Factors influencing the success of hand-reared juvenile brushtail possums (Trichosurus vulpecula) released into the wild

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WILDLIFE RESEARCH

Factors influencing the success of hand-reared juvenile brushtail possums (*Trichosurus vulpecula*) released into the wild

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ABSTRACT

Context. Wildlife is ubiquitous in urban environments, resulting in frequent interactions with humans and human infrastructure. The result of these interactions is often negative, in the form of road injury, orphaning of dependent young or eviction from natural home ranges. Wildlife rehabilitation programmes are devised to counter these negative interactions. However, the success of current management strategies is rarely assessed. **Aims.** We aimed to determine whether short-term survival of juvenile hand-reared common brushtail possums (*Trichosurus vulpecula*) released in the wild was a function of (1) intrinsic factors such as sex, individual personality and level of human habituation, and (2) extrinsic factors such as release practice (soft vs hard-release) or location (urban vs rural). We also evaluated the relationship between habituation to humans and time spent in care by possums, if presence of conspecifics during the rehabilitation process influenced the development of individual personality, and if this differed in hand-raised animals compared with wild individuals. **Methods.** We radio-tracked and monitored 20 hand-reared juvenile possums (10 females and 10 males) for up to 40 days after release in the wild. **Key results.** Eight possums (40%) survived until the end of the study, nine possums (45%) were killed by foxes or had to be returned into care and three possums (15%) had unknown fates (i.e. lost VHF signal). We found that more exploratory individuals and those less human-habituated were more likely to be successful in the wild in both rural and urban areas, whether or not they were hard or soft released. **Conclusions.** Our results suggest that personality is a key criterion to consider when evaluating the success of rehabilitation programmes. Behavioural traits of hand-reared brushtail possums differed significantly from those of wild individuals, showing that captive conditions can affect the development of personality. Hand-reared possums that spent more time in care were also more likely to display highly human-habituated behaviours. **Implications.** By demonstrating which factors influence success of hand-reared wildlife after release in the wild, our results help in evaluating current rearing and release practices, and improve the evidence base for developing best practice wildlife rehabilitation guidelines.

Keywords: exploration, hand-rearing, human habituation, marsupial, personality, post-release monitoring, survival, wildlife rehabilitation.

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Introduction

Anthropogenic activities such as urbanisation cause major changes to the natural environment (Tarsitano 2006), including habitat destruction and fragmentation (Marzluff and Ewing 2001; Pekin and Pijanowski 2012). These changes often increase the number of negative animal–human interactions, resulting in injured, orphaned or displaced wildlife (Russell *et al.* 2009). Rehabilitation programmes are established to mitigate these impacts and foster animals to a healthy state to release them back into the wild (Guy *et al.* 2013). Hand-rearing, the process of feeding and caring for a young animal (e.g. orphaned or injured) until it is independent and ready for release (Tribe and Brown 2000), is often required. Rehabilitation programmes have three main aims:

(1) animal survival post-release; (2) settlement or dispersal from release site; and (3) successful reproduction (Teixeira *et al.* 2007).

An integral component of rehabilitation programmes is the release stage, where animals are released from captivity into the wild. However, many rehabilitation programmes fail to monitor the post-release survival of animals, or to assess the factors influencing survival post-release. Monitoring of individuals after release in the wild is necessary to assess the success of rehabilitation programmes (Fischer and Lindenmayer 2000) and whether they are meeting the three rehabilitation aims.

Many factors can affect survival in the wild (Cope *et al.* 2022*a*). These factors can be both intrinsic (i.e. due to the characteristics of the individual, e.g. health status, body mass, sex and personality) or extrinsic (i.e. due to the characteristic of the release environment, e.g. quality of habitat or type of release method used) (Ario *et al.* 2018). For example, larger male little penguins (*Eudyptula minor*) had significantly higher survival rates than smaller females after release into the wild (Goldsworthy *et al.* 2000). The effect of personality on post-release survival of rehabilitated animals is species dependent (Bremner-Harrison *et al.* 2004; Rasmussen *et al.* 2021), with bold captive-raised swift foxes (*Vulpes velox*) dying quickly after release (Bremner-Harrison *et al.* 2004), but bold hand-reared Tasmanian devils (*Sarcophilus harrisii*) surviving well (Sinn *et al.* 2014).

Animals held in captivity can be stressed (Cope *et al.* 2022*b*) undergo behavioural changes compared with their wild counterparts, and may become habituated, tame or dependent on humans (McPhee and Carlstead 2010; Tolhurst *et al.* 2016). Human habituation of captive or hand-reared animals (McDougall *et al.* 2006) has been shown to increase with time in care (McPhee 2004), with animals displaying decreased responsiveness to humans. Negative behaviours associated with human-habituated wildlife include seeking refuge less, limited predator recognition and less reactivity to loud noises (McPhee and Carlstead 2010). These behaviours are seen as potentially detrimental to release success, and often result in predation of highly human-habituated individuals after release in the wild (Van Heezik *et al.* 1999; Rödl *et al.* 2007).

