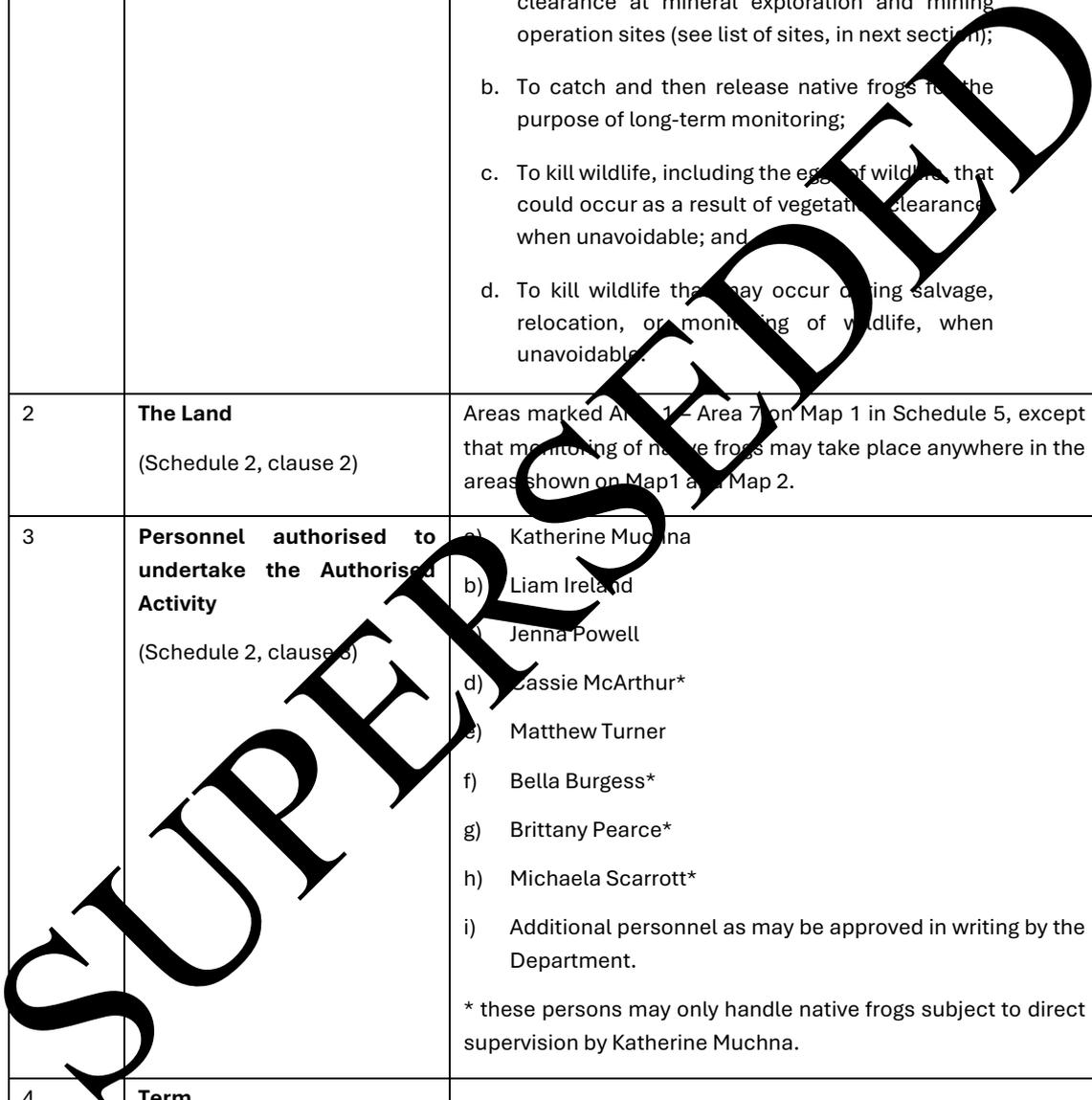


WAIHI NORTH PROJECT - WILDLIFE ACT APPROVAL

Wildlife Act Approval for wildlife located on public conservation land

SCHEDULE 1

1	<p>Authorised activity (Schedule 2, clause 2)</p>	<p>Activity:</p> <ul style="list-style-type: none"> a. To catch and temporarily possess the species listed in Schedule 4 for the purposes of salvage and relocation, prior to and during vegetation clearance at mineral exploration and mining operation sites (see list of sites, in next section); b. To catch and then release native frogs for the purpose of long-term monitoring; c. To kill wildlife, including the eggs of wildlife, that could occur as a result of vegetation clearance when unavoidable; and d. To kill wildlife that may occur during salvage, relocation, or monitoring of wildlife, when unavoidable.
2	<p>The Land (Schedule 2, clause 2)</p>	<p>Areas marked Area 1 – Area 7 on Map 1 in Schedule 5, except that monitoring of native frogs may take place anywhere in the areas shown on Map 1 and Map 2.</p>
3	<p>Personnel authorised to undertake the Authorised Activity (Schedule 2, clause 3)</p>	<ul style="list-style-type: none"> a) Katherine Muchna b) Liam Ireland c) Jenna Powell d) Cassie McArthur* e) Matthew Turner f) Bella Burgess* g) Brittany Pearce* h) Michaela Scarrott* i) Additional personnel as may be approved in writing by the Department. <p>* these persons may only handle native frogs subject to direct supervision by Katherine Muchna.</p>
4	<p>Term (Schedule 2, clause 4)</p>	<p>18 December 2025 to 18 December 2055</p>
5	<p>Approval Holder's address for notices (Schedule 2, clause 8)</p>	<p>The Approval Holder's address in New Zealand is:</p> <p>Physical: 22 Maclaggan Street, Dunedin 9016, New Zealand</p> <p>Postal: PO Box 5442 Dunedin 9054</p>



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		Phone: 03 479 4736 Email: NZ.Legal@oceanagold.com
6	Department's address for notices	The Department's address is: Physical: Hauraki District Office, 3/366 Ngati Maru Highway (SH25)Thames 3500; Postal: PO Box 343, Thames 3540 Phone: 0800 275 362 ; Email: thames@doc.govt.nz

NOTE: Interpretation

References in this Approval to the "Department" are to the Department of Conservation.

