Increasing generations in captivity is associated with increased vulnerability of Tasmanian devils to vehicle strike following release to the wild.

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Supplementary Methods

Release site selection and translocations

Because multiple biological and anthropogenic factors may influence post-release survival of devils, the STDP implemented a number of roadkill mitigation measures including remote site location, soft release tactics (feed stations), dampening dispersal trials (familiarization [following Shier 2006] or conspecific cueing [following Stamps 1988]), employment of virtual fence technology, signage on major roads, and public outreach. The results of these activities are to be reported elsewhere.

Narawntapu National Park: The first release of captive devils into a Tasmanian mainland site was conducted on the 25th of September, 2015, at Narawntapu National Park in the north of the State (Figure 1). Narawntapu was chosen as a release site due to its abundant devil population prior to DFTD arriving in 2008, with the site thereby inferred to provide suitable habitat. Following DFTD arrival, the wild incumbent population has reduced to approximately 15-20 animals (SF unpubl. data) with genetic diversity beginning to decline (CEG unpubl. data). Additionally, being a National Park, the risk of some threats such as roadkill, dogs and persecution are reduced relative to other sites in Tasmania.

Forestier Peninsula: The second and third releases occurred onto the Forestier Peninsula in the south-east of the State on the 18th of November, 2015, and the 25th of February, 2016, respectively. Forestier Peninsula has been a long-term study site for the STDP including monitoring DFTD spread and the effect of the disease on the incumbent population (e.g. Ujvari et al. 2014). In 2012, all devils were removed from Forestier Peninsula; two years of follow-up monitoring ensured that all devils had been removed. A barrier was erected at the isthmus between the town of Dunalley and the Forestier Peninsula to prevent the movement of diseased devils onto the now disease-free Forestier Peninsula. The Tasman peninsula, at the southern tip of Forestier Peninsula (Figure 1), still has a DFTD-free incumbent devil population. There appears to be limited movement between Forestier and Tasman Peninsula populations based on genetic analysis and trapping surveys (STDP unpubl. data).

Animal selection for releases

Devils were selected for release based on their age, sex, birth location, mean kinship relative to the remaining individuals in the insurance population (based on the Tasmanian devil studbook [Srb 2015]) and minimal mean kinship to other devils in their release group. Tasmanian devils in the insurance population are housed in three types of enclosure: intensive – zoo-based facilities containing one or two individuals; managed environmental enclosures (MEEs) – group housing with 7 to 10 individuals in less than 5 ha; and free-range enclosures (FREs) – group housing with 10 to 20 individuals in 10 to 22 ha (Hogg and Lee 2014; Hogg et al. 2016).

Narawntapu National Park: One of the STDP's goals for the first release of Tasmanian devils to mainland Tasmania was to assess the effectiveness of a DFTD immunisation protocol (Woods et al. 2007); all devils selected for this release were immunised as part of ongoing work for a different study. Two different cohorts were selected – individuals that had been captive-raised, i.e. they had lived in a DFTD environment until at least one year of age (N = 11), or came into the insurance population as pouch young (N = 1); the other cohort was captive-born, i.e. individuals from over-represented lines in the insurance population (Hogg et al. 2015) (N = 8). The Narawntapu release group totalled 20 individuals with a male:female sex ratio of 11:9, and an age structure of age 1 (N = 5); age 2 (N = 2); age 3 (N = 2); and age 4 (N = 11). The age 4 animals were all captive-raised, having been born in the wild (N = 12); while the younger cohort (ages 1 to 3) were captive-born in intensive facilities (N = 2) or FREs (N = 7).

Forestier Peninsula: Each of the two releases to Forestier Peninsula consisted of captive-born devils (except for one wild-born, captive-raised animal). The first release consisted of 39 individuals from intensive (N = 10), MEEs (N = 23) and FREs (N = 6); with a sex ratio of 27:22 males:females and an age structure of age 1 (N = 12); age 2 (N = 15); age 3 (N = 8); and age 4 (N = 4). The second release comprised dispersing-age juvenile devils of sex ratio7:3 males:females; males were born in FREs and females in an intensive facility.

Post-release monitoring

All released animals were uniquely microchipped with ISO-standard companion animal injectable glass transponders, and had a unique bleach mark painted on their rump to enable visual identification of each devil on camera. Post-release monitoring involved the use of trapping, remote sensing cameras and microchip scanners setup at bait stations. Bait stations involved wallaby, possum or kangaroo carcasses being staked to the ground, and a camera set up to record visitations by devils. Narawntapu National Park had five bait stations set up in an east – west direction across the park, which were rebaited every week. Forestier Peninsula had eight bait stations that were rebaited weekly. Bait stations and the resultant camera and microchip scanners, remained in place for 12 weeks. Trapping trips took place over seven days using specially made devil traps made from polypipe tubing usually used for drainage (Mooney and Ralph, unpublished design). Forty traps were set out across the release site, with traps baited with lamb flaps or wallaby pieces. Traps were checked daily, and four trapping trips occurred over the 12 week post-release period. Each devil caught was given a full body health check including taking weight and recording any wounds. We used these three forms of monitoring (cameras, microchip scanners and trapping), to construct movement patterns of each devil following release. In addition, the Save the Tasmanian Devil Program has a local "hotline", which has a 24 hour response, to take public reports of roadkill and welfare cases of animals with advanced DFTD (state-wide).