The release method employed in rehabilitation programmes can also affect survival. The two common methods used for releasing individuals in the wild are soft (delayed) and hard (immediate) release (Parker *et al.* 2012). Soft release involves a period of acclimatisation to the new environment, where animals are maintained in semicaptive conditions and food and shelter are provided in an enclosed safe area within the release site before final release (Campbell and Croft 2001). Hard-release methods do not include the provision of food and shelter, and rehabilitated animals are immediately released in the wild without any further support (Campbell and Croft 2001). The effectiveness of each method can vary with the species being released, and should therefore be explored in rehabilitation programmes. For instance, swift foxes survive longer if soft release is used (Sasmal *et al.* 2015), but for stitchbirds (*Notiomystis cincta*), hard release is more effective (Richardson *et al.* 2015).

Although the survival of animals post-release may be affected by a number of intrinsic and extrinsic factors, most studies only consider/monitor one or two factors (Bannister *et al.* 2020). Our study aimed to: (1) determine both intrinsic (i.e. sex, individual personality, level of human habituation) and extrinsic (i.e. release method, release environment) factors affecting survival of hand-reared juvenile brushtail possums (*Trichosurus vulpecula*), during the first 40 days after release, when animals are most vulnerable (Hamilton *et al.* 2010; Bannister *et al.* 2020); and (2) provide improved management recommendations based on our results to help inform wildlife rehabilitation organisations and future release programmes. We also explored factors that can affect the development of individual characteristics, such as how time in care, presence of conspecifics during rehabilitation and captivity can influence the development of personality and habituation to humans. Findings of the study highlight potential downfalls and opportunities to ameliorate rehabilitation management processes.

Materials and methods

Study species

Brushtail possums (hereafter possums) are Australian nocturnal arboreal marsupials, well adapted to urban landscapes (Eymann *et al.* 2006). They have one of the widest distributions of any Australian marsupial (Helgen and Jackson 2015), but populations have significantly declined in the last two centuries, with severe reduction in density and distribution range (Kerle *et al.* 1992; Kerle 2001). Despite their relatively high presentation rates in wildlife rehabilitation programmes (WIRES 2019), there is limited understanding of the factors affecting the fate of individuals after rehabilitation and release in the wild (but see May *et al.* 2016).

Study design

Pre-release stage

Possums were sourced in collaboration with a volunteer wildlife care organisation (Wildlife Information, Rescue and Education Service Incorporated; WIRES) in New South Wales, Australia. Animals remained with their volunteer wildllife rehabilitator until the release stage.

The pre-release stage of the study involved collecting information on intrinsic (i.e. body mass at rescue and release, sex, individual personality and level of human

habituation) and extrinsic factors (i.e. release method used and release environment) that may affect survival of individual possums. We also collected other life-history information for each possum such as their date of rescue (used here to calculate the total days spent in care) and whether they were raised with any conspecific.

Behavioural tests. We quantified personality traits by employing an open-field test, specifically designed for possums (see Mella *et al*. 2016). Possums were placed in an open-field apparatus, consisting of a vertical structure with four levels and adjoining holes to allow movement (Fig. 1). We placed a container with food in each level to encourage feeding in a novel environment [a measure of boldness in Mella *et al*. (2016)]. Possums were initially placed into the lowest level of the arena, and were then filmed from a distance for 5 min using a video camera (JVC GZ-EX555BSD HD camcorder). Behaviours relative to exploration and boldness were later quantified using the programme J-Watcher (Blumstein and Daniel 2007). All tests were performed at night (between 2000 and 2400 hours) when possums are active (Helgen and Jackson 2015). Each test was performed twice (with at least 1 week between tests), at a maximum 7 days before possums were released in the wild.

Human habituation. Many possums in the study exhibited behaviours that would be considered atypical for wild possums and indicative of some level of human habituation. These behaviours included climbing and resting on the handler when returned to their enclosures. Because these behaviours might affect survival in the wild (Elsbeth McPhee 2004), we quantified each possum's level of human habituation (i.e. low, medium and high) by scoring behaviours in response to the presence of humans during capture and handling (Table 1).

Body mass, microchipping and collaring. Before being released, each possum was weighed to determine release mass (5 kg; Pesola, Switzerland), and uniquely marked using a microchip (Bio-mark ISO FDX-B) for future identification. At least 3 days before release (to allow acclimation), we equipped possums with VHF collars (UG V5 VHF collar; Sirtrack, New Zealand, <3% body weight; Fig. 2*c*), fitted with mortality signal and a cotton thread weak link [as in Cawthen and Munks (2011)]. The weak link ensured each collar would come off with time as the thread weakened with weathering. This was necessary because all possums in the study were juveniles and still growing at the time of release. The weak link is designed to last a maximum of 45 days and is recommended for short-term monitoring studies of juvenile individuals (Cawthen and Munks 2011).

