

ARTICLE

Addressing prey naivety in native mammals by accelerating selection for antipredator traits

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Abstract

Harnessing natural selection to improve conservation outcomes is a recent concept in ecology and evolutionary biology and a potentially powerful tool in species conservation. One possible application is the use of natural selection to improve antipredator responses of mammal species that are threatened by predation from novel predators. We investigated whether long-term exposure of an evolutionary naïve prey species to a novel predator would lead to phenotypic changes in a suite of physical and behavioral traits. We exposed a founder population of 353 burrowing bettongs (*Bettongia lesueur*) to feral cats (*Felis catus*) over 5 years and compared the physical and behavioral traits of this population (including offspring) to a control (non-predator exposed) population. We used selection analysis to investigate whether changes in the traits of bettongs were likely due to phenotypic plasticity or natural selection. We also quantified selection in both populations before and during major population crashes caused by drought (control) and high predation pressure (predator-exposed). Results showed that predator-exposed bettongs had longer flight initiation distances, larger hind feet, and larger heads than control bettongs. Trait divergence began soon after exposure and continued to intensify over time for flight initiation distance and hind foot length relative to control bettongs. Selection analysis found indicators of selection for larger hind feet and longer head length in predator-exposed populations. Results of a common garden experiment showed that the progeny of predator-exposed bettongs had larger feet than control bettongs. Results suggest that long-term, low-level exposure of naïve prey to novel predators can drive phenotypic changes that may assist with future conservation efforts.

KEYWORDS

Australia, burrowing bettong, naiveté, natural selection, predation

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INTRODUCTION

Strong selection has been shown to cause rapid change in a range of organisms (Bonnet et al., 2022) including bacteria (MacLean & Millan, 2019), coral (Quigley et al., 2020), reptiles (Stuart et al., 2014) and insects (Cook & Saccheri, 2013). Selection pressures can be environmental or can be deliberately human-induced such as selective breeding of domestic animals for physical appearance or for production-traits. Recently, researchers have discussed the use of selection to drive phenotypic change for biodiversity conservation (Manning et al., 2021; Moseby et al., 2014; van Oppen et al., 2018). Harnessing selection to improve conservation outcomes has been attempted in a number of studies including attempts to instill taste aversion behavior in marsupials (Kelly & Phillips, 2019) and heat tolerance genes in coral (Crow, 2021), but such studies have primarily been conducted under controlled conditions in captivity. Studies that attempt to drive selection in wild organisms are rare, possibly due to the complexity of dealing with potentially opposing selective pressures in the wild. Patterns of selection in the wild can be complex and change over time but understanding how selection operates in this context is a necessary step toward harnessing natural selection as a conservation tool.

Selection in the wild can occur in response to a range of pressures on survival including predation, competition, resources, and climate change (Bonnet et al., 2022; Gordon et al., 2015; Johnson & Zúñiga-Vega, 2009; Kingsolver et al., 2001). These drivers of selection can change over time or location and be triggered by anthropogenic change, for example, the change in skull morphology recorded in urban foxes (Parsons et al., 2020). One historic example of selection causing rapid phenotypic change is the peppered moth (*Biston betularia*), which evolved to all black coloration to improve camouflage against polluted surfaces (Cook & Saccheri, 2013). Predation pressure is a strong driver of selection and predators can induce changes in prey phenotypes directly, through non-random predation, and indirectly by thinning the prey population thereby reducing competition and altering selection pressures (Johnson & Belk, 2020).

Predation is a leading cause of reintroduction failure, particularly in Oceania (Morris et al., 2021). In Australia, the impact of predation has been exacerbated by the introduction of novel predators to an environment where prey species have not co-evolved the required anti-predator traits. Prey populations that interact with novel predators typically fare poorly after reintroduction due to high rates of predation (Clayton et al., 2014; Moseby et al., 2011; Short, 2009). In an effort to counteract this evolutionary mismatch, trials of accelerated selection

have been initiated whereby threatened mammalian prey species are exposed to feral cats in the wild under controlled conditions to determine whether this can lead to improved survival in the wild (Moseby et al., 2016). Early results have been promising (Blumstein et al., 2019) with improved survival in one species (greater bilby *Macrotis lagotis*; Ross et al., 2019) and changes in physical (Moseby et al., 2018) and behavioral traits (Saxon-Mills et al., 2018; Tay et al., 2021; West et al., 2018) over time in another (burrowing bettong *Bettong lesueur*).

However, animals in arid environments are also under strong selective pressures due to food limitations, especially during droughts. In environments where food is limiting, a smaller body size may confer a survival advantage due to lower energetic requirements (Damuth, 1981). Here we compare the phenotypic changes over 5 years in the aforementioned population of burrowing bettongs exposed to feral cats (*Felis catus*) compared to a control population that was not exposed to cats and explore the divergence in traits relative to the different selective pressures operating on the two populations. Both bettong populations occurred within the Arid Recovery Reserve in South Australia, but the existence of predator-proof paddocks permitted us to expose one group to cats for 5 years, whereas the other remained predator-free, but lived at a higher density where competition for food was likely to be the main selective pressure. Significant changes in physical and behavioral traits (including pes length and flight initiation distance [FID]) were reported for this population after 18 months (Moseby, Letnic, et al., 2018), but we were unable to determine if changes were due to selection. A severe drought that occurred during the study affected both populations and thus presented the opportunity to determine its effect on selection by allowing us to compare the traits of bettongs before and after the drought. We compared the change in phenotypic traits of the populations over a longer time frame of 5 years and calculated selection gradients for traits in individuals that survived and died in each treatment. Finally, to determine if shifts in the physical traits of bettongs were due to natural selection we conducted a common garden experiment in which we reintroduced progeny from both populations into a common environment and then compared the physical traits of their offspring. We predicted that selection for physical traits would be stronger than behavioral traits. Specifically, we predicted that there would be selection for larger physical traits in the cat-exposed paddock due to increased predation pressure and that these traits would be heritable, that is offspring would also exhibit larger physical traits than control bettongs once placed into a common garden experiment.

MATERIALS AND METHODS

Study species

Burrowing bettongs (mean body weight 1500 g, Arid Recovery, unpublished data) are bipedal, omnivorous, and nocturnal marsupials belonging to the family Macropodidae (Figure 1) that live communally in burrows (Moseby, Blumstein, et al., 2020; Sanders et al., 1997). Once widespread across Australia, burrowing bettongs became extinct on mainland Australia in the 20th century but continued to persist on three islands in Western Australia. Burrowing bettongs are considered to be highly susceptible to predation by introduced predators, the feral cat and red fox (*Vulpes vulpes*) (Radford et al., 2018), and attempts to release them into areas with predators on mainland Australia have failed due to predation (Bannister et al., 2016; Christensen & Burrows, 1995; Moseby et al., 2011). The susceptibility of bettongs to predation is highlighted by the fact that on mainland Australia bettongs have only been successfully reintroduced into fenced-sanctuaries that are free of cats and foxes (Moseby et al., 2011; Short & Turner, 2000).

Study site

We studied bettongs at the Arid Recovery Reserve (30°29' S, 136°53' E), a private conservation reserve situated approximately 20 km north of Roxby Downs, in arid South Australia, with low annual rainfall (mean 139.2 mm, median 132.6; www.bom.gov.au Olympic Dam 1997–2022). Habitat consists of dunes dominated by *Acacia ligulata*, *Dodonaea viscosa* and *Zygochloa paradoxa*, interspersed with swales of predominantly *Maireana astrotricha* and *Atriplex vesicaria*, with some *Acacia aneura*. The 123 km² reserve is surrounded by a 1.8 m floppy-top fence (Moseby & Read, 2006) and is divided into six paddocks (range 7.9–34.5 km²) that are enclosed by predator-proof fencing (Figure 1). Four of these paddocks are free of introduced mammalian predators (feral cats, foxes) and dingoes (*Canis lupus dingo*) and introduced rabbits (*Oryctolagus cuniculus*). The remaining two paddocks are experimental paddocks and contained introduced rabbits. We studied bettongs in one of the four predator-free paddocks and one of the two experimental paddocks. The predator-free paddock was termed the “Control Paddock” (14 km²).