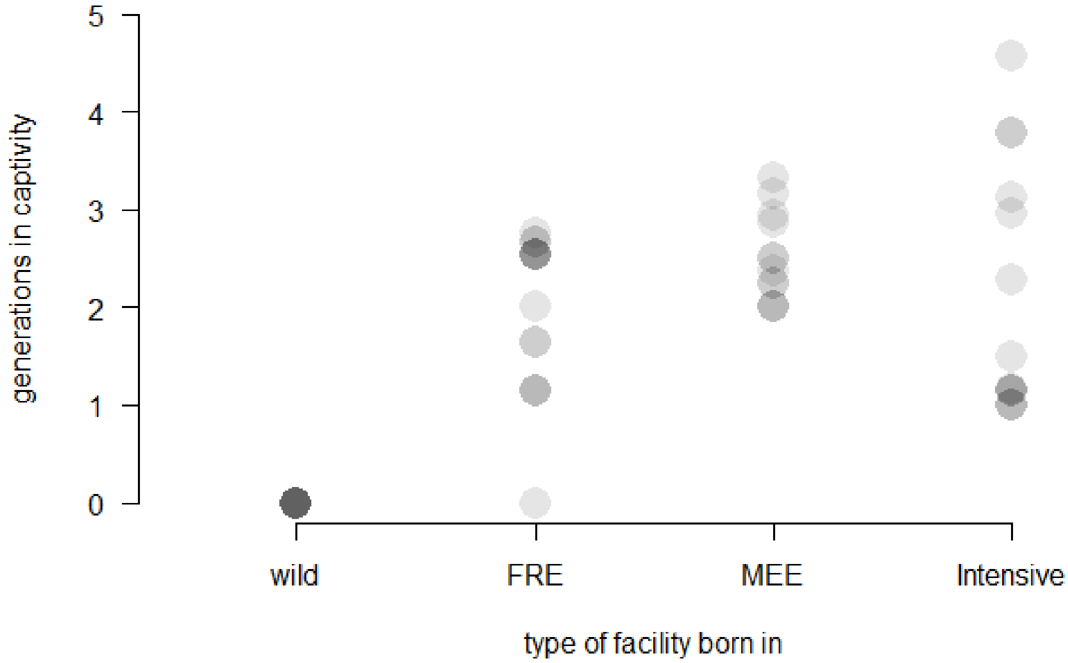
For our analysis we classified all released animals as "known road strike", "known survivor" and "unknown". Animals notified through the Save the Tasmanian Devil Program hotline were collected to enable confirmation of individual ID. All roadkill reports occurred within six weeks post-release, with the exception of one Forestier release animal killed by a vehicle after 14 weeks, and one Narawntapu release animal killed after nine months. The long period prior to roadkill reports for the latter two animals suggests that these events are unlikely to have been associated with initial exploratory behavior, therefore these two animals were considered to have "survived" the initial establishment period. Including these animals as "road strike" did not qualitatively change our results (data not shown). Other animals were designated as "known survivor" if they were observed via trapping, camera and microchip data (from both sites) up until the 4th of June, 2016 (most recent data at time of writing). Animals that were not recorded on any data type, beyond 1 day following release, were recorded as "unknown" and excluded from the analysis.

Model selection

To facilitate inference across predictors on different measurement scales, model predictors were standardised by subtracting the mean and dividing by 2 SD (following Gelman 2008). Model selection was conducted under an information theoretic approach, taking all submodels of the global model and averaging the top 2 AIC_C (corrected Akaike information criterion) by the conditional average method using the R-package MuMIn (Bartoń 2009). We report the final model effect sizes and their 95% confidence intervals (based on 1.96 × adjusted SE), as well as the relative importance of each parameter (RI): the posterior probability that a parameter is included in the best model, evaluated by summing the Akaike weights of all submodels containing that parameter. To facilitate interpretation, model fitted values were backtransformed onto the natural scale and 95% confidence intervals generated by parametric bootstrapping.

Literature cited in Supplementary Text

Supplementary Material



Supplementary Figure S1 Relationship between the type of facility an animal is born in, and its generational depth (*N* = 50). Four sites of origin are shown: wild, FRE (free-range enclosure; group housing with 10 to 20 individuals in 10 to 22 ha), MEE (managed environmental enclosure; group housing with 7 to 10 individuals in less than 5 ha) and intensive (zoo-based facilities containing one or two individuals), which together represent a continuum of increasing intensiveness and decreasing enclosure size (increasing housing density) (see also Hogg et al. 2015).