Fig. 1. A brushtail possum in the open-field test apparatus.

Table 1. Levels of human habituation for brushtail possums with corresponding behaviours in response to capture and handling.

Level of human habituation	Behaviours observed
Low	• Extensive avoidance and escape behaviours or aggressive behaviours observed while attempting to capture the animal (e.g. hiding in nest box, screeching, and aggressive biting or distressed climbing in enclosure while human present) • Biting and screeching while being handled • Retreats immediately after being handled
Medium	• Some avoidance and escape behaviours or aggressive behaviours observed while attempting to capture animal • Will not seek but neither actively avoid humans and may approach due to association with food • Some biting and/or screeching while being handled
High	• No aggressive or evasive behaviours observed while attempting to capture animal • Will seek human contact. Possum can be picked up without resistance. Answers to human calls, approaches and climbs on humans • Interacts with human in enclosure after being handled and does not retreat. Sometimes needs to be encouraged to climb away

Fig. 2. (*a*) aviary and fresh food supplemented as part of the soft release, (*b*) a nest box in a tree used as part of the hard release, and (*c*) a possum pair fitted with VHF collars before release in the wild.

Post-release stage

Possums were released in groups of three to six individuals, depending on the number WIRES defined as ready for release at that time. Becasue the possums came into care at different times and stages of development, they were not all ready for release at the same time. For this reason, we had different release dates for each group of possums. WIRES employs a 'buddy system', where young possums that come into care are paired with other joeys of similar body mass, with the aim to reduce stress and dependence on the human foster wildlife rehabilitator (WIRES 2017). All individuals used in the study had at least one 'buddy' they grew up with and with which they were released.

Release sites. The NSW Government guidelines for release of rehabilitated fauna (OEH 2011) suggest that large numbers of individuals should not be released at a single location to avoid saturating the environment. Therefore, we had five different release sites and each group of possums was randomly allocated to a release location. Release sites were selected by WIRES but possum buddies were randomly allocated a release location. These included both urban (i.e. areas surrounding residential or metropolitan infrastructures with limited natural corridors) and rural sites (i.e. less suburban with a high tree cover percentage and backing onto a conservation area or national park).

Release methods. Possum buddies were randomly allocated a release treatment (i.e. soft or hard release), then transported to their release site and released at dusk (1800–2000 hours). Soft-release possums were kept for a week in a portable aviary (3 m H × 2 m L × 2 m D) under dense non-isolated trees at the release site, with food supplementation (native foliage) to allow acclimatisation to local conditions (Fig. 2*a*). In the second week, the aviary door was opened and food supplementation was gradually reduced, to encourage the possums to explore beyond the familiar aviary. In the third week, the aviary was removed from the release site. All possums had vacated the aviary before its removal.

Hard-release possums were placed in the environment in nest boxes, which were left at the site at the time of the release, with no other provisions or support. Nest boxes were placed at least 200 m away from the soft-release aviary, about 5 m high in non-isolated native trees (Fig. 2*b*). We released hard-release possums in the wild at the same time as soft-release possums (i.e. when the soft-release aviary door was opened in week 2).

Survival. Following release, possums were tracked using radio-telemetry until collar loss or mortality (maximum 40 days). Because our study used short-term monitoring and juvenile possums, we could not use dispersal and reproduction as proxies for success. Therefore, we used short-term survival as indication of successful release. If a possum was alive at the end of the 40-day monitoring period or until its collar came off, it was recorded as a successful release (survival = yes). If a possum died or we had to take it back into care after release (because it became injured or sick), we recorded it as a failed release (survival = no). Animal remains found during post-release monitoring were assessed for cause of death following Augee *et al.* (1996).

Statistical analysis

Behavioural tests

We quantified personality traits using behaviours in the open-field test related to boldness and exploration as in Mella *et al.* (2016). The personality data were collated into a large database comprising existing data on possum personality (total $N = 99$ individual possums), using the same methodology (after Mella *et al.* 2016). The database included measurements of personality for wild possums collected at different locations and during different studies in the Sydney metropolitan area. This allowed us to compare personality between the wild individuals studied in other projects and the possums raised in captivity in this study; these differences may also affect survival of hand-raised possums in the wild.

To test whether the personality of the hand-reared possums in this study differed from that of wild possums, we used a two-sample independent *t*-test in R (R Core Team 2020) assuming unequal variances. Because the possums in our study were all juveniles and personality can change with age (Petelle *et al.* 2013), we only included data from wild juvenile possums ($N = 10$). We also tested the effect of the WIRES buddy system (i.e. *pair number* as fixed effect) on *personality traits* (*boldness* and *exploration*) using a one-way ANOVA test (R Core Team 2020) for each trait.