References in this Approval to the "Manager" are to the person for the time being holding the office of Operations Manager, Hauraki District, Hauraki - Waikato - Taranaki Region, the Director Operations, Hauraki-Waikato-Taranaki Region, of the Department of Conservation, and includes any person authorised by the Operations Manager/Director to act on their behalf in respect of this Approval.

SUPERSEDED

SCHEDULE 2

STANDARD TERMS AND CONDITIONS OF THE APPROVAL

1. Interpretation

- 1.1. The Approval Holder is responsible for the acts and omissions of its employees, contractors or agents. The Approval Holder is liable under this Approval for any breach of the terms of the Approval by its employees, contractors or agents as if the breach had been committed by the Approval Holder.
- 1.2. Where obligations bind more than one person, those obligations bind those persons jointly and separately.

2. What is being authorised?

- 2.1. The Approval Holder is only allowed to carry out the Authorised Activity on the Land described in Schedule 1, Item 2.
- 2.2. Any arrangements necessary for access over private land or leased land are the responsibility of the Approval Holder. The Department does not warrant that such access can be obtained.
- 2.3. The Approval Holder must advise the Manager prior to carrying out the Authorised Activity (where possible, one week prior).
- 2.4. The Approval Holder and Authorised Personnel must carry a copy of this Approval with them at all times while carrying out the Authorised Activity.
- 2.5. The Approval Holder must comply with any reasonable request from the Department for access to any wildlife.
- 2.6. The Approval Holder may publish research results.
- 2.7. The Approval Holder must immediately notify the Manager of any taxa found which are new to science. In addition, the Approval Holder must lodge holotype specimens and a voucher specimen of any new taxa with a recognised national collection.

3. Who is authorised?

- 3.1. Only the Approval Holder, its employees, contractors and agents and the Authorised Personnel described in Schedule 1, Item 3 are authorised to carry out the Authorised Activity, unless otherwise agreed in writing by the Manager, such agreement is not to be unreasonably delayed or withheld.

4. How long is the Approval for - the Term?

- 4.1. This Approval commences and ends on the dates set out in Schedule 1, Item 4.

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5. What are the liabilities?

- 5.1. The Approval Holder agrees to exercise the Approval at the Approval Holder's own risk and releases to the full extent permitted by law the Department and the Department's employees and agents from all claims and demands of any kind and from all liability which may arise in respect of any accident, damage or injury occurring to any person or property arising from the Approval Holder's exercise of the Authorised Activity.
- 5.2. The Approval Holder must indemnify the Department against all claims, actions, losses and expenses of any nature which the Department may suffer or incur or for which the Department may become liable arising from the Approval Holder's exercise of the Authorised Activity.
- 5.3. This indemnity is to continue after the expiry or termination of this Approval in respect of any acts or omissions occurring or arising before its expiry or termination.

6. What about compliance with legislation and Department's notices and directions?

- 6.1. The Approval Holder must comply with all statutes, bylaws and regulations, and all notices, directions and requisitions of the Department and any competent authority relating to the conduct of the Authorised Activity. Without limitation, this includes the Conservation Act 1987 and the Acts listed in the First Schedule of that Act and all applicable health and safety legislation and regulation.

7. Are there limitations on public access and closure?

- 7.1. The Approval Holder acknowledges that the public conservation land being part of the Land is open to the public for access and that the Department may close public access to that public conservation land during periods of high fire hazard or for reasons of public safety or emergency.

8. When can the Approval be terminated?

- 8.1. The Manager may terminate this Approval at any time in respect of the whole or any part of the Land, and/or the whole or any part of the Authorised Activity if:
 - (a) the Approval Holder breaches any of the conditions of this Approval; or
 - (b) in the Manager's opinion, the carrying out of the Authorised Activity causes or is likely to cause any unforeseen or unacceptable effects in relation to protected wildlife.
- 8.2. If the Manager intends to terminate this Approval in whole or in part, the Manager must give the Approval Holder such prior notice as, in the sole opinion of the Manager, appears reasonable and necessary in the circumstances.

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9. How are notices sent and when are they received?

- 9.1. Any notice to be given under this Approval by the Department is to be in writing and made by personal delivery, post, or email to the Approval Holder at the physical or email address specified in Schedule 1, Item 5. Any such notice is to be deemed to have been received:
- (a) in the case of personal delivery, on the date of delivery;
 - (b) in the case of post, on the 3rd working day after posting;
 - (c) in the case of email, on the date receipt of the email is acknowledged by the addressee by return email or otherwise in writing.
- 9.2. If the Approval Holder's details specified in Schedule 1, Item 5 change then the Approval Holder must notify the Department within 5 working days of such change.

10. What about the payment of costs?

- 10.1. The Approval Holder must pay the standard Department of Conservation charge-out rates for any staff time and mileage required to monitor compliance with this approval and to investigate any alleged breaches of the terms and conditions of it.

11. Biosecurity

- 11.1. The Approval Holder must take all precautions to ensure weeds and non-target species are not introduced to the Land; this includes ensuring that all tyres, footwear, gaiters, packs, clothes, and equipment used by the Approval Holder, its staff and clients are cleaned and checked for pests and weeds before entering the Land.

12. Are there any Special Conditions?

- 12.1. Special Conditions are specified in Schedule 3. If there is a conflict between this Schedule 2 and the Special Conditions in Schedule 3, the Special Conditions will prevail.

13. Can the Approval be varied?

- 13.1. The Approval Holder may apply to the Department for variations to this Approval in line with clause 7(2) of Schedule 7 of the Fast-track Approvals Act 2024.