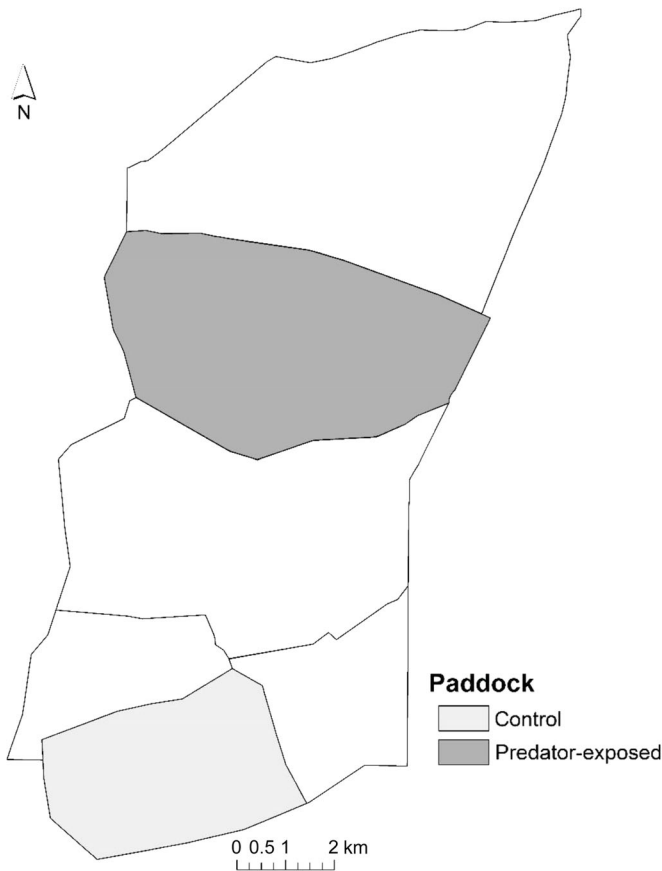


FIGURE 1 Treatment locations within the Arid Recovery Reserve indicating the predator-exposed and control paddocks (left), and burrowing bettong *Bettongia lesueur* (right, photo credit: Tom Hunt).

where a bettong population was established in 1999. The experimental paddock was termed the “Predator-exposed Paddock” (24 km²) where 353 bettongs were reintroduced in 2014 and cats were added within a few months of release (Figure 1, further details are outlined in Moseby et al., 2018). Track transects were used to monitor cat activity (see methods below) and the cat population increased over time due to in situ breeding and good seasonal conditions.

Behavioral and physical attributes

We compared the change in behavioral and physical attributes of bettongs in the control and predator-exposed paddocks from 2014 to 2019 inclusive, using trapping, track counts and radio-tracking. Initial tests were conducted within the control paddock prior to moving a portion of the bettongs into the predator-exposed paddock. The bettong population within each paddock was then tested every 3–12 months depending on the attribute.

Trap docility score

We defined docility as an individual’s reactivity to being trapped and handled (Bonnot et al., 2014; Petelle et al., 2013; Réale et al., 2000). During each trapping event, we quantified bettong behavior while we removed them from the trap. We used our extensive experience of trapping and handling bettongs to select a priori behaviors that were clearly indicative of more reactive individuals. We dichotomously scored whether animals moved in the trap during observer approach (1 = yes, 0 = no), made noise (1 = yes, 0 = no), moved immediately from the trap into a capture bag when the door of the trap was opened (1 = yes, 0 = no) and whether they moved in an agitated manner once they were secured in the bag (1 = yes, 0 = no). We then summed these scores to give a trap docility score between 0 = docile and 4 = non-docile. Scorers were trained with non-study animals to consistently approach traps and score bettong behavior. During scoring, illumination was provided by focusing a weak beam of light on the ground from behind the trap. Further details can be found in West et al. (2018).

Latency to leave trap

We recorded the latency (quantified in seconds) from when the trap door was opened to when each bettong was fully inside the capture bag. The time to leave the

trap was calculated using multiples of three because the observer blows (short sharp breaths aimed at the animal to encourage the animal to move into the bag) were administered at 3 s intervals.

Flight initiation distance

We scored FID as a measure of antipredator behavior. We measured FID for individuals at 0 months (prior to release) and then at a range of intervals up to 45 months after release. Some individuals were measured up to three times during the same time period and individual ID was included in models as a random effect. Because bettongs are nocturnal, traditional FID protocols (Blumstein et al., 2015; Cooper Jr. & Blumstein, 2015; Runyan & Blumstein, 2004) for diurnal species could not be used, so we first located radio-collared subjects using telemetry. Once a radio signal was located a single observer with a headlamp approached the bettong at a walking pace of 0.5 m/s until the animal fled and then measured the distance (in m) from the observer to the location from which the bettong fled (West et al., 2018). Some bettongs remained just ahead of the observer and out of sight (deduced from a continuing waning of the signal on approach); these approaches were recorded as “never seen.” Because the furthest distance that a bettong could reliably be seen with a headlamp was 40 m we estimated a minimum FID of 40 m for these never seen bettongs.

Physical traits

A subsample of the bettong population in both treatments was trapped every few months and weighed (body mass), sexed, measured to the nearest mm for hind foot length (hind foot length), tail width, and head length, and given a unique ear tag upon first capture. Tail width was recorded because bettongs store fat in their tail and thus tail width can give an indication of body condition (Staker, 2014).

Selection pressure and gradients

To identify possible drivers of selection we measured rainfall, cat activity and bettong density at each site. We assessed selection pressure by comparing physical and behavioral attributes over time between control and predator-exposed populations. We further monitored changes in bettong density and cat activity between these populations to assess time periods when selection was

most likely to occur and measured selection gradients relative to survival in these periods. Selection gradients can be used as a measure of strength and possible direction of natural selection on various traits (Palacio et al., 2019).

Bettong density

To estimate bettong density over time in the two treatments we used data from annual cage trapping conducted between 2016 and 2020. Cage traps baited with peanut butter and rolled oats were used to capture bettongs in a trapping session lasting 4–5 days. A total of 84–160 traps were laid out approximately 200 m apart along roads in each paddock. Bettongs that were caught were identified using unique ear tags with new tags given to new individuals.

Bettong density was calculated using spatially explicit capture-recapture (SECR) methods. SECR utilizes spatial data from individuals within an animal population to model distributions of animal home ranges and an observation model to determine the probability of detecting an individual at different detectors (e.g., traps) throughout the range (Borchers & Efford, 2008; Efford et al., 2009). A capture history file (location, day caught, individual, sex) and trap data file (trap location, coordinates, and days traps were open) were created. We used the *secr* package (Efford, 2016) to analyze our capture history data to determine total estimated population size. Each paddock represented a closed state space and no areas were excluded. For each year and treatment, we first tested models using the half-normal, exponential or hazard detection functions using model selection using Akaike information criterion (AIC) to determine the best detection function for the data. We then compared our null model ($D \sim 1$, $g_0 \sim 1$, $\sigma \sim 1$) where D represents the density, g_0 the probability of capture when the trap is at the activity center and σ is a spatial parameter related to home range size (Efford, 2004), with models allowing for our g_0 or σ or both to vary with sex (h2) of the individual, and behavior (“b” and “B”) providing a total of eight models for comparison using AIC (Appendix S1: Table S1). Where several models had delta AIC values < 2 , we used model averaging to estimate our parameters (Burnham & Anderson, 2002). Additionally, for the predator-exposed treatment, dunes were clumped in one section of the paddock, so we also included a habitat covariate on density based on whether traps fell in a region of sand dunes or swale.

