Genetic analysis of scat samples to inform conservation of the Tasmanian devil

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ABSTRACT

Recent advances in molecular genetics have enabled a great deal of information about species to be obtained from analysis of non-invasively collected samples such as scat. Scat provides genetic information via residual host DNA on the outside of the scat, via characterising the genetic makeup of intestinal microbes that are present in the scat, or by examining the DNA remnants of prey items that have passed through the animal's digestive tract. In this review, we provide a case study to demonstrate how these approaches are being used to better understand the threatened Tasmanian devil in the landscape, and to support the species' conservation. Scat analysis enables us to quantify the genetic diversity of remote populations, where trapping is logistically challenging. We are beginning to learn how conservation management impacts the microbiome of threatened species, and investigate how various management strategies may be impacting the diverse array of bacteria and viruses that devils, like all animal species, are host to. We are using scat samples to better understand the interaction between devils and other animals in their environment by learning more about what they eat. We explore the strengths and challenges of these approaches by comparing our work to that conducted in other species. Finally, we provide specific examples of how our results are being integrated into conservation strategy for the devil.

Key words: carnivores, conservation management, diet, ecology, faecal sampling, genetic diversity, metabarcoding, microbiome, population biology, virome

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Introduction: faecal DNA sampling in wildlife conservation

The conservation of threatened biodiversity presents several basic questions: which species are present? How many individuals are there? How are populations distributed (e.g. what are the barriers to movement)? What are the threats to species persistence? For many species, gathering such information directly can be a challenge, especially for animals that are reclusive, difficult to catch via conventional methods, or that persist in remote areas or at low densities. For these species, emerging technologies have a lot to offer. One particularly promising field is the use of environmental DNA (eDNA) sampling, which can involve the analysis of DNA mixtures present in environmental samples such as soil, water or even air, to determine which species are present (Thomsen and Willerslev 2015). A related approach is the analysis of DNA from animal scats, nesting material, shed feathers, hair and so on, often referred to as "non-invasive DNA sampling", as animals do not need to be captured for sampling. Non-invasive samples may be obtained from known individuals (e.g. collecting scats after witnessing the animal defecate), or from unknown individuals (e.g. collected from the landscape). The clear advantage of this approach is the opportunity for research to occur in remote areas or on endangered species without the need for direct human contact with the focal species (Waits and Paetkau 2005). There are several important caveats to non-invasive DNA sampling, primarily related to the rapid degradation of biological molecules once exposed to the elements (see Taberlet *et al.* 1999, and below). Nevertheless, scat samples can provide a range of valuable data, enabling conservation programs for threatened species (such as Eurasian otter [*Lutra lutra*; Hajkova *et al.* 2011], snow leopard [*Panthera uncia*; Janečka *et al.* 2008] and European pine marten [*Martes martes*; Kubasiewicz *et al.* 2016]), and management

programs for invasive species (such as the oriental weatherloach [*Misgurnus anguillicaudatus*] in Australia [Hinlo *et al.* 2018], or giant constrictors [*Python*, *Boa* and *Eunectes* spp.] in the USA [Hunter *et al.* 2015]).

In this review, we provide an illustration of the latest genetic and genomic approaches to the analysis of scat samples in conservation, using our work on the Tasmanian devil (*Sarcophilus harrisii*) as a case study (Figure 1a). Throughout, we demonstrate some of the research questions that we are addressing using scat samples, as well as highlighting how the latest technologies are making this work possible. We provide insight into the pros and cons of various methods, including limitations of each approach. Finally, we demonstrate how this work can generate tangible benefits for the conservation of species like devils.