SCHEDULE 3

SPECIAL CONDITIONS

Compatibility with Access Arrangement and Concession Documents

1. The Special Conditions in the following documents apply within their respective areas of effect:
 - a. Wharekirauponga Access Arrangement [reference number]
 - b. Favona Access Arrangement [reference number]
 - c. Northern Concession [reference number]
 - d. Willows Area Concession [reference number]
 - e. HDC and TCDC Land Use Consents.

Management and Monitoring Plans

2. All Activities authorised by this Approval must be undertaken in accordance with the relevant conditions of the Access Arrangements, Concessions, and Consents listed in clause 1 and with the relevant management and monitoring plans or any amended versions:
 - a. Within the Coromandel Forest Park:
 - i. The Terrestrial Ecology Management Plan as included in the WUG Ecology and Landscape Management Plan that has been certified under condition C5 of Schedule One: Conditions Common To The Hauraki District Council And Waikato Regional Council Resource Consents;
 - ii. The Native Frog Monitoring Plan that has been certified by the Department under the Wharekirauponga Access Arrangement and Northern Concession;
 - iii. The Native Frog Salvage Release Plan as certified by the Department under the Wharekirauponga Access Arrangement and Northern Concession;
 - b. Outside the Coromandel Forest Park:
 - i. The Willows Site section of the WUG Ecology and Landscape Management Plan that has been certified under condition C5 of Schedule One: Conditions Common To The Hauraki District Council And Waikato Regional Council Resource Consents;
 - ii. The Lizard Management Plan as included in the Waihi Area Ecology and Landscape Management Plan that has been certified under condition C5 of

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Schedule One: Conditions Common To The Hauraki District Council And Waikato Regional Council Resource Consents.

NOTE: Any change to the Access Arrangements, Concessions and Consents and their conditions will not constitute a change to the conditions of this Approval, unless or until this Approval is varied in accordance with requirements of the conditions of this Approval and/or the Fast-track Approvals Act 2024 as appropriate.

3. Any references to wildlife in conditions of the Access Arrangements, Concessions, and Consents shall apply to this Approval as if they are references to all species of absolutely protected wildlife, with any necessary modifications.
4. All Activities authorised by this Approval that are undertaken in accordance with the certified management and monitoring plans listed in Condition 2 must also be undertaken in accordance with relevant current DOC approved frog protocols or DOC SOPs. The current versions at the time of approval are attached as follows: the Frog grabbing protocol for New Zealand frogs set out in Schedule 8, the Frog buccal swabbing protocol set out in Schedule 9; and the Hochstetter's frog survey and searching protocol set out in Schedule 10 of this Approval.
5. In accordance with Conditions C8A –C8D of Schedule One, Conditions Common To The Hauraki District Council And Waikato Regional Council Resource Consents, and certification conditions in the Access Arrangements and Concessions, the Approval Holder may make amendments to any of the management plans referred to in Condition 2 at any time subject to any relevant certification requirements.

Lizard Capture and Handling

6. Lizards must only be handled by Authorised Personnel named in Schedule 1, or under the direct supervision of the Authorised Personnel in accordance with Schedule 2, clause 3.1.
7. Lizard capture and allocation must be undertaken between 1 October and 30 April when lizards are most active.
8. Capture and handling of lizards must involve only techniques that minimise the risk of infection or injury to the animal, following methods described in the Herpetofauna inventory and monitoring toolbox <http://www.doc.govt.nz/our-work/biodiversity-inventory-and-monitoring/herpetofauna/>.
9. The Approval Holder must ensure all live capture traps are covered to protect lizards from exposure and minimise stress. Damp leaf litter or other material must be provided to reduce desiccation risk and the bottom of the pit-fall trap must be perforated to allow drainage of water.

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10. The Approval Holder must ensure all live capture traps, (e.g. pitfall traps and G-minnow traps), are checked daily within 12 hours of sunrise.
11. The Approval Holder must sterilise any instruments that come in contact with the lizards and/or are used to collect or measure lizards between each location. A separate holding bag must be used for each animal. All gear should be thoroughly cleaned and dried between sites.
12. The Approval Holder must ensure lizards are temporarily held individually in a suitable container (e.g. breathable cloth bag) and held out of direct sunlight to minimise the risk of overheating, stress, and death.

Frog Capture and Handling

13. The Approval Holder must ensure that personnel undertaking frog handling and capture activities are accompanied by a suitably qualified herpetologist as per clause 2 of Schedule 1.
14. Frog capture and handling methods shall follow those described in the Herpetofauna inventory and monitoring toolbox <http://www.doc.govt.nz/our-work/biodiversity-inventory-and-monitoring/herpetofauna/>, the current Frog Hygiene Protocol, and those listed below to minimise the risk of injury or death:
 - a. Catch frogs by gently scooping and holding the frog in cupped, gloved hands, or by gently holding the middle of the frog between 1st and forefingers and thumb. Do not squeeze the frog and never hold it by the legs or head.
 - b. Frogs should be placed in a safe location to avoid accidental trampling. If holding frogs during the day, they must be held out of direct sunlight and bright day light to minimise the risk of overheating, drying out, stress and/or death.
 - c. For surveying/monitoring, release frogs at the original capture point and check bags/containers to ensure every frog has been released. If releasing frogs during the daytime, they should be released next to the cover object under which they were found and gently tapped with a gloved hand to encourage them to return under the refugia.
 - d. New gloves and new bags should be used for each individual frog found.
15. Frogs captured for relocation must be held individually in rigid bodied plastic containers with adequate aeration (perforated lid) containing a wet paper towel (use water from nearest stream). Frogs must be transported in chilly bins with low temperatures (e.g. <12 C) maintained for the period frogs are temporarily held.
16. Frogs must be checked every 6 hours (except for one 8 hour period per 24 hours; this 8 hour period must be during the hours of darkness).
17. Containers must be cleaned and rinsed between individual frogs following the current Frog Hygiene for Handling Protocol.

