Time to adjust: changes in the diet of a reintroduced marsupial after release

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Abstract An important component of reintroduction is acclimatization to the release site. Movement parameters and breeding are common metrics used to infer the end of the acclimatization period, but the time taken to locate preferred food items is another important measure. We studied the diet of a reintroduced population of brushtail possums *Trichosurus vulpecula* in semi-arid South Australia over a 12 month period, investigating changes over time as well as the general diet. We used next-generation DNA sequencing to determine the contents of 253 scat samples, after creating a local plant reference library. Vegetation surveys were conducted monthly to account for availability. Dietary diversity and richness decreased significantly with time since release after availability was accounted for. We used Jacob's Index to assess selectivity; just 13.4% of available plant genera were significantly preferred overall, relative to availability. The mean proportion of preferred plant genera contained within individual samples increased significantly with time since release, but the frequency of occurrence of preferred plants did not. Five genera (*Eucalyptus*, *Petalostylis*, *Maireana*, *Zygophyllum* and *Callitris*) were present in more than half of samples. There was no difference in dietary preferences between sexes (Pianka overlap = 0.73). Our results suggest that acclimatization periods may be longer than those estimated via reproduction, changes in mass and movement parameters, but that under suitable conditions a changeable diet should not negatively affect reintroduction outcomes. Reintroduction projects should aim to extend post-release monitoring beyond the dietary acclimatization period and, for dry climates, diet should be monitored through a drought period.

Keywords Acclimatization, Australia, brushtail possum, diet, marsupial, reintroduction, translocation, *Trichosurus vulpecula*

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Introduction

Acclimatization is defined as both physical and behavioural responses to changes in environmental factors or conditions. In a reintroduction context, acclimatization can be defined as the period of time that released animals take to exhibit normal physiological processes or condition and normal behaviour, and may include producing young, maintaining or increasing body mass, establishing normal activity and movement patterns (often a stable home range), sheltering in suitable locations, locating conspecifics and consuming a typical diet (Armstrong et al., 2017; Stadtmann & Seddon, 2018). If many individuals fail to do so, the reintroduction will not succeed. Typically, the acclimatization of released animals is measured by monitoring changes in body mass, post-release movement, reproductive status and the cause of death for released animals that have died (Hardman & Moro, 2006; Hamilton et al., 2010; Short & Hide, 2015). Starvation or malnutrition can occur when released animals fail to locate food resources soon after release (Islam et al., 2008; Jule et al., 2008); thus, post-release diet is an important, yet often overlooked, component of reintroduction biology. In addition to short-term survival, the availability of stable or seasonally-reliable food resources is critical for reproduction and long-term persistence (Nolet et al., 2005; Moorhouse et al., 2009; Carlson et al., 2014).

The post-release acclimatization period depends on a species' dietary breadth and movement patterns, and could take days, weeks or months. For example, European mink took c. 1 month after release to shift from an atypical to a typical diet (Põdra et al., 2013), and Gilbert's potoroos *Potorous gilbertii* increased the number of truffles in their diet with time since release, presumably as they were able to locate new food sources (Bougher & Friend, 2009). The acclimatization period is often unknown a priori and sometimes assigned arbitrarily as a fixed period (Armstrong et al., 2017). If post-release effects are not accounted for, the perceived likelihood of reintroduction failure may be much higher (Panfylova et al., 2016). Some post-release diet studies have assessed diet at various time points after release, rather than monitoring changes over time. For example, 15

months after release, the diet of captive-bred houbara bustards *Chlamydotis undulata* was the same as their wild-born counterparts (Bourass & Hingrat, 2015), but the time taken for the birds to adjust their diets was unknown. Many practitioners provide supplementary food or water during the acclimatization period in an attempt to ease the transition, but the usefulness of this has been debated (Rickett et al., 2013; Moseby et al., 2014). Understanding the diet of a reintroduced species and how it changes over time is useful in assessing whether dietary components are available in sufficient quantities for long-term population persistence. Dietary studies can contribute to both understanding the ecology of a species and improving reintroduction success of future projects.

We investigated the diet of a reintroduced population of brushtail possums *Trichosurus vulpecula* in semi-arid South Australia over a 12 month period following reintroduction. The brushtail possum is a widely distributed Australian marsupial, but has disappeared from > 50% of its historical range since European settlement, with declines most pronounced in the arid zone (Kerle, 1984; Kerle et al., 1992). Predominantly arboreal, brushtail possums usually forage in the canopy, consuming plants such as *Eucalyptus*, *Acacia*, *Agonis* and *Santalum*, but sometimes forage on the ground, consuming grasses, herbs and fungi (Fitzgerald, 1984; Kerle, 1984; Evans, 1992; How & Hillcox, 2000). Dominant plant species in their diet vary with location and environment. brushtail possums occasionally consume invertebrates and birds' eggs (Foulkes, 2001; Cruz et al., 2012). In the arid zone, these possums prefer plants that are high in moisture, nutrients and dry matter digestibility, with low levels of toxins (Foulkes, 2001). The species was introduced to New Zealand, where its diet has since been well studied (e.g. Brown et al., 1993; Owen & Norton, 1995), but because of differences in habitat as well as a differing niche we only discuss our findings in the context of the species' native distribution.

