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Season, weight, and age, but not transmissible cancer, affect tick loads in the endangered Tasmanian devil

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ABSTRACT

The Tasmanian devil (*Sarcophilus harrisii*) is a carnivorous marsupial threatened by a transmissible cancer, devil facial tumour disease (DFTD). While we have a good understanding of the effect of the transmissible cancer on its host, little information is available about its potential interactions with ectoparasites. With this study, we aimed to determine the factors driving tick loads in a DFTD affected Tasmanian devil population, using long-term mark-recapture data. We investigated the effect of a range of life history traits (age, weight, sex, body condition) and of DFTD (time since DFTD arrival and presence of tumours) on the ectoparasitic tick load of the devils. Mixed effect models revealed that tick load in Tasmanian devils was primarily driven by season, weight, body condition and age. Young devils had more ticks compared to older or healthier devils. The reduction in Tasmanian devil population size over the past 14 years at the studied site had little effect on tick infestation. We also found that devils infected by DFTD had a similar tick load compared to those free of observable tumours, suggesting no interaction between the transmissible cancer and tick load. Our study highlights seasonality and life cycle as primary drivers of tick infestation in Tasmanian devils and the need for further investigations to integrate devil stress and immune dynamics with ectoparasite counts.

1. Introduction

Despite their impact on host fitness, parasites are beneficial in the promotion of biodiversity and are necessary for a balanced and healthy ecosystem (Hudson et al., 2006). Because parasites have key roles in ecological and evolutionary processes, they should also be considered as important for biodiversity conservation (Carlson et al., 2017; Kwak et al., 2020). Indeed, on one hand, there are specialist parasites that can only thrive in restricted species and conditions, and thus depend on the survival of the host species that they co-evolved with. The decline or extinction of host species could, therefore, induce the loss of the associated parasitic fauna (Spencer and Zuk, 2016). On the other hand, some parasites could pose a threat for endangered species by reducing their fitness, via the transmission of diseases or weakening the health of their hosts, which would affect conservation efforts and could cause

population declines (Gómez and Nichols, 2013). For example, arthropod vectors, like ticks, transmit a plethora of animal pathogens (bacteria, viruses, protozoa and helminths), and pose as significant threat to vertebrate hosts, including wildlife, pets, livestock and humans (reviewed in Beard et al. (2021b)). Thus, monitoring parasites in the wild is important, especially when they or their hosts are endangered, rare, or endemic species (McCallum and Dobson, 1995). Tasmanian devils are the largest extant carnivorous marsupial, and the apex predator in Tasmania's ecosystems (Hollings et al., 2015). The main threat to the species is the spread of a lethal transmissible cancer that causes devil facial tumour disease (DFTD) (Pearse and Swift, 2006). DFTD was first detected in 1996 in Northeast Tasmania and has now spread across the island (Fig. 1), causing an 82% decline in local densities and reducing the total population to ~16,000 individuals (Cunningham et al., 2021).

Tasmanian devils host a variety of parasites, both endoparasites such

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as nematodes, platyhelminths or protozoa, as well as ectoparasites, including mites, fleas and ticks (Wait et al., 2017). Among those parasites, ticks are of particular interest, as they are vectors of disease agents to humans and animals, and can use wild animals as reservoir hosts (Jonjejan and Uilenberg, 2004). Indeed, ticks can serve as vectors to blood parasites, such as the protozoa *Hepatozoon* spp., *Theileria* spp., *Babesia* spp., or bacteria *Rickettsia* spp. which spread via the bloodstream (Beard et al., 2021a, 2021b; Dantas-Torres et al., 2012; Egan et al., 2020; Munns, 2018; Vilcins et al., 2005; Vilcins et al., 2009). Both pathogen types have been shown to impact host immune responses and survival (Djokic et al., 2018; Norval et al., 1991; Sahni et al., 2019; Ujvari and Madsen, 2006). Additionally, in Australia, some tick species (i.e. *Ixodes holocyclus* and *Ixodes cornuatus*) are of particular concern as they can cause paralysis in humans and some domestic animals, not usually observed in native hosts (Eppleston et al., 2013). As ticks have the potential to carry debilitating pathogens to both humans and domestic animals, it is important to monitor tick infestations and their interactions with native marsupials (Beard et al., 2021b).

Co-infections and co-infestations with different pathogens or parasites occur not only within the same host (Petney and Andrews, 1998) but also in the vector, as seen in ticks from bare-nosed wombats (*Vombatus ursinus*) (Beard et al., 2021a). Indeed, multiple parasites co-infecting the same host can interact in synergistic or antagonistic ways and impact host susceptibility to other parasites, parasite transmission, symptoms, and duration of the infection (Vaumourin et al., 2015). For example, canine vector-borne co-infections by *Ehrlichia canis* and *Hepatozoon canis* have been shown to impair immune function and host immune responsiveness (Baneth et al., 2015).

While the impact of co-infections with multiple parasites on host immune system has previously been studied, little is known about whether being affected by transmissible cancers (where cancer cells are the infectious agents behaving like a parasitic species) (Dujon et al., 2020; Pearse and Swift, 2006) would influence susceptibility to parasites. Tasmanian devils provide a unique opportunity to study the associations between a transmissible cancer cell line and ectoparasites such as ticks, in order to determine the impact of co-infestation on an endangered species (Dujon et al., 2021).