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18. Buccal swabbing may only be undertaken if it is certified by the Manager in consultation with the DOC Frog Recovery Group as an approved technique for the individual identification of native frogs, or if it is undertaken as part of a research project with animal ethics approval and DOC authorisation. If undertaken, buccal swabbing must be undertaken by suitably qualified herpetologists or ecologists certified as approved handlers by the Manager to undertake buccal swabbing of native frogs.
19. The Approval Holder must take all practicable steps to minimise trampling and disturbance of frogs and their habitat by:
 - a. Using the same marked access routes for access to the site.
 - b. Avoiding survey of habitat that may result in crushing or collapse of delicate refugia, e.g. stream seepages with small, stacked pebbles that could collapse entirely if searching is attempted.
 - c. Returning all captured and handled frogs to their original capture point (unless the frog is being relocated) using a system of release that avoids the risk of liberated frogs being disturbed or trampled.

Ownership and holding of Absolutely Protected Wildlife

20. This Approval gives the Approval Holder the right to hold absolutely protected wildlife for no longer than 12 hours in accordance with the terms and conditions of the Approval, but the wildlife remains the property of the Crown. This includes any dead wildlife, live wildlife, any parts thereof, any eggs or progeny of the wildlife, genetic material and any replicated genetic material.
21. Unless expressly authorised by the Manager in writing, the Approval Holder must not donate, sell or otherwise transfer to any third party any wildlife, material, including any genetic material or any material propagated or cloned from such material, collected under this Approval.

Death of Wildlife

22. If any protected wildlife is found dead; the Approval Holder must contact the Manager's Waikato Office at thames@doc.govt.nz, with known details of the animal's history. Then, if the Manager requests it, the body must be sent to Massey University Wildlife Post Mortem Service for necropsy.
23. In that eventuality; the Approval Holder must, if requested by the Grantor:
 - a. Ensure that the body is to be chilled if it can be delivered within 24 hours, or frozen if it will take longer than 24 hours to deliver.
 - b. Ensure appropriate measures are taken to minimise further deaths.

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- c. Discuss with the Manager whether it is necessary to halt all further handling until full investigations of death(s) occur.
- d. Pay for any costs incurred in investigation of the death.

Euthanasia

24. The Approval Holder must not euthanise any wildlife unless the Approval Holder:
 - a. Obtains the recommendation of a veterinarian where euthanasia is on animal welfare grounds; or
 - b. Carries out the euthanasia under direction from the Manager and in consultation with a veterinarian (as applicable).

Records

25. All survey, salvage and release records must be made available for inspection at reasonable times by officers of the Manager.

Lizard and Frog Survey and Salvage Reporting

26. Independent of any reporting required under the conditions of any Access Arrangement or Concession; a report is to be submitted in writing to the Manager by 1 October each year for the life of this Approval (covering the period from 1 July – 30 June period); summarising the matters listed in a. to c. of this Concession, and mentioning approval number [insert this WAA reference number]. Each report must include:
 - a. the species and number of any animals collected and released;
 - b. the GPS location (and detailed map) of the collection point(s) and release point(s);
 - c. results of all surveys, monitoring or research.
27. Completed Amphibian and Reptile Distribution System (ARDS) cards for all herpetofauna sightings and captures must be sent to the Herpetofauna Database Administrator, PO Box 1042, Wellington 6143, or via email to herpetofauna@doc.govt.nz (A copy of the ARDS card is included as Attachment 1 to this Approval).