Human habituation

First, we tested for correlations between continuous intrinsic factors (i.e. *rescue mass, time in care* and *personality* traits) by calculating Pearson's correlation coefficients in R (R Core Team 2020), to choose which variables to include in our model. Because there was a strong negative correlation between *rescue mass* and *time in care* (Table 2), we only included *time in care* in the final model. We then used multinomial Generalised Linear Models (GLM) to test the effect of *sex*, *time in care*, *boldness* and *exploration* on the level of human habituation of hand-reared possums.

Survival

To identify the effect of intrinsic factors (i.e. *sex, boldness*, *exploration* and *human habituation*) and extrinsic variables (i.e. *release type* and *release environment*) on *survival* (yes/no), we used GLMs with a binomial distribution and a logit link function. Variables that were not significant at a $P > 0.25$ were excluded from the final model (as per Winer *et al.* 1991). The final model included only *exploration* and *human habituation*.

Ethical statement

Use of animals was approved by University of Sydney (permit # 2014/707) Animal Ethics Committee and NSW National Parks and Wildlife Services NSW (permit # SL100443).

Results

We released 20 hand-reared juvenile possums (10 females and 10 males) within the Sydney metropolitan area: seven possums at urban release sites (four males and three females) and 13 (six males and seven females) at rural release sites. We used a soft-release method for nine individuals (five males and four females) and a hard-release method for the remaining 11 (five males and six females). We used both methods at each urban and rural site.

Behavioural tests

We identified significant measures of personality from behaviours quantified during the study. *Time spent eating in*

Table 2. Pearson correlation coefficients (*P*-values in brackets) for intrinsic factors of brushtail possums.

	Boldness	Exploration	Rescue mass	Release mass	Time in care
Boldness	1				
Exploration	0.22 (0.36)	1			
Rescue mass	0.24 (0.30)	0.16 (0.51)	1		
Release mass	−0.34 (0.15)	0.01 (0.98)	−0.04 (0.88)	1	
Time in care	−0.02 (0.93)	−0.04 (0.88)	**−0.71 (0.0005)**	0.12 (0.63)	1

Significant correlations are marked in bold.

Table 3. Generalised linear mixed models for behavioural measures in brushtail possums ($n = 99$).

	Behaviour	Model including PossID	−2LL	Diff LL	AIC	ΔAIC
Exploration	Time spent in unpreferred level in novel environment	No	674.49	7.50	686.49	15.50
		Yes	666.99		670.99	
Boldness	Eating in a novel environment	No	719.61	80.23	731.61	88.23
		Yes	639.38		643.38	

A significant measure of personality is indicated by differences in Log-likelihood and AIC values >2. −2LL = Log-likelihood ratio test; Diff LL = difference in Log-likelihood; AIC = Akaike information criterion value; ΔAIC = difference in AICs between models. All models include *test number*, *study*, *body mass* and *sex* as fixed effects. Time spent in an unpreferred level in the open-field apparatus was a measure of exploration and time spent eating in a novel environment was a measure of boldness.

a novel environment was a measure of *boldness* and *time spent in unpreferred levels* in the open field test was a measure of *exploration* (Table 3). These personality traits have been validated for possums and other animals in previous studies (Dammhahn and Almeling 2012; Raña 2017; Wat *et al.* 2020*a*).

The personality of hand-reared juvenile possums differed significantly from that of wild juvenile possums quantified in previous studies (Fig. 3). Hand-reared juvenile possums were significantly bolder [$t(20) = 3.25$, $P = 0.004$; Fig. 3*a*] and more explorative [$t(19) = 4.75$, $P = 0.0001$; Fig. 3*b*] than wild juvenile possums.

Possums 'buddied' by WIRES during weaning (assigned *pair number*) showed increased likelihood of similar personality traits. *Pair number* significantly affected both boldness ($F_{10} = 22.94$, $P = 0.00003$) and exploration ($F_{10} = 9.49$, $P = 0.001$).

Human habituation

Human habituation was significantly affected by *time in care* ($\chi^2_2 = 9.61$, $P = 0.008$). Possums that were in care for longer periods of time had higher human habituation scores (Fig. 4). *Sex* ($\chi^2_2 = 1.40$, $P = 0.50$) and personality, *boldness* ($\chi^2_2 = 2.32$, $P = 0.31$) and *exploration* ($\chi^2_2 = 1.74$, $P = 0.42$), did not significantly affect the level of human habituation.

Survival

Eight possums (five males and three females) survived (40%), nine possums (four males and five females) did not survive (45%) and three possums (one male and two females) had unknown fate (15%). Successful possums survived at least 2 weeks in the wild before collars came off (average of 23.9 ± 10.2 days in the study for successful possums). Failed releases included possum individuals that had to return into care because they were struggling in the wild ($n = 4$; 20%) or died ($n = 5$; 25%) before the end of the study (average of 10.1 ± 9.6 days in the study for failed possums). Possums with unknown fate either experienced collar failure or the collars came off early in the study period (average 5.6 ± 2.1 days in the study for unknown possums).