While currently 79 tick species are recognised in Australia (reviewed in Beard et al. (2021b)), only three species of ticks have been recorded

on Tasmanian devils: *Ixodes feicalis*, *Ixodes tasmani* (common marsupial tick), and *I. cornuatus* (southern paralysis tick) or *I. holocyclus* (paralysis tick) (Wait et al., 2017). A recent study by Munns (2018) found that *I. tasmani* was the sole species observed on Tasmanian devils at our study site, in northwest Tasmania, while various levels of co-infestations of *Ixodes* sp., *I. cf. feicalis*, *I. feicalis* and *I. cf. tasmani* were observed in Tasmanian devils from the Western, Eastern and Northern parts of Tasmania. A comprehensive study conducted on bare-nosed wombats at the same location also only identified the presence of *I. tasmani* (Beard et al., 2021a). These tick species have a broad range of hosts, including a number of marsupials (Beard et al., 2021a, 2021b), domestic animals, and cattle (Barker and Walker, 2014; Beveridge and Spratt, 2003). Little is known regarding the life cycle specificities of *I. feicalis*. *I. tasmani*, is suggested to be a nidicolous species that can complete multiple life cycles per year (Murdoch and Spratt, 2006).

The prevalence (number of hosts infested with ticks) and intensity (number of ticks per infested host) of ticks, and their effect on host fitness has been studied in some marsupials (Beard et al., 2021a, 2021b; Gemmell et al., 1991; Murdoch and Spratt, 2006), but the current knowledge underlying tick infestation in Tasmanian devils is limited (Wait et al., 2017). Epidemiological studies have linked parasite prevalence with an increased cancer incidence in animals and humans (Thomas et al., 2012; Vickers et al., 2015). For example, ear mites (*Otodectes cynotis*), a predisposing factor for ceruminous gland tumours, causes high cancer rates in the endangered Santa Catalina Island foxes (*Urocyon littoralis catalinae*) (Vickers et al., 2015), while infection with the protozoan parasite *Toxoplasma gondii* was associated with a close to 2-fold increase in the risk of brain cancers in humans (Thomas et al., 2012). Other studies have shown that parasites can also negatively regulate cancer development and progression by activating immune responses, such as inducing apoptosis and impairing metastasis and angiogenesis (reviewed in Callejas et al. (2018); Jacqueline et al. (2017)). Similar interactions might exist in devils, and the extent to which co-infection with DFTD and ticks might impact cancer progression in devils is unknown. For example, human cancer studies have shown that tumours both directly and indirectly pose elevated energetic demands on the host via increasing inflammation and causing changes in body composition by the activation of brown adipose tissue (reviewed in Purcell et al. (2016)). Increased energetic burden may limit the host's

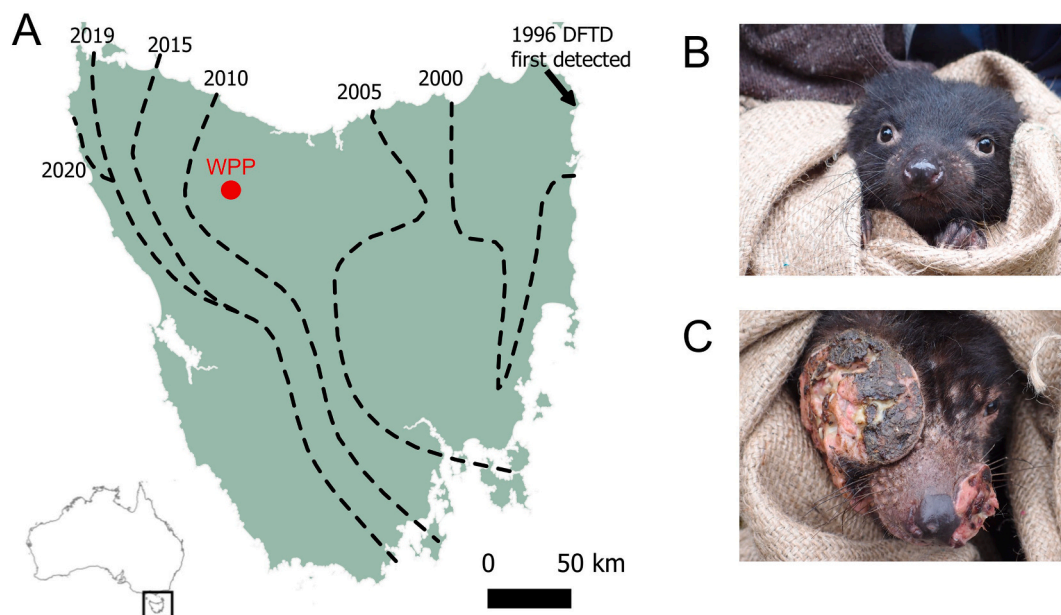


Fig. 1. Devil Facial Tumour Disease spread in Tasmania. (A) Map of Tasmania showing the increase of the geographical extent of the disease over time. West Pencil Pine (WPP) is indicated in red. (B) Picture of a healthy Tasmanian devil. (C) Picture of a Tasmanian devil suffering from advanced DFTD. Pictures by Frédéric Thomas. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