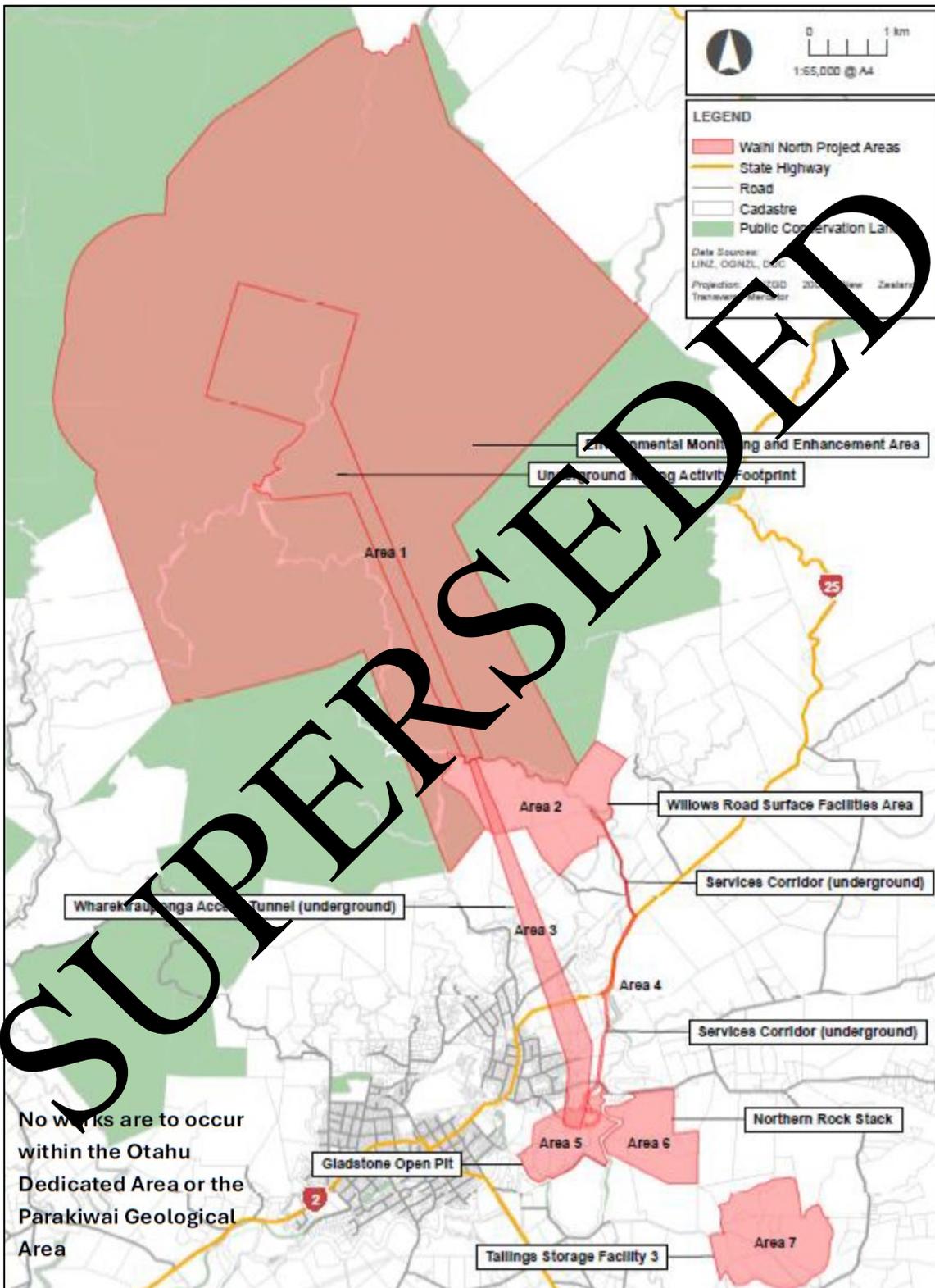
SCHEDULE 4

Common Name	Scientific Name
Northern striped gecko	<i>Toropuku inexpectatus</i>
Pacific gecko	<i>Dactylocnemis pacificus</i>
Elegant gecko	<i>Naultinus elegans</i>
Forest gecko	<i>Mokopirirakau granulatus</i>
Common gecko	<i>Woodworthia maculata</i>
Striped skink	<i>Oligosoma striatum</i>
Ornate skink	<i>Oligosoma ornatum</i>
Copper skink	<i>Oligosoma aeneum</i>
Moko skink	<i>Oligosoma moco</i>
Archey's frog	<i>Leiopelma archeyii</i>
Hochstetter's frog	<i>Leiopelma hochstetteri</i>
Stag beetle	<i>Geodorcus auratus</i>

SUPERSEDED

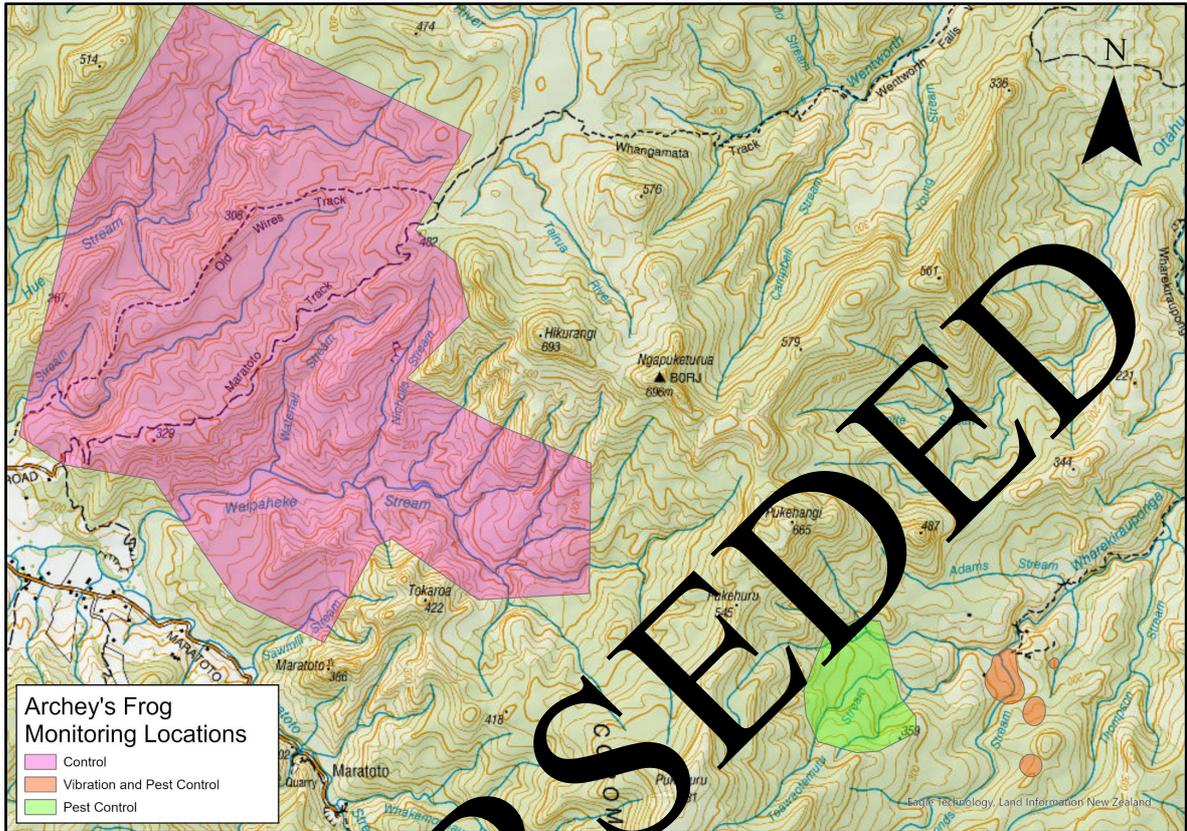
SCHEDULE 5:

Map 1



SCHEDULE 5:

Map 2



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SCHEDULE 7:

Frog hygiene for handling protocol

Generic Frog Hygiene and Handling Protocol

Background/aims:

- To minimise any possible spread of chytrid fungus and other pathogens to, within and/or between monitoring sites
- To avoid artificially increasing contact between frogs
- To implement the highest level of hygiene protocol that is effective and practicable in the field

Principles:

- Contamination can be managed/reduced through hygiene.
- New or disposable equipment is not a source of infection.
- Use of disinfectants will kill zoospores on equipment and clothing.
- Use of disinfectants will kill zoospores on footwear which has been first scrubbed clean to remove dirt.
- New or disinfected equipment/clothing/footwear should be used at every new site.
- New or disinfected equipment should be used for each frog, where practicable.
- When working in areas in or near where there are native frogs, hygiene protocols should be followed as if chytrid fungus and ranavirus are present and novel pathogenic organisms may be present.

