SHORT COMMUNICATION

Feral pig (*Sus scrofa*) predation of a green and golden bell frog (*Litoria aurea*)

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Abstract: Feral pigs (*Sus scrofa*) are a threat to New Zealand's biodiversity. Predation of frog species by feral pigs is a notable problem in other countries where pigs have been introduced. Our study aimed to determine through analysis of stomach contents if feral pigs are consuming frogs in the Waitakere Ranges, Auckland. Auckland Council contract pig hunters collected 274 feral pig stomach samples. Of these samples, 184 were screened for frog consumption via both dissecting microscope and DNA analyses. A single frog was identified by morphological and DNA analysis, the introduced green and golden bell frog (*Litoria aurea*). Anecdotal evidence from the hunter records also suggests that nine other individual frogs were found in the stomach of the same pig (n = 10 total). This evidence of frog predation by feral pigs should be considered along with the many other negative impacts of feral pigs in New Zealand, and management programmes for pigs should be considered in areas of biodiversity value.

Keywords: diet; feral pig; green and golden bell frog; *Litoria aurea*; predation; *Sus scrofa*

Introduction

Invasive species are one of the biggest threats to New Zealand ecosystems (Lockwood et al. 2013). The invasive feral pig (*Sus scrofa*), is descended from domestic pigs released in New Zealand by European settlers in the 18th century (King 2005). Feral pigs pose many direct and indirect threats to biodiversity conservation (Barrios-Garcia & Ballari 2012; Hone 2012; Krull et al. 2013a; Krull et al. 2013b). However, the direct threat of feral pig predation on native herpetofauna in New Zealand has been understudied.

Feral pigs feed mainly on plants, but are opportunistic omnivores, and animals can also form a significant part of their diet (Thomson & Challies 1988; Massei et al. 1996; Loggins et al. 2002; Giménez-Anaya et al. 2008). Feral pigs have been implicated in the decline of *Powelliphanta hochstetteri* (an indigenous giant land snail) and of breeding colonies of the New Zealand white-capped mollymawk (*Diomedea cauta steadi*; Coleman et al. 2001; Flux 2002). Challies (1975) also found evidence of both yellow-eyed penguin (*Megadyptes antipodes*) and Auckland Island prion (*Pachyptila desolata*) in pig stomachs and described evidence of predation of Auckland Island shag (*Leucocarbo colensoi*) by pigs.

Frogs have been detected in pig stomachs in other countries (e.g. Schley & Roper 2003; Wilcox & Van Vuren 2009; Jolley et al. 2010) and Richards et al. (1993) suggest that feral pigs, through either direct predation or habitat disturbance, may have contributed to the declines in populations of Australian endemic tropical rainforest frogs. This may also be of concern in New Zealand forests, as feral pigs can destroy the habitat of the endemic Hochstetter's frog (*Leiopelma hochstetteri*) by trampling and rooting on the edge of streams, and are also likely to kill Hochstetter's frogs opportunistically (Baber et al. 2006). This is of key concern, as Hochstetter's frog is listed as vulnerable (IUCN 2014) and as the 39th most evolutionarily distinct and globally endangered amphibian (EDGE 2008). This study aimed to determine if feral pigs are predators of frogs, particularly endemic species such as the Hochstetter's frog, via both morphological and DNA analyses.

Materials and methods

Animal ethics was not required for this study as the Auckland Council were culling pigs as part their scheduled pig management programme in the Waitakere Ranges, Auckland. Auckland Council contract pig hunters collected a total of 274 pig stomach samples as part of the cull requirements through all seasons from October 2008 until September 2011. The hunters collected a sample by slicing through the abdomen of the pig, opening the stomach with a knife and stirring the contents. An 80-ml sample container was then three-quarters filled with a subsample of mixed stomach contents and topped up with 75% ethanol for preservation.