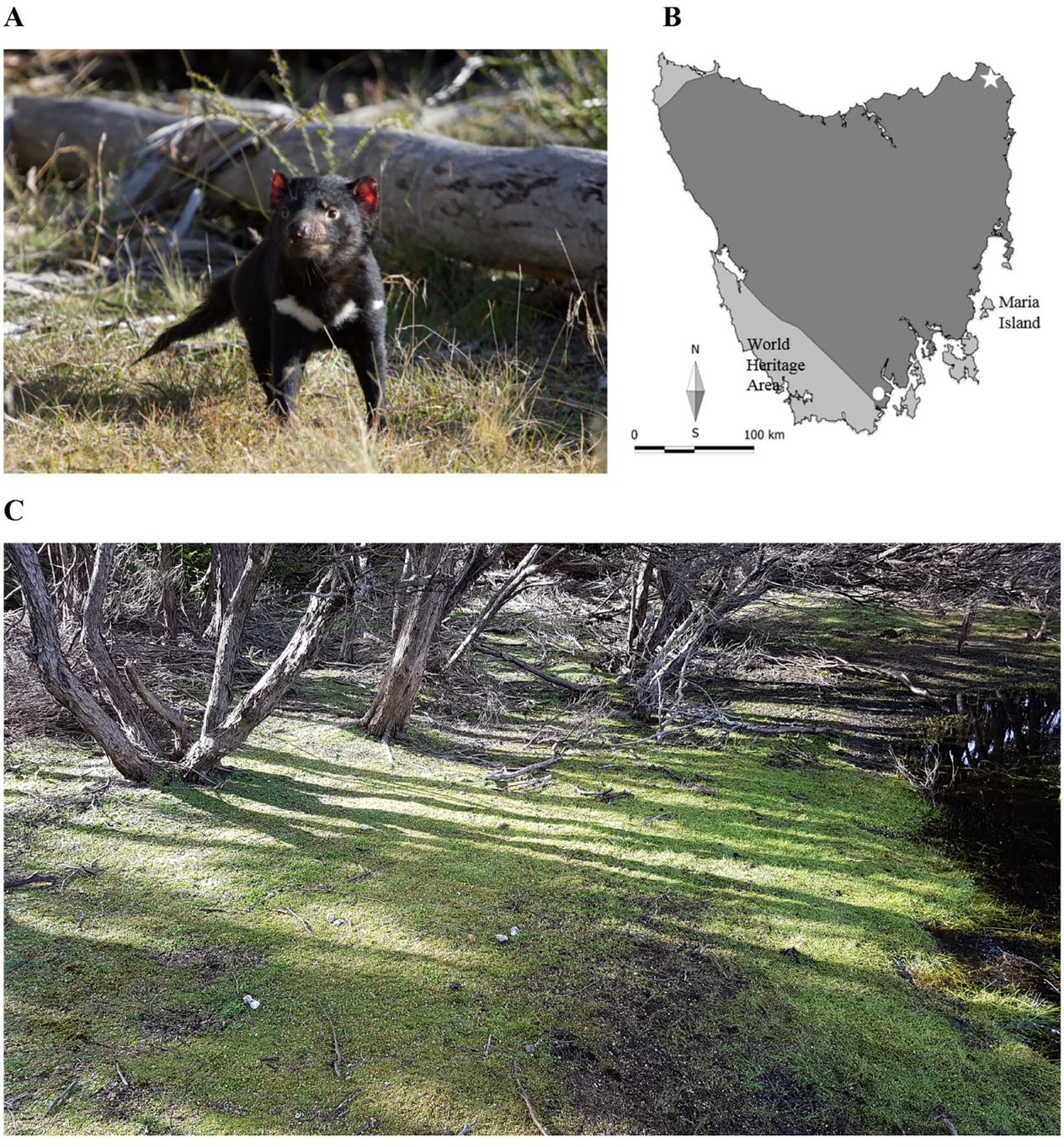

Figure 1: A) Tasmanian devil on Forestier Peninsula (photo credit: Save the Tasmanian Devil Program). B) The spread of DFT1 (dark grey) as of 2018, the light grey areas are currently disease free. The white star indicates where DFT1 was first observed and the white circle where DFT2 was first observed. C) devil latrine site in SW Tasmania; at least 11 devil scats are visible in this photo (photo credit: C. Hogg).

The Tasmanian devil

Our study species, the Tasmanian devil, is the world's largest extant carnivorous marsupial and endemic to the island state of Tasmania. The species is currently listed as Endangered due to devil facial tumour disease (DFTD) (Hawkins *et al.* 2008). DFTD is a recently emerged contagious cancer with a high fatality rate (Pearse and Swift 2006); the species has declined approximately 80% across its range since the disease emergence (Lazenby *et al.* 2018). There are two forms of DFTD, DFT1 was first observed in the north-east of the state in 1996 (Hawkins *et al.* 2006; Loh *et al.* 2006), and DFT2 was first observed in the south-east of the state in 2012 (Pye *et al.* 2016) (Figure 1b). DFT1 has since spread throughout devil populations towards the south and west and is now detectable across most of Tasmania (Figure 1b).