capacity to partition resources among physiological processes and to respond to intrinsic and extrinsic challenges (Jacqueline et al., 2016). As similar immune pathways are involved in protection against infectious agents and cancer cells, any disequilibrium in immune system homeostasis may enhance or constrain cancer cell proliferation (Dunn et al., 2004) and alter parasite prevalence and impact (Jacqueline et al., 2017). For example, chronic infections by parasites may suppress T cell immunosurveillance and facilitate cancer growth, as seen in human cancers (reviewed in van Tong et al. (2017)). By generating an inflammatory environment DFTD infection could also interfere with transmission and progression of other parasites through partial cross-immunity or immune facilitation, as documented between non-oncogenic pathogens (Gupta et al., 1998; Mideo, 2009). In particular, tick loads (such as *I. feicalis*, *I. tasmani*, and *I. cornuatus* or *I. holocyclus*, (Wait et al., 2017)) could potentially be influenced by DFTD, and if devils with DFTD display an increased number of ticks, they could be more exposed to tick-borne pathogens (such as *Rickettsia* spp., *Hepatozoon* spp. *Theileria* spp., *Babesia* spp., (Munns, 2018; Vilcins et al., 2009)), which could further impair their health.

The aim of the study was to assess the effects of season, host demography, density and DFTD status (healthy or infected) on tick load. Based on 13 years of mark-recapture data, statistical modelling was used to identify the drivers of tick intensity in a Tasmanian devil population. The effects of season, time since DFTD arrived to the study site, host age, sex, weight, body condition and DFTD status on tick loads were modelled and assessed.

2. Materials and methods

2.1. Study site and data collection

Tasmanian devils were captured at West Pencil Pine (WPP), a sub-alpine region in northwest Tasmania (41° 31' S, 145° 46' E) (Fig. 1). The trapping site is a 25km² area situated on private production forestry land that includes native and non-native eucalypts for commercial harvesting, dominated by shining gum (*Eucalyptus nitens*) (Fraik et al., 2019). Rainfall is highest in July and lowest in February (decile 5 (median) rainfall: 247.4 mm and 84.6 mm respectively) and mean minimum temperature is lowest in July and highest in February (0.8 °C and 7 °C respectively) in the area (recorded at the closest weather station: Waratah (Mount Road), site number: 097014, latitude: 41° 44' S, longitude: 145° 53' E, Elevation: 609 m, www.bom.gov.au). The devil population at this site has been regularly monitored since DFTD was first detected in 2006. The animals were captured following a mark-recapture framework for 10 nights (see Hamede et al. 2012). The capture sessions were conducted four times a year, to coincide with major life-history events of the devils: February (summer), when the juveniles just became independent and the mating season begins; May (autumn), around the end of the mating season; August (winter), when females usually have young in their pouches; and November (spring), when females are usually in late lactation (Hamede et al., 2012). All devils were individually marked with a microchip (Allflex, New Zealand), and their sex, age, weight, body size, were determined. Disease status was assessed by histopathological examination of tumour biopsies or by visual inspection of tumours (see Hawkins et al. 2006) for visual detection methods), and when tumours were present, their maximum width, length and depth were measured to the nearest mm using a calliper. An estimation of tick load on the host was determined by tallying the number of ticks recorded on the head, ears, tail, limbs and groin (with most of them being located on the ears and tail). As our study used a database collected over 13 years by several researchers, recording the life stages and exact species of individual ticks was beyond the scope of the study, and thus were not determined. Thirty-five animals for which tick number, weight, head width or tumour volume had not been recorded were excluded from the analysis. Individuals younger than two years old (the usual age of sexual maturity (Jones et al., 2008)) were

classified as juveniles, the others were considered as adults. Tumour volume (V), expressed in mm³, was calculated using the ellipsoid volume formula: $V = \frac{4\pi}{3} \times \left(\frac{\text{length}}{2}\right) \times \left(\frac{\text{width}}{2}\right) \times \left(\frac{\text{depth}}{2}\right)$. When animals had several tumours, the sum of the volumes of all tumours was calculated.

2.2. Population size estimation

The population size was estimated using the MARK Software and RMark package (Laake, 2013), using a Jolly-Seber POPAN open population model, with constant recapture rates and time variation in both survival rates and probability of entry parameters.

2.3. Residual body mass computation

To estimate the body condition of the devils, a residual body mass index was calculated (Schulte-Hostedde et al., 2005). This index is based on the relationship between weight and body size, here represented by the head width (measured in mm). A mixed effect model was fitted to take into account the multiple measurements per individual, and the residuals of the linear regression between the log-transformed weight and the head width were computed for each individual. Individuals with a positive residual body mass are considered to be in better body condition than the ones with a negative index.