Of the 274 samples, only 184 could be analysed due to issues with sample collection and storage (mainly samples drying out). All 184 samples were examined for evidence of frog consumption in 2014 under a dissecting microscope (Olympus SZ61, with Olympus DP25 digital camera, Olympus Corporation) at between 6.7 × and 45 × magnification on disposable dissection trays, following Egeter et al. (2015a). Where frog remains were identified, we used DNA analysis to provide additional evidence. DNA from tissue samples (c. 25 mg) was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's instructions. DNA was amplified using the universal vertebrate primers 12Sai/12Sbi and associated PCR conditions described by Simon et al. (1994). Bidirectional sequencing was carried out using an

ABI 3730xl DNA Analyser (Applied Biosystems) and resultant sequences were inspected, trimmed, and ambiguities removed based on chromatograms using Bioedit (Hall 1999). Mega6 (Tamura et al. 2013) was used to create sequence alignments (using the MUSCLE algorithm; Edgar 2004) and to conduct BLAST searches (Zhang et al. 2000).

Results

Of the 184 pigs subjected to stomach analysis, 135 had GPS locations provided by hunters. Of these, 132 were killed within 1 km of a stream, and 99 (73.3 %) within 1 km of a stream known to be inhabited by Hochstetter's frogs (based on data from Moreno 2009). However, it is likely that many of the other streams near to pig kills are also inhabited by Hochstetter's frogs as there have been only a limited number of surveys for frogs in the Waitakere Ranges (e.g. Bradfield 2005; Moreno 2009).

A single frog was identified from morphological analysis – the introduced green and golden bell frog (*Litoria aurea*). This prey item had been swallowed whole (Fig. 1) and was identified based on the near complete rear inter-digital webbing and terminal discs being wider than the digits (Courtice & Grigg 1975). BLAST results (100% query cover) provided further evidence that this was indeed a green and golden bell frog (99% identity; Accession No. AY819398.1), rather than either of the two other frog species known to be present in the study area, *Litoria ewingii* (87 % identity; Accession No. FJ965884.1) and Hochstetter's frog (80 % identity; Accession No. DQ283217.1). The stomach sample containing the frog was from a 57-kg boar killed on 29 May 2009 in the sand dunes at Whatipu Beach not far from Taranaki Stream, in the Waitakere Ranges, Auckland (E 1732880, N 5901569 New Zealand Transverse Mercator Grid). The hunter who gathered this sample noted that there were 10 frogs in the pig's gut, but unfortunately only collected a single frog in the sample for analysis. The hunter did not write any further notes about this sample.

Discussion

The proportion of pig stomachs containing vertebrates as prey varies among studies, ranging from 0 to c. 40% (excluding carrion; Everitt & Alaniz 1980; Wood & Roark 1980; Thomson & Challies 1988; Massei et al. 1996; Taylor & Hellgren 1997; Loggins et al. 2002; Giménez-Anaya et al. 2008; Wilcox & Van Vuren 2009; Jolley et al. 2010). Indeed, there are few reports in the literature of feral pigs preying upon frogs, although it has

Figure 1. Green and golden bell frog (*Litoria aurea*) found in a feral pig (*Sus scrofa*) stomach obtained from the Waitakere Ranges, Auckland, New Zealand.

been observed in the USA and Europe (Schley & Roper 2003; Giménez-Anaya et al. 2008; Wilcox & Van Vuren 2009; Jolley et al. 2010). A review of 21 feral pig diet studies from Western Europe concluded that amphibians are consumed relatively rarely and not in large volumes (Schley & Roper 2003). The present study is the first to report predation of frogs by feral pigs in New Zealand. This brings the number of introduced species in New Zealand reported to prey on frogs to nine, six of which are mammalian species (Egeter et al. 2015b).