Tasmanian devils are roughly the size and shape of a small dog; adult males weigh 8-14 kg and females 5-9 kg (Rose *et al.* 2017). The species has evolved a body shape that allows them to scavenge, hunt, crunch bones and travel long distances (Owen and Pemberton 2005). In addition, although devils are generally considered a solitary species, they do interact around carcasses and with their neighbours via latrines (Figure 1c). Latrines are a specific area, often on a track or where tracks merge, where devils leave their scats, i.e. 'their calling cards' (Owen and Pemberton 2005). Some latrines have been documented as persisting for over 30 years (D. Pemberton, *pers. comm.*), showing their value to devils.

After the extinction of the thylacine (*Thylacinus cynocephalus*), devils are now the top carnivore in the Tasmanian ecosystem. Their population decline due to DFTD has the potential to cause substantial ecological consequences for the native environment (Hollings *et al.* 2014). In 2006, the Save the Tasmanian Devil Program (STDP) established an "insurance population" for the devil, housing a DFTD-free population of devils in zoos and wildlife parks across Tasmania and mainland Australia (CBSG 2008). The goal of the insurance population has been to preserve 95% of the wild-sourced devil genetic diversity for 50 years in captivity (Hogg *et al.* 2015; Hogg *et al.* 2017b). To maintain the integrity of the insurance population, only DFTD-free animals were brought in to the program; when the population was established, this meant primarily capturing devils from the west of Tasmania, ahead of the disease front (Hogg *et al.* 2015). In more recent years, as we have learnt more about DFTD, devils have been brought in from DFTD-affected locations by following strict quarantine procedures (Hogg *et al.* 2017b). Now, the STDP is using the insurance population for releasing devils back to Tasmania, to support dwindling wild populations (Fox and Seddon 2019).

Using scat for population genetics

Managing threatened species in the wild often requires knowledge of the magnitude and distribution of genetic diversity among populations. The information is used to generate conservation priorities, such as identifying which populations to direct specific conservation actions towards (such as threat abatement), and planning translocations (Segelbacher *et al.* 2010). Over the last two decades, scats have become an increasingly popular source material for monitoring genetic diversity and to support conservation planning (Taberlet *et al.* 1999; Piggott and Taylor 2003; Rodgers and Janečka 2013). Methodological research to optimize scat storage and DNA extraction techniques across multiple taxa are well documented and highlight the growing popularity in using non-invasive sampling for research (e.g. Panasci *et al.* 2011; Pearson *et al.* 2015; Woodruff *et al.* 2015). In conservation planning, DNA from scats has been used to address key questions at the between- and within-population levels. For example, in the koala (*Phascolarctos cinereus*) genetic diversity across some populations has been homogenized and reduced by translocation (Wedrowicz *et al.* 2018). Using scats, the genetic profile of a remnant population of koala was examined in order to place this population into the broader species context (Wedrowicz *et al.* 2018). The large sample size obtained in that study, and thus the population genetic results, could only have been achieved with non-invasive sampling, given the arboreal and cryptic nature of the koala. Within populations, non-invasive genetic sampling using scats can allow researchers to infer relationships among individuals (e.g. wolf, *Canis lupus*; Stansbury *et al.* 2016). Generating population genetic data at this level can provide information regarding inbreeding and inbreeding depression in wild populations.

For devils, one intriguing site of a potentially important devil population is the remote and mountainous south-west region of Tasmania, forming part of the Tasmanian Wilderness World Heritage Area (Figure 1b). Due to the landscape features, it is possible that devils in the south-west are largely isolated from other populations. We conducted a study to determine whether devil DNA could be obtained from scats collected from the environment, and whether those scats collected from south-west Tasmania could provide insight into the genetic diversity of the devils there. Scat analysis was identified as a useful approach, because of the logistical difficulties of trapping devils in the remote south-west, to obtain tissue samples.