Of the four possums returned into care, two required veterinary treatment due to health issues including dermatitis and injuries. The remaining two possums returned to WIRES

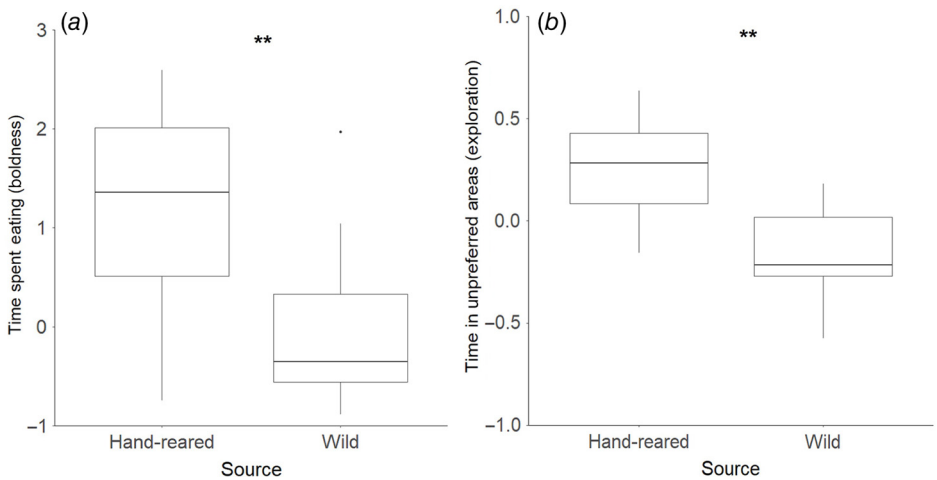

Fig. 3. Differences in indices of two personality traits (*a*) boldness and (*b*) exploration, between hand-reared and wild juvenile brushtail possums. Asterisks indicate significance at $P \leq 0.01$.

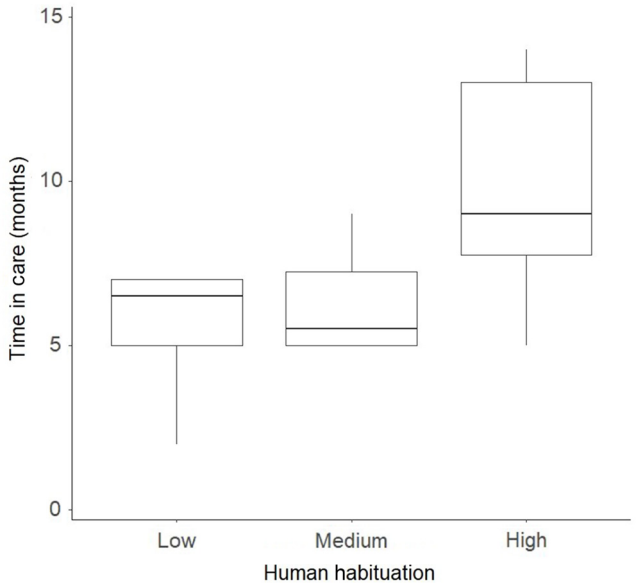

Fig. 4. Relationship between time in care and level of human habituation for juvenile hand-reared brushtail possums.

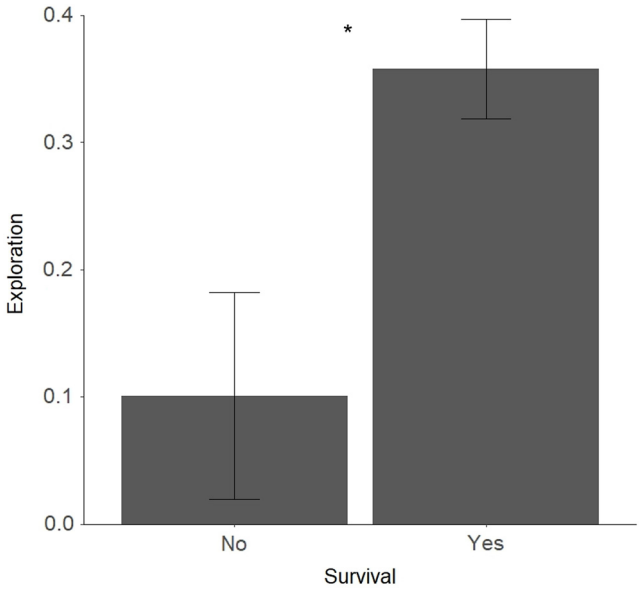

Fig. 5. Mean ± s.e. of failed and successful releases in relation to the personality trait, exploration, of hand-raised brushtail possums. Asterisk indicates significance at $P \leq 0.05$.

care due to repeated engagement with humans and incapacity to self-sufficiently forage.

The remains of dead possums were retrieved within 48 h to ensure minimal decomposition. In three cases, the head and a limb remained still attached to the collar, and in two other instances the remains were buried in the ground or found near fox den sites and the collars had visible bite marks and scratches. Fox (*Vulpes vulpes*) predation inherently involves removing the head of prey, eating gut content and burying remains in soft soil (Augee *et al.* 1996). Therefore, the five dead possums were recorded as fox kills.