Protocol:

Site hygiene:

- Clean between sites by ensuring that soil and other organic matter is removed from all gear including footwear, gaiters, rainwear, clothing, packs, frog handling/measuring equipment and any other equipment used in the area including storage bins.
- Disinfect between sites including footwear, gaiters, rainwear, clothing, packs, from handling/measuring equipment and any other equipment used in the area including storage bins (Table 1)
 - All clothing must be freshly laundered using hot water, Sterigene, F10 Veterinary disinfectant or Virkon S (including outer clothing).

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- Apply disinfectant solution either via a soaking spray, a very wet wipe-down solution or submersion to achieve good coverage and the correct contact time.
- Due to rapid evaporation, alcohol sprays will need to be repeatedly reapplied to ensure the full 2 minute contact time before air drying.
- Wherever a chemical disinfectant is used (e.g. Sterigene, bleach, F10) this must be rinsed off in clean (tap) water after the appropriate contact time.
- Plan ahead to allow drying times
- Footwear and gaiters must be cleaned and disinfected at the point of entry to a frog field site.
- Dogs: Clean all soil from within the recesses of the dog's paws and from their coat before entering a site and after leaving. At home, use clean water and a dog-friendly soap or shampoo to thoroughly wash all soil off the dog's paws and coat. Dermcare Malaseb shampoo (antifungal and antibacterial dog shampoo, available from vets) can be used prior to entering high conservation value sites. Follow product label directions for use.

Frog handling hygiene:

- A new glove(s) must be used for catching and handling each frog (the same glove can be re-used on the same frog if that glove remains isolated from other frogs and/or their body fluid).
- Each frog must be held in a separate plastic bag (one plastic bag is used per capture and then disposed of).
- Each frog must be weighed and measured in the plastic bag.
- If frogs are too small to be measured in a plastic bag then callipers should be disinfected between frogs using alcohol wipes.
- A new stage platform cover must be used for photographing each frog.
- All stage platform covers must be soaked in 70% ethanol for 2 minutes and air dried between frogs.
 - covers are disinfected daily, sufficient covers must be available for each night so that a clean one can be used for each frog
 - if there are not sufficient covers then they must be cleaned with alcohol wipes.
- The mirror stage must be disinfected with either 70% ethanol (contact time at least 2 minutes then air dried) or Sterigene or similar product (rinsed thoroughly and air dried) between sites and wiped with alcohol wipes or 70 % ethanol between successive nights at the same site.
- Alcohol wipes must contain 70% alcohol (either ethanol or isopropyl alcohol) and 30% water. Wipe surface for 2 minutes (more than one alcohol wipe may be needed if the first dries). Some alcohol wipes have other additives which will remain when the surface is dried and which are toxic to frogs - these must not be used.
- Minimise handling time to reduce stress and to avoid side effects of stress.

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- Sick or dead frogs should be collected and held separately from all other frogs until delivered to the appropriate recipient or buried. All equipment should be thoroughly cleaned and disinfected after use.
- Wherever a chemical disinfectant is used (e.g. Sterigene, bleach, F10) this must be rinsed off after the disinfection time. Ethanol can be air dried.

Table 1: Disinfection strategies for frog field studies (minimum times and concentrations) that will kill chytrid fungus and ranavirus

Purpose	Disinfectant	Concentration	Mix	Time	Rinse required	References
Disinfecting cloth (e.g. clothing, cloth bags)	sterigene	50mL per 4 kg laundry load (do not use detergent, do not overfill)		Normal wash time	Yes	(product label)
	Hot wash and complete drying	60°C or greater		10 minutes	No	3
Disinfecting footwear	Sodium hypochlorite (household bleach, 4% concentration)	0.5%	5ml bleach in 1 litre water	1 minute	Yes	2,3,4
	Sterigene (Trigene)	1%	10ml in 1 litre water (1:100)	1 minute	Yes	3,5, Product label
	F10 Veterinary disinfectant	1%	10ml in 1 litre water (1:100)	1 minute	Yes	3,5, Product label
	Virkon S	1%	10g virkon in 1 litre water	1 minute	Yes	2,3,4, Product label
Disinfecting collection equipment, gloves and containers	Sodium hypochlorite (household bleach, 4% concentration)	0.5%	5ml bleach in 1 litre water	1 minute	Yes	2,3,4
	Sterigene (Trigene)	1%	10ml in 1 litre water (1:100)	1 minute	Yes	3,5, Product label

¹ WARNING – Virkon is a corrosive substance which will corrode gear over time

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F10 Veterinary disinfectant	1%	10ml in 1 litre water (1:100)	1 minute	Yes	3,5, Product label
Virkon S1 above ¹	1%	10g virkon in 1 litre water	1 minute	Yes	2,3,4, Product label
Ethanol (including alcohol wipes)	70%	Apply liberally	2 minutes	Air dry	1,3,4
Isopropyl alcohol (including alcohol wipes)	70%	Apply liberally	2 minutes	Air dry	1
Heat	60°C of greater		15 minutes	No	

Notes

- Salt solution is not effective on either chytrid fungus or ranavirus
- Leaving gear to dry is only effective against chytrid fungus not ranavirus
- Give everything a good spray, not just a sprinkle
- Items can be rinsed in clean (tap) water after the appropriate contact time, but it is important that they are left to dry thoroughly
- The activity of household bleach begins to reduce once diluted, so this solution must be made fresh each day. Other solutions will last longer after dilution; refer to the manufacturer's instructions. Use alcohol from a small sealed container and replace regularly. Check expiry dates on the concentrated products and don't use expired disinfectants
- Concentration is important. Diluting products to the correct concentration is key to its efficacy.

