)**

## 2 Potential threats to GGBF during construction

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Injury or death to GGBFs may potentially occur as a result of direct and indirect impacts during construction of the proposal as outlined below.

### 2.1 Direct impacts

- Depending on timing of works, hibernating GGBFs may be run over or injured by vehicles moving around the proposal area
- GGBFs that may be sheltering in grass being impaled or injured during the installation of fences
- GGBF entering the proposal area overnight and seeking refuge under machinery, vehicles, construction materials or stockpiles, may be injured or killed when these items are moved for use
- GGBF sheltering in grass or reeds, on tree branches, underneath rocks, debris or timber, or within culverts and waterbodies, may be killed or injured during vegetation removal and earthworks
- Loss of native vegetation and habitat
- Increased barrier to GGBF movement due to proposed concrete barrier in median between carriageways adjacent to Long Pond
- Draining and infilling of existing drainage lines or wet areas.

These potential impacts would be mitigated through the use of permanent frog exclusion fences and pre-clearing surveys for GGBFs as outlined in Section 3.

### 2.2 Indirect impacts

- Chemical, fuel or solvent spills contaminating the soil or waterways being used by GGBFs and causing death or injury to frogs or tadpoles
- Sediment laden water may be discharged and potentially contaminate GGBF habitat
- Wind-blown dust, Quick Lime particles or industrial fumes causing burns or injury to frogs or contaminating water that is being used as a breeding site
- Soil, mulch or other landscaping materials containing spores of Chytrid Fungus (*Batrachochytrium dendrobatidis*) causing illness or death to frogs and tadpoles
- Water containing spores of Chytrid Fungus being imported onto the work site, causing illness or death to frogs and tadpoles
- Water containing the introduced Eastern Gambusia being spread into adjacent waterways
- Use of herbicides on the work site
- Noise impacts.

Measures to mitigate these potential impacts are outlined in Section 3.

## 3 GGBF mitigation measures

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The following management measures would be implemented to minimise potential impacts on the GGBF and must be incorporated into the CEMP:

- Environmental induction training
- Site hygiene management
- Temporary frog exclusion fencing
- Permanent frog exclusion fencing
- Pre-clearing surveys for GGBF (nocturnal and diurnal)
- GGBF relocation procedures
- Construction works procedures (including timing of works)
- Reporting procedures.

### 3.1 Environmental induction training

All personnel and contractors would undergo environmental induction training before commencing work on site. Information to be addressed during this training would include:

- GGBF profile and identification
- Identification of GGBF habitat areas. Construction personnel would be prohibited from entering GGBF habitat areas located outside defined construction or operation areas
- Site hygiene management in accordance with the Hygiene Protocol
- Procedures to be followed in the event that GGBFs are found or injured.

#### 3.1.1 Chytrid Fungus (*Batrachochytrium dendrobatidis*)

The accidental introduction or spread of pathogens such as Chytrid Fungus has the potential to adversely affect frog populations worldwide. In Australia, Chytrid Fungus has impacted on native frog species causing the extinction of one species and suspected to have caused the extinction of three others. The '*Infection of frogs by amphibian Chytrid fungus causing the disease Chytridiomycosis*' is listed as a Key Threatening Process under the TSC and EPBC Acts.

Typical symptoms of frogs infected with Chytridiomycosis include lethargy, accumulation of sloughed skin over the body, emaciation, half-closed eyes, redness on the underside of the body and legs. Chytrid Fungus is a virulent and highly contagious frog disease that kills frogs and tadpoles. Chytrid Fungus is a water-borne fungus that may be spread as a result of handling frogs or through cross contamination of water bodies.

#### 3.1.2 Training

To reduce the likelihood of introducing or spreading pathogens such as Chytrid fungus, all construction personnel and contractors would be trained in site hygiene management in accordance with the *RTA Biodiversity Guidelines – Protecting and Managing Biodiversity on RTA Projects* (Guide 7- Pathogens) (RTA 2011) as part of environmental induction training.

#### 3.1.3 Best practice hygiene protocols for Chytrid fungus

In accordance with *EPBC Act Policy Statement 3.19 Significant impact guidelines for the vulnerable Green and Golden Bell Frog (*Litoria aurea*)* the contractor would prepare a hygiene protocol to be implemented for the proposal. The protocol would aim to ensure that new sources of Chytrid are not introduced into the proposal area through the movement of contaminated machinery and materials over long distances along with the proper use and disposal of waters.

Table 1 presents a range of best practice hygiene protocols to reduce the risk of the introduction or spread of Chytrid on the proposal. The NSW National Parks and Wildlife Service *Hygiene Protocol for the control of Disease in Frogs (2008)* should be utilised in conjunction with the site specific hygiene protocol (Appendix A).

**Table 1 Best practice hygiene protocols to be implemented for the proposal to prevent the spread or introduction of Chytrid**

<b>Best Practice Hygiene Protocols</b>	<b>Chytrid (<i>Batrachochytrium dendrobatidis</i>)</b>
<b>Work programs</b>	<ul style="list-style-type: none"> <li>Minimise work during excessively wet or muddy conditions.</li> </ul>
<b>Restrict access</b>	<ul style="list-style-type: none"> <li>Set up exclusion zones with fencing and signage to restrict access into potential frog habitat areas outside of the proposal area.</li> </ul>
<b>Inductions</b>	<ul style="list-style-type: none"> <li>All personnel (including visitors) to be inducted on Chytrid management measures for the site through toolbox talks and site inductions.</li> </ul>
<b>Vehicles and machinery</b>	<ul style="list-style-type: none"> <li>Vehicles initially entering the proposal area must not be tracking soil/mud and/or vegetative material. If soil/mud and/or vegetative material are found on these vehicles, they must be cleaned in a hard stand area within the site compound area. Any organic waste collected during the washdown process would be removed from site.</li> <li>Restrict vehicles to parking within proposal area and site compound parking areas (eg within designated parking areas only).</li> <li>Provide a bunded area for the wash down of earth moving plant</li> <li>Provide parking and turn-around points on hard, well-drained surfaces.</li> <li>Vehicles leaving the site must be washed down with a high-pressure washer containing Farmcleanse (in accordance with the manufacturer recommended dosages) or methylated spirits diluted in town water (70:30 ratio).</li> </ul>
<b>Personnel and equipment</b>	<ul style="list-style-type: none"> <li>For high risk activities including establishing permanent frog exclusion fencing, pre-clearing surveys and clearing and grubbing, ALL personnel are to disinfect their footwear with methylated spirits diluted in town water (70:30 ratio) prior to commencing work and prior to leaving the proposal area at the completion of the day's activities (Photo 5).</li> <li>Disinfect hands or change gloves between the handling of individual frogs and between each site.</li> <li>Only handle frogs when necessary and always use gloves. Use the 'one bag-one frog' or 'one container-one frog' approach.</li> </ul>
<b>New material</b>	<ul style="list-style-type: none"> <li>Source landscaping materials from a supplier that is certified to be disease-free.</li> </ul>
<b>Disposing of material</b>	<ul style="list-style-type: none"> <li>To avoid cross contamination, generally avoid transferring water between two or more separate waterbodies.</li> </ul>
<b>Further information</b>	<ul style="list-style-type: none"> <li><i>Hygiene protocol</i> for the control of disease in frogs, Information Circular Number 6 (Wellington and Haering 2008) (Appendix A)</li> </ul>

No studies are known to have been undertaken within the proposal area to determine the presence of Chytrid Fungus in Long Pond. However, if Chytrid Fungus is present (likely), the infection rate and intensity would be expected to increase in the cooler months, when the metabolic rate of frogs slows down and they are more susceptible to infection and mortality.



**Photo 5** Boot wash down to prevent the spread of Chytrid on shoes on the Sapphire to Woolgoolga project, Northern Region (Photo: Josie Stokes, Roads and Maritime).

### 3.2 Temporary frog exclusion fencing

It is highly likely GGBFs may enter the works area from off-site habitat under suitable weather conditions. Frog protection measures are therefore required to protect GGBFs from injury or death during construction of the proposal.

Additionally, given the proximity of the proposal area to core and supplementary GGBF habitat (Long Pond) the majority of the works area would be considered potential GGBF habitat; particularly the aquatic vegetated sections and ponds between Tourle Street Bridge and Pacific National Intersection.