2.4. Statistical analysis

Since individuals were captured several times, the number of ticks per individual were not independent, therefore, a series of generalized linear mixed models (GLMMs) was used (Zuur et al., 2009a). These models contain both fixed effects and random effects, and the individuals (recognised via their unique microchip identifier) are considered as a random intercept effect: the model fits a different intercept for each individual, while the calculated slopes are the same for all individuals. The variables initially included in the analyses were the capture month, population size, host age group, sex, weight, residual body mass, host disease status and time since appearance of DFTD. However, preliminary exploration of the data indicated that population size (computed using the POPAN model) and time since DFTD appearance were strongly correlated (Spearman's rho $r_s = 0.91$). This indicates that the time elapsed since the arrival of DFTD to the study site (in 2006) is a good proxy of population size. We also observed that population size estimates had relatively large confidence intervals for the last years of the time series because the population size shrunk due to DFTD-induced mortality, which made population size more difficult to estimate. Therefore, raw population size data was not included in the analyses and, instead, we used the number of years since the arrival of DFTD as a proxy of population size, as it had no associated confidence interval (i.e. it is a precise measurement) which made it more suitable to use as an explanatory variable in a GLMM (Zuur et al., 2009a). As the Tasmanian devils were surveyed every four months, the sampling design allowed us to consider the month of year as a continuous variable (rather than categorical summer/winter variable) and to include cyclicity in the model (the sampling frequency being twice the seasonal cycle frequency). This also allowed us to account for more inter-individual variability (variance) between Tasmanian devils compared to a simpler summer/winter categorisation. A zero-inflated Poisson distribution model was used so as to account for over dispersion and the elevated proportion of measurements with a null count of ticks (34.8%) (Zuur et al., 2009b). Tumour volume was transformed as $\log(x + 1)$ to ensure normality of residuals. DFTD status was coded as a binary factor (healthy and infected). Capture months were transformed as $\cos(2\pi \cdot \text{month}/12)$ to model seasons as a cosine wave and obtain a linear relationship between number of ticks and capture month (as per Stoltwijk et al. 1999).

2.5. Model selection and validation

The number of ticks in Tasmanian devils was first quantified using a model including all the explanatory variables, as well as interactions between month and each continuous variable (time since DFTD first detection, weight, residual body mass and tumour volume). This model was then simplified via deletion tests as described by [Crawley \(2015\)](#). The least significant terms were removed so as to only keep the explanatory variables that significantly improved the fit of the model. To estimate the fit of the models and determine the best ones, the Akaike Information Criteria (AIC) and the Akaike weight (wi) were used ([Symonds and Moussalli, 2011](#)). For all models, model validation tests were run to identify potential violations of the assumptions of the models. Homogeneity of variances was investigated from scatter plots of residuals versus fitted values and residuals against each explanatory variable in the model. Normality of residuals was interpreted from quantile–quantile plots and from residual histograms. In addition, effect sizes were visualized by computing the marginal effects of each variable included in the final model. Marginal effects measure the effect that a change in a given explanatory variable has on the predicted number of ticks, when all the other covariates are held constant. All model assumptions were fulfilled. The package glmmTMB ([Brooks et al., 2017](#)) was used to implement the zero-inflated Poisson GLMMs, and MuMIn ([Bartoń, 2020](#)) and bbmle ([Bolker et al., 2020](#)) were used to compute AIC scores and Akaike weights. Since the computation of *p*-values is not reliable for mixed effect models (see [Halsey et al. \(2015\)](#); [Zuur and Ieno \(2016\)](#)), effect sizes are considered significant when the 95% confidence intervals do not overlap. All analyses were implemented using R version 3.6.3 ([R Core Team, 2020](#)) and RStudio 1.3.1056 ([RStudio Team, 2020](#)).

3. Results

Altogether, 630 animals were captured between 2007 and 2019, for a total of 2133 observations. Observations for which there were missing values in one of the measurements were removed, therefore the analysis was conducted on 595 Tasmanian devils (320 females and 275 males), for a total number of 1732 capture events (985 for females, 747 for males). There were as many observations of juvenile and adult devils ([Table 1](#)). 62.2% of the devils were recaptured at least once over the course of the study. It was observed that 65.2% of the devils had ticks when they were captured.

3.1. Model selection

To model the number of ticks in Tasmanian devils, several models including different combinations of explanatory variables and of interactions were formulated ([Table 2](#)). The best model included devils' residual body mass, weight, age group and tumour volume, season, time

Table 1
Mean, standard deviation, and range of the weight and tick loads of the Tasmanian devils included in the analysis. s.d = standard deviation.

	Weight (kg)			Tick load (number of ticks per devil)		
	Mean	s.d	Range	Mean	s.d	Range
Juveniles (n = 866)	5.6	1.6	[1.0, 13.0]	8.2	10.1	[0, 72]
Female adults (n = 513)	6.4	0.89	[4.1, 9.7]	4.8	7.3	[0, 78]
Male adults (n = 353)	8.4	1.3	[5.1, 12.4]	3.9	6	[0,33]
All adults (n = 866)	7.2	1.4	[4.1, 12.4]	4.4	6.8	[0, 78]
Overall (n = 1732)	6.4	1.7	[1.0, 13.0]	6.3	8.8	[0, 78]

Table 2
Model selection based on AIC and Akaike weight criteria. The models are sorted from best to worst fit.

Competing models ¹	Age	Weight	Month	Weight * Month	Time since DFTD appearance	Time since DFTD appearance * Month	Tumour volume	Residual body mass	Sex	Cancer	Tumour Volume * Month	Residual body mass * Month	AIC ²	ΔAIC	oi ³
1													9758.52	0.00	0.34
2					+		+	+					9759.18	0.66	0.24
3						+	+	+					9760.05	1.53	0.16
4						+	+	+	+				9760.80	2.29	0.11
5						+	+	+		+			9761.45	2.93	0.08
6						+	+	+			+		9762.70	4.18	0.04
7						+	+	+				+	9764.11	5.59	0.02
8						+	+	+				+	9764.70	6.18	0.02

A plus sign (+) means that the explanatory variable is included in the considered model. An asterisk (*) represents an interaction between two explanatory variables.
¹ Fitted zero-inflated Poisson generalized linear mixed models.
² Akaike Information Criteria (AIC), a lower score indicates a better fit of the model.
³ Akaike weight of each tested model relative to all the models fitted for the analysis.

since DFTD first detection, and the interactions between time since DFTD first detection and season, and between weight and season (Table 2).