A preliminary assessment of devil diversity at the site was obtained by processing seven scat samples that were opportunistically collected from a hiking trail in south-west Tasmania and sent to our group at the University of Sydney for population genetic analysis. One challenge of DNA analysis from environmental samples is the propensity for the DNA to degrade quickly, leading to high risk of errors in the analysis; multiple technical repeats for each sample must therefore be conducted in the laboratory (Taberlet *et al.* 1999; Waits and Paetkau 2005). In our study, DNA was extracted from each sample in duplicate, and multiple PCRs undertaken for each extraction. We also accounted for the possibility that the scat may have been misassigned

to species (i.e. the scats were not from devils), by using a positive control sample from an Eastern quoll (*Dasyurus viverrinus*, in the same family as devils, Dasyuridae) and a feral cat (*Felis catus*), both of which are present in Tasmania (Ramsey *et al.* 2018). Each sample was genotyped at a minimum of 15 microsatellite markers (following Jones *et al.* 2003; Gooley *et al.* 2017), ten of which were species-specific for the Tasmanian devil, having been designed using the Tasmanian devil genome (Gooley *et al.* 2017). Our preliminary findings confirmed that (1) amplification of markers designed using the devil genome can be used as a species identification method, as these markers failed to amplify with quoll and cat samples (2) devils are present in the south-west of Tasmania, and (3) the south-west population showed evidence of novel genetic diversity.

From a devil management perspective, the south-west population could be used in future as a source for founder intakes into the insurance population, providing a source of additional genetic diversity; a result obtained as a result of genotyping host DNA from scat samples. Going forward, creating a reliable methodology for obtaining genotypes from scat could reduce the financial and time costs of monitoring trips for the Tasmanian devil by reducing the need to trap and sample directly from devils. This preliminary data also helped support the need for a much larger survey effort to trap devils in the south-west (see Carlyon 2018).

More broadly, the biggest challenge facing population genetic analysis with scat or other non-invasive samples is the low quality and low concentration of host DNA acquired from the specimen (Broquet *et al.* 2007). Practical solutions include performing a preliminary pilot study to determine the genotyping error rate, optimal storage technique for samples, optimal timeframe to collect samples to prevent DNA degradation, and optimization of extraction techniques and amplification methods. These studies can be conducted in a variety of ways: Schultz *et al.* (2018) collected scats from captive koalas, aged them for different periods of time, and compared genotyping results to data obtained via invasive methods (blood samples). Similarly, Woodruff *et al.* (2015) showed that microsatellite amplification was most successful in samples from the Sonoran pronghorn (*Antilocapra americana sonoriensis*) that are collected within 7 days of defecation. In the coyote (*Canis latrans*), Panasci *et al.* (2011) found that preserving scat samples in ethanol allowed for greater microsatellite amplification compared to lysis buffer. Identifying sensitivities in the sample-to-data pipeline is essential to realistic study design planning and appropriate interpretation of findings.

Using scats for dietary analysis

Ecologists, conservationists and wildlife managers use dietary studies to understand the impacts of predators on other species and the roles of animals within their ecosystems (e.g. Lyngdoh *et al.* 2014). Keystone or top-order carnivores have broad direct and indirect influences on the structure and stability of an ecosystem, as well as its trophic regulation (Ritchie and Johnson 2009; Estes *et al.* 2011). For example, leopard (*Prionailurus bengalansis*) and Asiatic golden cats (*Catopuma temminckii*) in Southwestern China (Xiong *et al.* 2017) predate heavily on pikas (*Ochotoma spp.*), which are known to degrade forests and grasslands at high densities (Ma 2015). Pikas also perform useful ecological functions of seed dispersal and increasing plant diversity at low densities (Paine and Beck 2007), so to maintain ecosystem functions, top-order carnivores, such as the leopard and Asiatic golden cats, that keep lower trophic species at sustainable levels, must be preserved. Understanding the interactions between species is important information that can help inform conservation programmes.

Traditionally, a dietary analysis involves the identification of hard parts and indigestible material, such as hair or bones, in both stomach contents and scats (Tollit *et al.* 1997; Figure 2). However, the ability to taxonomically assign indigestible material requires a high level of training

Figure 2: Representative Tasmanian devil scats. A) Typical scats often contain vertebrate prey remains (fur, feathers, pieces of bone). B) Some scats show clear evidence of a devil feasting on a particular type of prey, such as the grubs visible in this scat. C) Devils can be fairly indiscriminate in their diet, this devil chomped on an unfortunate pair of hiking boots, as seen by the shoelaces in the scat! (photo credits: A & C, A. Lee; B, C. Hogg, respectively).

and skill, and prey items can rarely be identified to species level (Pompanon *et al.* 2012). Morphological or traditional methods cannot therefore provide enough taxonomic resolution to gain a complete picture of the ecological impact of a predator (Barba *et al.* 2014).

