WAIHI NORTH PROJECT - WILDLIFE ACT APPROVAL CONDITIONS

Wildlife Act Approval for wildlife located on public conservation land

SCHEDULE 1

1	Authorised activity	A.	A. Activity:					
	(including the species, any approved quantities and collection methods) (Schedule 2, clause 2)		a)	To catch, salvage and relocate native frog and lizard species listed in Schedule 4 prior to 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 take or destroy the eggs of the following wildlife species when unavoidable:				
				 i. Pīwakawaka / New Zealand fantail (Rhipidura fuliginosa); ii. Kāhu / Australasian harrier (Circus 				
				approximans); iii. Korimako / Bellbird (Anthornis melanura); iv. Riroriro / Grey warbler (Gerygone igata); v. Keruru / NZ pigeon (Hemiphaga				
				novaeseelandiae); vi. Kotare / Kingfisher (Todiramphus sanctus);				
				vii. Tauhou / Silvereye (<i>Todiramphus sanctus</i>); viii. Miromiro /Tomtit (<i>Petroica</i> macrocephala);				
				ix. Tūī (<i>Prosthemadera novaeseelandiae</i>);x. Warou /Welcome swallow (<i>Hirundo neoxena</i>);				
				xi. Pōpokotea / Whitehead (<i>Mohoua</i> albicilla);				
				xii. Kākāriki/ Yellow-crowned parakeet (Cyanoramphus auriceps);				
				xiii. Ruru / Morepork (<i>Ninox novaeseelandiae</i>); xiv. Kākā (<i>Nestor meridionalis</i>);				
				xv. Pīpīwharauroa / Shining cuckoo (<i>Chrysococcyx lucidus</i>); and				
			۹/	xvi. Stag beetle (Geodorcus auriculatus sp).				
			d)	To kill the wildlife species listed in A(c)(i – xv) above, and / or long-tailed bats (<i>Chalinolobus tuberculatus</i>) when unavoidable				
			e)	Any accidental / unintentional harm to wildlife that could arise from any of the activities undertaken in relation to the Waihi North Project.				

		B. Methodology:
		 a) Within the Coromandel Forest Park: Except when instructed otherwise by the Grantor, the methods set out in:
		 i. The Terrestrial Ecology Management Plan as included in the Wharekirauponga Underground Mine Ecology and Landscape Management Plan and included in Part II of the application documents; and
		ii. Native Frog Monitoring Plan.
		b) Outside the Coromandel Forest Park: Methods set out in the Willows Site section of the Wharekirauponga Underground Mine Ecology and Landscape Management Plan and included in Part II of the application documents, and the Lizard Management Plan section of the Waihi Area Ecology and Landscape Management Plan and included in Part II of the application documents.
2	The Land	Areas marked Area 1 – Area 7 on Map 1 in Schedule 5, except
	(Schedule 2, clause 2)	that monitoring of native frogs may take place anywhere in the areas shown on Map1 and Map 2.
3	Personnel authorised to undertake the Authorised Activity (Schedule 2, clause 3)	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 Grantor. * these persons may only handle native frogs subject to direct supervisions and training by Katherine Muchna.
4	Term (Schedule 2, clause 4)	[insert date of approval] to [insert date 30 years from date of approval]
5	Approval Holder's address for notices (Schedule 2, clause 8)	The Approval Holder's address in New Zealand is: Physical: 22 Maclaggan Street, Dunedin 9016, New Zealand Postal: PO Box 5442 Dunedin 9054 Phone: 03 479 4736
		Email: NZ.Legal@oceanagold.com

0	Grantor's notices	address	for	The Grantor's address for all correspondence is:
				[Hauraki District Office, 3/366 Ngati Maru Highway (SH25)
				Thames 3500 (physical);
				PO Box 343, Thames 3540 (postal);
				Phone: <u>0800 275 362</u> ;
				Email: thames@doc.govt.nz]

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. In granting this approval the Grantor does not warrant that such access can be obtained.
- 2.3. The Approval Holder must advise the Department of Conservation's local Operations Manager(s) ("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 Grantor for access to any wildlife.
- 2.6. The Approval Holder may publish authorised research results.
- 2.7. The Approval Holder must immediately notify the Grantor 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 Grantor, 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.