Personality and human habituation significantly affected *survival*. More exploratory ($\chi^2_1 = 6.46$, $P = 0.011$, Fig. 5) and less human-habituated possums ($\chi^2_2 = 10.76$, $P = 0.005$, Fig. 6) survived more after release. *Boldness* ($\chi^2_1 = 0.08$, $P = 0.780$), *release type* ($\chi^2_1 = 0.53$, $P = 0.467$), *release environment* ($\chi^2_1 = 0.004$, $P = 0.950$) and *sex* ($\chi^2_1 = 0.56$, $P = 0.455$) did not affect *survival*.

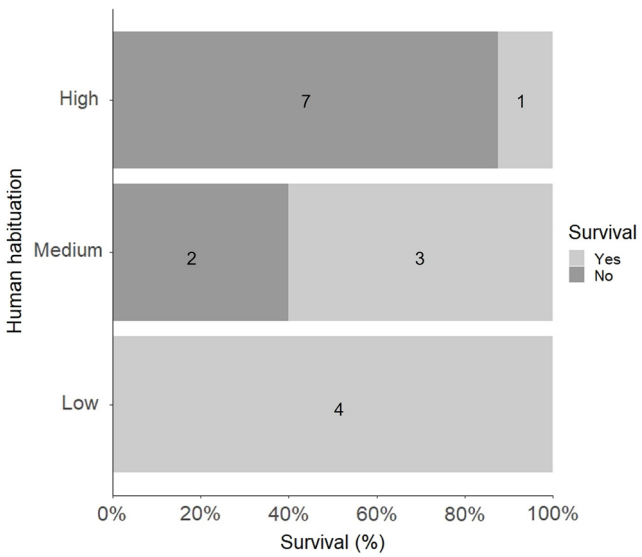

Fig. 6. Survival of brushtail possums in relation to their level of human habituation. Sample size for each group is indicated in bold.

Discussion

Our study explored intrinsic (those related to the characteristics of individuals) and extrinsic factors (those related to the characteristic of the environment) that may affect post-release survival of hand-raised possums. Our findings suggest that intrinsic behavioural factors such as individual personality and level of human habituation are of key importance to predict survival after rehabilitation.

Effect of intrinsic factors on survival

Possums that were more exploratory had a significantly better chance of survival in the wild. This pattern is similar to that found for survival of great tits (Dingemanse *et al.* 2004). More exploratory possums may be more efficient in finding resources, such as food and refuge; in wild adult possums, more exploratory individuals have greater diet diversity (Herath *et al.* 2021), and the exploration trait affects home range and core area characteristics (Wat *et al.* 2020*b*).

Survival of individual possums also depended on their level of human habituation. The majority (n = 9) of habituated possums failed in the wild (i.e. had to be taken back into care or were killed by predators), whereas all possums with a low human-habituation score (n = 4) were successful releases (i.e. survived until the end of the study). Possums rescued at lower body weights required longer time in care, and this in turn affected the level of human habituation of the individuals. WIRES will rear pouch young possums with a body mass of >120 g, with ears detached from the body and eyes open (WIRES 2017). However, we suggest these guidelines should be reconsidered, unless hand-rearing strategies can be adapted to minimise the extent of human habituation. Highly habituated possums frequently engaged with humans during monitoring, suggesting some sort of dependence on human support, a trait not desirable in wild animals. Fearfulness towards humans (measured as avoidance and/or stress related behaviours) before release in the wild was the strongest predictor of survival after translocation for adult possums (May *et al.* 2016). Although human habituation is commonly noted as a factor to consider during the rehabilitation of native wildlife (Tribe and Brown 2000), only a few studies have assessed its impact on release success (Elsbeth McPhee 2004). Based on our findings, time spent in care should be minimised and/or behaviours linked to human habituation should be monitored and preferably prevented during the rehabilitation process.

We found that sex had no effect on the short-term survival of hand-reared juvenile possums, which is consistent with observations for Tasmanian devils [*Sarcophilus harrisii*; Sinn *et al* (2014)]. However, male and female juvenile possums have different dispersal patterns (Bannister *et al.* 2019), so 40 days may be insufficient to detect these differences and their effect on survival. Sex can affect survival of released individuals of other species (e.g. little penguins in Goldsworthy *et al.* 2000), and should therefore always be considered. For possums, other factors appear to be more influential in predicting success in the wild.