We also conducted regular track counts to determine bettong and cat activity trends on sand dunes within each treatment. We used trends in activity to determine periods of high cat activity which we used as a proxy for

predation pressure. Although predation pressure is also influenced by other factors such as abundance of alternative prey (Nordberg & Schwarzkopf, 2019) and population demography of predators (Moseby et al., 2021), we assumed that it also provides an indication of selection pressure from predation. The method for track counts is described in Moseby et al. (2011) and involved dragging an iron bar behind an all terrain vehicle (ATV) to clear old tracks along a transect on sand dunes (predator-exposed = 11.8 km, control = 11.2 km) before counting fresh tracks the following morning. Track counts have been shown to be a strong predictor of density in bettongs at the study site (Moseby, Lollback, & Lynch, 2018) and we expect this to be similar for cats as well.

We fitted radio collars to bettongs in each treatment ($n = 22$ predator-exposed, $n = 20$ control) for up to 6 months in August–November 2017 and June–November 2018 to monitor survival and attempted to identify causes of mortality using carcass remains and known body condition prior to death.

Data analysis: Selection pressure

We used linear mixed models (LMM) to test for a priori hypotheses of the effects of sex, treatment, months since exposure (a measure of time since bettongs were placed in an enclosure with predators), an interaction between treatment and months since exposure, on the response of four physical traits: hind foot length, head length, body mass, and tail width. Similarly, we used treatment and months since exposure with an interaction as fixed effects, and included sex and percentage moon cover (a measure of predation risk) as control variables for testing their effect on three behavioral traits: trap behavior score, FID, and latency to leave traps. For trap docility score and latency to leave trap, we fitted generalized linear mixed models (GLMM) with a Poisson or a negative binomial distribution. Months since exposure was recorded for all traits as a continuous variable and we tested whether this was a linear or non-linear relationship for each response variable by including month squared. Physical traits were measured at the same time (May) annually but behavioral traits were measured at more variable intervals. Since multiple measurements were conducted on the same individual at various times for both physical and behavioral traits, we included animal ID as a random effect. We fitted models in R (R Core Team, 2020) using the package *lme4* (Bates et al., 2015). We used likelihood ratio tests to determine whether control variables, squared variables or random factors were important to retain in the model. All models were checked for model fit by plotting residuals using the

DHARMA package (Hartig, 2020). As in most cases we had mixed models, we used bootstrapping with a 1000 simulations in the package parameters (Ludecke et al., 2020) to calculate 95% confidence intervals (CI) and *p*-values. We used the bootstrap results with the emmeans package (Lenth, 2020) to calculate estimated marginal means for plotting our results.

Data analysis: Selection gradients

We measured selection gradients before and after periods of significant population decline that occurred in both the cat exposed and non-cat exposed (control) bettong populations as measured by trapping and track transects (cat-exposed October 2017-49% track activity decline due to predation; control October 2018-86% track activity decline due to drought—see *Results*). We tested for a relationship between survival and several traits (hind foot length, tail width and head length) in each of our two treatments (control and predator-exposed) separately. For each population, we recorded each trait measurement from each individual and survival as a binary trait (No = 0, Yes = 1) for two key periods. First, in both treatment groups, we looked at survival from 2014 to 2017 to assess selection prior to the major population crashes. Second, we calculated the survival of individuals that survived the major population crash using data from the preceding trapping period and opportunistic captures for the predator-exposed group (if alive in May–October 2017, were they alive anytime from May 2018 to 2020) and the control group (if alive May–October 2018, were they alive anytime from May 2019 to 2020). We included trapping periods in several years after the crash period in case they were not initially trapped in the first annual trapping period after the crash. We restricted our analysis to adults only (body mass \geq 900 g).

We followed the protocol for selection analysis by Palacio et al. (2019) and Wood and Brodie (2016) using the Lande and Arnold approach (Lande & Arnold, 1983) by first creating a relative absolute fitness measure for each population in each of the two episodes by dividing individual survival by the population mean survival for each episode. We standardized all traits to have a mean of zero and standard deviation of one, and checked for collinearity between traits using variance inflation factors. Due to the lack of normality in most cases we used a two-step approach to determine selection gradients from a linear and quadratic model. Linear models included traits of hind foot length and tail width for each episode and our quadratic model included an interaction between these two traits, in addition to linear and quadratic variables. We fitted separate models for head length, because

there were substantially fewer data points for this trait. In each model we included animal ID as a random factor because some individuals were measured multiple times. We used likelihood ratio tests to determine whether the model fit was improved with the inclusion of the random factor or correlated traits and removed these when it was not. Model fit was checked by plotting residuals and identifying potential outliers. Due to the lack of normality in residuals in many cases, we used bootstrapping to create standard errors and 95% CI from 1000 bootstrap samples for all of our parameters. We used GAM (generalized additive models) models from the package mgcv (Wood, 2011) to visualize our selection gradients and to infer stabilizing or disruptive selection from any significant results. All graphs were made using ggplot2 (Wickham, 2016).

Common garden experiment

In order to determine whether changes in physical traits that occurred in the bettong population from the predator-exposed population were due to phenotypic plasticity or had a genetic basis, we conducted a common-garden experiment by moving animals from the control and predator-exposed population into an 8 ha fenced pen and comparing traits of the offspring. Nine adult females (with three young at foot) from the control population, and seven adult females (with one young at foot), and three adult males from the predator-exposed populations were moved into the pen in May and June 2020. A further two adult females from the control population were added in December 2020 to supplement the population and increase sample size. The soft-release pen was fenced to prevent incursion or excursion of other bettongs; supplementary feed and water was provided several times a week. We monitored adult females for up to 16 months via regular cage trapping to monitor pouch young size (microchipped at emergence) to ensure we could accurately identify pairs of mothers and offspring upon emergence from the pouch. Offspring were fitted with VHF collars when large enough (>900 g). All individuals in the pen were weighed and measured on multiple occasions. Offspring born in the pen remained until at least 6 months after pouch emergence (~ 9 –10 months old) when they were considered adult (Short & Turner, 1999; Tyndale-Biscoe, 1968). Once offspring had reached maturity some individuals were moved out of the pen to limit potential inbreeding or reduce the density of the population within the enclosure. Since female bettongs undergo embryonic diapause where they retain and delay a fertilized embryo while rearing a young in the pouch (Tyndale-Biscoe, 1968), we assumed that the first two young born from the control adults would be sired by males from the control population before

potentially breeding with predator-exposed males while in the enclosure. Since only adult males from the predator-exposed population were present, all offspring born to predator-exposed females were considered predator-exposed offspring.

We compared differences between the two populations for three traits: hind foot length, tail width, and head length, but not body mass due to highly variable weight changes in growth at this age. We included only the first two offspring from the control population (including the pouch young brought into the pen who were within one-month of emergence; $n = 13$), and all offspring born to the predator-exposed adult females ($n = 8$) that survived to 6 months' post pouch emergence (in all but one case this was only the first two offspring). We did not include any individuals that could not be confidently assigned to a mother. We used measurements from individuals recorded at 6 months or nearest to this age as hind foot length and head length tend to plateau at and even before this age (Tyndale-Biscoe, 1968). We used linear mixed models to compare each trait between populations and included sex as a control variable, but removed this when not significant. We included animal ID as a random effect to account for multiple measurements from the same individual.

RESULTS

Rainfall over the study period was below average in 2014 (study site 100.6 mm) and average in 2015 (study site 140 mm) and above average in 2016 (241.7 mm). Annual rainfall declined to well below average over the following 3 years (Figure 2a). The study area experienced drought conditions in 2018 and 2019 when less than 52 mm of rain was recorded each year.

Population changes

At the start of the study in 2014, track densities of bettongs were higher in the control paddock (over 200 tracks per km) than the predator-exposed paddock (<50 tracks per km) (Figure 2c). However, the two populations reached similar levels by 2017 before a sharp decline in the predator-exposed population in October 2017. A similar decline was recorded in the control population 12 months later in October 2018.

Density estimates from trapping followed a similar trend to track counts, as expected. Bettongs within the predator-exposed treatment increased in population size from the 353 released in late 2014 to over an estimated 1000 in May 2017 (Figure 2d). The population then

experienced significant declines between October 2017 and February 2018 (Figure 2), despite average rainfall conditions. The decline in the predator-exposed paddock was attributed to cat predation due to the increasingly high density of cats recorded in the paddock at that time (2.08 cats per square km, Moseby, McGregor, & Read, 2020, Figure 2) combined with the lack of a significant change in body condition or weight in bettongs captured in this paddock over the same period (Figure 4). Bettongs were also found in the stomachs and scats of feral cats removed from the predator-exposed paddock in late 2017 (H. McGregor, personal communication).