Supplementary Table S1 Evidence of behavior changes in response to captivity. Comments in parentheses indicate data collected over multiple generations.

	Behavior affected								
Species	Antipredator	Foraging	Competitive ability; aggression	Locomotion; activity	Spatial orientation; refuge use	Maternal care	Mate choice	Social behavior	References
Environmental effects of captive environment on behavior (captive versus wild)									
Rainbow darter (*Etheostoma caeruleum*)	*	*							Crane, Lampe & Mathis, 2015
Brown trout (*Salmo trutta*)		*	*					*	Sundstrom, Bohlin & Johnsson, 2004; Sundstrom & Johnsson, 2001; Sundstrom, Lohmus & Johnsson, 2003
Coho salmon (*Oncorhynchus*)			*						Swain & Riddell, 1990
Steelhead trout (*Oncorhynchus mykiss*)	*		*	*					Berejikian, 1995; Berejikian, Mathews & Quinn, 1996; Lee & Berejikian, 2008
Wild cod (*Gobiusculus flavescens*)		*							Steingrund & Ferno, 1997
Rhea (*Rhea americana*)	*								de Azevedo & Young, 2006
Parrots and Passerines (various species)	*								Carrete & Tella, 2015
Kangaroo rat (*Dipodomys heermanni*)	*								Yoerg & Shier, 1997
Black-tailed prairie dog (*Cynomys ludovicianus*)	*								Shier & Owings, 2006; Shier & Owings, 2007
Behavior modified in response to generations in captivity									

Invertebrates									
Coonstripe shrimp (*Panadalus danae*)	*(10)								Marliave, Gergits & Aota, 1993
Fish									
Crimson spotted rainbowfish (*Melanotaenia duboulayi*)								* (many)	Kydd & Brown, 2009
Atlantic salmon (*Salmo salar*)		*(many)							Thodesen *et al.*, 1999
Masu salmon (*Oncorhynchus masou*)	*(30)								Yamamoto & Reinhardt, 2003
Brown trout (*Samo trutta*)		*(many)							Sanchez *et al.*, 2001
Herps									
Mallorcan midwife toad (*Alytes muletensis*)	*(9-12)								Kraaijeveld-Smit *et al.*, 2006
Otago skink (*Oligosoma otagense*)				*(1-3)					Connolly & Cree, 2008
Birds									
Red junglefowl (*Gallus gallus*)	* (4)								Campler, Jongren & Jensen, 2009; Hakansson & Jensen, 2008
Mammals									
Golden lion tamarin (*Leontopithecus rosalia*)	*	*		*	*	*			Kleiman *et al.*, 1990
House mouse (*Mus musculus*)							*(3)		Slade *et al.*, 2014
Old field mous (*Peromyscus poloniotus*)	*(1-3)			*(1-3)	*(1-3)				McPhee, 2003; McPhee, 2004
Norway rat (*Rattus norvegicus*)									
Behavior loss in captivity									
Ratsnake (*Elaphe obsoleta*)		*							DeGregorio *et al.*, 2013
Kangaroo rat	*								Yoerg & Shier, 1997

(*Dipodomys heermanni*)		
Red-legged partridge (*Alectoris rufa*)	*	Gaudioso *et al.*, 2011

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Supplementary Table S2 Chart indicating the type of facility inhabited, across the lifespan, by devils released to the wild (green = wild, blue = FRE [free-range enclosure; group housing with 10 to 20 individuals in 10 to 22 ha], pink = MEE [managed environmental enclosure; group housing with 7 to 10 individuals in less than 5 ha], orange = intensive [zoo-based facilities containing one or two individuals]). Data are sorted first by road-kill outcome, then by generation (G).