3.2. Effect of season on the number of ticks

Seasonality is the main driver of the number of ticks in the WPP population (Table 3, Fig. 2). Tasmanian devils host about ten times more ticks during summer than winter, (Fig. 2.A-2.E), and the peak of abundance is predicted in December (Australian summer), with a predicted marginal count of 11.28 ticks per devil (mean = 11.30, 95% CI [10.43,12.24]), while the lowest number of ticks is observed in May (Australian autumn) and July (Australian winter) (mean = 1.18, 95% CI [1.02,1.36]).

3.3. Effect of the weight on the number of ticks

The devils' weight is a significant contributing factor that drives the number of ticks (slope estimate = 0.90, 95% CI [0.87,0.93]). The predicted tick load decreases when the weight increases. The contribution of the body weight to the total number of ticks is three times more important for light animals (1 kg) compared to heavier ones (10 kg; Fig. 2.B). Overall, lighter devils have a higher tick load than heavier devils at any month of the year. In addition, there is a significant interaction between season and weight of the devils on the number of hosted ticks (Fig. 2.A, Table 3).

3.4. Effect of the residual body mass

The residual body mass of the devils, representative of their body condition, does not appear to significantly impact tick loads (slope estimate = 0.74, 95% CI [0.55,1.01]). Low residual body mass values (i.e. poor body condition indexes) are associated with a predicted number of ticks that is 1.5 times higher than for individuals with high residual body mass values (Fig. 2.C).

3.5. Effect of DFTD on the number of ticks

Tumour volume has only a relatively small effect on the number of ticks (slope estimate = 1.01, 95% CI [1.00,1.02]), as devils with a high tumour load are predicted to have an additional 0.5 ticks compared to tumour-free devils (Fig. 2.D).

3.6. Effect of the time since DFTD arrival on the number of ticks

The less significant terms of the model were the time since DFTD arrival and the interaction between time since DFTD arrival and season (Table 3). The interaction between time since DFTD first detection and

season is negligible, as seasonal variation follows nearly the same pattern when time after DFTD first detection is both accounted for, or not (Fig. 2.E). Over the entirety of the 14 years since the first detection of DFTD to the study site, the average number of ticks per devil only increased by 0.6 ticks (Fig. 2.F), which suggests that the time since DFTD first detection does not have a pronounced impact on the number of ticks.

3.7. Effect of the age on the number of ticks

The age group of the devils seems to have a significant impact on the number of ticks (slope estimate = 1.31, 95% CI [1.21,1.41]). Indeed, juveniles are expected to have 1.15 additional ticks than adults, on average (Fig. 2.G).

4. Discussion

The results strongly suggest that the number of ticks in Tasmanian devils is mainly driven by seasonality, host weight, body condition, and host age. Tumour volume, as well as the interaction between weight and season, appear to have a marginally significant effect on the number of ticks. There is a very limited effect of time since DFTD first detection (a proxy of population size) on tick load, and no apparent interaction with the season, while disease status and sex of the animals do not impact tick loads, and were excluded from the model during the model selection process.

Overall, Tasmanian devils have more ticks during spring and summer, with a peak intensity between November and January. While tick species were not determined in our study, we assumed that the studied ticks belonged to the *I. tasmani* species, as this is the sole species previously reported at our study site (Beard et al., 2021a; Munns, 2018). It is important to note that the study by Munns (2018) was conducted in Autumn 2018 and involved nine Tasmanian devils, while Beard et al. (2021a) examined only ticks species from bare-nosed wombats between January 2020 and 2021, therefore, we cannot exclude the potential presence of other tick species during other seasons and years at our study site. The observed seasonal variations in tick numbers are consistent with previous findings for *I. holocyclus* in bandicoots (*Isodoom macrourus*) (Doube, 1979; Gemmell et al., 1991; Roberts, 1960), but, interestingly, is in contrast to a study on *I. tasmani* in possums (*Trichosurus vulpecula*) in New South Wales where higher tick prevalence was observed during winter compared to summer (Murdoch and Spratt, 2006). It is possible that due to local environmental conditions ticks only breed once per year in the WPP area. Conditions such as an appropriate climate, high humidity, type of vegetation, and vegetation use, are thought to influence tick activity, questing, and abundance (Wall et al., 2007), thus, geographic and host differences may explain the dichotomy between our and previous studies. Being an exophilic, non-nidicolous exoparasite (Murdoch and Spratt, 2006), *I. tasmani* can maximise contact with hosts and minimise the risk of desiccation by hiding in the buffered micro-environment of devils' dens. Tasmanian devils, being nocturnal, may be exposed to ticks in their burrows during daytime or to questing ticks at night when they are searching for food (Carroll et al., 1998; Durden et al., 1996). Our study site is on a sub-alpine region, where the highest average precipitation and lowest temperatures occur during autumn and winter (June – September). Based on climatic conditions at our study site, we propose that devils most likely encounter ticks in their burrows, as observed in common brushtail possums (*T. vulpecula*), where a female acquired multiple ticks without leaving her den (Murdoch and Spratt, 2006). In addition, during the warmer and drier periods of summer, questing may occur at night when ticks can avoid desiccation while searching for a host (Barker and Walker, 2014). Oorebeek and Kleindorfer (2008) suggested that, in parallel with climate, host availability influences the seasonal population dynamics of ticks and also showed that tick prevalence corresponded with the breeding season of birds, when host density was highest. For Tasmanian devils, we show that

Table 3

Slope values for the explanatory variables of the best model.