Bettongs were in high abundance in the control paddock in 2016 but unfortunately, no estimate was made for the control population in 2016 due to the high capture rate and very low recapture rate. We therefore correlated actual captures with the secr estimates from 2017 to 2020, to estimate abundance based on minimum captures in 2016. Bettongs in the control paddock were in high abundance during the decline in the predator-exposed paddock and were in such high numbers that nearly 400 were removed from the paddock in early 2018 to reduce the impacts that their herbivory was having on vegetation (Figure 3, Linley et al., 2017; Moseby, Lollback, & Lynch, 2018). Despite this removal, the population estimate of bettongs in 2018 (after removal) was similar to 2017 pre-removal levels. A catastrophic drought-driven decline in the control paddock occurred in October 2018 (Figure 2c,d), 12 months after the predator-exposed bettong population decline. During this period, 82% (18 of 22) of radio-collared bettongs died in <5 months with carcasses found intact on the surface. Body condition during this time was poor and body mass declined, suggesting they died from starvation (Figure 4). Field observations included seeing bettongs moving lethargically while attempting to forage outside during the day. This compared with only three deaths (15%) of radio-collared bettongs in the predator-exposed paddock over the same time period, with predation suspected in at least two of these instances (based on the fact that carcasses were partially consumed, animals were in good condition prior to death and some cats captured within the predator-exposed paddock had bettong remains in their stomach). Track counts confirmed the population fluctuations derived from capture mark recapture analysis with the most significant decline in the predator-exposed bettong population occurring between October 2017 and March 2018 and later for the control population in late 2018 (Figure 2c,d).

The density of cat tracks on track transects increased over time in the predator-exposed paddock (Figure 2) reaching a peak in early 2018. Due to the significant decline in bettong abundance around that time, and the

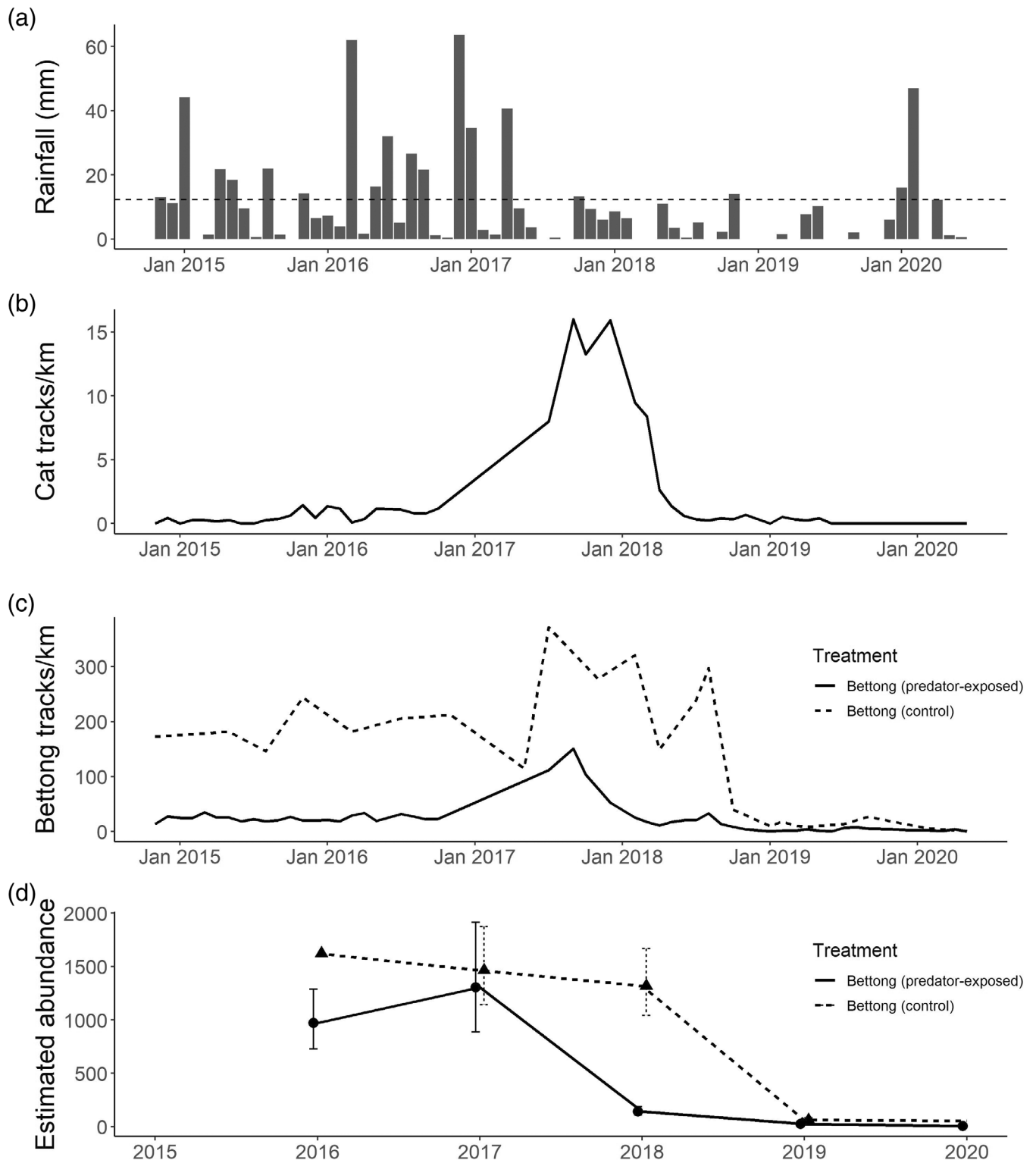


FIGURE 2 (a) Monthly rainfall for the study site with mean monthly rainfall shown as dashed line (Bureau of Meteorology [BOM] 1997–2021), (b) tracks counts of cats (predator-exposed treatment), (c) track counts of bettongs (predator-exposed and control treatments), and (d) estimated abundance of bettongs with 95% CI. Periods of selection analysis shown by lines on the bettong track graph.

presence of bettongs in cat scats and stomachs, most of the cats were removed in March and April 2018.

Because the climatic conditions were similar at both sites, the differential timing of population decline in the

two treatments suggests that different selection pressures may have been operating on each population. High mortality of bettongs and the crash in population from September 2018 in the control paddock where cats were