Using DNA sequencing to identify dietary components, we investigated whether brushtail possum diet changed with time since release, taking into account variation in food availability assessed using monthly vegetation surveys. We hypothesized that after accounting for availability, the diet would initially contain a high diversity and richness of food items as possums explored their new environment, but that this would decrease over time as preferred foods were located, thus increasing selectivity. The diet should stabilize, relative to availability, once possums have acclimatized to the release site. We investigated whether possums favoured certain plant height classes, which can relate to predation risk, and evaluated sex differences in the diet. We also investigated any effect of rainfall, given the influence it can have on arid zone systems.

Study area

Brushtail possums were reintroduced to c. 20% of the 93,400 ha Ikara-Flinders Ranges National Park (Fig. 1) in semi-arid South Australia in June 2015 (Bannister et al., 2020, Moseby et al., 2020). The species became regionally extinct in the 1940s (Kerle et al., 1992). Prior to being declared a National Park in 1970, significant land degradation occurred as a result of overgrazing by domestic stock (from the 1850s) and introduced herbivores (Mincham, 1996; Robinson, 2012). The Bounceback Project was initiated in 1992 and involved the removal of introduced foxes *Vulpes vulpes*, goats *Capra hircus* and rabbits *Oryctolagus cuniculus* (Alexander et al., 1997; Robinson, 2012), but goats and rabbits persist (Smith, 1996; Brandle et al., 2018) along with overabundant native macropods (*Macropus rufus*, *Macropus robustus* and *Macropus fuliginosus*), arresting the regeneration and recruitment of many plants. Vegetation types consist of open woodlands (*Callitris glaucophylla* interspersed with *Eucalyptus intertexta*), or *Eucalyptus camaldulensis* floodouts (where floodwater infrequently overflows), *E. camaldulensis* creeklines, mallee (*Eucalyptus* spp.), shrubland and grassland.

Methods

We studied the brushtail possums' diet over 12 months (August 2015–July 2016), commencing 1 month after animals had been released, to avoid monitoring during a post-release period of containment and supplementary feeding for some animals. Scats were collected from trapped possums (baited with peanut butter and rolled oats or apple and peanut butter), with an average of 44 ± SE 3 days between collections from the same individual. We recorded the location of collected scats using a GPS (Fig. 1) and documented the identity, sex, age, body mass, body condition and reproductive status of each possum. Because possums use shelter sites, we collected scats from some sites multiple times, from either the same or different individuals. Samples were temporarily stored at −4 °C after collection and were then transferred to a −20 °C freezer where they were stored until being analysed. Unless specified otherwise, methods and results refer to the consumption of plants only; the consumption of birds and invertebrates is discussed separately.

To determine the environmental availability of plant genera, we conducted monthly vegetation surveys. Surveys were conducted within the two habitat types used by possums for shelter: creekline and woodland (including floodouts). Each survey involved recording the presence and a visual estimate of per cent cover for each plant species within a 50 m radius of a shelter tree, usually also a trap site. When identification was not possible in the field a photo and sample were taken for subsequent identification.
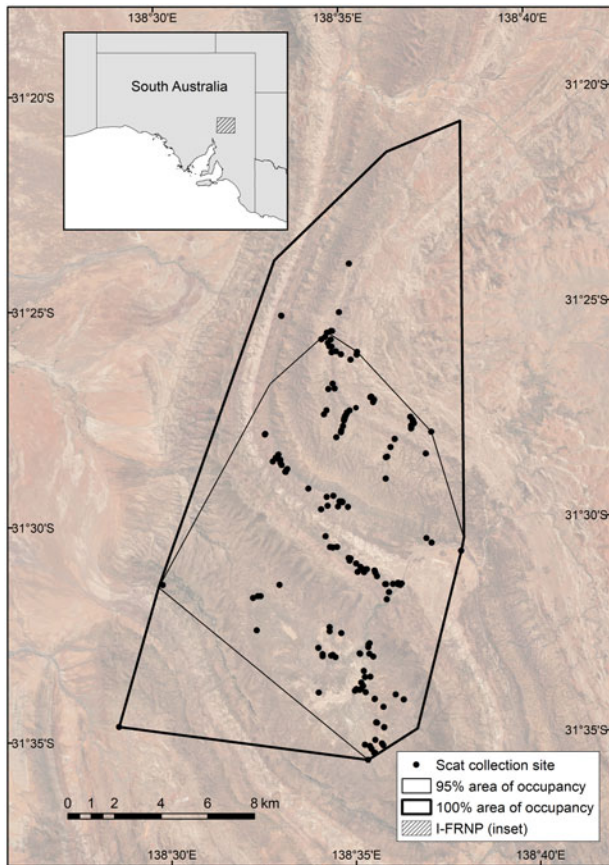
FIG. 1 Sites where brushtail possum *Trichosurus vulpecula* scats were collected within Ikara-Flinders Ranges National Park (I-FRNP), the known area of occupancy (95 and 100% minimum convex polygons) for possums during the study period, and the location of the National Park within South Australia.