Advances in molecular genetics have revolutionised dietary studies from scat by enabling genetic identification of all prey items, including soft-bodied species and unidentifiable hard parts. This technique, referred to as "metabarcoding", involves sequencing a mixture of DNA fragments, such as those from prey species in a scat, by targeting specific genes. The genes that are used have sequences that are sufficiently conserved across species that they can be successfully picked out of the DNA mix using universal primers, but variable enough so that species can be differentiated (e.g. 12Sv5 [Shehzad *et al.* 2012; Xiong *et al.* 2017]). These new technologies enable the identification of thousands of DNA fragments corresponding to multiple species from numerous faecal samples in a single processing run (Valentini *et al.* 2009), which is both cost effective and efficient. For example, a total of 63 prey items were detected in 270 samples from the Australian fur seal (*Arctocephalus pusillus*) including 4 cartilaginous fish species that were not detected using traditional morphological techniques (Deagle *et al.* 2009).

An important drawback to the metabarcoding technique is the availability of published sequences against which to compare the results. If a species has not been sequenced at the gene being targeted, it cannot be identified within the scat. In cases where the sequence for a given prey species is not available in public databases, new computational tools can attempt to assign the DNA sequence to a higher taxonomic level, such as family (Boyer *et al.* 2016). Known ecological information about the study site can then be incorporated to narrow down the sequence assignment. For example, a database of locally occurring species was used to refine a contested sequence from two possible genera to one for the diet of the critically endangered golden-crowned sifaka (*Propithecus tattersalli*) (Quéméré *et al.* 2013). Another important caveat is the risk of contamination during sampling, DNA extraction and analysis with a sensitive technique such as metabarcoding. When collecting scats for dietary analysis, avoiding contamination in the field can be a challenge as any material the scat comes into contact with has the potential to be amplified using next-generation sequencing (Goldberg *et al.* 2016). To minimise the risk of contamination, all field equipment should be sterilised between each collection, fresh sterile gloves must be worn with each new sample and all samples should be stored in individual sealed containers (Goldberg *et al.* 2016). Protection against contamination doesn't end with sample collection. Dietary studies using metabarcoding should follow previously established protocols in the laboratory as with other low-quality and low-quantity DNA samples such as ancient DNA and non-invasive host genetic sampling (Goldberg *et al.* 2016). A dedicated clean laboratory is the ideal solution to minimising contamination in metabarcoding samples. These laboratories have strict one-way flow rules pertaining to all equipment, samples and personnel. In short, all equipment such as centrifuges, pipettes and lab coats must not leave the clean lab space once sterilised, no other DNA extractions or PCRs, the products of which float freely in the air, are to be performed in this space and no personnel are permitted to enter the clean laboratory without a shower and fresh clothes if they have been into any other laboratories on the same day (Goldberg *et al.* 2016). When following the strict anti-contamination protocols described above, little to no contamination was found from laboratory processes in a diet study of 357 scat samples from 16 bat species (Galan *et al.* 2018).

We used metabarcoding to examine the diet of devils, as the top-order carnivore in Tasmania. One component of devil conservation strategy was to introduce a DFTD-free population of devils to Maria Island, Tasmania (Figure 1b) (Thalmann *et al.* 2016; Wise *et al.* 2016). The Maria Island devil population has grown (McLennan *et al.* 2018), and is now used as a source for supplementing wild sites where devils have declined. However, Maria Island is a national park and home to many other protected species. As devils are not endemic to the site, ongoing monitoring is taking place to determine whether devils are having a negative impact on the other species on the island (STDP *pers comm*). On Maria Island, Tasmanian devils have been observed preying on vulnerable bird species, such as the short-tailed shearwater (*Ardenna tenuirostris*) and the little penguin (*Eudyptula minor*). Previous scat analyses have used morphological techniques to identify some species such as macropods (STDP *pers. comm.*) but items such as feathers and egg-shell could not be taxonomically assigned to the species level. As the devil is a generalist and opportunistic carnivore, a broad spectrum of the diet, namely soft-tissue species, may be being overlooked using this method (Tollit *et al.* 1997). To develop management plans for vulnerable species on the island it is important to fully characterise the devil diet. In collaboration with the STDP, our research group is currently undertaking metabarcoding analysis of devil diet via scats on Maria Island.