In accordance with *EPBC Act Policy Statement 3.19 Significant impact guidelines for the vulnerable Green and Golden Bell Frog (*Litoria aurea*)*, temporary frog exclusion fencing would be installed prior to the commencement of construction. An example design of temporary frog exclusion fencing is provided in Photos 6 and 7. The location and extent of temporary frog exclusion fencing (indicative location and extent depicted on Figure 1) will be determined in consultation with the **Project Ecologist** and a suitably qualified frog specialist with experience with GGBF populations in the Hunter Region.

Temporary frog exclusion fencing provides a barrier which minimises the risk of GGBF entering the construction works area and being injured or killed. The **Site Environmental Representative** would ensure relevant signage is erected on the temporary frog exclusion fencing to alert staff to the “environmentally sensitive area” and to prevent personnel from entering these areas. The signage should be highly visible.

In some locations, temporary frog exclusion fencing may need to include frog-proof gates to allow vehicle and personnel access to the proposal area. If frog-proof gates are installed, they must be shut at the end of each day.

The **Site Environmental Representative** would include the inspection of temporary frog exclusion fences as part of the daily checklist for personnel, to ensure they are functional and not torn or holed. Damage to the fence from machinery during construction would be fixed within the working day. The **Site Environmental Representative** would ensure any repairs to the fence are made before dusk to minimise the risk of frogs entering the works area overnight.

It is anticipated that a minimum of one nocturnal and diurnal pre-clearing GGBF survey would be undertaken prior to the installation of temporary frog exclusion fencing.



**Photo 6** Close up view of temporary frog fencing (Photo: Shaun Walsh, Roads and Maritime).



**Photo 7** Temporary frog fencing that has been installed on a Roads and Maritime project. (Photo: Shaun Walsh, Roads and Maritime).

### 3.2.1 Marking of GGBF habitat beyond the temporary frog exclusion fencing

Potential GGBF habitat (eg Long Pond and the small ponds to the south of Cormorant Road opposite the Pacific National Access Road – Figure 1) beyond the temporary frog exclusion fencing must be marked on maps as “environmentally sensitive areas” and included in the CEMP.

Construction personnel would be prohibited from entering GGBF habitat areas beyond the temporary frog exclusion fencing without approval from the **Site Environmental Representative**. The establishment of the GGBF habitat areas would be conducted in accordance with the relevant measures outlined in the Hygiene Protocol.

## 3.3 Pre-clearing surveys for GGBFs

### 3.3.1 Prior to construction or disturbance

A pre-clearance survey will be undertaken by the **Project Ecologist** prior to the identified potential GGBF habitat areas are disturbed (including frog fence installation or clearing/grubbing). Pre-clearance surveys will consist of one night time calling and spotlighting event and one diurnal habitat investigation, 24 hours prior to the disturbance of habitat to determine whether the area is free of GGBFs (as best as can be determined).

Nocturnal pre-clearing surveys of the proposal area may include the following methods:

- Spotlighting (in the lower branches of trees, Typha, macrophytes, ephemeral water bodies and grass)
- Spotlighting of frog exclusion fencing once installed (particularly along the ground on the inside and outside of the fence)
- Active searching of potential sheltering habitat within the proposal area (hand-turning rocks, timber, bricks and other debris)
- Call playback.

Diurnal (day-time) pre-clearing surveys would be carried out by the **Project Ecologist** to reduce the risk of injury or death to GGBFs that may be sheltering or utilising habitat within the proposal area.

This may include searching:

- Frog exclusion fencing
- Vegetation (including groundcover) that is scheduled to be removed
- Under rocks, debris, timber and other construction materials, within the site.

If GGBFs are detected during a pre-clearance survey, the handling and rescue procedure outlined below must be followed, and the pre-clearance survey undertaken again, until the ecologist can determine that the area is free of GGBFs and only then can works be undertaken.

A pre-clearance survey approving works will be valid for a single connected habitat area for a period of 1 week, in which time another pre-clearance survey must be undertaken before clearing is to be continued.

The validity of pre-clearance survey can be retained indefinitely if a frog-exclusion fence is installed and maintained around the perimeter of the potential habitat area prior to the pre-clearance survey and must be undertaken prior to any disturbance. A pre-clearance survey in an area isolated by a frog-exclusion fence is only valid as long as the frog-exclusion fences integrity is not compromised.

All surveys for GGBFs would be undertaken in accordance with the relevant measures outlined in the Hygiene Protocol.

All GGBFs collected during pre-clearing surveys would be weighed, measured, sexed and checked for signs of injury/disease by the **Project Ecologist** before being relocated to the adjacent Long Pond habitat in accordance with the GGBF relocation procedures (Section 3.5).

### 3.3.2 Pre-start up checks for GGBFs during construction

During construction, only selected trained staff would be responsible for checking on and around plant and equipment for any GGBFs that may have moved into the construction works area

overnight. The base of the frog exclusion fencing should also be checked as part of this procedure for any frogs that may be sheltering near or under the base of the fencing.

The pre start-up check for GGBFs would be added to the form for the pre-start checklist, and a fortnightly review/audit of these forms would be undertaken by the **Roads and Maritime Environment Representative** as part of the environment inspections.

The GGBF relocation procedure would be followed for any live frogs found as part of the pre start-up check by the **Roads and Maritime Environment Representative** or **Project Ecologist**. A flowchart outlining the key steps for the GGBF relocation procedure must be included in the CEMP.

The pre start-up check for GGBFs is an adaptive procedure that would be reviewed monthly by the **Site Environmental Representative**. If GGBFs are regularly found by personnel within the construction works area, the procedure would be amended and additional mitigation measures would be implemented to further minimise risk of injury or death to GGBFs.

### 3.4 Vegetation clearing within potential GGBF habitat areas

Prior to any clearing of potential GGBF habitat areas, a pre-clearance survey must be completed by an appropriately qualified ecologist. This must be undertaken before clearing commences within potential GGBF habitat areas. Once an area has been surveyed and any detected GGBF relocated, disturbance activities may commence.

### 3.5 GGBF relocation and handling procedures

If any GGBFs are encountered during construction, the procedure outlined below will be implemented (refer to Figure 2).

In the event that a GGBF is discovered during construction, works must cease in the affected area and the **Site Environmental Representative** must be contacted immediately. The representative will confirm the identification of the species, record the location of the GGBF and set up an exclusion zone around the location. The representative will immediately contact the **Project Ecologist** to capture and quarantine the GGBF until relocation can be undertaken.

The preferred relocation site has been identified as the existing GGBF habitat in Long Pond outside of the proposed frog exclusion fencing. The benefits of the identified relocation site include:

- Being likely to be already part of the relocated GGBFs existing habitat range
- Being geographically within the GGBFs range (Pyke & White, 2001) from the proposal area
- Having known observations recorded post 1995, which are therefore recognised as important populations.

The preferred relocation site is subject to change according to suitability and feasibility at the time. If needed for any reason, an alternative relocation area would be identified, in consultation with the **Project Ecologist**, and a suitably qualified frog specialist with experience with the GGBF populations within the Hunter Region this specialist would be approved by Roads and Maritime and consult with Office of Environment and Heritage (OEH) in providing a suitable alternative relocation site.

Any deceased GGBFs uncovered during construction, where the cause of death is not clear, should be quarantined and the **Project Ecologist** will be contacted to undertake any preservation required prior to donation for testing/research, as well as suitable disposal, if required.



**Figure 2 GGBF handling and relocation procedure**

Chytrid Fungus is a key threat to ongoing viability of GGBF and therefore any handling of GGBFs on site would be undertaken as per the NSW NPWS hygiene protocol for the control of disease in frogs (refer to Appendix A). This would include precautions to minimise the potential spread of Chytrid Fungus, such as:

- Handling frogs as little as possible as to avoid removing skin secretions
- Keeping frogs moist
- No use of insect repellent or hand creams before handling frogs
- Hands should be cleaned and disinfected between handling individual frogs or a new pair of disposable gloves should be used per individual sample.

The **Site Environmental Representative** contact number would be displayed around the site and included within the project's Inspection and Test Plans, and on relevant site cards.