Effect of extrinsic factors on survival

Survival in the wild did not depend upon the release method used (as in Bannister *et al.* 2020). It appears that the best release method varies among species, with Swift foxes (Sasmal *et al.* 2015), Western burrowing owls (*Athene cunicularia hypugaea*) and dormice (*Muscardinus avellanarius*) (Bright and Morris 1994), benefiting from a gradual release method (e.g. food and shelter provided). Other species, (such as passerine birds, Richardson *et al.* 2015) prefer a hard release (e.g. immediate independence and no support), and some, such as eastern barred bandicoots (*Perameles gunnii*) (de Milliano *et al.* 2016), hare-wallabies (*Lagorchestes hirsutus* and *Lagostrophus fasciatus*) (Hardman and Moro 2006), western quolls (*Dasyurus geoffroii*) (Jensen *et al.* 2021), and eastern grey kangaroos (*Macropus giganteus*) (Campbell and Croft 2001), show no clear advantage with either method. There is currently no standardised set of techniques for the release of rehabilitated possums, so we suggest that either method can be used with no detrimental consequences.

A quarter (n = 5) of our possums were killed by foxes shortly after release. The release sites used in our study were selected by wildlife rehabilitators. To the best of our knowledge, information on the presence or absence of predators is rarely incorporated when selecting potential release sites. In the future, wildlife rehabilitators could use local databases (e.g. www.feralscan.org.au) to assess where predators are present (West 2017). The use of these types of databases would allow an assessment of the likelihood of encountering a feral predator at a selected release site and could help determining potential release locations (in terms of predator absence). Discussions with local land managers at various levels of government may also prove helpful in identifying sites where some level of fox control has been applied. Our study showed that an appropriate release site is most likely an area where feral predators are controlled. Because there appears to be an acclimation period after release (3–4 weeks) during which animals are most vulnerable to predation (Hamilton *et al.* 2010; Bannister *et al.* 2020), at sites where predators are controlled, possums would have more time to acclimate to natural conditions, without the additional pressure of dealing with risk of predation.

Another aspect of rehabilitation programmes that can determine survival is habitat quality at the release site (Cheyne 2006; Moorhouse *et al.* 2009). We used release environment (i.e. urban or rural) as a proxy for habitat quality, using percentage tree cover and level of urban development to classify release sites. We found no significant effect of release environment on survival for possums. However, our sample size was limited. More exploratory possums living on the boundary of a National Park and urban areas are more likely to use urban environments than less exploratory ones (Wat *et al.* 2020*b*). Thus, although it may seem preferable to release animals at rural sites that may provide more natural habitat rich of food resources and refuge (Fischer and Lindenmayer 2000; Cheyne 2006; Moorhouse *et al.* 2009), the effect of personality on space use by individuals should also be considered in future rehabilitation projects.

Effect of captivity on personality

Hand-reared possums had significantly different personality traits than wild individuals. Many studies have shown that personality traits can differ in captivity due to the change in selection pressures on behaviour (Price 1999; Elsbeth McPhee 2004; O'Regan and Kitchener 2005). In our study, captive juvenile possums were considerably more exploratory and bolder than wild juvenile possums in the

open-field test. Hand-reared animals may lose some of the natural inhibition that juvenile possums display in the wild, and this is likely to increase their vulnerability to predators. Captive-bred grey partridges (*Perdix perdix*) show poor vigilance behaviour compared with wild individuals and, as a result, suffer high predation rates post-release (Rantanen *et al.* 2010).

The personality of the possums was also influenced by the buddy system, which is commonly used by wildlife rehabilitators. Possums that were 'buddied' and grew up together had similar personalities. The buddy system is used to limit imprinting on humans and encourage social learning from conspecifics (Dindo *et al.* 2011; Schwartz *et al.* 2016), but our results show it can also affect the development of personality traits of individuals, and suggest that it might limit behavioural variability in hand-reared captive possums. The particular selection pressures (i.e. predator absence, food quality or housing facilities) driving this pattern are currently unknown. but could be explored in future studies. The difference in selection pressures between wild and captive settings (McPhee and Carlstead 2010), and the consequent behavioural differences of individuals experiencing these conditions, could contribute to the high failure rate of rehabilitation and reintroduction programmes (Fischer and Lindenmayer 2000; Guy *et al.* 2013).

Management implications

Our study highlights the detrimental impact of introduced predators on hand-reared juvenile possums, with the European red fox being responsible for all possum mortalities (most occurring within 3 days post-release). Wild possums regularly have to deal with predation risk (Mella *et al.* 2014), whereas hand-reared or captive bred animals often lack predator detection and avoidance behaviours (Moseby *et al.* 2012). The exclusion of foxes was a key factor in the successful reintroduction of brushtail possums in Western Australia (Short and Hide 2014) and in the Flinders Ranges in South Australia (Moseby *et al.* 2020). The presence of predators at the release site appears to be a critical factor responsible for the failure of many release programmes (Fischer and Lindenmayer 2000; Moseby *et al.* 2011; Short 2016). Our failed release rate (45%) is comparable to the mortality rate reported in a translocation study on possums, where 42% of individuals died due to predation by introduced predators soon after release (Pietsch 1995). This indicates that the risk of predation is particularly high after release to a new environment.