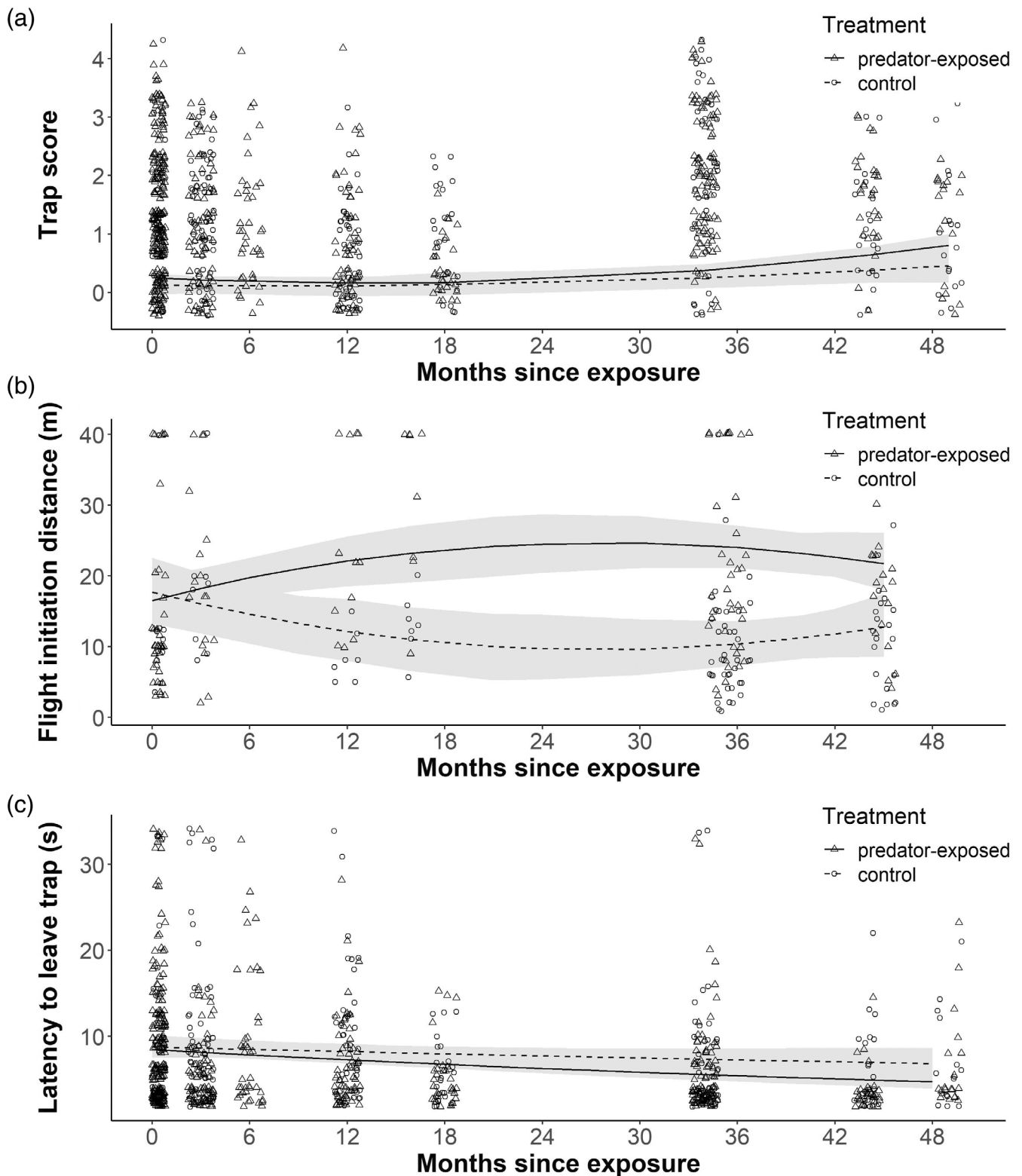


FIGURE 3 Predicted relationship over time between predator-exposed and control treatment populations of bettongs for three behavioral traits: (a) trap score, (b) flight initiation distance, and (c) latency to leave trap. Shaded areas represent 95% CI and raw data are included. Predictions are based on mean moon cover and averaged over sex where these predictors were included in the model.

absent was associated with extreme drought conditions (Figure 2) suggesting the decline was likely caused by competition for food due to high bettong density and food

limitation. In comparison, selection pressure in the predator-exposed paddock was likely highest between 2017 and 2018 and likely caused by cat predation because

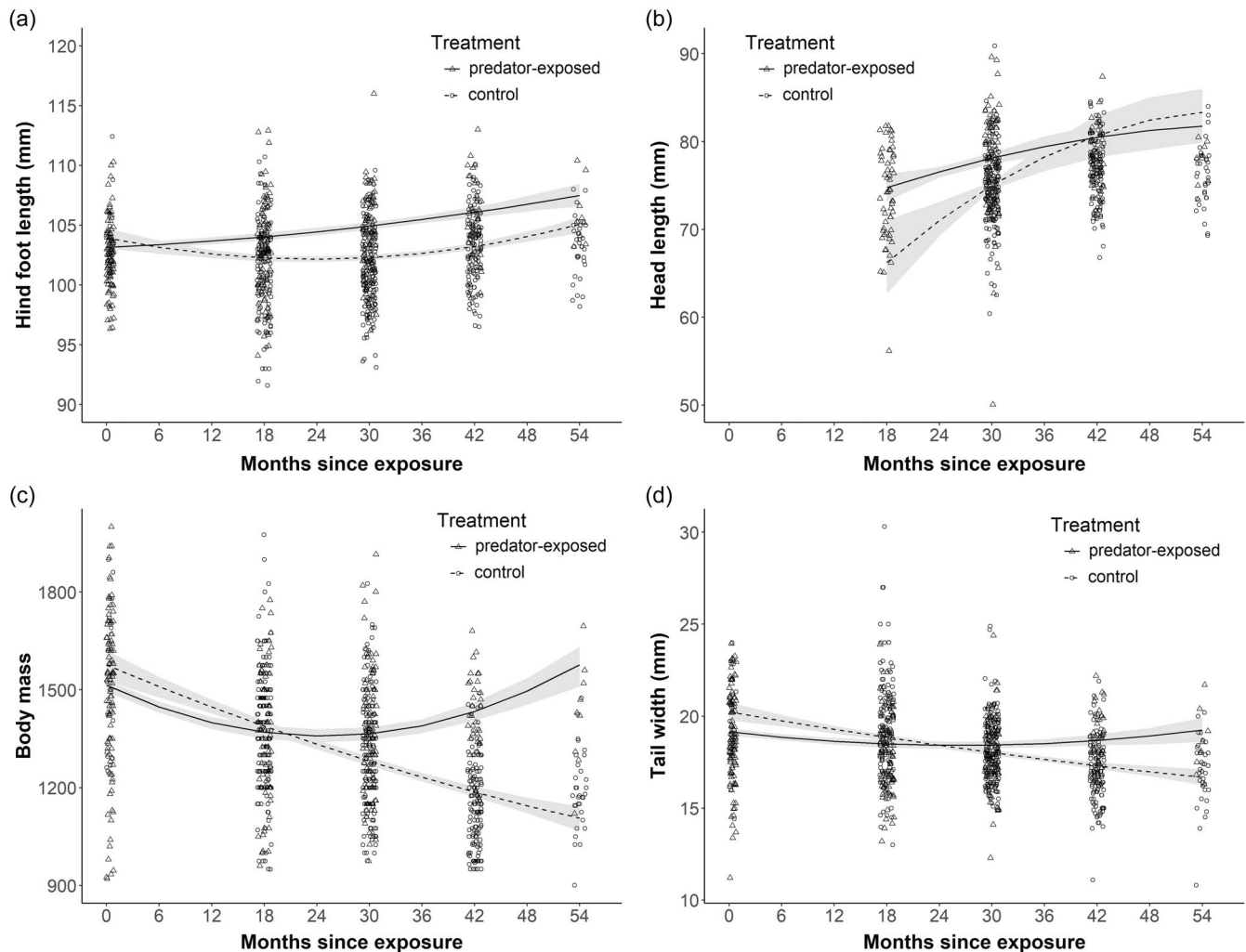


FIGURE 4 Predicted trends of physical traits between two populations of bettongs (control and cat-exposed) over time for (a) hind foot length (mm), (b) head length (mm), (c) body mass (g), and (d) tail width (mm). Shaded areas represent 95% bootstrapped CI and raw data is also plotted. Predictions are averaged over sex.

normal rainfall conditions were experienced in 2017, bettong population densities were relatively low in comparison to the control population and cat track counts reached very high levels. Although nearly all cats were removed from the predator-exposed paddock after the decline, the population decline continued possibly due to additional pressure on the predator-exposed population in late 2018 as drought conditions worsened.

Selection pressure in traits over time

Behavioral traits

FID was the only behavioral trait to change significantly over time between the two populations (Table 1). Initially similar in both populations, FIDs increased in the predator-exposed population as bettong became harder to approach and decreased in the control population

(Figure 3b). FIDs were also longer on nights with more moon visibility. The maximum FID possible was 40 m due to difficulties in observing animals at night. Although our measure of docility (trap score) appeared to increase in cat-exposed bettongs, suggesting they became less docile over time, it did not significantly differ between the two populations (Figure 3a). Similarly, individuals from both populations left the trap more quickly after capture over time (Figure 3c).