References

1. Ranaviruses: Brunner, J, Sesterhenn, T (2001) Disinfection of Ambystoma tigrinum virus (ATV) Froglog 48, 2
2. Brown LK, Baldwin CA, Gray MJ, Miller DL. Efficacy of select disinfectants at inactivating Ranavirus. Dis Aquat Organ. 2009 Apr 6;84(2):89-94. doi: 10.3354/dao02036. PMID: 19376278.
3. CHHWG (2017) Canadian Herpetofauna Health Working Group. 2017. Decontamination Protocol for Field Work with Amphibians and Reptiles in Canada. 7 pp + ii. <http://www.cwhcrcsf.ca/docs/HHWG%20Decontamination%20Protocol%202017-05-30.pdf>
4. Van Rooij P, Pasmans F, Coen Y, Martel A (2017) Efficacy of chemical disinfectants for the containment of the salamander chytrid fungus Batrachochytrium salamandrivorans. PLoS

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- ONE 12 (10): e0186269. Efficacy of chemical disinfectants for the containment of the salamander chytrid fungus *Batrachochytrium salamandrivorans* | PLOS ONE
5. Webb R., Mendez D., Berger L. & Speare R. (2007). Additional disinfectants effective against the amphibian chytrid fungus *Batrachochytrium dendrobatidis* - PubMed (nih.gov) *Batrachochytrium dendrobatidis*. Dis Aquat Org, 74, 13-16. Wildlife Health Australia factsheet, *Ranavirus in Australian Amphibians 2016*. *Ranavirus_in_wild_Australian_amphibians.pdf* (wildlifehealthaustralia.com.au)
 6. Sterigene MDS Microsoft Word - STERIGENE MDS Info (cwnl.co.nz)
 7. *Ranavirus: ranavirusesinfection-with.pdf* (woah.org) Chytrid fungus: Fungicidal effect of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis* - PubMed (nih.gov)

SUPERSEDED

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Schedule 8:

Amphibian chytrid fungus swabbing protocol

Swabbing protocol for New Zealand frogs.

Prepared by Leigh Marchall 22/12/04. Adapted from protocol prepared by Alex Hyatt (CSIRO).

1. MWE MW100-100 is recommended swab (NZ distributor NZ Medical Supplies 09 259 4062 nzms@nzms.co.nz). If using alternatives, validation is required.
2. A fresh pair of gloves and a fresh swab should be used for each frog.
3. Swab comprehensively (e.g. repeat 2-3 times) on the ventral surface of the frog, including underside of the thighs, feet and legs.
4. Place the swab back into the container. It does not require drying (but air dried is better as it reduces microbe grow) or preserving. Ensure that swabs are not contaminated by water (i.e. keep dry).
5. Label swab with frog's individual identification, location, date and name of swab taker.
6. Swabs should be stored at 4 degrees or frozen to inhibit growth of other organisms. Swabs can be stored for up to 6 months before diagnostics without compromising results.
7. Submit sample to qualified diagnostic laboratory for analysis (until the laboratory has been decided, swabs should be stored as above).



Figure 1. A frog being swabbed according to this method.

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Schedule 9:

Frog buccal swabbing protocol

Buccal Swab Collection Protocol for New Zealand Frogs

Prepared by Amanda Haigh (ahaigh@doc.govt.nz) November 2008. Department of Conservation, Hamilton, NZ

Introduction

Buccal swabbing involves collecting mucosal cells from the buccal (mouth) cavity of a frog and is being trialled/used as a non-destructive method of collecting DNA. This protocol outlines the materials, handling and sampling protocol for collecting buccal swabs from NZ frogs. Buccal swab collection requires careful delicate handling and manipulation of the frog – a light touch and gentle pressure is all that is needed during swabbing manipulations. Only persons experienced at handling frogs and that have received training in buccal swab collection should collect buccal swabs from native frogs.

Training

All persons wishing to collect buccal swabs from *Leiopelma* spp. should first receive training. Training (demonstration and practice) should be completed using an introduced *Litoria* spp. preferably of similar size to the study species.

Materials

1. Sterile micro cotton swabs (with flexible wire shaft)
2. New unused gloves (unpowdered) nitrile or vinyl
3. Sterile guitar picks (rough edges removed)
4. Storage container for swabs (as required)
5. Ampoules of sterile water
6. Plastic bags for handling frogs
7. Head torch & hand torch
8. Headset magnifier (with light) or eye loupe magnifier (optional)

Handling and restraining the frog

1. Each frog should be handled using a **new unused** pair of nitrile or vinyl gloves
2. Hold the frog by restraining it gently so the head exits the hand between fingers and/or thumb (Figure 1). If the head needs further immobilisation, gently clasp the back of the head behind the eyes (Figure 2). Face the head toward the sample collector.
3. Alternatively, cut the corner off a clean unused plastic bag, place the frog into the bag and **gently** manoeuvre the head out through the opened corner. Hold the frog's body on the

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outside of the plastic bag exposing the head toward the sample collector for easy sample collection.

4. The person opening the frog's mouth and collecting the swab should also wear a **new unused** pair of gloves
5. The frog is now ready for sample collection.

Collection of swab

1. For each sample use a **new unused sterile micro swab** and a **new unused sterile** guitar pick.
2. Once the frog is restrained, very gently insert the guitar pick into the mouth several mm (Figure 2). The frog should respond and slightly open its mouth. Then insert the guitar pick further into the mouth cavity and **very gently** press down until the frog's mouth is sufficiently open to insert the swab (~10 mm) (Figure 3).
3. **Do not use strong force** at any time when attempting to open a frog's mouth; this could cause injury to the frog.
4. Holding the wire shaft close to the cotton tip end of the swab (for maximum control), gently slide swab cotton tip into frog's open mouth. **Without applying pressure, very gently** wipe/roll the swab over the buccal surfaces for 30 seconds and/or until swab is moist with mucus.
5. Use a head torch/headset magnifier (as necessary) to provide additional lighting and magnification to assist with swabbing.
6. Replace swab into dry sterile storage tube with **no medium**.
7. It is important that the swab does not touch any other surfaces apart from the frog's mouth and buccal cavity during swabbing. If a swab is touched on any other surface, it must be discarded and another swab collected from that frog.
8. Label swab with species, location, individual ID code and date
9. Keep cool and freeze asap on return from the field.