Plant samples were also collected for a DNA reference library. The availability of perennial species was averaged across the year as differences between months were probably a result of the varied locations of survey sites. The per cent of canopy that was new growth was not accounted for. The availability of annuals was calculated monthly. Where there was any doubt about the life history of a species, it was considered annual. Because the vegetation survey method used resulted in recording eucalypts (used as shelter sites) in all surveys, the availability of eucalypts was assessed using transect data from another study conducted simultaneously (Moseby et al., 2020); the proportion of belt transect segments (100 m long, 20 m wide, location randomly selected, stratified by habitat type) containing eucalypts was calculated, based on 27.5 km worth of transects surveyed within possum habitat.

Local plant DNA-barcode reference library

We developed a plant DNA reference library for the study site, which included 165 plant species (Supplementary Table 1), as well as the bait used in traps (apple, peanut butter and oats). Leaf samples were freeze-dried for 2 days prior to analysis. The dual locus barcoding approach of Wilkinson et al. (2017) was used to develop the local reference library for the *rbcL* (Kress & Erickson, 2007) and *ndhJ* (Schmitz-Linneweber et al., 2001) barcodes and sequenced on the MiSeq platform (Illumina, San Diego, USA) at the University of Adelaide.

Scat analysis: plants

We used a modification of the two-step PCR strategy described by Bell (2011) for the amplification of *rbcL* and *ndhJ* barcodes from scat samples. Scats (2–3 per sample) were freeze-dried and homogenized with tungsten-carbide beads in a TissueLyser (Qiagen, Melbourne, Australia) and DNA was extracted using an ISOLATE II Plant DNA Kit (Bioline, London, UK) according to the manufacturer's instructions. The first amplification was performed in 20 µl reaction volume consisting of 1× MyFi Buffer (Bioline, Sydney, Australia), 0.2 nM of each forward and reverse primer, 1.6 U MyFi Polymerase (Bioline) and 20 ng of DNA. PCRs were performed on a RotorGene RG-6000 machine (Corbett Life Science, Melbourne, Australia) using the following thermocycling conditions: for *rbcL*, 95 °C for 1 minute followed by 35 cycles of 95 °C for 15 seconds, 55 °C for 15 seconds, 72 °C for 15 seconds; and for *ndhJ*, 95 °C for 1 minute followed by 35 cycles of 95 °C for 15 seconds, 50 °C for 15 seconds, 72 °C for 15 seconds. Amplification products were then purified using the Agencourt AMPure XP system (Beckman Coulter, Sydney, Australia) at a ratio of 0.8× beads to PCR product.

The second PCR was performed using Nextera 96 index adapter sequences (Illumina, San Diego, USA) to add identifying sequences to the amplification products from the first PCR. This was achieved by adding the following into a 12.5 µL reaction volume: 1× MyFi Buffer (Bioline), 1.6 U MyFi Polymerase (Bioline), 0.4 nM of paired Nextera 96 Index Sequences and 4 µL of purified PCR product. The amplification conditions consisted of 95 °C for 1 minute followed by 5 cycles of 95 °C for 5 seconds, 55 °C for 10 seconds and 72 °C for 10 seconds. Amplified products were then performed using the Agencourt AMPure XP PCR Purification beads at a ratio of 0.6× beads to PCR product and quantified by qPCR with reference to known PhiX standards (Illumina) using the SYBR FAST qPCR Kit (Kapa Biosystems, Wilmington, USA) on a RotorGene RG-6000 machine (Corbett Life Science, Melbourne, Australia).

The pooled library was then diluted and a 16 pM aliquot was paired-end sequenced on a MiSeq V3 sequencer, using a 600-cycle Version 3 kit (Illumina) according to the manufacturer's instructions. The MiSeq Bcl output files were demultiplexed and converted to fastq files using *MiSeq Reporter 2.6* (Illumina).

The *ndhJ* locus raw sequences were merged using *BBMerge Paired Read Merger 37.64* (Bushnell et al., 2017) and aligned to the local DNA reference database using a pairwise match of $>99\%$ in *Geneious 11.1.5* identified to genus level. Sequences $<99\%$ similar were identified to genus, but the confidence was lower. For the *rbcL* sequences, reads were trimmed and quality-filtered, and only reads of 300 bp with QF >30 were used for alignment to the *rbcL* reference database with a pairwise match of $>99\%$ (Bushnell et al., 2017).