Physical traits

Hind foot length, body mass, and tail width changed over time differently as a function of treatment while changes in head length had no such interaction (Table 1). Bettongs from the predator-exposed population had larger hind foot lengths and head lengths, but relatively stable body mass and tail width (Figure 4). In the control

TABLE 1 Results of linear mixed models (LMM), generalized linear models (GLM), and generalized linear mixed models (GLMM) comparing changes in physical and behavioral traits between two bettong populations over time.

Response	Fixed effects	β	<i>t</i> value	LCI	UCI	<i>p</i>
Physical traits						
Hind foot length, <i>n</i> = 2076 (1451)	Intercept	102.51	545.29	102.14	102.88	<0.001
	Sex—male	1.27	7.79	0.98	1.56	<0.001
	Treatment (control)	0.81	1.79	−0.01	1.67	0.066
	Months	0.03	1.94	0	0.07	0.048
	Months ²	0	2.08	0	0	0.039
	Treatment (control): Months	−0.18	−5.45	−0.25	−0.12	<0.001
	Treatment (control): Months ²	0	3.57	0	0	0.002
Head length, <i>n</i> = 1176 (976)	Intercept	76.22	246.81	75.56	76.8	<0.001
	Sex—male	1.19	4.36	0.69	1.72	<0.001
	Treatment ^a (control)	−4.98	−6.51	−6.46	−3.39	<0.001
	Months ^a	0.2	4.95	0.12	0.28	<0.001
	Months ^{2a}	0	−2.04	−0.01	0	0.046
	Treatment (control): Months ^a	0.28	2.6	0.06	0.5	0.01
	Treatment (control): Months ^{2a}	−0.01	−2.01	−0.01	0	0.04
Body mass, <i>n</i> = 2094 (1456)	Intercept	1503.88	136.3	1481.82	1524.43	<0.001
	Sex—male	20.69	2.23	3.17	39.54	0.023
	Treatment (control)	61.82	2.37	11.91	110.94	0.014
	Months	−12.56	−11.39	−14.79	−10.44	<0.001
	Months ²	0.25	9.33	0.2	0.31	<0.001
	Treatment (control): Months	1.4	0.61	−2.66	5.33	0.514
	Treatment (control): Months ²	−0.21	−5.09	−0.29	−0.13	<0.001
Tail width, <i>n</i> = 2086 (1458)	Intercept	19.08	166.78	18.85	19.3	<0.001
	Sex—male	0.16	1.61	−0.02	0.35	0.102
	Treatment (control)	1.08	3.94	0.58	1.58	<0.001
	Months	−0.06	−4.7	−0.08	−0.03	<0.001
	Months ²	0	3.64	0	0	<0.001
	Treatment (control): Months	−0.03	−1.26	−0.07	0.01	0.2
	Treatment (control): Months ²	0	−1.74	0	0	0.071
Behavioral traits						
FID, <i>n</i> = 285 (112)	Intercept	16.13	8.64	12.51	19.86	<0.001
	Sex—male	−3.37	−2.19	−6.57	−0.46	0.032
	Moon visibility	4.03	2.03	0.22	8	0.05
	Treatment (control)	1.23	0.4	−4.72	6.73	0.686
	Months	0.59	2.53	0.12	1.02	0.016
	Months ²	−0.01	−19.73	−0.02	0	0.044
	Treatment (control): Months	−1.19	−3.42	−1.84	−0.51	0.004
	Treatment (control): Months ²	0.02	2.69	0.01	0.04	0.009
Trap score, <i>n</i> = 1096 (663)	Intercept	0.16	2.96	0.06	0.27	0.004
	Moon visibility	0.16	1.9	0	0.32	0.053
	Treatment (control)	−0.13	−1.31	−0.31	0.03	0.14
	Months	−0.01	−1.72	−0.03	0	0.064

(Continues)

TABLE 1 (Continued)

Response	Fixed effects	β	<i>t</i> value	LCI	UCI	<i>p</i>
	Months ²	0	2.71	0	0	0.005
	Treatment (control): Months	0.01	0.71	-0.02	0.04	0.46
	Treatment (control): Months ²	0	-0.94	0	0	0.317
Latency to leave trap, <i>n</i> = 1096 (663)	Intercept	2.09	60.04	2.02	2.16	<0.001
	Treatment (control)	0.03	0.47	-0.15	0.21	0.639
	Months	-0.01	-6.48	-0.02	-0.01	<0.001
	Treatment (control) × months	0.01	2.26	0	0.02	0.024

Note: β Values show untransformed coefficients. All 95% CI (LCI and UCI) and *p*-values were measured using 1000 bootstrap samples. Significant values are in bold. Abbreviation: FID, flight initiation distance; GLM, generalised linear model; LCI, lower confidence interval; UCI, upper confidence interval.

^aMonths were centered at the mean.

population of bettongs, hind foot length increased but at a lower rate, head length increased at a similar rate to predator-exposed individuals, but control bettongs exhibited sharper declines in body mass and tail width. The only significant effect of sex was for head length where males had larger heads than females (Table 1).

Selection gradients in physical traits

Selection was evident for several traits in each population of bettongs, and in most cases was always linear with evidence for disruptive selection only in tail width (Table 2). Prior to the population crash, increasing hind foot length was associated with increased survival in the predator-exposed population, but hind foot length was not associated with improved survival in control populations (Figure 5). Greater head length was also associated with higher survival in the predator-exposed population in the pre-crash period (Appendix S1: Figure S1), but there was no relationship in the control populations (Table 2). Survival prior to either of the high population crash events was significantly lower for bettongs with thicker tails in both control and predator-exposed populations (Figure 6), but the reverse was true for the control population after the major drought period.

Common garden

Offspring born from the predator-exposed population had significantly larger hind feet than those from the control population upon reaching adulthood ($\beta = -2.60$, $z = -2.85$, $p = 0.004$). However, we detected no difference in tail width ($\beta = -0.52$, $z = -0.78$, $p = 0.436$) or head length ($\beta = -1.13$, $z = -0.86$, $p = 0.388$) between the two populations. There were no differences by sex for any trait; hence, we removed it from the final models.

DISCUSSION

Accelerating natural selection could assist with improving conservation outcomes for prey that have been ontogenetically or evolutionarily isolated from predators. We aimed to determine whether exposure of a naïve prey to a novel predator could result in significant phenotypic changes in behavior and physical traits and if these changes could be due to selection. A key finding of our study was that predator-exposed bettongs had larger hind feet and longer FIDs than control bettongs and these traits diverged over the duration of the study. Importantly, survival trends and the common garden experiment indicated potential evidence of selection and heritability respectively, in physical traits. Our results extend initial work at the study site during the first 18 months after cat exposure that found phenotypic differences between cat exposed and control bettongs (West et al., 2018). Furthermore, our results support other studies that have found rapid adaptive evolution in animals in response to anthropogenic-induced environmental change (Bonnet et al., 2022).

FID was the only behavioral trait to significantly change after cat exposure. Bettongs that were exposed to cats were harder to approach and fled at twice the approach distance after several years compared to control bettongs. This difference in FID continued to increase over the 5 years of the study suggesting continual learning and improvement in predator avoidance behavior. Unfortunately monitoring future changes in FID will be difficult due to the maximum detection distance being reached for many of the predator-exposed bettongs. Conducting FIDs on nocturnal species is problematic and most studies use FIDs for diurnal species that are easy to observe (Blumstein et al., 2015; Cooper Jr. & Blumstein, 2015; Runyan & Blumstein, 2004). The inability to detect changes in other behavioral traits may be due to the difficulties in finding and measuring appropriate behavioral

TABLE 2 Results of the selection analysis for control and predator-exposed populations during episodes of pre- and post-crash population declines of bettong physical traits (hind foot length HFL, tail width, and head length) using linear and quadratic equations to highlight selection gradients (β_i) and selection type (γ_{ii} or γ_{ij}) with associated standard errors and bootstrapped 95% CI.