### 3.6 Hold points

The proposal will use an internal permit system for critical activities, requiring approval to release by the nominated authority prior to the commencement of the activity. A hold point applies to the following GGBF works:

- Clearing of vegetation
- Works adjacent to, or near core GGBF habitat (refer to Figure 1).

### 3.7 Mitigation measures to address indirect impacts

A summary of construction mitigation measures to be implemented for the proposal are provided in Table 2.

#### 3.7.1 Construction spills and air quality impacts

Chemical, fuel or solvent spills have the potential to contaminate waterways and adjacent GGBF habitat, particularly in the immediate vicinity of Long Pond and the ponds opposite the Pacific National Access Road, which may cause death or injury to frogs and tadpoles.

Fuel and chemical storage sites would be stored in site compound areas in accordance with the procedures for fuel and chemical storage for the proposal.

Wind-blown dust, industrial fumes or construction particulates would be contained through the use of silt screens, where possible. Volatile substances would not be permitted in areas of GGBF habitat. These substances must only be used in suitable locations and stored in a manner that is appropriate for the substance.

Water tankers would be used to settle dust in exposed areas (see Section 3.7.2 for mitigation measures that address importation of water to the proposal area).

#### 3.7.2 Importation of water to the proposal area

Water that may be used on site to suppress dust may contain spores of Chytrid Fungus. It is important that any off-site water used in dust suppression has been chlorine-treated as it will be less likely to contain Chytrid Fungus spores. Chlorine-treated town water and/or rainwater collected in sediment basins within the proposal area would be used for dust suppression.

The Eastern Gambusia predated on GGBF tadpoles and is listed as a Key Threatening Process under the TSC Act. Several waterbodies sighted in the proposal area (Long Pond and the ponds

opposite the Pacific National Access Road) were observed to contain high densities of Eastern Gambusia and additional sources of this species should not be introduced.

Imported water would not be used on site unless it has been demonstrated to be fish-free. If water is sourced from existing, open water bodies in the area, it must be screened through 600 micron mesh before being used.

### 3.7.3 Discharging water from sediment basins

Sediment laden water that may be discharged from construction sediment basins has the potential to contaminate GGBF habitat. The Erosion and Sediment Control Plan would be reviewed by the **Project Ecologist** to ensure it is consistent with the objectives of this GGBF Management Plan. Water from construction basins would meet any Environmental Pollution Licence requirements and would be either re-used on site for dust suppression or would be discharged off site.

### 3.7.4 Importation of landscaping materials to the proposal area

Soil, mulch, tubestock and landscaping materials may contain spores of Chytrid Fungus. The '*Infection of frogs by amphibian Chytrid fungus causing the disease Chytridiomycosis*' is listed as a Key Threatening Process under the TSC and EPBC Acts.

In accordance with EPBC Act Policy Statement 3.19 *Significant impact guidelines for the vulnerable Green and Golden Bell Frog (Litoria aurea)* a Hygiene Protocol would be developed and implemented for the proposal. Site landscaping materials would be sourced from a supplier that is certified to be disease-free. Any imported materials will comply with Australian Standards, as well as any material import standards outlined in the CEMP.

### 3.7.5 Use of herbicides

The use of herbicides should be avoided around aquatic habitats and in the vicinity of potential GGBF habitat within the proposal area. Where herbicides are to be used on other areas within the proposal area, ensure spray drift cannot reach aquatic habitats and potential GGBF habitat. Ensure surface sprays cannot enter aquatic habitats and potential GGBF habitat (eg Long Pond and the ponds opposite the Pacific National Access Road) in solution via surface water run-off.

## 3.8 Reporting procedures

For the duration of the proposal, the **Site Environment Representative** and **Project Ecologist** must keep records of the number, location and time/date of all GGBFs detected and relocated during the pre-clearing surveys and pre-start up checks during of the proposal.

The **Project Ecologist** would supply a post-construction report which details all information to the **Roads and Maritime Environmental Representative**.

## 3.9 Additional recommendations

Additional recommendations during the construction of the proposal that are consistent with the objectives of this GGBF Management plan include:

- The **Site Environment Representative or Project Ecologist** must be on site during the removal of potential GGBF sheltering/overwintering habitat (eg large boulders, culvert pipes, concrete rubble and pond mud) to capture any GGBFs
- The **Project Ecologist** would be on call to assist with the relocation procedure for any GGBFs found during construction. If the **Project Ecologist** is not available, the **Site Environment**

**Representative** must call the **Roads and Maritime Environment Representative** or **Roads and Maritime Environment Branch Biodiversity Specialist**

- Clearing and grubbing works are to be timed (where possible) when GGBFs are at their most active (September to April), and can move away from potential disturbances in accordance with *EPBC Act Policy Statement 3.19 Significant impact guidelines for the vulnerable Green and Golden Bell Frog (Litoria aurea)*. If clearing and grubbing works are scheduled to be undertaken outside the activity period for GGBFs, the **Project Ecologist** and/or **Roads and Maritime Environment specialists** must be consulted as additional mitigation measures (eg more intensive pre-clearing surveys) may need to be implemented
- In accordance with this GGBFMP, tadpole surveys would be undertaken by the **Project Ecologist** prior to the de-watering of any waterbodies that are to be drained for construction (eg culverts) to minimise the risk of harming any GGBF tadpoles if they are present. If GGBF tadpoles are present, they would be relocated to Long Pond by the **Project Ecologist**. The relocation of GGBF tadpoles is to be undertaken in accordance with requirements of Section 3.6.

## 4 Long-term mitigation measures and compensatory habitat creation

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Roads and Maritime will implement the following mitigation measures to minimise potential long-term impacts to the GGBF.

### 4.1 Permanent frog exclusion fencing

An increase in the width of the road surface along Cormorant Road, together with the installation of a vertical concrete barrier along the central median will effectively fragment areas of known habitat associated with Long Pond and sub-optimal habitats to the south of Cormorant Road. It is likely that without appropriate ameliorative measure, GGBFs could become trapped on the road, increasing the extent of vehicle strikes. Permanent frog protection measures are therefore required to protect GGBFs from this hazardous barrier. It is proposed that permanent frog exclusion fencing would be installed along the southern edge of the Long Pond (Figure 1). The location, extent and type of permanent frog exclusion fencing will be determined in consultation with the **Project Ecologist** and a suitably qualified frog specialist with experience with GGBF populations in the Hunter Region.

It is envisaged that permanent frog exclusion fencing would slowly replace temporary fencing during construction of the proposal. Permanent frog exclusion fencing would ideally be installed in its entirety during the transition from construction phase to the operational phase of the proposal.

### 4.2 Implement Eastern Gambusia (*Gambusia holbrooki*) control

The existing Long Pond and the ponds opposite the Pacific National Access Road contain relatively high densities of the predatory Eastern Gambusia. As detailed in DSE (2011) *Guidelines for Eastern Gambusia Removal*, there are a range of actions that can be taken to control Eastern Gambusia including:

- Do nothing
- Drain/ dry the site
- Poison the site
- Remove Eastern Gambusia.

Whilst drying or poisoning a site has been shown to be a successful eradication strategy, this is unlikely to be a suitable option given the requirement of aquatic habitat for the GGBF and the likely sensitivity of GGBF to any poisons whatsoever.

NSW Department of Industry and Investment indicates that currently, there are minimal effective control options for managing Eastern Gambusia in open waterways. Once pest fish become established in a waterway it can be almost impossible to eradicate them. In the *Threat Abatement Plan for Predation by Gambusia holbrooki*. (NSW National Parks and Wildlife Service 2003) it is indicated that chemical, biological and physical controls are the main options. Physical controls such as netting and draining appear to be preferred options. This is supported by the Victorian DSE (2011) which indicates that these are the main potential controls. There are complexities with potential impacts upon other native species with each of these approaches.

It is considered that given that physical removal is the preferred option, this would occur via seine netting (preferably in winter / spring) as outlined in Appendix B (DSE (2011) *Guidelines for Eastern Gambusia Removal*).

The proposed implementation of Eastern Gambusia control is to be undertaken within six months of construction being completed, during a period of dry weather when water levels are naturally low.

### 4.3 Compensatory GGBF habitat creation

About 0.80 ha of suitable GGBF habitat will be removed by the proposal. Rehabilitation of existing poor quality habitat or unsuitable habitat to provide compensatory habitat for the GGBF would be located and designed in accordance with '*Best Practice Guidelines GGBF habitat*' (DECC 2008) and with advice/input from GGBF experts.