Scat analysis: invertebrates and birds

We analysed possum scats for the presence of invertebrate and bird DNA using a similar approach as described for plant DNA, using invertebrate (Hebert et al., 2004a; Foottit et al., 2008) and bird (Hebert et al., 2004b) specific COI primers. Amplification products were assessed by visualization following electrophoresis on a 1% agarose gel and products sequenced on the Illumina MiSeq platform using a 600-cycle v3 kit (Illumina), as described previously.

Data analysis

We conducted data analysis using *R 3.5.0* (R Development Core Team, 2018). Genera making up $<1\%$ of reads within a sample were removed from analyses to limit the inclusion of material resulting from incidental ingestion or environmental contamination. Bait items were also omitted from results. The plant component of the diet of brushtail possums in the Ikara-Flinders Ranges National Park was investigated by calculating the frequency of occurrence in scats for each genus (the number of scats containing each genus), both monthly and pooled across the 12 months. We also calculated relative abundance of genera within samples by measuring the proportion of each genera contained within each sample (i.e. the number of reads for a particular genus, divided by the total number of reads for that sample). We calculated an average pairwise Pianka's niche overlap index to test for sex effects (Pianka, 1974).

To investigate the influence of time since release on diet, we calculated selectivity, frequency of occurrence in scats and proportions within scats monthly. Selectivity was calculated using Jacob's Index (Jacobs, 1974), which assesses plant preference or avoidance relative to environmental availability:

$$\text{Jacob's Index } (D) = \frac{r_i - p_i}{r_i + p_i - 2r_i p_i}$$

where r_i = proportion of genera i within the diet (frequency of occurrence within scats), and p_i = proportion that genus i is available (frequency of occurrence within the environment). Maximum preference is a Jacob's Index value of +1, and maximum avoidance is −1. Genera present in $<5\%$ of both scats and vegetation surveys were omitted from the selectivity analysis to avoid skewing selectivity results because of low detectability.

We created linear mixed-effects models using the package *lme4* (Bates et al., 2015) to investigate the frequency of occurrence of plants in scats, proportions within scats, dietary richness (i.e. number of genera) and dietary diversity (using Shannon's index of diversity) over time (Table 1). Model fit was assessed using Q-Q plots. A generalized linear model with a Poisson distribution was used to test whether the number of non-perennial genera available was related to rainfall (log-transformed), which could explain dietary changes.

The height of plants (<0.5 m, 0.5–2.5 m or >2.5 m) falling into each preference category (calculated overall rather than monthly) was calculated along with the overall frequency of occurrence in scats for plants in those height classes, to assess whether possums were selecting certain height classes.

Results

We collected a mean of $21.1 \pm$ SE 2.8 scat samples monthly during the study period, from a total of 55 adult possums (26 female, 29 male) of known identity, totalling 253 samples. A mean of $5.3 \pm$ SE 0.7 and $4.0 \pm$ SE 0.6 samples were collected from individual females and males, respectively, over the study period. Samples were collected from across the possums' area of occupancy (Fig. 1). A total of 112 vegetation surveys were conducted (mean number of surveys per month 9.3, range 7–12) during the same period, in both of the habitat types possums used for shelter; 57 in woodland habitat and 55 in creekline habitat. Ikara-Flinders Ranges National Park received 466 mm of rain during the study period, comparable to the 440 mm annual mean (Bureau of Meteorology, 2018). The number of annual genera available was not significantly related to rainfall in the preceding month ($\chi_1^2 = 2.7$, P = 0.098; Supplementary Fig. 1), although there was a positive trend.

Time since release

Both dietary richness and diversity decreased with time since release (Fig. 2, Table 2). The frequency of occurrence in scats changed over time and with preference grouping, and the proportion of each genus within scats changed significantly over time based on preference grouping (Table 2).

General diet One hundred and one plant genera were detected in at least one sample, and 88 were present in at least five samples. Twelve genera were present in $>20\%$ of

TABLE 1 Linear mixed-effects models, with a Gaussian distribution, used to investigate brushtail possum *Trichosurus vulpecula* diet over time.

