

Draft assessment report to amend the *List of Specimens taken to be Suitable for Live Import* to include Growling Grass Frogs, for the purpose of scientific research only

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| <p>1. Provide information on the taxonomy of the species, including any subspecies that occur naturally outside Australia.</p> | <p>a) Family Hylidae b) Genus <i>Litoria</i> c) Species <i>raniformis</i> (i.e. <i>Litoria raniformis</i>) d) <i>No subspecies described</i> e) Keferstein 1867 (ITIS Global 2016) f) Common name: Growling Grass Frog, also known as Southern Bell Frog, Green and Golden Frog, Warty Swamp Frog g) The species was endemic to Australia (NSW, VIC, TAS, SA), but was introduced to New Zealand in 1867, where it is now widely distributed throughout the North and South Islands (Gill and Whitaker 2001). h) This species is not a GMO (genetically-modified organism)</p>  <p>Adult <i>Litoria raniformis</i> (photo by Geoff Heard)</p> |
| <p>2. Provide information on the status of the species under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES),</p> | <p>This species is not listed in CITES Appendices I, II or III (CITES 2015). <i>Litoria raniformis</i> is listed as Vulnerable under the Commonwealth <i>Environment Protection and Biodiversity Conservation Act 1999</i> (EPBC Act; Department of the Environment 2016).</p> |

and its conservation status under the Environment Protection and Biodiversity Conservation Act 1999.

3. Provide information on the possible impacts that imported specimens could have on the native population of the same species, and on other components of the Australian environment. This may include an assessment of:

- any possible phenotypic or behavioural changes that may have occurred in these specimens as compared to those naturally occurring in Australia
- any adaptations to differing climatic conditions in the country of export.
- any possible parasites or pathogens that these specimens may carry as compared to those naturally occurring in Australian populations.

Litoria raniformis was introduced to the South Island of New Zealand in 1867 and 1868; genetic analyses have revealed that individuals involved in the introductions originated from Melbourne populations (Vörös et al. 2008). Voros (2008) found individuals from Melbourne and New Zealand were genetically quite similar when comparing a partial sequence from the mitochondrial COI gene, with one shared haplotype (genetic type) and six unique haplotypes separated from the most common, shared haplotype by only 1 or 2 mutational steps. *Litoria raniformis* is now widespread across the North and South Islands of New Zealand. The species has not established non-native populations elsewhere.

The specimens proposed for import are not likely to have an impact on native populations of the same species. There is no evidence in the literature that individuals from New Zealand are phenotypically different (i.e. different in appearance) from the wild individuals occurring in Australia. Similarly, there is no evidence that populations in New Zealand have modified their diet or habitat use, or modified their social behaviour. Pyke *et al.* (2002), for example, showed that the closely-related *Litoria aurea*, which has also been introduced to New Zealand, has similar habitat preferences, in NSW, VIC, and NZ.

Litoria raniformis inhabits some areas of New Zealand that are colder compared to its native Australasian range. For example, one notable, very large population in Central Otago on the South Island of New Zealand survives winter temperatures which often exceed -10°C (Bishop 2008). However, there is no evidence that New Zealand *L. raniformis* have physiologically adapted to such conditions. For example, Cree (1984) studied *L. raniformis* in Christchurch, NZ, and found that at 23°C, tadpoles were active and that only 5% died over a 16 day period. At 15°C, however, *L. raniformis* tadpoles did not feed or swim, and 80% died over 16 days. In the same study, juvenile *L. raniformis* were acclimated to 23°C, 15°C, or 7°C for a minimum of 9 days. The species was active at 15°C and 23°C, but at 7°C, all individuals were inactive; they did not feed and moved lethargically when provoked. These observations suggest that New Zealand *L. raniformis* are not adapted physiologically to colder conditions. This conclusion will be explicitly tested as part of this research project (see Section 5).

If live specimens from New Zealand established populations in Australia, the overall impact on native Australian species would be minimal. The species is unlikely to cause environmental degradation, property damage, or impact primary industry. Its impact on native Australian species with similar environmental requirements would be no