Compensatory habitat for the GGBF population at Long Pond will include replacement of 0.52 ha of Exotic Grassland disturbed habitat that exists within and adjoining Long Pond with native aquatic vegetation including Typha. This will replace the areas of Typha and associated habitat to be removed within the proposal area and ensure a similar level of habitat exists for the GGBF post-development.

Refer to Figure 1 for proposed areas of habitat restoration.

## 5 Summary of mitigation measures

**Table 2 Summary of construction and operation mitigation measures to be implemented to minimise impacts on the GGBF**

Proposal phase	Potential impact	Mitigation measure	Description
Construction	Direct mortality	Temporary frog exclusion fencing	The presence of this fence will discourage and minimise frogs from moving into construction areas where they may be injured and killed.
		Pre-clearing surveys	Diurnal and nocturnal pre-clearing surveys will be undertaken by a qualified Ecologist to remove frogs.  Daily pre-start up checks will be undertaken to collect and move any frogs that have moved into construction areas, to prevent frogs being injured or killed when these items are moved for use.  Internal permit systems would be developed for critical activities, including vegetation clearing.
		Critical activity 'Hold Points'	Critical activities would require approval to release by the nominated authority prior to the commencement of the activity.
		Restriction in time of vegetation clearing	Clearing of vegetation after a period of heavy rainfall would be avoided (where possible).  Clearing is such times would otherwise result in works occurring during periods when frogs are unlikely to be moving around in the proposal area, reducing their chances of moving into works areas once they have commenced.  Clearing and grubbing works are to be timed (where possible) when GGBFs are at their most active (September to April), and can move away from potential disturbances.
	Habitat disturbance/ indirect mortality	Implementation of a Hygiene Protocol to minimise the possible impacts of Chytrid Fungus	The protocol would aim to ensure that new sources of Chytrid Fungus are not introduced into the proposal area through the movement of contaminated machinery and materials over long distances, together with the proper use and disposal of waters.
		Environmental induction training	All personnel and contractors would complete environmental induction training before commencing construction activities. Information disseminated would include, GGBF identification and profile, identification of GGBF habitat areas, site hygiene management procedures, and procedures should GGBF be identified during construction activities.  Construction personnel would be prohibited from entering GGBF habitat areas beyond the temporary frog exclusion fencing without prior approval from the Site Environmental Representative.

Proposal phase	Potential impact	Mitigation measure	Description
		GGBF handling and relocation procedures	This procedure nominates key personnel and their responsibilities should a GGBF be encountered during construction activities. It specifically addresses the requirement for cessation of construction activities in the immediate vicinity of the sighting and appropriate relocation any individual(s). Appropriate handling and relocation of encountered GGBF would ensure
Operation	Direct mortality	Permanent frog-exclusion fencing	The presence of this fence will discourage and minimise frogs moving into the road corridor where they may be injured or killed.
	Loss of known habitat	Compensatory habitat within the study area (Long Pond)	The proposal will provide compensatory habitat within the study area post construction to mitigate for the removal of habitat within long pond (refer to Section 4.2).
	Increased presence of Eastern Gambusia	Implement Eastern Gambusia control	Implementation of Eastern Gambusia control is an additional longer term mitigation measure aimed at physical removal of this species during a period of dry weather when water levels are naturally low. Ideally, this process would be completed within six months of completion of construction activities.

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# Appendix A

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Threatened Species Management  
Information Circular No. *Hygiene Protocol for  
the Control of Disease in Frogs*



# Threatened Species Management Information Circular No. 6



hygiene protocol for the  
control of disease in

# frogs

April 2008

Department of **Environment & Climate Change** NSW



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Department of Environment and Climate Change (NSW)  
59-61 Goulburn Street  
(PO Box A290)  
Sydney South 1232

Phone: (02) 9995 5000 (switchboard)  
Phone: 131 555 (environment information  
and publications requests)  
Phone: 1300 361 967 (national parks information  
and publications requests)  
Fax: (02) 9995 5999  
TTY: (02) 9211 47 23  
Email: [info@environment.nsw.gov.au](mailto:info@environment.nsw.gov.au)  
Website: [www.environment.nsw.gov.au](http://www.environment.nsw.gov.au)

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This hygiene protocol is an adaptation of the Declining Amphibian Population Task Force (DAPTF) Fieldwork Code of Practice and the recommendations of Speare et al. (1999) and has drawn on recommendations from earlier guidelines prepared by Environment ACT.

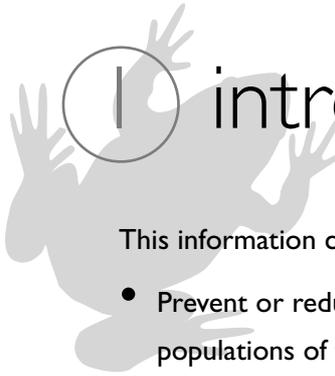
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# hygiene protocol for the control of disease in

# frogs

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# 1 introduction

This information circular outlines measures to:

- Prevent or reduce disease causing pathogens being transferred within and between wild populations of frogs.
- Ensure captive frogs are not infected prior to release.
- Deal safely with unintentionally transported frogs.
- Assist with the proper identification and management of sick and dead frogs in the wild.

## 1.1 Who should read this document?

This protocol is intended for use by all researchers, wildlife consultants, fauna surveyors and students undertaking frog field-work. In addition, the protocol should be read by Department of Environment and Climate Change (DECC) personnel, frog keepers, wildlife rescue and carer organisations, herpetological/frog interest groups/societies, fauna park/zoo operators/workers and other individuals who regularly deal with or are likely to encounter frogs.

This protocol outlines the expectations of the DECC regarding precautionary procedures to be employed when working with frog populations. The intention is to promote implementation of hygiene procedures by all individuals working with frogs. New licences and licence renewals will be conditional upon incorporation of the protocol. The DECC recognises that some variation from the protocol may be appropriate for particular research and frog handling activities. Such variation proposals should accompany any licence application or renewal to the DECC.

## 1.2 Background

### 1.2.1 Amphibian Chytrid Fungus

The apparent decline of frogs, including extinctions of species and local populations, has attracted increased international and national concern. Many

potential causes for frog declines have been proposed (eg see Pechmann et al., 1991; Ferrero and Bergin, 1993; Pechmann and Wilbur, 1994; Pounds and Crump, 1994; Pounds et al., 1997). However, the patterns of decline at many locations suggest that epidemic disease maybe the cause (Richards et al., 1993; Laurance et al., 1996; Alford and Richards, 1997). Recent research has implicated a water-borne fungal pathogen *Batrachochytrium dendrobatidis* as the likely specific causative agent in many of these declines both in Australia and elsewhere (Berger et al., 1998; 1999). This agent is commonly known as the amphibian or frog chytrid fungus and is responsible for the disease Chytridiomycosis (Berger et al., 1999).

*B. dendrobatidis* is a form of fungus belonging to the phylum Chytridiomycota. Most species within this phylum occur as free-living saprophytic fungi in water and soil and have been found in almost every type of environment including deserts, arctic tundra and rainforest and are considered important primary biodegraders (Powell 1993). *B. dendrobatidis* is a unique parasitic form of Chytridiomycete fungi, in that it invades the skin of amphibians, including tadpoles, often causing sporadic deaths with up to 100% mortality in some populations. Chytridiomycosis has been detected in over 40 species of native amphibian in Australia (Mahony and Workman 2000). However, it is not currently known whether the fungus is endemic or exotic to Australia.