Treatment and episode	Trait	β_i	SE	Lower CI	Upper CI	γ_{ii} or γ_{ij}	SE	Lower CI	Upper CI
Control									
Pre-crash	HFL	-0.01	0.03	-0.07	0.05	-0.08	0.04	-0.16	0.01
	Tail	-0.15	0.03	-0.21	-0.10	-0.09	0.03	-0.15	-0.02
	Head	-0.03	0.02	-0.07	0.01	0.00	0.01	-0.02	0.04
Post-crash	HFL	-0.28	0.24	-0.76	0.19	-0.52	0.39	-1.28	0.25
	Tail	0.76	0.32	0.13	1.40	0.28	0.41	-0.53	1.08
	Head	-0.02	0.02	-0.06	0.01	0.00	0.01	-0.02	0.03
Predator-exposed									
Pre-crash	HFL	0.16	0.03	0.09	0.22	-0.02	0.04	-0.11	0.07
	Tail	-0.16	0.03	-0.22	-0.10	-0.16	0.05	-0.25	-0.07
	Head	0.21	0.05	0.12	0.30	-0.07	0.05	-0.18	0.03
Post-crash	HFL	0.12	0.18	-0.24	0.45	0.24	0.30	-0.29	0.94
	Tail	0.07	0.15	-0.23	0.36	-0.32	0.19	-0.69	0.06
	HFL:Tail					0.43	0.20	0.05	0.80
	Head	-0.14	0.16	-0.46	0.18	0.03	0.16	-0.16	0.49

Note: Significant values are in bold.

responses that are indicative of predator avoidance. For example, although we found no difference in trap docility or latency to leave the trap, a recent study on escape behavior in the same bettong populations recorded significant differences in escape behavior between control and predator-exposed bettongs when animals were released into a runway (Tay et al., 2021). Predator-exposed bettongs fled faster than control bettongs after release and exhibited heightened responses. Similarly, behavior at feed trays with different predator scents also found differences in wariness between predator-exposed and control bettongs (Saxon-Mills et al., 2018; West et al., 2018). Although temperament has been linked to survival in predator environments in other species (e.g., Bremner-Harrison et al., 2004; May et al., 2016), determining docility of bettongs in cage traps may not be representative of normal antipredator behavior and effort should be focused on finding other ways of recording behavior during normal activity.

Hind foot length and head length in bettongs that were exposed to cats were both longer and, in the case of hind foot length, increased at a faster rate relative to control bettongs. Although these phenotypic changes could be due to phenotypic plasticity without differences in survival, our selection analysis found higher survival in animals with longer hind foot length and larger head length in predator-exposed bettongs during the first 4 years of cat exposure, suggesting that selection may have occurred. Predators can trigger rapid selection in other mammals (Réale & Festa-Bianchet, 2003) and our results

are supported by a review of phenotypic selection in natural populations which found selection on morphological traits to be significantly stronger than on behavioral ones (Kingsolver et al., 2001). Furthermore, the results of our common garden experiment showed that hind foot length was greater in the progeny of predator-exposed bettongs than the progeny of control bettongs. Although the possibility of trans-generational plasticity cannot be ignored (Tariel et al., 2020), the results do suggest that this trait is heritable and that predator exposure could potentially lead to evolutionary changes in mammalian prey exposed to predators.

The shifts in hind foot length and head length in predator-exposed bettongs could be related to larger body size which may confer a survival advantage when exposed to predators. Another study at our study site found higher survival in bettongs with larger hind feet after release into an area with cats (Bannister et al., 2021) supporting the hypothesis that smaller animals are more susceptible to cat predation possibly due to cats targeting small individuals or because larger hind feet may convey an escape advantage. Adult bettongs weigh approximately 1.5 kg, constituting a prey item at the upper end of the prey size range for an adult feral cat (body-weight 3–6 kg) in the study region (Moseby et al., 2021). A study on the related Eastern Bettong, *Bettongia gaimardi*, also found higher survival in larger individuals when animals were released into an area where cats and foxes were present (Evans et al., 2021). Other studies have found predators drive

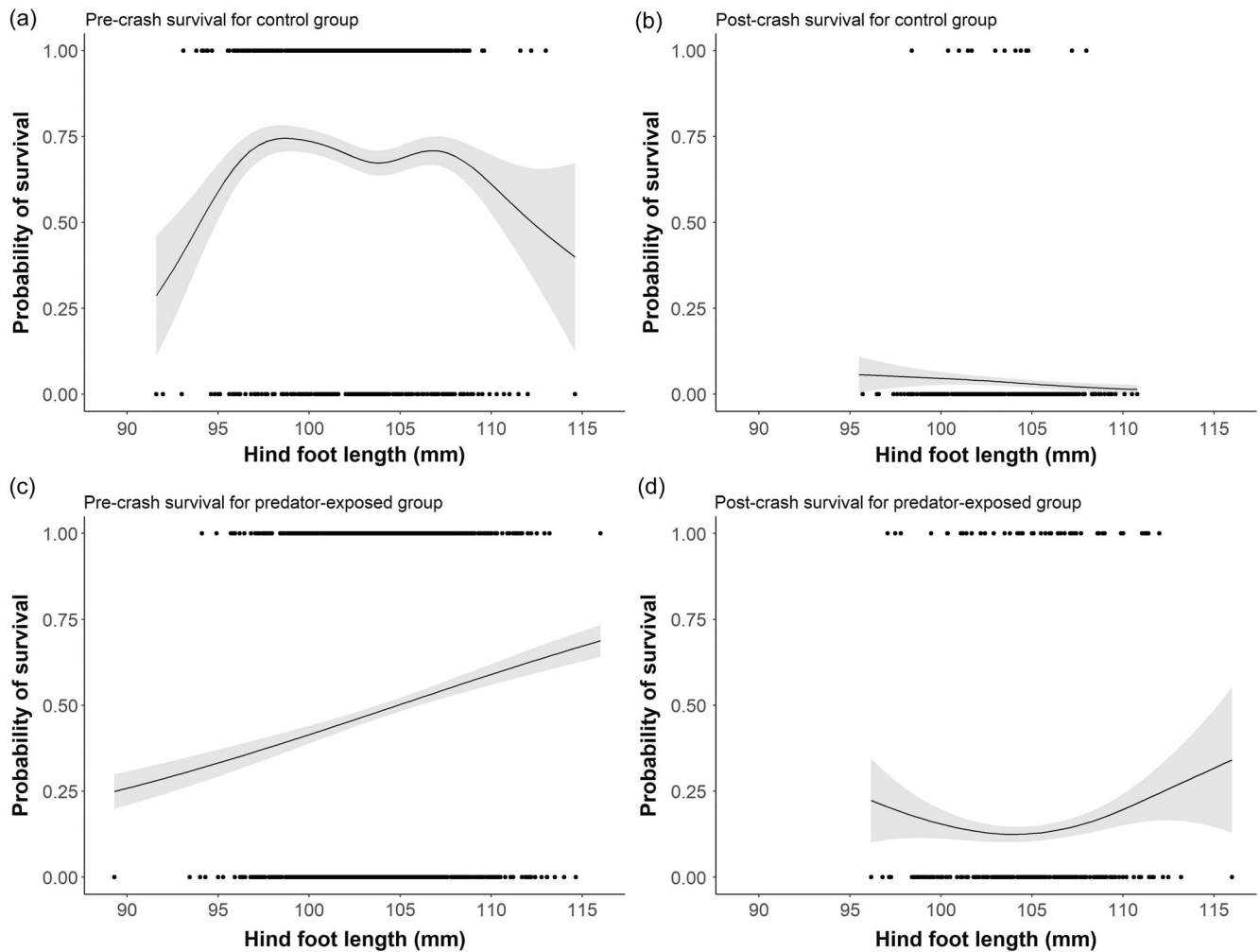


FIGURE 5 Probability of survival for the control (a) and predator-exposed (b) populations for individuals with varying hind foot length in episodes prior to any population crash in 2017 (2014–2017) and in post-crash events for control population (c) (survival over drought in late 2018) and predator-exposed population (d) (survival over high cat density in late 2017).

selection by targeting young, sick, or weak individuals (Genovart et al., 2010) and that predation risk can vary individually based on body mass (MacLeod et al., 2006), age (Wright et al., 2006), personality (Bremner-Harrison et al., 2004) and sex (Fitzgibbon, 1990).