Scats have been collected from across the island, including localities that are not routinely trapped due to the logistical challenges of placing traps in those areas. Every effort to avoid degradation and contamination of these samples was undertaken including freezing samples same day as collection, disinfecting all field equipment coming into contact with the samples and performing DNA extractions in a dedicated laboratory. A dedicated clean lab at the University of Sydney was established by repurposing a lab where no DNA work had been previously been undertaken. Laboratory surfaces were fully cleaned using a 50% bleach solution as this is known to destroy nucleic acids at this concentration (Champlot *et al.* 2010). A portable UV lamp was used to sterilise all equipment, fume hoods and bench surfaces. To ensure

no outside material contaminated the laboratory, pipettes and other equipment required for the metabarcoding work were purchased new, all windows were sealed with plastic sheeting, and the one-way flow procedure described above was employed. Using both a bleach solution and the portable UV lamp, all equipment and surfaces were sterilised between processing of each sample. The creation of a dedicated laboratory enabled us to perform a pilot study using 12 Tasmanian devil scats from Maria Island using a 12Sv5 metabarcoding technique. Although this work is still underway, preliminary data support previous observations that devils are opportunistic generalists, and that a wide variety of species contribute to their diet (EM, unpubl. data). We are now sequencing a much larger sample of Maria Island devil scats to generate a holistic overview of the impact of devils on other species in the national park.

Understanding population health using scat microbiome sequencing

The animal gut is home to an enormous diversity of microbes, including bacteria, viruses, fungi and archaea, collectively called the gut microbiome (Cho and Blaser 2012). Recent advances in sequencing technology have enabled us to study the gut microbiome in great detail, revealing new genera and species of non-culturable organisms and unravelling complex host-microbe relationships and their functional importance to host health and wellbeing (Kinross *et al.* 2011). Increasing numbers of studies continue to shed light on the gut microbiome's contribution to host nutritional status (Kau *et al.* 2011), immunity (Round and Mazmanian 2009), physiological development (Sommer and Bäckhed 2013) and even behaviour (Heijtz *et al.* 2011). Perturbations to the microbiome, known as dysbiosis, can lead to a range of health problems including increased susceptibility to infection, obesity, and inflammatory bowel disease (Turnbaugh *et al.* 2006; Kamada *et al.* 2013). Given its importance to host health, there is growing interest in studying the gut microbiome in wildlife species as a tool to understand species biology (for example the role of the gut microbiome in host health), the co-evolutionary relationships between host and microbes, and to support conservation. For example, a number of studies have compared the microbiome of captive animals and their wild counterparts, providing an insight into how intensive management practices, such as captive rearing, may alter the microbiome of threatened species (Nelson *et al.* 2013; Clayton *et al.* 2016; McKenzie *et al.* 2017). In many cases, species living in captive environments exhibit microbiomes that lack diversity or are highly dissimilar to what is found in the wild, providing evidence of dysbiosis (Kohl *et al.* 2014; Cheng *et al.* 2015).

Our study species, devils, also shows microbiome perturbations or dysbiosis in captivity (Cheng *et al.* 2015). The devil insurance population is a large captive management program that houses devils in a range of enclosures, including intensive sites (typical zoo facilities) and a variety of free-range sites (i.e. larger enclosures with multiple males and females housed together) (Hogg *et al.* 2017b). The gut microbiome of devils living in the wild versus captivity (intensive captive and free-range enclosures) was first characterized in 2015 using 16S rRNA amplicon sequencing (Cheng *et al.* 2015). As seen in other host species (Kohl *et al.* 2014), the gut microbiomes of captive devils were highly dissimilar to those of wild devils and had significantly lower microbial diversity (Cheng *et al.* 2015). Of captive devils, those living in free-range enclosures had a microbiome more similar to wild devils, suggesting that this method of housing is preferable from the perspective of managing devils and their microbiome in captive settings (Cheng *et al.* 2015).

Microbiome perturbations in captivity may affect host physiological functions and fitness, especially if depleted of beneficial microbes. For instance, captive black howler monkeys (*Alouatta pigra*) lack butyrate producing bacteria (e.g. *Butyrivibrio* spp.) in their gut microbiome (Amato *et al.* 2013), which are thought to provide energy for mammalian colon cells and have various other health implications (Donohoe *et al.* 2011). As such, microbial perturbations could potentially lead to reduced reintroduction success if captive individuals released into the wild suffer compromised health (Redford *et al.* 2012; Bahrndorff *et al.* 2016). In the grouse (*Tetrao urogallus*), anatomical alternations in the gastrointestinal tract (e.g. shorter small intestines and caeca) (Liukkonen-Anttila *et al.* 2000) and microbiome disturbances observed in individuals living in captivity (Wienemann *et al.* 2011) are thought to be responsible for poorer digestion and nutrient absorption. This may in turn explain the high mortality of captive birds upon reintroduction to the wild (Seiler *et al.* 2000).