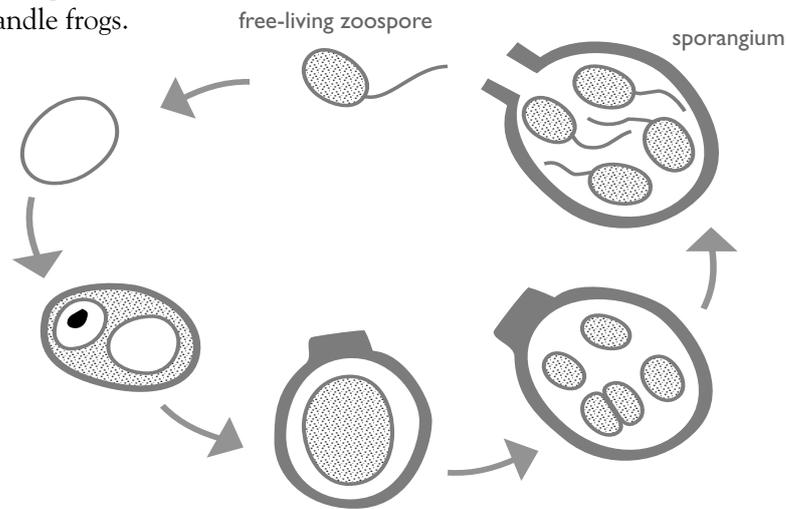
The infective stage of *B. dendrobatidis* is the zoospore and transmission requires water (Berger et al., 1999). Zoospores released from an infected amphibian can potentially infect other amphibians in the same water. More research is needed on the dynamics of infection in the wild. *B. dendrobatidis* is known to be susceptible to seasonal temperature changes, dehydration, salinity, water pH, light, nutrition and dissolved oxygen (Berger et al., 1999).

### 1.3 Objectives

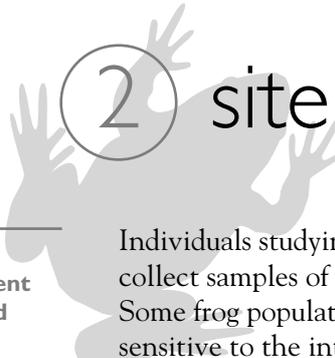
The objectives of the hygiene protocol are to:

- Recommend best-practice procedures for DECC personnel, researchers, consultants and other frog enthusiasts or individuals who handle frogs.

- Suggest workable strategies for those regularly working in the field with frogs or conducting fieldwork activities in wetlands and other aquatic environments where there is the potential for spreading pathogens such as the frog chytrid fungus.
- Provide background information and guidance to people who provide advice or supervise frog related activities.
- Provide standard licence conditions for workers engaged in frog related activities.
- Inform Animal Care and Ethics Committees (ACEC) for their consideration when granting research approvals.



Life cycle of frog chytrid fungus from infective free-living zoospore stage to sporangium (adapted from L. Berger).



## 2 site hygiene management

A checklist of risk management procedures and recommended standard hygiene kit is provided in Appendix I. Please note Footnote I on page 4.

Individuals studying frogs often travel and collect samples of frogs from multiple sites. Some frog populations can be particularly sensitive to the introduction of infectious pathogens such as the frog chytrid fungus. Also, the arrangement of populations in the landscape may make frogs particularly vulnerable to transmission of infectious pathogens. Therefore, it is important that frog workers recognise the boundaries between sites and undertake measures which reduce the likelihood of spreading infection.

Where critically endangered species or populations of particular risk are known to occur, this protocol should be applied over very short distances ie a single site may need to be subdivided and treated as separate sites.

When planning to survey multiple sites, always start at a site where frog chytrid fungus is not known to be present before entering other infected areas.

### 2.1 Defining a site

Defining the boundary of a site maybe problematic. In some places, the boundary between sites will be obvious but in others, less so. 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. It may be that a natural or constructed feature forms a logical indicator of a site boundary eg a road/ track, a large body of water such as a river or the sea, a marked habitat change or a catchment boundary.

**As a guiding principle, each individual waterbody should be considered a separate site.**

When working along a river or stream or around a wetland or a series of interconnecting ponds it is reasonable, in most instances, to treat such examples as a single site for the purposes of this protocol. Such a case would occur in areas where frogs are known to have free interchange between ponds.

Where a stream consists of a series of distinctive tributaries or sub-catchments or where there is an obvious break or division then they should be treated as separate sites, particularly if there is no known interchange of frogs between sites.

### 2.2 On-site hygiene

When travelling from site to site it is recommended that the following hygiene precautions be undertaken to minimise the transfer of disease from footwear, equipment and/or vehicles.

#### Footwear

**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 that contains *benzalkonium chloride* as the active ingredient. Disinfecting solutions should be prevented from entering any water bodies.

Rubber boots such as 'gum boots' or 'Wellingtons' are recommended because of the ease with which they can be cleaned and disinfected.

Several changes of footwear bagged between sites might be a practical alternative to cleaning.

## 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 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 in 2.4 below.

## Vehicles

Where necessary, vehicle tyres should be sprayed/flushed with a disinfecting solution in high-risk areas.

Transmission of disease from vehicles is unlikely to be a problem. However, if a vehicle is used to traverse a known frog site, which could result in mud and water being transferred to other bodies of water or frog sites, then wheels and tyres should undergo cleaning and disinfection. This should be carried out at a safe distance from water bodies, so that the disinfecting solution can infiltrate soil rather than run-off 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 will prevent the transfer of pathogens from/to vehicle floor and control pedals.

## 2.3 Handling of frogs in the field

The spread of pathogenic organisms, such as the frog chytrid fungus, may occur as a result of handling frogs.

Frogs should only be handled when necessary.

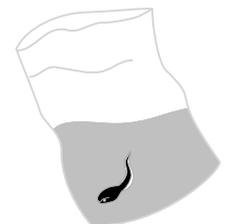
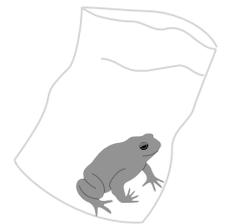
Where handling of frogs is necessary the risk of pathogen transfer should be minimised as follows:

- Hands should be either cleaned and disinfected between samples or a new pair of disposable gloves used for each sample<sup>1</sup>. This may be achieved by commencing with a work area that has a dish containing a disinfecting solution and paper towels.
- A 'one bag – one frog' approach to frog handling should be used especially where several people are working together with one person processing frogs and others doing the collecting. Bags should not be reused.
- A 'one bag – one sample' approach to tadpole sampling should be used. Bags should not be reused.

Researchers who use toe clipping or Passive Integrated Transponder (PIT) tagging are likely to increase the risk of transmitting disease between frogs due to the possibility of directly introducing pathogens into the frogs' system. This can be minimised by using:

- Disposable sterile instruments
- Instruments disinfected previously and used once
- Instruments disinfected in between each frog

Disinfecting solutions containing *benzalkonium chloride* are readily available from local supermarkets. Some brands include Toilet Duck, Sanpic, New Clenz and Pine Clean.



<sup>1</sup> As a principle, this protocol assumes that not all frogs in an infected pond will be contaminated by the frog chytrid fungus. The infective load of a body of water may not be high enough to cause cross contamination of individual frogs in the same pond. Therefore care should be taken to use separate gloves and bags and clean hands for each sample, to avoid transmission of high infective loads between individuals.

Open wounds from toe clipping and PIT tagging should be sealed with a cyanoacrylate compound such as *Vetbond*® to reduce the likelihood of entry of pathogens. The DECC ACEC further recommends the application of topical anaesthetic *Xylocaine*® cream and *Betadine*® disinfectant (1% solution) before and after any surgical procedure. This should then be followed by the wound sealant.

All used disinfecting solutions, gloves and other disposable items should be stored in a sharps or other waste container and disposed 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

## 2.4 Disinfection Methods

Disinfecting agents for hands and equipment must be effective against bacteria and both the vegetative and spore stages of fungi. The following agents are recommended:

- Chloramine and Chlorhexidine based products such as *Halamid*®, *Halasept*® or *Hexifoam*® are effective against both bacteria and fungi. These products are suitable for use on hands, footwear, instruments and other equipment. The manufacturers instructions should be followed when preparing these solutions.
- Bleach and alcohol (ethanol or methanol), diluted to appropriate concentrations can be effective against bacteria and fungi. However, these substances may be less practical because of their corrosive and hazardous nature.

When using methanol either:

- immerse in 70% methanol for 30 minutes or
- dip in 100% methanol then flame for 10 seconds or boil in water for 10 minutes

Fresh bleach (5% concentration) may be also effective against other frog pathogens such as Rana Virus.

Some equipment not easily disinfected in these ways can be effectively cleaned using medical standard 70% isopropyl alcohol wipes – *Isowipes*®.