Explanatory variable ¹	Slope estimate ²	95% CI ³
Month ⁴	1.81	[1.37,2.39]
Age group	1.31	[1.21,1.41]
Residual Body Mass	0.74	[0.55,1.01]
Weight	0.90	[0.87,0.93]
Weight * Month	1.07	[1.06,1.11]
Time since DFTD appearance * Month	1.04	[1.02,1.06]
Tumour volume ⁵	1.01	[1.00,1.02]
Time since DFTD appearance	1.01	[0.99,1.03]

¹ Explanatory variables included in the best model.

² Estimate of the slope for each variable. A slope equal to 1 indicates that the variable has a negligible effect in the model.

³ 95% confidence interval calculated from the model.

⁴ cos-transformed month.

⁵ log-transformed tumour volume.

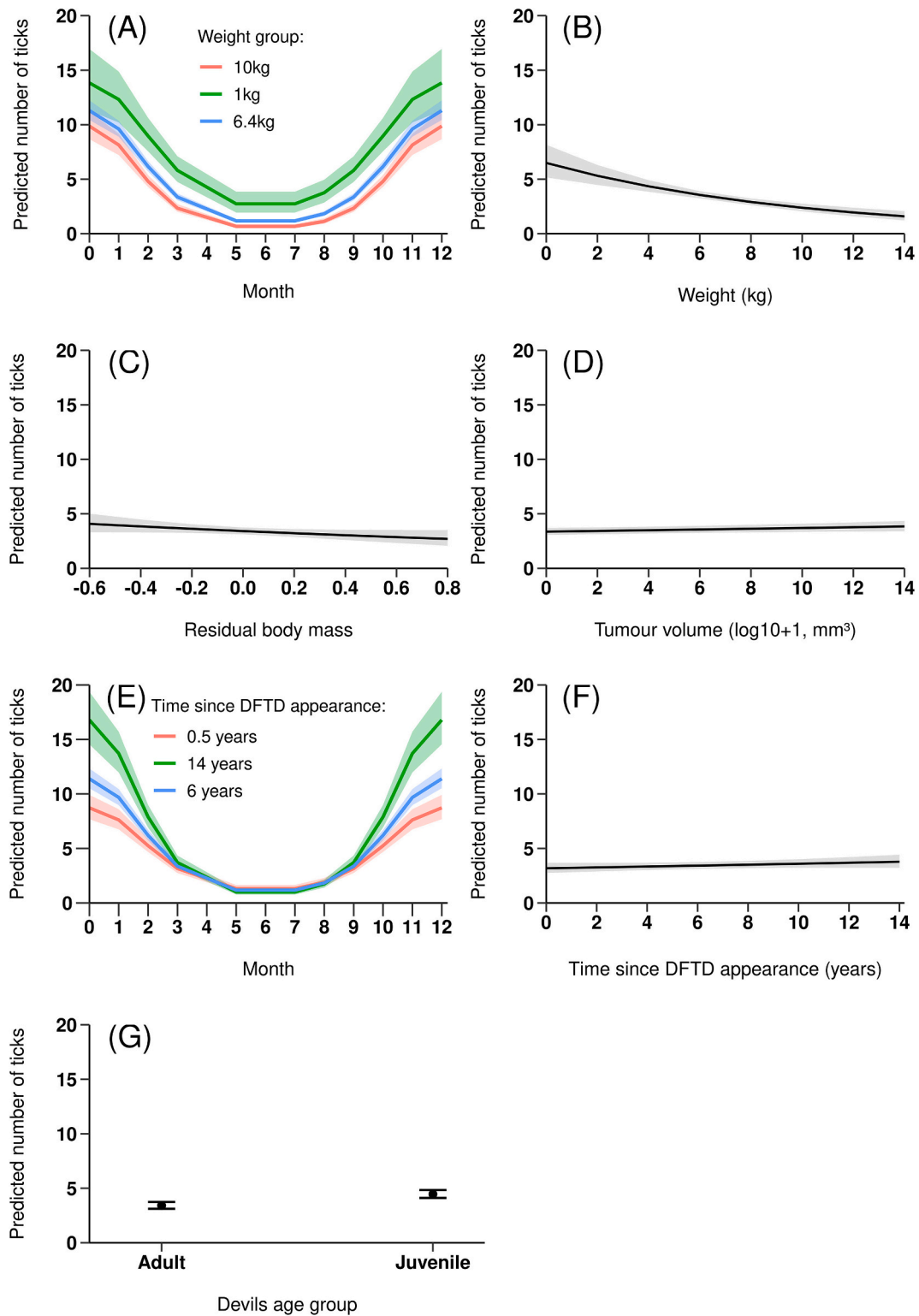


Fig. 2. Marginal effects of the explanatory variables on the number of ticks estimated using a mixed effect model. Plots of the marginal effects of each explanatory variable from our best model and their predicted impact on the number of ticks. For each plot, all other covariates from the model are held constant and equal to the population average value. (A) Marginal effects of the interaction between the month of capture and the weight (in blue, 6.4 kg corresponds to the populations' average weight), (B) of the devils' weight, (C) of the devils' residual body mass, (D+) of the devils' tumour volume, transformed as $\log_{10}(\text{tumour volume}+1)$, (E) of the interaction between the month of capture and the time since DFTD first detection, (F) of the time since DFTD first detection (here used as a proxy of population size), (G) of the devils' age. The bands in (A-F) and the black bars in (G) represent the 95% confidence interval of the marginal effects. The difference between Adult and Juvenile age groups in (G) is significant (confidence intervals do not overlap). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

population size (here estimated using the time elapsed since the first detection of DFTD, see material and methods) does not have a strong effect on tick numbers; but the young start to venture out of the den in late spring / early summer, and late summer is when they become independent (Guiler, 1970b). Spring and summer thus coincide with a peak in the number of juveniles, that seem to be the preferred hosts for ticks, which could also explain why average tick loads are overall higher during these seasons. It is also very likely that ticks are transferred from mothers to young devils in the den, and that this is an important contributor of tick loads in young and dispersing devils. A previous study observed only adult ticks during autumn at our study site (samples were only collected during autumn), but found both nymph and adult ticks infesting Tasmanian devils throughout the year at other locations in Tasmania (Munns, 2018). Laboratory experiments demonstrated that *I. tasmani* can complete a full life cycle within four months, although the life cycle can be accelerated under artificial conditions (Oliver, 1989). Field data by Munns (2018) indicate the potential presence of all life stages throughout the year. We, therefore, propose that the observed seasonal variation in tick infestation is most likely driven by the seasonal variations in host activity at our study site.

The significant seasonal variation in tick loads can have further implications for the host's immune system, as seen in juvenile northern brown bandicoots (*Isodon macrourus*) (Gemmell et al., 1991). Bandicoots released into tick-infested enclosures had lower growth rate, reduced haematocrit value, but higher level of white blood cells compared to bandicoots in tick-free enclosures. These results indicate a negative impact of tick infestation on the health of juvenile northern brown bandicoots (Gemmell et al., 1991). Furthermore, seasonal variation in parasite load has also been suggested to cause seasonal changes in host immune function in some bird species (Møller et al., 2003). In the case of Tasmanian devils, it has been shown that they undergo temporary immunosuppression during periods of stress, such as the mating season (Kreiss et al., 2008), and it is known that the immune response of the devils attenuates during their life, in particular because of physiological changes at puberty (Cheng et al., 2017; Cheng et al., 2019). Varying tick loads could further contribute to shaping the immune function of the host, and result in devils having an immune system more or less efficient according to the season and period of life, which could affect the response against DFTD or other pathogens. Future studies would be necessary to investigate seasonal changes in devil immune function and tick infestation.