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 Grantor and the Grantor'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 Grantor against all claims, actions, losses and expenses of any nature which the Grantor may suffer or incur or for which the Grantor 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 Grantor'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 Grantor 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 Grantor 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 Grantor 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 Grantor'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 Grantor intends to terminate this Approval in whole or in part, the Grantor must give the Approval Holder such prior notice as, in the sole opinion of the Grantor, appears reasonable and necessary in the circumstances.

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

- 9.1. Any notice to be given under this Approval by the Grantor is to be in writing and made by personal delivery 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 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 Grantor 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 and equipment used by the Approval Holder, its staff and clients are cleaned and checked for pests 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 Grantor 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]

Management and Monitoring Plans

- 2. All Activities authorised by this Wildlife Act Approval must be undertaken in accordance with the following management and monitoring plans, or any amended versions that may be made under Condition 4:
 - 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;
 - The Native Frog Monitoring Plan that has been certified under condition C5 of Schedule One: Conditions Common To The Hauraki District Council And Waikato Regional Council Resource Consents;
 - The certified Native Frog Monitoring Plan must be provided to the Department of Conservation no later than 4 years prior to the commencement of WUG stoping activities;
 - The Native Frog Salvage Release Plan as included 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.
 - b. Outside the Coromandel Forest Park:
 - 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 Schedule One: Conditions Common To The Hauraki District Council And Waikato Regional Council Resource Consents.
- 3. All Activities authorised by this Wildlife Act Approval that are undertaken in accordance with the certified management and monitoring plans listed in Condition 2 must also be undertaken in accordance with the Frog swabbing 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.
- 4. In accordance with Conditions C8A –C8D of Schedule One: Conditions Common To The Hauraki District Council And Waikato Regional Council Resource Consents the Approval holder may make amendments to any of the management plans referred to in Condition 2 at any time.

Ownership and holding of Absolutely Protected Wildlife

- 5. 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.
- 6. Unless expressly authorised by the Grantor 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

- 4. If any protected wildlife is found dead; the Approval Holder must contact the Grantor's Hauraki Office on 07 867 9180, with known details of the animal's history. Then, if the Grantor requests it, the body must be sent to Massey University Wildlife Post Mortem Service for necropsy.
- 5. 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 delivery.
 - b. Ensure appropriate measures are taken to minimise further deaths.

- c. Discuss with the Grantor's Hauraki office, 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

- 6. The Approval Holder must not euthanize 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 Grantor and in consultation with a veterinarian (as applicable).

Records

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

Lizard and Frog Survey and Salvage Reporting

- 8. 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 proceeding 1 July 30 June period); summarising the matters listed in a. to c. of this Condition, 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 (or a detailed map) of the collection point(s) and release point(s);
 - c. results of all surveys, monitoring or research.
- 9. Completed Amphibian and Reptile Distribution System (ARDS) cards for all herpetofauna sightings and captures must be sent to the Herpetofauna Database Administrator, PO Box 10420 Wellington 6143, or via email to herpetofauna@doc.govt.nz (A copy of the ARDS card is included as Attachment 1 to this Approval).

Dispute Resolution

- 10. The parties agree to negotiate in good faith to resolve any differences which arise in connection with this Approval.
- 11. Failing resolution in accordance with Condition 53, any differences and disputes between the parties concerning this Approval, its interpretation, effect or implementation or any act or thing to be done in pursuant thereof (except as otherwise expressly provided) is to be referred to arbitration in New Zealand by a single arbitrator who is to be mutually agreed upon and,