For rare or endangered species, such as the devil, faecal sampling is an attractive entry-point for studies of the gut microbiome, as it is non-invasive, convenient and allows for repeated sampling over time (Ingala *et al.* 2018). In practice however, the study of gut microbiomes in wildlife species is often constrained by sample collection in the field (Menke *et al.* 2015; Hird 2017). Challenges faced by those wanting to study the gut microbiome of wildlife species include the need to obtain fresh faecal samples and store them under optimal conditions to minimise degradation and ensure accurate microbiome analysis (Menke *et al.* 2015; Ingala *et al.* 2018). For the devil, faecal samples are typically collected from animals shortly after defecation during routine health checks, or from the traps in which they were captured. This ensures only fresh faecal samples are collected. Rectal or cloacal swabs are an alternative for optimal sample freshness and have been utilised in other species (Waite *et al.* 2012; Budding *et al.* 2014; Alfano *et al.* 2015). Freezing of faecal samples intended for microbiome analysis in liquid nitrogen or a portable -80°C freezer immediately after collection minimises sample degradation in the field, although doing so in remote areas presents a significant logistical challenge.

Since 2015, the STDP has been releasing devils into wild sites across Tasmania to supplement declining natural populations (Fox and Seddon 2019). The initial findings of dysbiosis in captive devils has since prompted our recent research investigating the effects of translocation on the gut microbiome of release devil, and more specifically, to determine whether captive release devils can reacquire a "wild-type" microbiome upon reintroduction to the wild. Preliminary results suggest that the devil gut microbiome is not static, as we observed significant compositional and diversity changes over the course of translocation (RC *unpubl. data*). Importantly, the microbiome of released devils showed a shift towards the microbiome of incumbent devils within the first six months after translocation, indicating that microbiome perturbations from captivity are not necessarily permanent (Chong *et al.* 2019a). The health consequences of microbiome perturbations in devils are still largely unknown, but research shows that, in this species at least, reacquisition of wild-type microbiome can occur within a short period of time.

The examples described above primarily represent studies of the bacterial microbiome, but the gut is also home to a complex community of viruses. Beyond being the causative agents of diseases, emerging views suggest that the abundance of viruses inhabiting the host gut (the gut/faecal virome) may play a larger role in host health than previously thought (Cadwell 2015). For example, resident viruses known as bacteriophages may influence the composition and functions of the bacterial microbiome, thereby affecting host health (Duerkop *et al.* 2012; De Paepe *et al.* 2014). In addition, in-depth characterisation of the host-associated virome is important for virus discovery and may contribute to the health management and conservation of threatened wildlife species. In a recent study, gut virome characterisation of wild and domestic canids using metagenomics identified viruses of potential conservation relevance, including a novel bocavirus species (Conceição-Neto *et al.* 2017). These results made significant contributions to the current knowledge on canid virology and will aid conservation through better screening processes. Previously, virome studies have been hindered by a lack of standardised protocols. Unlike bacteria, for which the 16s rRNA gene is conserved across species and readily used for species identification, viruses are highly divergent and lack universally conserved genes. Many viruses are also unculturable. The recent advances in sequencing technology have made the characterisation of complex viral communities feasible through the use of shotgun sequencing methods (Edwards and Rohwer 2005).

The faecal virome of the devil was characterised for the first time using an innovative combined metagenomics and meta-transcriptomics approach (Chong *et al.* 2019b). As a result, 24 novel, marsupial-associated viruses were discovered, including some from families of important pathogens including bocaviruses, papillomaviruses and astroviruses. Known mammalian pathogens such as rabbit haemorrhagic disease virus 2 (RHDV2) were also detected in both captive and wild devils, though whether they were simply ingested in prey or replicating in the gut of devils remains unresolved. Similar to the gut bacterial microbiome, the faecal virome of captive devils showed significantly less diversity compared to the virome of wild devils (Chong *et al.* 2019b). The characterisation of the devil faecal virome has significantly improved our knowledge of viruses found within this endangered species. Understanding which viruses infect devils, and future studies into their pathogenic and zoonotic potential, will aid in assessing devil health and the risk of disease emergence.