Response variable	Explanatory variables[1]	Interaction	Random effect
Frequency of occurrence over time[2]	Preference, Time	Preference × Time	Genus
Proportion over time[3]	Preference, Time	Preference × Time	Genus
Dietary richness[4]	Time, Sex, No. of genera available	Time × No. of genera available	Possum ID
Shannon's index of diversity[5]	Time, Sex, No. of genera available	Time × No. of genera available	Possum ID

[1]Preference: preferred, no preference, non-preferred (derived from Jacob's Index results); Time: months since release; Number of genera available was determined from vegetation surveys.
[2]Number of scats in which genus was present.
[3]Proportion of genus within a scat (averaged from *rbcL* and *ndhJ* results; see Methods).
[4]Number of genera within an individual scat.
[5]Calculated as $(H') = \sum (p_i \, In(p_i))$, where p_i = the proportion of each genus within the individual scat (averaged from *ndhJ* and *rbcL* results; see Methods).

samples: *Eucalyptus*, *Petalostylis*, *Maireana*, *Zygophyllum*, *Callitris*, *Acacia*, *Silene*, *Austrostipa*, *Stackhousia*, *Melaleuca*, *Senna* and *Sonchus* (Table 3, Supplementary Tables 2 & 3). Only four of those were not readily available at the source site (Yookamurra Wildlife Sanctuary) prior to translocation: *Petalostylis*, *Silene*, *Stackhousia* and *Sonchus* (H. Crisp, pers. comm., 2017). The diet of males and females did not differ greatly during the 12 months (Pianka overlap overall = 0.73, monthly overlap values 0.65–0.84). Overall, nine (13.4%) genera were preferred, occurring in a significantly higher proportion of scats than expected based on their availability at the study site, 22 (32.8%) genera were consumed in the same proportion as their availability (neutral), and 36 (53.7%) genera were non-preferred, being consumed in significantly lower

FIG. 2 Dietary richness (a) and Shannon's index of diversity (b) for plant genera consumed by possums after their release (August 2015–July 2016). Both relationships are statistically significant.

proportion compared to their availability (Supplementary Table 4). Of the nine preferred genera, four were readily available at the source site (*Melaleuca*, *Zygophyllum*, *Pittosporum* and *Maireana*; H. Crisp, pers. comm., 2017).

Foraging habits Of the plant genera recorded in the possums' diet, most (72%) were < 0.5 m in height. However, these genera made up a relatively small proportion of the diet (frequency of occurrence in scats 11.4%), along with mid storey plants (0.5–2.5 m, frequency of occurrence in scats 9.5%), which made up 21% of plant genera consumed. The highest frequency of occurrence in scats was genera > 2.5 m in height (32.6%; Fig. 3), despite comprising only 7% of genera available. Although taller plants made up a small proportion of genera, per cent cover was relatively similar to that of small plants (> 2.5 m, mean % cover 41.7 ± SE 1.8; < 0.5 m, mean % cover 45.9 ± SE 5.6), with mid storey plants providing considerably lower per cent cover (0.5–2.5 m, mean % cover 13.4 ± SE 1.8). Mid storey genera including *Acacia*, *Pittosporum* and *Eremophila* were found in a higher proportion of scats with increasing time since release. No bird DNA was detected in any of the possum scats. We were unsuccessful in amplifying invertebrate DNA and thus the consumption of invertebrates by possums in the National Park remains unknown.

Discussion

The diet of reintroduced brushtail possums changed significantly with time since release, independent of changes in the availability of genera. Presumably, possums consumed a wider variety of foods (higher dietary richness and diversity) after release because the location of preferred and nutritious food plants was unknown. Flora at the source site (Yookamurra Wildlife Sanctuary) differed from the release site, although many of the same genera were available (H. Crisp, pers. comm., 2017). Over time, possums probably discovered sources of preferred plants within the release area.
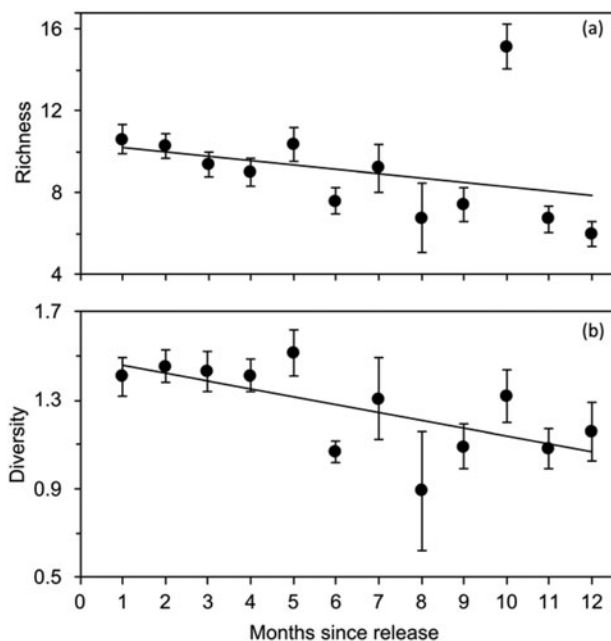
TABLE 2 Model results for assessing brushtail possum dietary changes over time since release.