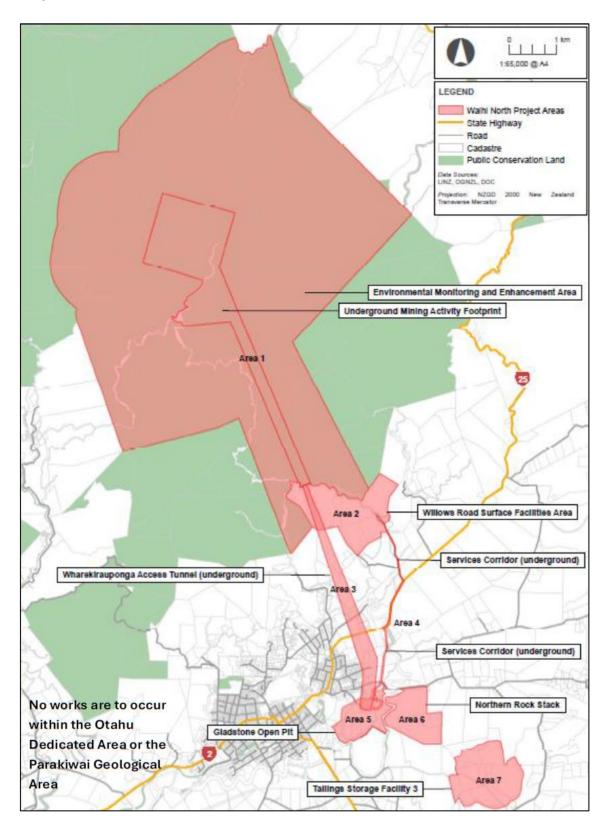
failing agreement, is to be appointed by the President of the New Zealand Law Society. In all other respects the provisions of the Arbitration Act 1996 shall apply.

SCHEDULE 4

Common Name	Scientific Name	NZ Threat Classification
Northern striped gecko	Toropuku inexpectatus	Threatened-Nationally
		Endangered
Pacific gecko	Dactylocnemis pacificus	Not Threatened
Elegant gecko	Naultinus elegans	At Risk-Declining
Forest gecko	Mokopirirakau granulatus	At Risk-Declining
Common gecko	Woodworthia maculata	Not threatened
Striped skink	Oligosoma striatum	At Risk-Declining
Ornate skink	Oligosoma ornatum	At Risk-Declining
Copper skink	Oligosoma aeneum	At Risk - Declining
Moko skink	Oligosoma moco	At Risk-Relict
Archey's frog	Leiopelma archeyii	At Risk- Declining
Hochstetter's frog	Leiopelma hochstetteri	At Risk-Declining

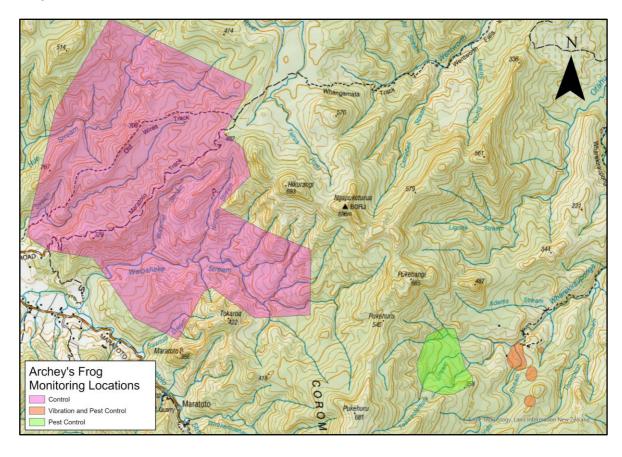
SCHEDULE 5:

Map 1



SCHEDULE 5:

Map 2



SCHEDULE 6: Amphibian and Reptile Distribution System (ARDS) card

ARDS CARD	NEW ZEALAND AMPHIBIAN/REPTILE DISTRIBUTION SCHEME Herpetofauna Administrator, RD&I, Department of Conservation, P.O. Box 10420, Wellington.								Card No:		
Date:						: Locality		ty Na	ıme:		
Observer: Initials Surname				Alt (m):	:					
Address:				F		Easting	Lasting		Northing		
				GPS							
				Series N		Map No.	Iap No. Easti		ing Northing		
Affiliation:				Area Office:		Cons	Conservancy:		Ecol. District:		
Species name		No.	Time	Habitat	Weathe	r Weathe	•	Ma	ior Hab	itat Types	
						y vveatne	ſ	Major Habitat Types			
e.g. Hoplodactylus n	naculatus	6	18:00	16, D, E	6,2,1	Light	Light		 Beech Forest Podocarp forest 		
						1 Fine/S		3 Broadle 4 Exotic f 5 Scrub			
						2 Part Cl 3 Overca	st				
						4 Showe 5 Rain	r'S	6 7	Sub-alpi Alpine		
						6 Night	1:4	8	1		
							7 0-½ Moonlit 8 ½-1 Moonlit		10 River terrace 11 Fresh water		
Voucher specim	nen(s)	Yes	/No	Specify:				11	1 ICSII W	atti	
Photograph(s)		.,				=	Temperature				
			/No			1 Hot 2 Warm					
Extra notes on	reverse side		/No			3 Moder 4 Cool	1 4 2 2 1		Micro habitats		
Notes:								14 15	Scree Bare roc	"	
rvotes.								16	Beach	B Trunk	
							1 Calm 2 Light broom 2 D Und			C Branches	
										D Under stones	
							3 Mod breeze 4 Gusty E Und F Open			E Under wood	
Identified by:						F Open ground G Crevices					
Authority used:									H H		