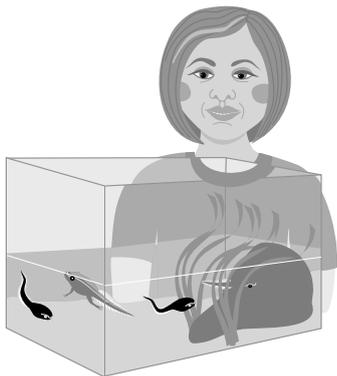
# 3 captive frog hygiene management

## 3.1 Housing frogs and tadpoles

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

When it is necessary for frogs or tadpoles to be collected and held for a period of time, the following measures should be undertaken:

- Animals obtained at different sites should be kept isolated from each other and from other captive animals.
- 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.
- Prior to housing frogs or tadpoles, ensure that tanks, aquaria and any associated equipment are disinfected.
- Tanks and equipment should be cleaned, disinfected and dried immediately after frogs/tadpoles are removed.



Careful maintenance of your enclosures will ensure a safe and hygienic environment for captive frogs and tadpoles.

When contemplating a release of captive bred tadpoles for conservation purposes a Translocation Proposal should be submitted to the DECC and pathological screening for disease should be undertaken (see also DECC Translocation Policy). Tadpoles can be tested by randomly removing 10 individuals at 6 weeks and again at 2 weeks before anticipated release. Testing could be undertaken by the pathology section at Taronga Zoo, Newcastle University, CSIRO Australian Animal Health Laboratories at Geelong and James Cook University at Townsville. Such an arrangement would need to be negotiated by contacting one of these institutions well before the anticipated release date. (see Appendix 2 for contact details)

DECC have licenced NSW Schools to allow students and/or teachers to remove tadpoles for classroom life cycle studies. They are authorised to remove individuals from only one location, each school also requires endorsement from Department of Education and Training Animal Care and Ethics Committee and comply with this protocol.

Tadpoles collected for these purposes are to be obtained from the local area of the school and are not to be obtained from DECC Reserves. As soon as tadpoles have transformed, froglets must be returned to the exact point of capture. Tadpoles from different locations are not to be mixed.

Antifungal cleansing treatments to clear tadpoles of the frog chytrid fungus are currently being trialed. In the future, such a treatment may be an added procedure required prior to froglet releases.

Detailed information on safely maintaining frogs in captivity is provided in Voigt (2001).

## 3.2 Tadpole treatment

In most instances:

**Release to the wild of tadpoles held or bred in captivity should be avoided.**



### 3.3 Frog treatment

The rigour with which frogs must be treated to ensure pathogens are not introduced to native populations means that any proposal for the removal of adult frogs (particularly threatened species) from wild populations should be given careful consideration.

When it is essential for frogs to be removed from the wild, the following should apply.

Individuals to be released should be quarantined for a period of 2 months and monitored for any signs of illness or disease.

Frogs must not be released if any evidence of illness or infection is detected. If illness is suspected, further advice must be sought from a designated frog recipient (Appendix 2) as soon as possible to determine the nature of the problem. Chytridiomycosis can be diagnosed in live frogs by microscopical examination of preserved toe clips or from shedding skin samples. Research is still in progress on the development of a simple technique for the detection of Chytridiomycosis and a treatment for infected frogs.

Current methods which may be used include:

- A technique for the treatment of potentially infected frogs is to place the frogs individually in a 1mg/L benzalkonium chloride solution for 1 hour on days 1, 3, 5, 9, 11 and 13 of the treatment period. Frogs are then isolated/quarantined for two months. This and other possible treatments are documented in Berger and Speare (1998)
- *Betadine*© and *Bactone*© treatments have also been used on adult frogs with some success (M. Mahony, Newcastle University pers. comm.)
- *Itraconazole*© is an expensive drug

which has been used successfully (Lee Berger CSIRO Australian Animal Health Laboratory pers. comm.). Information on this method is available on the Website <http://www.jcu.edu.au/school/PHTM/frogs/adms/attach6.pdf>.

Frogs undergoing treatment should be housed individually and kept separate from non-infected individuals.

### 3.4 Displaced frogs

Displaced frogs are those native frog species and introduced Cane Toads (*Bufo marinus*) which have been unintentionally transported around the country with fresh produce, transported produce and landscaping supplies. Procedures to be undertaken when encountering introduced/displaced native frog species (as well as Cane Toads) are as follows.

#### 3.4.1 Banana box frogs

'Banana Box' frog is the term used to describe several native frog species (usually *Litoria gracilentata*, *L. infrafrenata*, *L. bicolor* and *L. caerulea*) commonly transported in fruit and vegetable shipments and landscaping supplies. In the past, well meaning individuals have attempted to return these frogs to their place of origin but this is usually impossible to do accurately. There is risk of spread of disease if these frogs are transferred from place to place.

It is strongly recommended that:

**Displaced Banana Box frogs should be treated as if they are infected and should not be freighted anywhere for release to the wild unless specifically approved by DECC.**

When encountering a displaced frog:

- Contact a licensed wildlife carer organisation to collect the animal. The frog should then undergo a quarantine period of 2 months along with an approved disinfection treatment.
- Post-quarantine, the frog (if one of the species identified above) may be transferred to a licensed frog keeper. All other species require the permission from DECC Wildlife Licensing and Management Unit (WLMU) prior to transfer. Licensed carer groups are to record and receipt frogs obtained and disposed of in this way.
- Licensed Frog Keepers are to list these frogs in their annual licence returns to DECC.

Frogs held by licensed frog keepers are not to be released to the wild except with specific DECC approval.

Displaced frogs may be made available to recognised institutions for research projects, display purposes or perhaps offered to the Australian Museum as scientific specimens once approval has been provided by the DECC WLMU.



Frogs are often unintentionally transported with fresh produce and landscaping supplies. They are collectively known as 'banana box' or displaced frogs.

### 3.4.2 Cane toads

**Cane toads are known carriers of the Frog chytrid fungus and should not be knowingly transported or released to the wild.**

If a cane toad is discovered outside of its normal range, it should be humanely euthanased in accordance with the recommended NSW Animal Welfare Advisory Council procedure (see Appendix 3). Care should be taken to avoid euthanasia of native species due to mistaken identity.

### 3.4.3 Local frog species

**Frogs encountered on roads, around dwellings and gardens or in swimming pools should not be considered as displaced frogs.**

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.

Incidences of frogs spawning or tadpoles appearing in swimming pools should be referred to a wildlife carer/rescue organisation for assistance (see Appendix 4).

Contact the Frogwatch Helpline if you are unsure whether a frog is a local species or displaced.

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An NPWS information brochure titled 'Cane Toads in NSW' provides further information on cane toads and assistance with identification of some of the commonly misidentified native species. This information is also available on the DECC website.

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# 4 sick or dead frogs

Unless an obvious cause of illness or death is evident (eg predation or road mortality): Sick or dead frogs encountered in the wild should be collected and disposed of in accordance with the procedures described in section 4.2 below.

## 4.1 Symptoms of sick and dying frogs

Sick and dying frogs exhibit a range of symptoms characteristic of chytrid infection. Symptoms may be expressed in the external appearance or behaviour of the animal. A summary of these symptoms are described below. More detailed information can be found in Berger et al., (1999) or at the James Cook University Amphibian Disease website at: <http://www/jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm>.



### Appearance (one or more symptoms)

- darker or blotchy upper (dorsal) surface
- reddish/pink-tinged lower (ventral) surface and/or legs and/or webbing or toes
- swollen hind limbs
- very thin or emaciated
- skin lesions (sores, lumps)
- infected eyes
- obvious asymmetric appearance

### Behaviour (one or more symptoms)

- lethargic limb movements, especially hind limbs
- abnormal behaviour (eg a nocturnal, burrowing or arboreal frog sitting in the open during the day and making no vigorous attempt to escape when approached)
- little or no movement when touched

Great barred frog (*Mixophyes fasciolatus*) with severe Chytrid infection — note lethargic attitude and sloughing skin. Photo: L. Berger

## Diagnostic behaviour tests

Sick frogs will fail one or more of the following tests:

test	healthy	sick
Gently touch with finger	Frog will blink	Frog will not blink above the eye
Turn frog on its back	Frog will flip back over	Frog will remain on its back
Hold frog gently by its mouth	Frog will use its forelimbs to try to remove grip	No response from frog

## 4.2 What to do with sick or dead frogs

A procedure for the preparation and transport of a sick or dead frog is given below<sup>2</sup>. Adherence to this procedure will ensure the animal is maintained in a suitable condition for pathological examination and assist the DECC and researchers to determine the extent of the disease and the number of species affected.