Applications of scat DNA analysis for devil conservation

There has been much discussion in the literature recently around the research-implementation gap (for recent examples from the Australasian region, see Ottewell *et al.* 2016; Hogg *et al.* 2017a; Taylor *et al.* 2017). For devils, the inception of the 'Tools & Tech' project (Hogg *et al.* 2017a) has facilitated a focus on integrating research into real-time conservation management. All three of the major research themes described in this review contribute to the 'Tools & Tech' adaptive management framework (Hogg *et al.* 2017a), in which researchers and conservation managers work closely together to generate novel conservation insights and provide tangible management recommendations.

It is well recognised that the Tasmanian devil insurance metapopulation was founded on individuals from a small number of locations in Tasmania and that some of these individuals were related (Hogg *et al.* 2015; Hogg *et al.* 2018). To maintain genetic diversity within the insurance metapopulation and ensure that it is representative of wild populations, remote regions of the devil's range need to be surveyed to determine whether new alleles are present. The work undertaken in the south-west of Tasmania showed that scat collection is a useful tool for assessing such remote areas, to ascertain whether resource intensive trapping efforts should be undertaken.

Islands are commonly used as refuges for species under threat (Ostendorf *et al.* 2016), although most target species are birds or small mammals, e.g. New Zealand saddlebacks (*Philesturnus carunculatus*; Parker and Laurence 2008), hihi (*Notiomystis cincta*; Ewen *et al.* 2011) and mala (*Lagorchestes hirsutus*; Langford and Burbidge 2001). However, introducing a carnivore to an island that had no carnivores previously was always going to cause changes within the ecosystem (Wise *et al.* 2019). As a national park, Maria Island is home to a suite of fauna both endemic to the island (e.g. short-beaked echidna [*Tachyglossus aceleatus*] and little penguin [*Eudyptula minor*]) as well as introduced species (e.g. Cape Barren goose [*Cereopsis novaehollandiae*]). Tasmanian devils were not resident on Maria Island prior

to their introduction in 2012 (Thalmann *et al.* 2016), and there were no other predators at this time except for feral cats (DPIPWE 2012). Solely basing management decisions on traditional scat analysis only provides prey information on species who have hard parts, or fur, in the scat. Metabarcoding provides a more comprehensive method of diet analysis, as noted in brown bears (*Ursus arctos*; De Barba *et al.* 2014) and Australian fur seals (Deagle *et al.* 2009), and so will be invaluable for the management of devils on Maria Island. Devils on Maria Island are an essential part of the Tasmanian devil insurance metapopulation (Hogg *et al.* 2017b) and are a source population for translocations to mainland Tasmania (Fox and Seddon 2019; Grueber *et al.* 2019). Understanding the impact that devils are having on the island ecosystem will support the long-term management of devils, and other species, on Maria island.

One of the key objectives for the Tasmanian devil insurance metapopulation is to ensure that not only the genetic diversity of devils is maintained, but also the diversity of their commensal biota (Lees and Andrew 2012). An animal's microbiome plays an integral role in health (Kinross *et al.* 2011), immune function (Kau *et al.* 2011) and even behaviour (Cryan and Dinan 2012), and the long-term consequences of a changing microbiome to translocated individuals is unknown. As described above, our initial study into the differences between wild and captive microbiomes (Cheng *et al.* 2015) generated the impetus for our follow-up project examining changes in gut microbiome during reintroduction and translocation (Chong *et al.* 2019a). By working hand-in-hand with our conservation partners at the STDP, this research is informing future management practices in respect to translocations. Furthermore, use of scat sampling coupled with modern sequencing technology has enabled us to study the devil gut virome in great detail for the first time, leading to the discovery of many never-before-seen viruses in this endangered species.

Conclusion

Emerging molecular genetics technologies are enabling researchers to learn more about threatened species through creative use of environmental DNA and novel sampling techniques. We have provided an overview of diverse projects utilising genetic analysis of devil scats, to learn more about this endangered carnivore, its ecological impact, and its health, to improve the conservation of the species. The results are already having a positive impact on devil conservation, and we look forward to further benefits as new results emerge.

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Ethics statement

Scat samples used herein were collected opportunistically (i.e. without handling animals), or by the Save the Tasmanian Devil Program staff during their conservation operations, under their standard operating procedures.

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