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).

- 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.
- o 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 reused 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.
 - o covers are disinfected daily, sufficient covers must be available for each night so that a clean one can be used for each frog
 - o 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.

- Sick or dead frogs should be collected and held separately from all other frogs until delivered to the appropriate recipient. 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	References
					required	
District attend	akani da na	FOrest war A lan		NI I	V	0 (
Disinfecting	sterigene	50mL per 4 kg		Normal	Yes	6 (product
cloth (e.g.		laundry load (do		wash time		label)
clothing, cloth		not use				
bags)		detergent, do not				
		overfill)				
	Hot wash and	60°C of greater		15 minutes	No	3
	complete drying					
Disinfecting	Sodium	0.5 %	5ml bleach	1 minute	Yes	2,3,4
footwear	hypochlorite		in 1 litre			
	(household bleach,		water			
	4% concentration)					
	Sterigene (Trigene)	1%	10ml in 1	1 minute	Yes	3,5, Product
			litre water			label
			(1:100)			
	F10 Veterinary	1%	10ml in 1	1 minute	Yes	3,5, Product
	disinfectant		litre water			label
			(1:100)			
	Virkon S ¹	1%	10g virkon in	1 minute	Yes	2,3,4, Product
			1 litre water			label
Disinfecting	Sodium	0.5%	5ml bleach	1 minute	Yes	2,3,4
collection	hypochlorite		in 1 litre			
equipment,	(household bleach,		water			
instruments	4% concentration)					
and						
containers	Sterigene (Trigene)	1%	10ml in 1	1 minute	Yes	3,5, Product
			litre water			label
			(1:100)			

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

F10 Veterinary	1%	10ml in 1	1 minute	Yes	3,5, Product
disinfectant		litre water			label
		(1:100)			
Virkon S1 above ¹	1%	10g virkon in	1 minute	Yes	2,3,4, Product
		1 litre water			label
Ethanol (including	70%	Apply	2 minutes	Air dry	1,3,4
alcohol wipes)		liberally			
Isopropyl alcohol	70%	Apply	2 minutes	Air dry	1
(including alcohol		liberally			
wipes)					
Heat	60°C of greater		15 minutes	No	7

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. Ranavirus: Brunner, J, Sesterhenn, T (2001) Disinfection of Ambystoma tigrinum virus (ATV) Froglog 48, 2
- 2. Bryan 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: 19476278.
- 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%202017-0530.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

- 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 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 effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid Batrachochytrium dendrobatidis PubMed (nih.gov)

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)

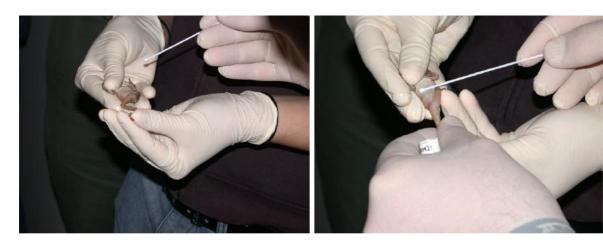


Figure 1. A frog being swabbed according to this method.

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 holding 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

- outside of the plastic bag exposing the head toward the sample collector for easy sample collection
- The person opening the frogs 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 mucous.
- 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.

Schedule 10:

Hochstetter's frog survey and searching protocol

Hochstetter's frog 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 clear 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.
- 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 2m 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 experiences frog observer. Training, at a minimum, must include

- Observations of live 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



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