Separating out the effects of an increase in overall body size from any individual effects of hind foot length and head length is difficult. Body mass was not a good indicator of overall body size as it declined in the control population over time despite no decline in head or hind foot length. In a previous study, Tay et al. (2021) found bettongs in the predator exposed group exhibited faster escape behavior than control bettongs, possibly due to longer hind feet, but whether this was due to an overall increase in body size is unclear. Future research should include a wider range of body measurements to determine if they exhibit similar changes over time. Additionally, the relationship between age and body size may need further exploration. We addressed this by only

including adult bettongs in our selection analysis and choosing an adult weight cut off that occurred after body growth measurements had plateaued. However, if predators are selecting individuals based on smaller body sizes, then young bettongs may be even more susceptible to cat predation. Although this is unlikely to lead to selection, it may impede efforts to use selection to improve antipredator traits.

Evidence for selection for larger hind foot size and head length was only found during the period prior to the population crash in the predator-exposed bettongs. The failure to detect selection during the sharp decline in bettong abundance could have been influenced by the low numbers of bettongs that survived the population crash creating a small sample size for analysis, or the predation pressure being too high to allow selection to occur. The high density of cats in the paddock at the time led to a very high mortality rate of bettongs based on the reduced density estimates over time, and the

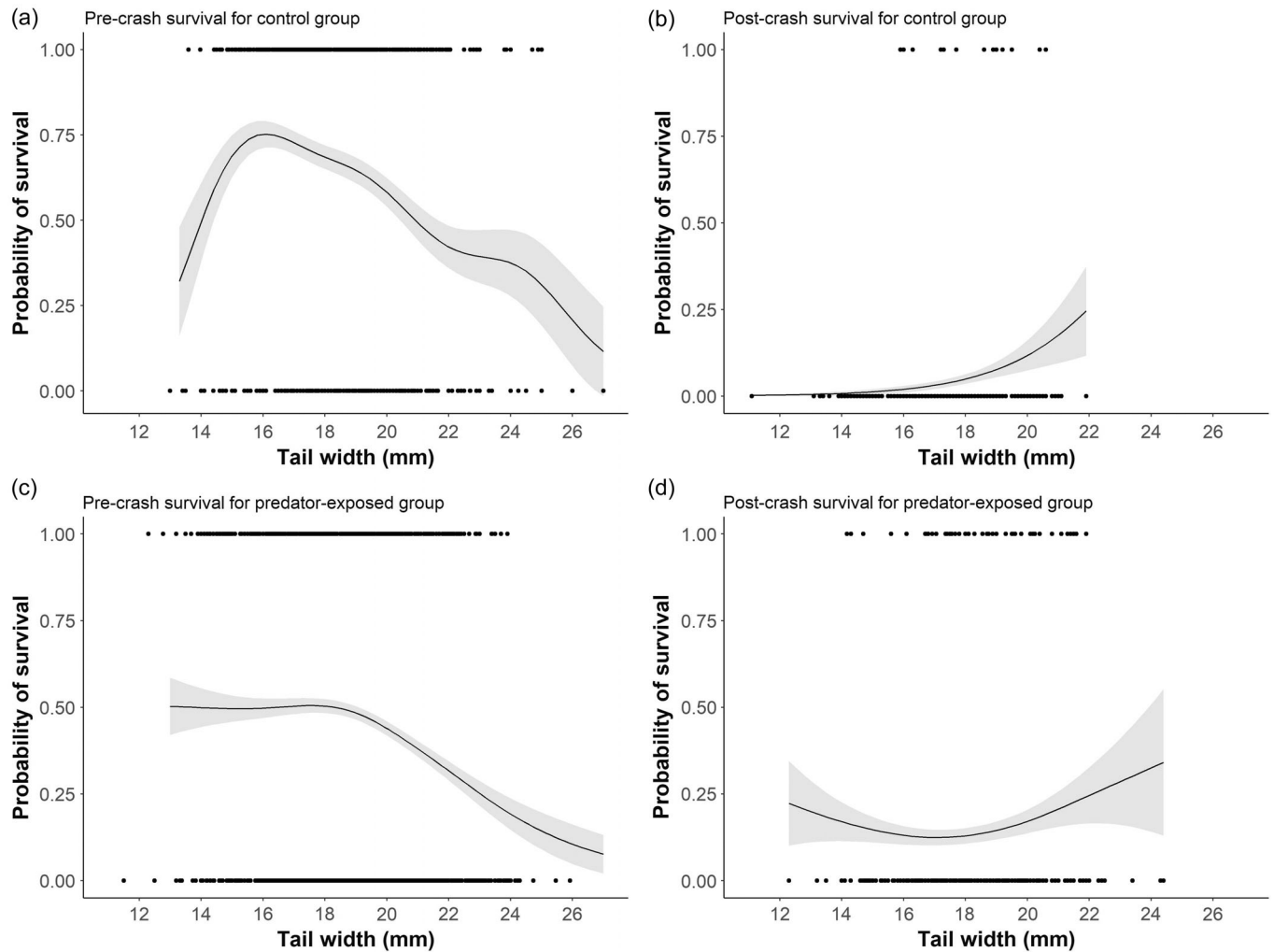


FIGURE 6 Probability of survival for the control (a) and predator-exposed (b) populations for individuals with varying tail width in episodes prior to any population crash in 2017 (2014–2017) and after the post-crash events for control population (c) (survival during drought in late 2018) and predator-exposed population (d) (survival during high cat density in late 2017).

lack of differential survival may have precluded natural selection.

Tail width is an indication of body condition in bettongs as fat is stored in the tail during good seasons (Staker, 2014). Thus, higher survival in bettongs with thinner tails in both treatments was a perplexing finding. However, similar trends in both treatments suggest that the link between survival and tail-width was unlikely to be related to cat exposure. Tail width may also be correlated with age which we were not able to estimate effectively for individuals in our study.

Rabbits were present in the cat-exposed paddock but not the control paddock and thus the effect of the presence of rabbits on the project results could not be accounted for. However, rabbits are primarily herbivorous, whereas bettongs are omnivorous and previous studies have found little dietary overlap or evidence of interspecific competition (Robley & Short, 2001). Rabbits are an important food

source for feral cats in the region (Read & Bowen, 2001) and may have reduced the predation pressure on bettongs at certain times. Without this alternative food source, predation on bettongs and selection for traits may have been stronger.

Predation is one of the most significant causes of reintroduction failure (Fischer & Lindenmayer, 2000; Moseby et al., 2011; Short, 2009). In the case of novel predators, predation impacts can be exacerbated by prey naivety due to an absence of shared evolutionary history between novel predators and native prey (Banks & Dickman, 2007; Carthey & Blumstein, 2018). Our results have implications for changing traits of prey to enable them to increase survival when exposed to novel predators. Increased survival has already been recorded for predator-exposed bilbies from the same paddock (Ross et al., 2019) but releases of predator-exposed and control bettongs into new areas with cats did not show similar

trends (Bannister et al., 2021). The continuing divergence in traits between the two populations suggests that improved survival may be obtained in the future if cat exposure can be maintained at levels that enable population persistence. Our results suggest that accelerated natural selection may be a useful tool in combating anthropogenic disturbances over relatively short time frames.

AUTHOR CONTRIBUTIONS

Katherine Moseby, Mike Letnic and Daniel T. Blumstein conceived the ideas, and together with Rebecca West, Hannah Bannister and Leanne Van der Weyde designed the methodology. Rebecca West, Hannah Bannister, Leanne Van der Weyde and Katherine Moseby collected the data. Leanne Van der Weyde and Hannah Bannister analyzed the data. Katherine Moseby led the writing of the manuscript with assistance from Hannah Bannister and Leanne Van der Weyde. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

DATA AVAILABILITY STATEMENT

Data (Moseby, 2022) are available in the Open Science Framework at <https://doi.org/10.17605/OSF.IO/9H3D2>.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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