- Disposable gloves should be worn when handling sick or dead frogs. Avoid handling food and touching your mouth or eyes as this could transfer pathogens and toxic skin secretions from some frog species.
- New gloves and a clean plastic bag should be used for each frog specimen to prevent cross-contamination. When gloves are unavailable, use an implement to transfer the frog to a container rather than using bare hands.
- If the frog is dead, keep the specimen cool and preserve as soon as possible (as frogs decompose quickly after death making examination difficult). Specimens can be fixed/preserved in 70% ethanol or 10% buffered formalin.

Cut open the belly and place the frog in about 10 times its own volume of preservative. Alternatively, specimens can be frozen (although this makes tissues unsuitable for some tests). If numerous frogs are collected, some should be preserved and some should be frozen. Portions of a dead frog can be sent for analysis eg a preserved foot, leg or a portion of abdominal skin.

- The container should be labelled showing at least the species, date and location. A standardised collection form is provided in Appendix 5.
- If the frog is alive but unlikely to survive transportation (death appears imminent), euthanase the frog (see Appendix 3) and place the specimen in a freezer. Once frozen, the specimen is ready for shipment to the address provided below.
- 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.
- 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 (available from BOC/CIG Gas outlets).
- Place live or frozen specimens into a small styrafoam esky (available from K-Mart/Big W for approximately \$2.50).
- Seal esky with packaging tape and address to one of the laboratories listed in Appendix 4.
- Send the package by courier.

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Further information on sick and dying frogs is available on the Amphibian Disease Home Page at <http://www.jcu.edu.au/dept/PHTM/frogs/ampidis.htm> — in particular refer to 'What to do with dead or ill frogs'.

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<sup>2</sup>The measures described below are standard procedures and may vary slightly depending on the distance and time required to reach the intended recipient. Contact the intended recipient of the sick or dead frog prior to sending to confirm the appropriate procedure.



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# appendix I

## 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.

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 taken into account 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 (consider both vehicle wheels/tyres and control pedals) and 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 DECC Hygiene Protocol for the Control of Disease in 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 this protocol. Recommended contents of a field hygiene kit would include:

- Small styrofoam eski
- Disposable gloves
- Disinfectant spray bottle (atomiser spray) and/or wash bottle
- Disinfecting solutions
- Wash bottle
- Scraper or scrubbing brush
- Small bucket
- Plastic bags large and small
- Container for waste disposal
- Materials for dealing with sick and dead frogs (see section 4.2)



# appendix 2

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Always contact the relevant specialist prior to sending a sick or dead frog. In some cases, only wild frogs will be assessed for disease. Analysis may also attract a small fee per sample.

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## designated sick and dead frog recipients

Contact one of the following specialists to arrange receipt and analyse sick and dead frogs. Make contact prior to dispatching package:

Karrie Rose  
Australian Registry of Wildlife Health  
Taronga Conservation Society, Australia  
PO Box 20  
MOSMAN NSW 2088  
Phone: 02 9978 4749  
Fax: 02 9978 4516  
Krose@zoo.nsw.gov.au

Diana Mendez or  
Rick Speare  
School of Public Health,  
Tropical Medicine and  
Rehabilitation Sciences  
James Cook University  
Douglas Campus  
TOWNSVILLE QLD 4811

Phone: 07 4796 1735  
Fax: 07 4796 1767  
Diana.Mendez@jcu.edu.au  
Richard.Speare@jcu.edu.au

Michael Mahony  
School of Biological Sciences  
University of Newcastle  
CALLAGHAN NSW 2308

Phone: 02 4921 6014  
Fax: 02 4921 6923  
bimjm@cc.newcastle.edu.au

For information on frog keeping licences and approvals to move some species of displaced frog contact:

Co-ordinator, Wildlife Licensing  
Wildlife Licensing and Management Unit  
DECC  
PO Box 1967  
Hurstville NSW 1481  
Ph 02 9585 6481  
Fax 02 9585 6401  
wildlife.licensing@environment.nsw.gov.au

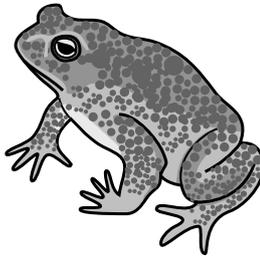
For information on the possible identity of displaced frogs contact:

Frog and Tadpole Society (FATS)  
Frogwatch Helpline  
Ph: 0419 249 728

# appendix 3

## NSW Animal Welfare Advisory Council methodology

The NSW Animal Welfare Advisory Council procedure for humanely euthanasing cane toads or terminally ill frogs is stated as follows:



- Using gloves, or some other implement, place cane toad or terminally ill frog into a plastic bag.
- Cool in the refrigerator to 4°C.
- Crush cranium with a swift blow using a blunt instrument.

Note: Before killing any frog presumed to be a cane toad, ensure that it has been correctly identified and if outside the normal range for cane toads in NSW (north coast) that local DECC regional office is informed.

# appendix 4

## licensed wildlife carer and rescue organisations

Following is a list of wildlife rehabilitation groups licensed by  
Department of Environment and Climate Change (NSW):

### **Northern NSW**

Australian Seabird Rescue  
For Australian Wildlife Needing Aid  
(FAWNA)  
Friends of the Koala  
Friends of Waterways (Gunnedah)  
Great Lakes Wildlife Rescue  
Koala Preservation Society of NSW  
Northern Rivers Wildlife Carers  
Northern Tablelands Wildlife Carers  
Tweed Valley Wildlife Carers  
Seaworld Australia  
WIRES branches in Northern NSW

### **Southern NSW**

Looking After Our Kosciuszko Orphans  
(LAOKO)  
Native Animal Network Association  
Native Animal Rescue Group  
Wildcare Queanbeyan  
WIRES branches in Southern NSW

### **Sydney, Hunter and Illawarra**

Hunter Koala Preservation Society

Ku-ring-gai Bat Colony Committee  
Kangaroo Protection Co-operative  
Native Animal Trust Fund  
Organisation for the Rescue and Research of  
Cetaceans (ORRCA)  
Sydney Metropolitan Wildlife Services  
Wildlife Aid  
Wildlife Animal Rescue and Care (Wildlife  
ARC)  
Waterfall Springs Wildlife Park  
Oceanworld  
Wildlife Care Centre, John Moroney  
Correctional Centre  
Koalas in Care  
WIRES branches around Sydney, Hunter and  
Illawarra

### **Western NSW**

Rescue and Rehabilitation of Australian  
Native Animals (RRANA)  
RSPCA Australian Capital Territory Inc.  
Wildlife Carers Network (Central West)  
WIRES branches in Western NSW  
Cudgegong Wildlife Carers

# appendix 5 — sick or dead frog collection form

## Sender details:

name: \_\_\_\_\_ address: \_\_\_\_\_ postcode: \_\_\_\_\_  
 phone: (w) \_\_\_\_\_ (h) \_\_\_\_\_ fax: \_\_\_\_\_ email: \_\_\_\_\_

## Collector details: (where different to sender)

name: \_\_\_\_\_ address: \_\_\_\_\_ postcode: \_\_\_\_\_  
 phone: (w) \_\_\_\_\_ (h) \_\_\_\_\_ fax: \_\_\_\_\_ email: \_\_\_\_\_

## Specimen details:

record no: \_\_\_\_\_ no. of specimens: \_\_\_\_\_ species name: \_\_\_\_\_ date collected: \_\_\_\_\_  
day/month/year

time collected: \_\_\_\_\_ sex: \_\_\_\_\_ status at time of collection: \_\_\_\_\_ date sent: \_\_\_\_\_  
male/female healthy(H)/ sick(S)/ dead(D) day/month/year

location: \_\_\_\_\_ map grid reference: \_\_\_\_\_  
(easting) (northing)

reason for collection: \_\_\_\_\_

## Batch details for multiple species collection:

species	no.	locality	(AMG)	date	sex	status (H/S/D)

habitat type: \_\_\_\_\_ vegetation type: \_\_\_\_\_ micro habitat: \_\_\_\_\_  
eg creek, swamp, forest eg rainforest, sedgeland eg creek bank, under log, amongst emergent vegetation, on ground in the open

unusual behaviour of sick frogs: \_\_\_\_\_  
eg lethargic, convulsions, sitting in the open during the day, showing little or no movement when touched.