During rehabilitation, possums are housed in aviaries in suburban settings with limited exposure to natural stimuli, and are often raised in the presence of pets, such as cats and dogs, which may cause habituation to potential predators, and a lack of danger perception in juvenile hand-reared possums. Possums are arboreal animals and although they can spend time on the ground to feed and to move between foraging patches, they generally prefer the safety of trees (MacLennan 1984). However, rehabilitated arboreal animals often spend more time on the ground than their wild counterparts (Basalamah *et al.* 2018) and this may lower survival prospects. Rehabilitated possums in our study might have spent more time on the ground (as in Bannister *et al.* 2018) than wild juvenile possums, and thus might have been more vulnerable to predation. Rearing guidelines should be developed to encourage wariness in hand-raised animals (Rantanen *et al.* 2010).

The threat of predation of hand-reared possums could potentially be minimised using a number of approaches. The potential link between habitation to humans and predation should be further explored to ascertain whether minimising habituation improves release survival. Another potential management technique is predator avoidance training before release. This usually involves exposing animals to predator stimuli (e.g. predator model, tactile, audio and/or olfactory stimuli) associated with a negative feedback (Moseby *et al.* 2012). Predator avoidance training can improve success rates of animals post-release (Van Heezik *et al.* 1999; West *et al.* 2018) and may be essential for hand-reared individuals lacking any previous predation risk experience. Simple and inexpensive forms of anti-predator training should be explored as a viable option for rehabilitation programmes. Limiting association with pets while in care is also fundamental.

During our study, we had to intervene and rescue four possums that were placed back into care. These individuals showed inappropriate behavioural responses to life in the wild, were habituated to humans, lacked independent foraging skills and would have most likely died without intervention. Reducing the extent of habituation would hopefully reduce the likelihood of this occurring in the future. If reduced habituation alone does not sufficiently reduce the incidence of inappropriate behavioural responses post release, additional approaches, such as pre-release locomotor and foraging training (Kleiman 1989), could potentially improve post-release success rates. Previous research has shown that facilitating and eliciting natural behaviours in captivity increases foraging and locomotion skills post-release in the wild (Stoinski and Beck 2004; Schwartz *et al.* 2016), and potentially improves release success (Humle *et al.* 2011). Because not all individuals that undergo rehabilitation seem to be suited for release, we urge the rehabilitation sector to consider the ethical consequences and implications of releasing animals that have a poor chance of post-release survival.

Studies also suggest that rehabilitation enrichment, in the form of facility modification, can have an impact on survival rates in the wild (Kelly *et al.* 2008). For example, pipistrelle bats (*Pipistrellus pipistrellus*) have increased survival rates when housed in large flight cages compared with wildlife

rehabilitators' homes (Kelly *et al*. 2008). For arboreal marsupials such as possums, locomotion and climbing skills are a requirement for foraging and predator avoidance in the wild. Ensuring appropriate rehabilitation aviaries large enough to encourage natural climbing and foraging behaviours may therefore be fundamental for their success post-release.

General conclusions and future directions

We found that intrinsic factors (those specifically related to the characteristics of the individuals) were more important in determining success of hand-reared possums than extrinsic factors (those related to the release method or site). Naturally, there could be a number of other factors that may affect post-release success that could not be explored here due to our small sample size. By identifying some of the factors that contribute to the success of release programmes, our study aimed to help inform management strategies for rehabilitation of wildlife. These can be implemented in the form of behavioural and management modifications (e.g. anti-predator and foraging training), and efficient selection of release method and release site. Although our results have possible applications for a wide range of wildlife, it is important to note that specific strategies may need to be tailored depending on the species considered (Moseby *et al*. 2014).

Our study also reiterates the importance of understanding animal personality in rehabilitation programmes, including how this can differ between hand-reared and wild populations. Future studies may provide more information on which aspects of personality are being altered by captivity and which are the most damaging when individuals are placed into a wild context. Our study has contributed to identifying the negative effects of time in care and human habituation on survival of wildlife.

Post-release monitoring of individuals can help us to determine factors impacting release success and understand important ecological interactions (e.g. invasive predator effects) (Moseby *et al*. 2021). It is therefore important that rehabilitation programmes invest in post-release monitoring as much as possible. Our study was short-term, so we were only able to monitor survival of a small number of individuals for a maximum of 40 days. Ideally, future studies should monitor released individuals for longer to determine long-term (e.g. 2 years) survival, dispersal/establishment and reproductive viability of released animals. Comparisons with wild conspecifics would also help to establish the extent to which rates and causes of mortality vary between hand-reared and wild animals.

The results of our study can be used to ensure that the limited funds available to wildlife rehabilitation organisations are spent in the most effective way for release programmes, and that the substantial time investment needed to foster wildlife to the release stage translates to the best chance of survival for the animals. Rehabilitation programmes are an important part of the welfare of native wildlife. With further scientific support, rehabilitation programmes could also positively contribute to the conservation of threatened species.

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Data availability. The data used to generate the results in the paper will be shared on the Sydney eScholarship Repository https://ses.library.usyd.edu.au.

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