Response variable	Explanatory variable 1	Explanatory variable 2	Explanatory variable 3	Interaction	Trend
Frequency of occurrence in scat	Time	Preference		Time × Preference	Frequency of occurrence changed significantly over time. Preferred genera present in higher number of scats; this did not change significantly over time.
β estimate (95% CI)	−0.004 (−0.006−−0.001)	0.140 (0.096–0.180)		0.004 (−0.002–0.009)	
ANOVA output	$\chi_1^2 = 5.9$ (P = 0.015*)	$\chi_1^2 = 185$ (P < 0.001*)		$\chi_1^2 = 1.8$ (P = 0.180)	
Proportion within scat	Time	Preference		Time × Preference	Preferred genera made up larger proportions of scats, & increased in proportions over time.
β estimate (95% CI)	−0.00028 (−0.001−< 0.001)	0.0029 (−0.009−0.015)		0.002 (< 0.001−0.004)	
ANOVA output	$\chi_1^2 = 0.3$ (P = 0.600)	$\chi_1^2 = 20.2$ (P < 0.0001*)		$\chi_1^2 = 6.0$ (P = 0.014*)	
Dietary richness	Time	No. of genera available	Sex	Time × No. of genera available	Richness significantly decreased with time. Genera available changed significantly over time. Richness was not significantly influenced by sex.
β estimate (95% CI)	1.81 (0.860–2.76)	0.17 (0.073–0.270)	−0.42 (−1.40–0.560)	−0.033 (−0.048−−0.018)	
ANOVA output	$\chi_1^2 = 10.8$ (P < 0.001*)	$\chi_1^2 = 1.4$ (P = 0.240)	$\chi_1^2 = 0.7$ (P = 0.400)	$\chi_1^2 = 18.9$ (P < 0.001*)	
Dietary diversity	Time	No. of genera available	Sex	Time × No. of genera available	Diversity significantly decreased with time. Diversity was not significantly influenced by sex.
β estimate (95% CI)	−0.051 (−0.160–0.062)	< 0.001 (−0.012–0.012)	−0.074 (−0.190–0.042)	0.00029 (−0.002–0.002)	
ANOVA output	$\chi_1^2 = 10.1$ (P = 0.002*)	$\chi_1^2 = 0.5$ (P = 0.470)	$\chi_1^2 = 1.5$ (P = 0.210)	$\chi_1^2 = 0.1$ (P = 0.750)	

*Significant at P < 0.05.

An investigation of the diet from the time of release, rather than 1 month later, may have uncovered stronger changes, but scats from the first month after release were not collected because some animals were fed supplementary food (Bannister et al., 2020). Trends suggested that either the acclimatization period had not ended 12 months after release, or that seasonal effects on food consumption were taking place. Both explanations suggest that the diet of this possum is flexible and varied, and thus it can adapt to changes in the availability of food resources. Although other studies have suggested that possum diet changes seasonally, most studies have not spanned > 12 months (Freeland & Winter, 1975; Fitzgerald, 1984; Statham, 1984; Cruz et al., 2012). In one exception, differences were found between the wet and dry season diet of brushtail possums in the tropics, with some sex effects also observed over the 29 month study period (Gloury & Handasyde, 2016). Although these studies suggest that the post-release dietary acclimatization in our study could have included some seasonal effects, more long-term dietary studies are needed to differentiate between normal fluctuations in possum diet and post-release effects.

Post-release acclimatization time is often measured using metrics such as reproduction, movement (establishment of a stable home range) and body mass (Bright & Morris, 1994;

Hardman & Moro, 2006; Facka et al., 2010). Possums released into Ikara-Flinders Ranges National Park retained all pouch young and continued to breed after release, despite initial loss of body mass (Bannister et al., 2020; Moseby et al., 2020). Mass was generally regained (or increased) within 30–60 days of release and possums settled into home ranges within 2–6 weeks (Bannister et al., 2020). However, our study suggests that although most post-release acclimatization parameters were met relatively quickly, changes in the diet were still occurring 12 months after release, and thus total acclimatization may have been incomplete, although not a barrier to short-term reintroduction success (Table 4). An alternative explanation is that the diet is highly variable and thus the concept of dietary acclimatization does not apply, but the direction of change suggests this is unlikely. Research usually focuses on the initial post-release period, when starvation is most likely, and during this period body condition may provide a more informative and rapid measure of foraging success than an investigation of diet (Soderquist, 1995). However, good food resources and successful breeding are vital for long-term population persistence through difficult conditions such as drought, and thus diet should be viewed as an important metric for measuring population viability and reintroduction success. For brushtail possums, total

TABLE 3 Genera detected in > 20% of adult brushtail possum scats and the mean proportion of those genera found in each scat using two primers (*ndhJ* and *rbcL*).