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| | <p>different to the impact of native <i>L. raniformis</i> populations. The species' prey, for example, would be the same as native Australian populations of the species.</p> <p>It is possible that some of the specimens would carry the amphibian chytrid fungus, <i>Batrachochytrium dendrobatidis</i>. The chytrid fungus is a waterborne pathogen, which infects the epidermis of amphibians and can impair osmoregulation and electrolyte balance, potentially killing susceptible individuals (Berger et al. 1998, Voyles et al. 2009). The first incidence of <i>B. dendrobatidis</i> in New Zealand was reported in Christchurch, in 1999 (Waldman et al. 2001).</p> <p><i>Batrachochytrium dendrobatidis</i> is already present in Australia. The pathogen first appeared in Australian amphibians in 1978, and the disease phase, chytridiomycosis, has been listed as a key threatening process for <i>L. raniformis</i>, with several lines of evidence suggesting it was a driver of population declines and extinctions (Clemann and Gillespie 2012, Heard et al. 2014). For instance, chytrid infections are common among <i>L. raniformis</i> in the populations north of Melbourne, where Heard et al. (2014) found severe infections reduce the monthly probability of individual survival by 1/3. <i>Litoria raniformis</i> populations in New Zealand appear to be more robust to the pathogen than Australian populations (Vörös et al. 2008). Relative to Australian populations, <i>L. raniformis</i> appears to occur in large, stable populations in New Zealand, and only a few dramatic declines have been reported (Bishop 2008).</p> <p>Sensitive diagnostic tests for <i>B. dendrobatidis</i> have been developed using quantitative PCR, enabling importers to test specimens for infection prior to import into Australia.</p> |
| <p>4. Provide information on the origin of the live specimens that you propose to import.</p> | <p>The live specimens (adult frogs) would be imported from multiple populations of <i>L. raniformis</i> from throughout the North and South Islands, New Zealand. This species is common and widespread throughout the North and South Islands and specimens would be from wild populations; the specimens would not have been captive bred or previously maintained in captivity.</p> |
| <p>5. Provide a summary of the proposed purpose of import.</p> | <p><i>Litoria raniformis</i> will be imported into Australia for scientific research purposes only. The aim of this research is to use the tadpoles of this species as a model system to understand how organisms respond to environmental variability experienced during development. Precipitation and temperature are key determinants of population dynamics, and global climate models predict increases in the frequency and intensity of droughts and thermal extremes. Such changes can significantly alter developmental trajectories of organisms, inducing inappropriate phenological responses, and causing population extinctions.</p> |

This project aims to develop a generalisable modelling framework for predicting effects of environmental variability on developmental strategies of organisms. This framework will deliver new insights into how environmental variability influences geographic variation in developmental strategies, and provide the needed tools to account for that variation in physiological models of species distributions and extinction risk forecasts under environmental change.

Tadpoles present exceptional systems for understanding how environmental variability influences developmental trajectories (Mueller et al. 2012). They exhibit remarkable plasticity in life-history, morphology, and behaviour in relation to variation in temperature and hydroperiod, but this plasticity is constrained by important life-history trade-offs between growth and maturity (Newman 1992, Warne and Crespi 2015). The growling grass frog system has particular advantages. *Litoria raniformis* are distributed along strong thermal gradients in eastern Australia, and previous studies hint at geographic variation in developmental strategies (Cree 1984, Mann et al. 2010, Heard et al. 2012). Furthermore, the origin and residence time of introductions to New Zealand are known (Bishop 2008, Vörös et al. 2008). These ‘experiments in nature’ (Diamond 1983) provide unparalleled opportunities to test our ability to predict development strategies from local microclimates under different conditions. Additionally, the species has a fast life-history; it is highly fecund (~ 4000 eggs on average) and develops rapidly (eggs hatch within days and tadpoles develop within 2-3 months). These traits make *L. raniformis* ideal for estimating growth and development in captivity.

We will score key life-history traits (e.g., growth rates, development rates, size at metamorphosis, morphology, feeding rates, activity levels, and swimming speeds) in tadpoles from multiple populations of *L. raniformis* from North and South Island, New Zealand, as well as from multiple Australian populations. This comparison will enable us to determine whether *L. raniformis* from New Zealand have adapted, via developmental plasticity or evolution, to novel environmental conditions encountered in New Zealand. From a conservation perspective, understanding why *L. raniformis* has been so successful in New Zealand may help shed light on causes of (and potential solutions to) declines in Australia.