				Type of facility inhabited at each age, prior to release				
ID	Sex	G	Road kill*	Age 0-1	Age 1-2	Age 2-3	Age 3-4	Age 4-5
1334	F	0.0000	0	green	green	orange	blue	green
1436	M	0.0000	0	green	orange	blue	blue	green
1437	M	0.0000	0	green	orange	blue	blue	green
1439	M	0.0000	0	green	orange	blue	blue	green
1447	M	0.0000	0	green	orange	blue	blue	green
1450	M	0.0000	0	green	orange	blue	blue	green
1456	F	0.0000	0	green	orange	blue	blue	green
1464	M	0.0000	0	green	orange	blue	blue	green
1763	M	0.0000	0	blue	green			
1341	F	1.0000	0	orange ‡	orange	orange †	green	
1342	F	1.0000	0	orange ‡	orange	orange †	green	
1346	F	1.0000	0	orange ‡	blue	blue	green	
1579	M	1.1500	0	blue	green			
1581	F	1.1500	0	blue	green			
1583	M	1.1500	0	orange	green			
1585	M	1.1500	0	blue	green			
1198	F	1.5000	0	orange	orange	orange †	green	
1591	F	1.6500	0	blue	green			
1688	M	1.6500	0	blue	green			
1434	M	2.0000	0	pink	pink	green		
1523	M	2.0000	0	pink	pink	green		
1555	F	2.2500	0	pink	pink	pink	green	
1810	F	2.2905	0	orange	green			
1641	F	2.3750	0	pink	green			
1749	M	2.5313	0	blue	green			
1762	M	2.5313	0	blue	green			
1764	M	2.7523	0	blue	green			
1628	F	2.9375	0	pink	green			
1360	M	2.9688	0	orange	orange †	green		
1816	F	3.7749	0	orange	green			
1818	F	3.7749	0	orange	green			
1449	M	0.0000	1	green	orange	orange	blue	green
1584	M	1.1500	1	orange	green			

1686	F	1.1500	1					
1592	F	1.1650	1					
1297	M	2.0000	1					
1476	F	2.0000	1					
1302	M	2.2500	1					
1300	M	2.5000	1					
1645	M	2.5000	1					
1744	M	2.5313	1					
1745	M	2.5313	1					
1750	M	2.5313	1					
1606	F	2.6667	1					
1608	M	2.6667	1					
1151	F	2.8750	1					
1662	M	3.1250	1					
1309	F	3.1667	1					
1629	F	3.3333	1					
1704	M	4.5714	1					
1445	F	0.0000	U					
1463	F	0.0000	U					
1145	F	1.0000	U					
1586	M	1.1500	U					
1537	M	1.5000	U		†			
1549	F	1.5000	U		†			
1690	M	1.6500	U					
1138	M	2.0000	U					
1225	F	2.0000	U				†	
1367	F	2.0000	U					
1526	M	2.0000	U					
1531	M	2.0000	U					
1533	M	2.2500	U					
1660	M	2.3750	U					
1635	F	2.5000	U					
1246	F	2.5234	U					
1631	F	2.6250	U					
1607	M	2.6670	U					
1649	F	2.7500	U					

* 0 = known to have survived, 1 = known to have been hit by a car, U = unknown fate

† devils were moved to a FRE for the 2 months prior to their release

‡ devils brought into the insurance population as pouch young, so although technically born in the wild, they did not become independent until they were already present in the insurance population.

Supplementary Table S3 Top model set (2 AIC_C) of generalized linear models for probability of fatal vehicle strike following release of captive Tasmanian devil (*Sarcophilus harrisii*); final model provided at Table 2.

Model*	AIC_C	ΔAIC_C	w_i
β_0 + G	63.117		0.244
β_0 + A + G	63.495	0.378	0.202
β_0 + S + G	64.491	1.374	0.123
β_0 + A + S + G	64.574	1.457	0.118
β_0 + Site(NNP) + G	64.851	1.735	0.103

* β_0 = model intercept; A = age; S = sex; G = generations in captivity; Site = release site, Forestier was the reference category

Supplementary Table S4 Final model results (after model averaging) under alternative assumptions regarding the 19 "unknown" fate animals excluded from the main analysis. Each model presents standardised effects of each predictor on the probability of fatal vehicle strike ($N = 69$)

Model	Predictor*	Effect size	Adjusted SE	95% CI	RI
Assuming all	β_0	0.228	0.261	-0.283; 0.739	
	G	1.541	0.679	0.211; 2.872	1.00
unknowns died	A	0.903	0.674	-0.418; 2.224	0.38
	Site(NNP)	0.552	0.678	-0.777; 1.881	0.20
Assuming all	β_0	-1.125	0.314	-1.741; -0.509	
	G	1.903	0.776	0.382; 3.424	1.00
unknowns survived	A	0.963	0.735	-0.478; 2.403	0.46
	S	0.704	0.622	-0.514; 1.923	0.40

* β_0 = model intercept; A = age; S = sex; G = generations in captivity; Site = release site, FP was the reference category

Supplementary Table S5 Final model (after model averaging) standardised effects of each predictor on the probability of fatal vehicle strike, excluding wild-born individuals where G = 0 (*N* = 40).

Predictor*	Effect size	Adjusted SE	95% CI	RI
β_0	-0.218	0.349	-0.901; 0.465	
G	1.306	0.780	-0.224; 2.836	0.74
A	1.199	0.795	-0.358; 2.756	0.60
S	0.868	0.757	-0.616; 2.352	0.32
Site(NNP)	1.119	1.073	-0.983; 3.222	0.23

* β_0 = model intercept; A = age; S = sex; G = generations in captivity; Site = release site, FP was the reference category