Genus	Frequency of occurrence[1]	*ndhJ*	*rbcL*	Jacob's Index ± SE[2]	P	Preference
Eucalyptus	78.5	38.1	8.8	−0.01 ± 0.2	0.15	Neutral
Petalostylis	72.3	3.2	32.2	1.00 ± 0.0	< 0.001	Preferred
Maireana	59.1	5.5	2.5	0.55 ± 0.1	< 0.001	Preferred
Zygophyllum	51.5	1.7	0.9	0.94 ± 0.0	< 0.001	Preferred
Callitris	50.7	3.2	18.4	−1.00 ± 0.0	< 0.001	Non-preferred
Acacia	36.5	3.6	2.8	−0.09 ± 0.2	0.48	Neutral
Silene	34.7	6.7	5.4	0.15 ± 0.2	0.48	Neutral
Austrostipa	33.6	0.0	2.0	0.21 ± 0.2	0.15	Neutral
Stackhousia	33.6	4.9	0.0	0.58 ± 0.2	0.03	Preferred
Melaleuca	31.4	1.9	5.2	1.00 ± 0.0	< 0.001	Preferred
Senna	29.6	2.3	2.2	−0.39 ± 0.1	0.03	Non-preferred
Sonchus	27.0	3.7	0.0	0.69 ± 0.1	< 0.001	Preferred

[1]% of scat samples containing the genus.
[2]Mean selectivity values for the study period and whether they significantly deviate from zero (P); negative values indicate avoidance (non-preferred) relative to availability, positive values indicate preference.

acclimatization was not required for successful post-release reproduction under non-drought conditions.

Post-release diet studies can be used to investigate the diet of species reintroduced to areas where knowledge of their local diet may be limited, or when the feasibility of the translocation is unknown. The diets of the red-tailed phascogale *Phascogale calura* and mala *Lagorchestes hirsutus* were investigated as part of trial releases to determine the feasibility of reintroductions to other nearby areas (Stannard et al., 2010; Clayton et al., 2015), and the diet of translocated Gilbert's potoroos was studied to determine whether sufficient fungi were present for population establishment and reproduction (Bougher & Friend, 2009). Dietary studies can thus be used to inform acclimatization time, the feasibility of population establishment, the suitability of additional release locations and the species' ecology. With some uncertainty as to the impacts of habitat degradation on possum survival in the National Park post-release, we found that food was sufficiently abundant and diverse to facilitate both survival and reproduction.

Eucalypts had both the highest frequency of occurrence in scats (78.5%) and the highest proportions within samples (making up an average of 23.5% of DNA extracted from scats) for scats collected in the National Park. However, possums cannot consume an exclusively eucalypt diet: eucalypts are high in fibre, relatively indigestible and contain terpenes (Foley & Hume, 1987; Boyle & McLean, 2004). A varied diet is therefore needed to meet their nutritional needs and energy demands (Marsh et al., 2006). The low number of preferred genera combined with the relatively high frequency of non-preferred genera in the brushtail possums' diet suggests they feed opportunistically in small amounts, while consuming large amounts selectively. Drought conditions, not experienced during our study,

may exacerbate the need for possums to locate easily digestible, moist food plants. Foulkes (2001) found that the moisture content of mature foliage was the only reliable predictor of possum occupancy in arid central Australia. Low availability of traditionally preferred plants such as *Santalum* may explain low consumption (without noticeable consequence), but the importance of such nutritious and moist plants (Foulkes, 2001) may differ under drought conditions. In arid systems drought can negatively affect reintroduction success, as occurred for black-tailed prairie dog *Cynomys ludovicianus* populations in the Chihuahuan Desert (Facka et al., 2010), and red-necked ostriches *Struthio camelus camelus* in Saudi Arabia (Islam et al., 2008). Possums are well known for their plasticity and ability to adapt to different environments (Kerle, 1984; Kerle et al., 1991) and in this study we demonstrated persistence in an area where previously identified preferred food plants are now uncommon. The diet of possums in the National Park should be monitored through the next drought period, to assess whether plants containing high moisture and nutrients are available in sufficient quantities to facilitate persistence.

The majority of the genera present in the possums' diet were ground cover plants < 0.5 m in height but their combined frequency of occurrence in scats was low, suggesting they were only occasionally eaten, despite being the most diverse height class. Palatability was not accounted for, and many herbs and grasses were present but dry/senesced at various times during the study period. In contrast, few tall genera (> 2.5 m height) were available compared to shrubs and annuals, but their frequency of occurrence within scats was high. This suggests that possums spend more time foraging in the canopy than on the ground, a result supported by previous studies of possum foraging and movement, where diet is dominated by canopy species (Foulkes, 2001;
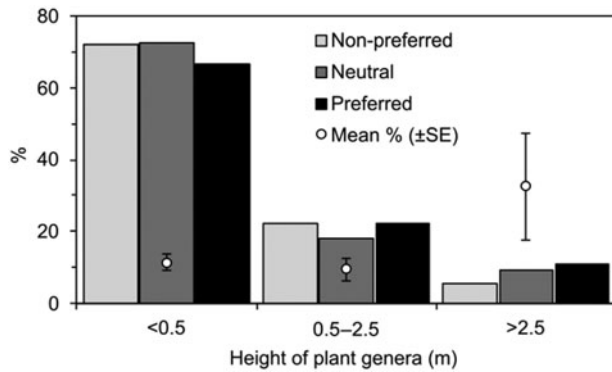
FIG. 3 The height distribution of plants grouped by dietary preference (Jacob's Index values compared to zero, calculated for the year), and the mean per cent of scats containing genera in those size classes. Genera contained in < 5% of both scats and vegetation surveys were omitted, probably reducing the number of non-preferred plants included.