Body weight is an important driver of tick loads in Tasmanian devils. Although Tasmanian devils are sexually dimorphic, with adult males being larger than females (7–13 kg and 4–9 kg respectively (Guiler, 1970a)), we found no sex specific differences in average tick load. The observation that light devils have more ticks than heavy devils could reflect the relationship between host age and weight, considering that young Tasmanian devils are on average smaller and lighter than adults, and have more ticks. It is known that the devils' weight varies throughout the year and usually reaches a maximum in spring (Guiler, 1970a). The interaction between weight and season appears to have a significant effect on the number of ticks. Indeed, we observed that the age category of the devils had little impact on the average tick load but found a significant interaction between the weight of Tasmanian devils and the season; lighter individuals (younger) have higher tick infestation during all times of the year compared to heavier (older) devils. This interaction can be an artefact due to the host life cycle (in early summer juveniles are weaned and leave the dens more often, hosting ticks transferred from their mother). A difference in weight in summer seems to have a stronger effect on the number of ticks than in winter. Indeed, in winter, the tick loads stay low and are similar for different weight values, which could reflect a predominance of the seasonal effect over the other factors: when there is an overall low number and activity of ticks because of the season, such as in winter when the other factors do not greatly contribute to the variation in tick loads.

Another conclusion of our study is that young devils have a higher

tick load than adults. This could be confounded with the weight, as generally young devils are lighter and adult devils are heavier. These findings agree with the common observation that young hosts are often more affected by ectoparasites (Lehmann, 1993). Among marsupials for example, juvenile bandicoots were shown to be particularly vulnerable to tick infestation (Gemmell et al., 1991). A reason for this could be that juveniles tend to spend more time in and/or around the dens, especially from August to November, which is where exophilic, nidicolous ticks (such as *I. tasmani*) quest for hosts (Barker and Walker, 2014; Munns, 2018; Murdoch and Spratt, 2006). It is also possible that juveniles have a naiver immune system than adults (see further explanation below), and are thus less capable of defending themselves against parasites, as it has been suggested for young ruminants (Colditz et al., 1996), or reptiles (Madsen et al., 2005). As Tasmanian devils are apex predators with a low extrinsic mortality risk (Hollings et al., 2015), ageing theories predict that selection will favour the investment into immune function maintenance and parasite and disease resistance in juveniles (Kirkwood and Austad, 2000). Thus, potentially only individuals able to resist high parasite loads may survive to adult age, as observed in water pythons (*Liasis fuscus*) (Madsen et al., 2005). In addition, Cheng et al. (2017) demonstrated that, in their second year of life, Tasmanian devils experience a shift from T-helper 1 immune response (involved in anti-cancer immunity) towards T-helper 2 immune response (more involved in antiparasitic immunity). Other major immunological changes occur during puberty, in particular a decline in T-cell repertoire diversity (Cheng et al., 2019). These observations, in combinations with those of Ujvari et al. (2016), suggest an age-related decline in immune functions of Tasmanian devils, that particularly impacts anti-cancer immune capacity in adults. Clearly, the immune responses involved in antiparasitic and anti-cancer immunity are very complex, and further studies would be necessary to decipher the underlying immunological mechanisms of tick infestations and DFTD infection.