NB: It is very important the frog is immobilised for swabbing to minimise any chance of injury.

Sterilisation

New guitar picks require sterilisation prior to use and should not be re-used. Sterilise by soaking in 70% ethanol for 1 minute then air dry, or 4% concentration of bleach for 15 minutes time, rinse thoroughly in sterile water and then air dry.

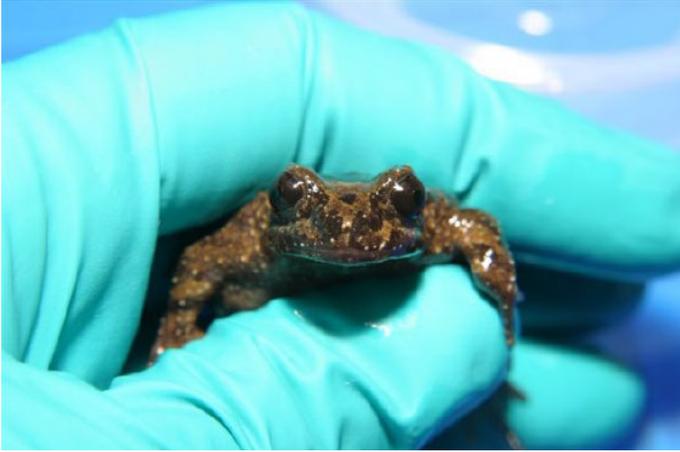


Figure 1: General holding position



Figure 2: Gentle head immobilisation / Guitar pick insertion



Figure 3: Opening mouth

Images for Figure 2 and 3 supplied by Auckland Zoo.

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Schedule 10:

Hochstetter's frog survey and searching protocol

Frog / pepeketua Recovery Group

June 2014

This best practice note covers searching for Hochstetter's frogs during the daytime. The purpose is to provide guidance that will minimise risk to frogs from trampling, habitat disturbance, disease transfer and stress.

When searching for frogs the DOC hygiene protocol (DOCDM-214757) must be followed, alongside any other hygiene protocols that are relevant to the site, e.g. for Kauri dieback.

Study design and preparation

- Because of the risk to frogs, any frog searches must have a real purpose and conservation benefit.
- The survey or monitoring method must be appropriate to the purpose of the study, e.g. for determining the range of a population a simple detection/non-detection survey of select streams may be appropriate, but for monitoring population changes or estimating abundance repeat surveys are needed. Contact the FRG for advice.
- Do not search for Hochstetter's during wet weather. Frogs not only occur along stream sides, but also away from streams. This is particularly the case during and after heavy rainfall. Searching during wet weather can also cause more habitat disturbance due to slippery rocks and more unstable ground.
- Limit repeated surveys at the same site to minimise habitat disturbance. For example, in one study, three surveys within one day of a stream transect done three years apart is considered the maximum that can be done without too much habitat disturbance.

Field protocols

- Before searching, have a system for knowing where to start and stop searching, and have on hand data sheets, pencil, torch, spare batteries, GPS and disposable gloves.
- Search by slowly moving upstream from the start point, carefully examining refugia for frogs (underneath rocks, logs and leaves, and inside crevices and tunnels). Working upstream is easier than downstream, and gentler on the ground underfoot. It also reduces the chance of disturbing frogs which may have been washed downstream during survey disturbance. It is also possible that frogs may hide due to human scent or other disturbances that may flow downstream.
- Carefully assess which objects can be picked up easily and avoid those that can't. Be careful not to accidentally drop the object.

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- Do not pick up an object that would cause other objects to subside. e.g. stream seepages with small, stacked pebbles that could collapse entirely if searching is attempted
- Replace all objects carefully to their original position.
- Before replacing the object run your hand lightly across the ground to check that no frogs have been missed. This is particularly important for inexperienced observers.
- If replacing an object poses a risk to a frog, gently pick up the frog/s underneath the object by gently scooping and holding the frog in cupped, gloved hands, or by gently holding the middle of the frog between 1st or 2nd forefingers and thumb. Do not squeeze the frog and never hold it by the legs or head. Then replace the object, and gently put the frog/s headfirst to where it/they can move under the object again. Do this as soon as possible to reduce the time they are held in hot, gloved hands. Be aware that there can be more than one frog under an object. Use powder free nitrile glove/s, and change glove/s between each frog.
- A torch must be used (there are often low light levels, and the frogs have cryptic colouration).
- Be aware that frogs, particularly small frogs, can be hiding amongst streamside vegetation, so avoid stepping on vegetation within about 2 m of the stream bank.
- Walk in the stream (feet in the water) as much as possible. Along stream sides walk where there is least likelihood of frog presence, e.g. on sand or large immobile rocks.
- Use the same marked access routes to transects, using routes that avoid frog habitat where possible.
- Unless it is part of survey method, avoid double checking/disturbing objects. One option is to chalk-mark objects after they are replaced.

Training

Before searching for frogs independently new observers must receive training by an experienced frog observer. Training, at a minimum, must include

- Observations of the frogs of a variety of sizes
- Demonstration of the variety of places and microhabitats frogs can be found
- How to safely lift and replace objects
- Direct (in person) observation by the trainer of the ability of the trainee to follow these guidelines to safely search for frogs



Photo credit: DOC

Figure 1. Hochstetter's frogs can be well camouflaged (there are three frogs in this photo)

SUPERSEDED