TABLE 4 The time taken by reintroduced brushtail possums to acclimatize, based on five measures. Additional data from Bannister et al. (2020) and Moseby et al. (2020).

Acclimatization measure	Time taken
Successfully reproduce	0 days
Select safe, suitable shelter sites	3 weeks
Establish a stable home range	2–6 weeks
Maintain or gain body mass	30–60 days
Consume a natural, stable diet, relative to availability	> 12 months

Cruz et al., 2012; Gloury & Handasyde, 2016). This could be a result of the avoidance of predators at ground level, the quality of food in the canopy, or a combination of both.

We found no significant sex effects in the diet of brushtail possums. Combined with the presence of pouch young at all times during the study period (Bannister et al., 2020; Moseby et al., 2020), this suggests that lactation demands do not extend to detectable differences in the diet of breeding and non-breeding individuals. We suggest post-release diet studies should, at least, span one full breeding season after release, to assess whether food resources are adequate for breeding and the survival of juveniles, and to investigate whether food requirements differ between breeding and non-breeding individuals, or by sex.

We were unsuccessful in identifying invertebrate DNA in brushtail possum scats and therefore do not know the importance of invertebrates in their diet at our study site. Employing a second analysis technique such as microhistological analysis could have benefited our study and facilitated the identification of invertebrates and other non-plant material in scats. Fungi intake was not measured, but was present in the diet of possums in south-west Australia (How & Hillcox, 2000). The use of two genetic markers, as per Wilkinson et al. (2017), facilitated the detection of a higher number of genera within the diet than the use of one marker alone, but the sensitivity of each marker to genera varied; some genera were only detected by one of the markers. Although DNA sequencing is increasingly being used to study animal diets (e.g. Hibert et al., 2013; Thomas et al., 2014), limitations include not being able to determine which parts of plants are being eaten, an often low resolution of diet content to family or genus, inaccurate proportions of genera within samples, and reliance on a comprehensive and accurate reference library (Hibert et al., 2013; Thomas et al., 2014). Some species identified as having a high frequency of occurrence in scats using DNA

(e.g. Petalostylis) were uncommon at our study site and may in fact be closely related genera. Diet studies of any method are not immune to error and we urge caution in relying solely on DNA studies of diet. Selectivity analyses are subject to the accuracies of the proportions given by the two markers, as well as plant availability data. Finally, with the benefit of hindsight, our study should have extended beyond 12 months, given that the possums' diet had not stabilized within this period. Monitoring the diet over multiple years and comparing to the diet of an established, non-reintroduced possum population would also allow seasonal effects to be discerned from effects of post-release acclimatization.

The interaction between diet and time since release in reintroductions has been largely overlooked. Our study demonstrates that a reintroduced population can, over time, decrease its dietary richness and diversity, while increasing the consumption of preferred foods. This acclimatization period is longer than that recorded using movement, body condition and reproductive data and suggests a range of indicators should be used to measure acclimatization at different time scales, to ensure ecological relevance. Although only a small number of genera made up the bulk of each sample, a large number of genera were ingested, suggesting some opportunistic feeding and a relatively high consumptive diversity. The availability and consumption of plant species at our site was not a barrier to reintroduction success in the short- or medium-term but drought conditions were not experienced and could impact longer-term establishment. We suggest that the acclimatization period should extend past the first drought period for arid zone species, to ensure that suitable food plants can be sourced under stressful conditions.

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Author contributions Experimental design: HB, RB, DP, KM; data collection: HB, RB, KM; laboratory analyses: AC, HB; writing: HB, AC; revision: all authors.

Conflicts of interest None.

Ethical standards Research was conducted under the Australian Code for the Care and Use of Animals for Scientific Purposes (2013), with ethics approvals from the South Australian Wildlife Ethics Committee (Project number 15/2014) and the University of Adelaide's Animal Ethics Committee (Approval number S-2015-091), and a Permit to Undertake Scientific Research from the Department of Environment, Water and Natural Resources (Permit number Y26420-1), and otherwise abided by the *Oryx* guidelines on ethical standards.

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