The fact that no Hochstetter's frogs were observed as prey in feral pig stomach contents may be because pigs are not preying upon this species; however, the present study cannot be considered extensive enough to draw this conclusion. Further sampling is required to assess the level of predation feral pigs may be exerting on Hochstetter's frogs, and we make the following recommendations for such future studies. 1) The collection and analysis of feral pig faecal samples would extend the length of time over which frogs could be detected as prey, particularly if pigs are consuming frogs during night hours. Passage rates through the entire pig gastrointestinal tract can range from 6 h to 7 days, depending on the items ingested (Li et al. 2014). 2) Further stomach or faecal analysis studies should be focused on hunting in seasons and weather conditions conducive to greater frog activity (i.e. when frogs spend more time outside of retreats). Jolley et al. (2010) identified 49 eastern spadefoot toads (*Scaphiopus holbrookii*) from a single pig stomach and hypothesised that pigs may focus their foraging patterns on specific prey items when conditions are optimal and rates of encounter are elevated (Jolley et al. 2010). This could also be the case in the Waitakere Ranges. Although Hochstetter's frogs have been observed emerging from retreats in all seasons (e.g. Green & Tessier 1990; Baber et al. 2006; Moreno 2009), Haigh et al. (2010) observed that emergence was highest during spring and summer and lowest in winter, and that it has a positive relationship with overnight minimum temperature, ambient air temperature, and relative humidity. 3) Where possible, entire stomach contents or faecal contents should be taken for analysis as it was noted in the present study that subsampling of stomach contents led to the exclusion of nine frogs from one stomach sample, and this could potentially have resulted in the exclusion of frogs from other samples. 4) Samples should be stored upright in tightly sealed containers to prevent degradation as this led to exclusion of 90 stomach subsamples in the present study.

No prey remains of Hochstetter's frog were observed, but the presence of a green and golden bell frog in the stomach of a feral pig (and anecdotal evidence of 9 other frogs in the stomach of a single pig) is cause for concern. Feral pigs are widely known to preferentially root in and around drainage lines (Mitchell & Mayer 1997; Vernes et al. 2001; Mitchell et al. 2007) and evidence of pig disturbance is often present at streams inhabited by Hochstetter's frogs (Baber et al. 2006), including those within the Waitakere Ranges (Egeter 2014). The abundance of Hochstetter's frogs varies widely among streams in the Waitakere Ranges, from zero to over 45 frogs/100 m stream reach (Green & Tessier 1990; Ziegler 1999; Bradfield 2005; Moreno 2009). However, accurate estimates of Hochstetter's frog density are exceedingly difficult to obtain, making it hard to estimate the impact pigs may have on this species, either through predation or habitat modification.

Feral pigs have often been implicated in the decline of amphibians in Australia (Richards et al. 1993; Hines et al. 1999; Hero & Morrison 2004) and the present study highlights the potential for negative impacts through direct predation by feral pigs on amphibians in New Zealand. Indeed, the green and golden bell frog is listed as vulnerable within its native Australian range (IUCN 2014). It is possible that pigs are not consuming native *Leiopelma* spp. and may be preferentially targeting introduced frog species, possibly due to their larger size, palatability, life-history or habitat preferences. This could even provide some benefit for native frogs as green and golden bell frogs are known to prey on Archey's frogs (*Leiopelma archeyi*; Thurley & Bell 1994). However, more comprehensive study would be required to elucidate this. Alternatively, native frogs may be consumed by feral pigs, and Archey's frogs may be more vulnerable than Hochstetter's frogs to pig predation, given their fully terrestrial life-history and their critically endangered status (IUCN 2014). Feral pigs may or may not exert a sustained predation pressure on frogs, but could potentially be responsible for localised population reductions or even extirpations, given the evidence that individual pigs will occasionally eat many frogs in a short time period (present study and Jolley et al. 2010).

The extent to which feral pigs are involved in the decline of New Zealand's native frogs remains unknown, but the evidence presented herein of feral pigs preying on frogs in New Zealand should be considered along with the many other negative impacts of feral pigs including the consumption of native land snails (Coleman et al. 2001), native bird species, particularly ground nesting shore birds (Challies 1975; Flux 2002), and the destruction of native habitats through their rooting behavior (Mitchell & Mayer 1997; Mitchell et al. 2007). Furthermore, adequate population monitoring techniques for Hochstetter's frogs could be employed to measure the success of any pig removal operations, for example, the photographic mark-recapture methods currently being used for Archey's frogs (Bradfield 2004; Haigh et al. 2007). Mounting evidence (Krull et al. 2013a, b) now supports the sustained control or (where possible) local eradication of this invasive species as a worthwhile exercise when trying to conserve at risk native species and preserve native habitats and ecosystems.

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