Our results suggest that population size in WPP does not affect tick loads. This is in contradiction with studies conducted in diverse host and parasite species that showed a positive relationship between host population size or host density and parasite abundance (Aneberg et al., 1998; Bagge et al., 2004). As the *Ixodes* ticks that parasitize devils are generalist species, they have a broad range of hosts in Tasmania (Barker and Walker, 2014), thus, it is possible that the number of available devils does not affect the population of ticks, as they can still thrive by feeding on other hosts inhabiting the same area (WPP holds a large density of browsers, including, but not limited to, wallabies, pademelons, possums, and bandicoots (Hamede, R. pers. com.)). As the same tick species was identified on bare-nosed wombats, a species that is abundant at the sampling location (Beard et al., 2021a) and exhibits very similar burrowing behaviour to devils, it suggests that bare-nosed wombats most likely present as alternative hosts for ticks when devil numbers decline. This would explain why the average number of ticks per devil did not significantly change throughout the years, despite the observed population decline. The interaction between season and population size has a negligible effect, although it is possible to notice that this interaction is stronger from November to January (Fig. 2.E).

The results suggest that in Tasmanian devils, the sex of the host does not influence tick loads. This is in contrast with the widespread observation of a sex-biased parasitism across a range of mammals, where males are often subject to higher parasite loads than females (Gallivan et al., 1995; Harrison et al., 2010; Zuk and McKean, 1996). Two causes, ecological and physiological, are usually invoked to support the sex-specific differences (Zuk and McKean, 1996). In devils, there is a sex-bias in the tolerance to DFTD, with males having a lower tolerance than females to the cancer that is considered as an obligate internal parasite (i.e. depends on the presence of the host to survive) (Ruiz-Aravena et al., 2018). This could indicate that Tasmanian devils are able to generate sex specific responses to cancer progression, but both sexes are equally impacted by ectoparasites, like ticks.

For the first time, we have shown that infection with DFTD in

Tasmanian devils does not increase tick loads. Furthermore, tumour volume only had a marginal effect on the number of ticks, with devils with larger tumour loads only predicted to have an additional 0.6 ticks. One explanation may be that our results are biased by the higher tick loads observed in juveniles, that have not yet been exposed to DFTD. Wells et al. (2017) demonstrated that devils with a relatively high fitness are the most likely to become infected by DFTD because they more often engage in fights for mating, which increases the risk of transmission of the disease between individuals. This could explain that the devils infected with DFTD are not necessarily the most susceptible to other pathogens or infections, including parasite infestation. As DFTD is a transmissible cancer, it favours the use of immune evasion strategies over immune suppression to avoid being recognised by the devils' immune system (Siddle et al., 2013). This strategy could explain the observed limited effect of DFTD on the number of ticks, which can be beneficial for the survival of the species. Only one type of ectoparasite was considered in this study, thus it cannot be excluded that DFTD could have an (reciprocal) impact on other types of parasites and pathogens, particularly on endoparasites, that may be more greatly influenced by host immune responses. As ticks act as vectors of blood pathogens (*Rickettsia* spp., *Hepatozoon* spp., *Theileria* spp., *Babesia* spp., (Munns, 2018)), further studies should perform analyses similar to the one presented here, but on tick-borne parasites (see for example detection of tick-borne pathogens in bare-nosed wombats (Beard et al., 2021a)), that may have a more significant impact on host physiology and capacity to fight pathogens (such as DFTD), in order to determine whether there is any association between extrinsic (environmental factors, ectoparasites) and internal factors (physiological status, DFTD presence) and endoparasite levels.

The current study thus contributes to our understanding of the relationships between Tasmanian devils and the ticks that infest them, and lays down the foundations of future research investigating the factors influencing the capacity of Tasmanian devils to respond to the physiological, immunological and evolutionary challenges posed by DFTD, which is also key for conservation programs (Wait et al., 2017).

Author contribution

Project administration, funding acquisition: BU, FT, RH; study design: AMD, BU, RH; data gathering: RH; statistical analysis: SB, AMD; writing – original draft: SB; writing – review and editing: SB, RH, FT, BU, AMD.

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Ethical standards

The research was carried out according to guidelines and regulations approved by University of Tasmania's Animal Ethics Committee (approval permit A0013326; A0016789) and Deakin University's Animal Ethics Committee (approval number AEX03–2017). Sampling permit was issued by the Department of Environment and Energy. Permission was also obtained from landowners to sample on private land.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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