Adults will be sourced from wild populations in New Zealand and Australia. These adults will be bred in an approved arrangement site at The University of Melbourne. As outlined in Section 3, New Zealand *L. raniformis* originated from Melbourne. Breeding is easily controlled; the species will not breed unless they have access to a large waterbody in which to lay their aquatic eggs. After the imported adults have been bred, they will be immediately euthanased; research will be conducted on their progeny (tadpoles), not on the imported adults. This will minimise the chance of parasite or disease transmission (e.g., chytrid), because the eggs and tadpoles will have no contact with the adults. At the conclusion of the study, all progeny of the imported frogs and all progeny of the native

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| | <p>Australian frogs will be euthanized and the carcasses will be destroyed. All animals (both imported and Australian adults and their progeny) will remain in the approved arrangement site; no animals will be released into the wild in Australia.</p> <p>Why does this research have to be conducted in Australia? Why, for example, couldn't we conduct these experiments in New Zealand? The primary reason (other than logistical constraints) is that environmental conditions that frogs experience – factors such as light, temperature, and food supply – profoundly influence their morphology, behaviour, physiology, and reproductive success. That sensitivity means that wild-caught frogs need to be bred, and their tadpoles raised, in the same place, at the same time, under identical laboratory conditions. This also means that we cannot simply bring tadpoles from New Zealand into Australia, because the early environmental conditions experienced as eggs and tadpoles in New Zealand would fashion their phenotypes; we need to import live adults. This “common-garden” design, aimed at eliminating external factors that would otherwise create differences between individuals from different populations, is a standard method in many scientific fields (e.g., crop production, forestry, evolutionary research). An additional issue is that the scientific data on <i>L. raniformis</i> has been collected almost entirely in Australia. There are hardly any existing studies that we could use to answer our research questions, especially given the wide range of traits we need to assess.</p> <p>The significance of this research is evidenced by the fact that it has been recently supported by a 2017 Australian Research Council Discovery Early Career Researcher Award to Dr Reid Tingley. The ARC uses a rigorous peer-review system, it funds a small proportion of submitted applications.</p> |
| <p>6. What conditions or restrictions, if any, could be applied to the import of the species to reduce any potential for negative environmental impacts (e.g. desexing specimens).</p> | <p>To reduce any potential for negative environmental impacts, imported specimens should be restricted as follows:</p> <ul style="list-style-type: none"> - <i>Litoria raniformis</i> specimens are imported for scientific research purposes only. - <i>Litoria raniformis</i> specimens are housed in secure enclosures within a secure facility and access restricted to authorised research personnel only. - Husbandry routines should reduce the potential spread of pathogens and follow the <i>Guidelines for minimising disease risks associated with captive breeding, raising and restocking programs for Australian frogs (Murray et al. 2011)</i>. These include using latex gloves when handling individuals and changing gloves between individuals and enclosures. All equipment should be sterilised in 70% ethanol between frogs and enclosures. Footwear should be sterilised before exiting the facility. - Each <i>L. raniformis</i> specimen should be swabbed to test for infection with the amphibian chytrid fungus prior to import. - <i>Litoria raniformis</i> specimens should be euthanased at the completion of the research. - <i>Litoria raniformis</i> tissue/specimens to be preserved in 96% ethanol and donated to Museum collections. |

7. State/territory controls on the species.

Victoria

The Department of Environment, Land, Water and Planning require a permit to import wildlife into the state and an advanced wildlife licence for keeping *L. raniformis* in captivity as a pet.

Queensland

All native wildlife is protected under the Department of Agriculture and Fisheries. A wildlife movement permit is required for bringing native or exotic animals into, or out of, Queensland. A recreational wildlife licence is required to keep amphibians at a residential property. In non-protected areas, a scientific or educational purposes permit is required to keep protected animals for these purposes. *Litoria raniformis* is not native to Queensland and potentially should not be imported there.

New South Wales

The Office of Environment and Heritage requires a Native Animal Keeper Licence (category A2) to keep *L. raniformis*. A licence is required to import or export protected native animals into the state.

Northern Territory

The Department of Primary Industry and Resources prohibits the import of all wildlife not native to the Northern Territory and the import of frog species not native to the Northern Territory is illegal.

Western Australia

In Western Australia, the Department of Parks and Wildlife require a licence to keep amphibians as pets. A licence is also required for to import fauna and for fauna research. *Litoria raniformis* is not native to WA and potentially should not be imported there.

South Australia

The Department of Environment, Water and Natural Resources requires a permit to import or keep native animals as pets in captivity, or to use animals in research.

Tasmania

The Department of Primary Industries, Parks, Water and Environment does not allow the private keeping of any imported wildlife other than caged birds in Tasmania. A wildlife exhibition licence or a scientific research facility is required to apply to import wildlife into Tasmania. A Herpetology Permit is required to keep Tasmanian native reptiles and amphibians.

Australian Capital Territory

The ACT Government Environment, Planning and Sustainable Development Directorate require a licence to keep wildlife under the *Nature Conservation Act 2014*.

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