dead frogs appearance: \_\_\_\_\_  
eg thin, reddening of skin on belly and/or toes, red spots, sore, lumps or discolouration on skin

deformed frogs: \_\_\_\_\_ dead/sick tadpoles: \_\_\_\_\_  
eg limb(s) missing, abnormal shape or length eg numbers/behaviour

unusual appearance of egg masses: \_\_\_\_\_ recent use of agricultural chemicals in area: \_\_\_\_\_  
eg grey or white eggs eg pesticides, herbicides, fertilisers

other potential causes of sickness/mortality/comments/additional information: \_\_\_\_\_

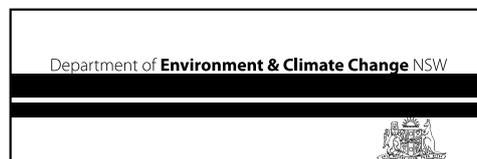


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**NSW  
NATIONAL  
PARKS AND  
WILDLIFE  
SERVICE**

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**General inquiries:** PO Box A290 South Sydney 1232  
**Phone:** 9995 5000 or 1300 361967  
**Fax:** 02 9995 5999 **Web site:** [www.environment.nsw.gov.au](http://www.environment.nsw.gov.au)



# Appendix B

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DSE (2011) Guidelines for Eastern  
Gambusia Removal



# Guidelines for physical removal of Eastern Gambusia

Bringing Native Fish Back

## The Alien fish threat

Alien fish species are recognised as one of eight major threats to native fish in the Murray-Darling Basin (MDB) and the control of these species is one of the key driving actions of the Murray-Darling Basin Authority's Native Fish Strategy.

Eastern Gambusia (*Gambusia holbrooki*), a native of the south-east of the United States of America and Mexico, was brought to Australia in 1925 to ostensibly control mosquito larvae (which it fails to do). The species has detrimental impacts on native fish fauna globally and is widespread in the MDB. Sixteen of the 37 native freshwater fish of the MDB have major niche overlaps with the Eastern Gambusia, suggesting these species are at high risk of impacts such as predation of eggs and/ or larvae, competition for food and habitat, and aggression (including fin nipping). In particular, the smaller native fish occupying the slower, still water habitats of the MDB wetlands are at high risk, including the Ambassids (glassfish), Nannoperchids (pygmy perch), Melanotaenids (rainbowfishes), Atherinids (hardyheads), Eleotrids (gudgeons) and Retropinnids (smelt).



Eastern Gambusia (*Gambusia holbrooki*) Photo  
Tarmo Raadik

## Eastern Gambusia project

The Eastern Gambusia removal project explored the feasibility of controlling Eastern Gambusia populations to densities where native fish communities could recover. The project:

- Reviewed current knowledge on Eastern Gambusia and its impacts
- Studied the responses of native fish communities in natural billabong systems in the MDB to the reduction of Eastern Gambusia
- Provided a framework to assess the feasibility and effectiveness of control options, and
- Developed a template for evaluating control options for other alien species in the MDB.

## Eastern Gambusia removal:

### What's possible?

With Eastern Gambusia now well established throughout the Murray-Darling Basin and demonstrating an ability to rapidly colonise sites, it is clear that currently, it is not feasible to undertake large scale Eastern Gambusia control or eradication across the MDB.

This does not, however, preclude management actions for this species. Instead, minimising the ecological impact of Eastern Gambusia at a localised scale may be an important conservation strategy, particularly in areas supporting threatened fish fauna, until further control measures are developed.

# Guidelines for Eastern Gambusia Removal

## What to Consider

A response to a presence of Eastern Gambusia at a site might include:

- Do nothing
- Drain/ dry the site
- Poison the site
- Remove Eastern Gambusia

Whilst drying or poisoning a site has been shown to be a successful eradication strategy, many regions or specific sites are not suitable to these techniques e.g. fragile ecosystems or the presence of threatened fish species. It may then be worth considering physical removal, which will minimise impacts on non-target biota.

## Physical removal

Physical removal of Eastern Gambusia before the species' spawning season can result in major reductions in Eastern Gambusia abundance, even resulting in complete eradication at some sites. The degree of success depends on site hydrology and hydrological connectivity, ecological value, habitat complexity, habitat size and climate.

**If physical removal at a particular site is likely to have low ecological benefit for a given investment, other mitigation strategies such as habitat restoration should still be considered**

A decision to remove fish will consider the likely effectiveness of the required effort and any negative impacts, including a possible increase in other exotic species (such as carp) as a result of removal actions.

A simple decision tool has been developed (see page 3) which should assist in assessing the likely effectiveness of removal actions at a particular site.

**Best results with removal are achieved with targeted, repeated effort in isolated, small sites, before Eastern Gambusia spawn.**

The success of removal activities (as assessed by the proportion of fish removed and ecological benefits) will depend on:

- Timing
- Hydrological Connectivity
- Ecological Values
- Size & features of site

## Timing

Best results are achieved by targeting Eastern Gambusia pre-spawning, in winter-early spring, with water temperatures  $<16^{\circ}\text{C}$  and photoperiod  $<12$  hours.

Consideration should be given to the capacity for sustained effort: unless complete eradication is achieved, suppression of Eastern Gambusia population size lasts little more than a month, therefore repeat removal must be undertaken at intervals such that removal is  $>$  the population growth rate.

## Hydrological Connection

If a site is permanently or frequently connected to adjoining habitats, repeated re-invasion will render any removal benefits as very short lived.

## Ecological Values

Sites supporting small threatened species (e.g. pygmy perch or glassfish) will yield greater ecological benefits from removal than effort at a site with lesser-impacted, more common species.

Eastern Gambusia removal may result in small improvements to carp populations, suggesting that Eastern Gambusia removal may also have unexpected benefits to other exotic species. Site specific ecosystem function must be considered before undertaking a removal program.

## Site Size and Features

Eastern Gambusia are strongly attracted to light and heat - it is important to identify and concentrate removal activities at areas of a site which are shallow and most exposed to direct sunlight.

Presence of structural habitat/ woody debris/ dense macrophytes can reduce the effectiveness of netting, but bait trapping may be quite effective in these sites. It should be noted that sites with abundant structural habitat may also support more cover and more separation between Eastern Gambusia and native fish fauna, and may therefore be in less need of Eastern Gambusia control.

Complete eradication is possible in very small sites (surface area  $<3\text{m}^2$ ) but in the current study eradication was also achieved in sites up to  $600\text{m}^2$ .



# Guidelines for Eastern Gambusia Removal

## What to do

- Undertake removal during winter and early spring.
- Target the shallowest, brightest-lit area of water.
- Targeted seine netting is by far the most effective method, especially for initial visits.
- Set bait traps with the trap entry area at the surface, in an area of water receiving the most direct sunlight.
- Sustained use of even dip nets can be useful if seine netting is not possible.
- Repeat targeted effort over several weeks.

## Resources required

- Light weight fine seine net
- Bait traps, possibly dip nets
- Polarised sunglasses
- Polypropylene barrier fencing across inflow points may help minimise re-colonisation if sites become connected.
- Thorough understanding of localised hydrology.

## Permits/ Approvals

Regulations specific to each state may also restrict the activities which may be used by non-permit holders. E.g. the type and number of nets, water bodies where they can be used and requirements for euthanizing and disposal of fish. Please contact your specific state body for details on removal activities and requirements for euthanizing and disposal of fish. Details of specific state regulations can be found at

[www.dpi.vic.gov.au](http://www.dpi.vic.gov.au) for Victoria;

[www.dpi.nsw.gov.au/fisheries/recreational/regulations](http://www.dpi.nsw.gov.au/fisheries/recreational/regulations) for NSW;

[www.dpi.qld.gov.au](http://www.dpi.qld.gov.au) for Queensland and;

[www.pir.sa.gov.au/fisheries](http://www.pir.sa.gov.au/fisheries) for SA.



*Above: Demonstrating Eastern Gambusia removal to school students. Photo Glenys Avard*



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