

# Chew-cards can accurately index invasive rat densities in Mariana Island forests

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## Abstract

Rats (*Rattus* spp.) are likely established on 80–90% of the world's islands and represent one of the most damaging and expensive biological invaders. Effective rat control tools exist but require accurate population density estimates or indices to inform treatment timing and effort and to assess treatment efficacy. Capture-mark-recapture data are frequently used to produce robust density estimates, but collecting these data can be expensive, time-consuming, and labor-intensive. We tested a potentially cheaper and easier alternative, chew-cards, as a count-based (quantitative) index of invasive rat densities in tropical forests in the Mariana Islands, an archipelago in the western North Pacific Ocean. We trialed chew-cards in nine forest grids on two Mariana Islands by comparing the proportion of cards chewed to capture-mark-recapture density estimates and manipulated rat densities to test whether the relationship was retained. Chew-card counts were positively correlated with rat capture-mark-recapture density estimates across a range of rat densities found in the region. Additionally, the correlation between the two sampling methods increased with the number of days chew-cards were deployed. Specifically, when chew-cards were deployed for five nights, a 10% increase in the proportion of cards chewed equated to an estimated increase in rat density of approximately 2.4 individuals per ha ( $R^2 = 0.74$ ). Chew-cards can provide a valid index of rat densities in Mariana Island forests and are a cheaper alternative

to capture-mark-recapture sampling when relative differences in density are of primary interest. New cost-effective monitoring tools can enhance our understanding and management of invaded islands while stretching limited resources further than some conventional approaches, thus improving invasive species management on islands.

### Keywords

Abundance estimation, capture-mark-recapture, Guam, invasive predator control, *Rattus*, Rota, spatial capture-recapture, tropical ecology

## Introduction

Invasive species jeopardize worldwide biodiversity (Liu et al. 2020), economies (Hanley and Roberts 2019), and human health (Mazza and Tricarico 2018) and cost billions of dollars annually in ecological damages, economic losses, and management efforts (Cuthbert et al. 2022), an amount that is continually increasing (Diagne et al. 2021). Minimizing costs to control invasive species makes combatting this problem more feasible at larger scales (Jardine and Sanchirico 2018). Primary ways invasive species management costs can be reduced are via effective pathway and vector management (e.g., screening protocols), early detection and rapid response, and improved approaches to mitigation and restoration (Green and Grosholz 2020).

Islands are often the focus of invasive species research and control efforts (Holmes et al. 2019). Housing an estimated quarter of Earth's plant and animal species, islands are biodiversity hotspots and targeting them can maximize conservation funds (Myers et al. 2000). However, isolation—the same feature that supports high endemism and richness (MacArthur and Wilson 1967)—makes islands remarkably susceptible to damage from biological invasions (Whittaker and Fernández-Palacios 2007). In particular, introduced predators can easily exploit native species that have evolved under limited predation pressure (Doherty et al. 2016).

Rats (*Rattus* spp.) are difficult to detect (Yackel Adams et al. 2011; Smart et al. 2021), easily transportable (Gatto-Almeida et al. 2021), highly fecund (Harper and Bunbury 2015; Clapperton et al. 2019), and adaptable (Duron et al. 2019). These anthropogenic commensal generalists (Dammhahn et al. 2017) are likely established on 80–90% of the world's islands (Towns et al. 2006)—of which at least 78% are documented to support highly threatened vertebrates (Spatz et al. 2017)—and represent one of the most damaging and expensive biological invaders (Harper and Bunbury 2015; Cuthbert et al. 2021). Invasive rats have a myriad of impacts on island ecosystems. As direct predators of many small vertebrates, rats have caused extinctions or severe declines in birds (Bond et al. 2019), reptiles (Donihue et al. 2021), and mammals (Hanna and Cardillo 2014). Cascading effects shift interspecific dynamics, causing further deterioration to island communities and exacerbating destruction caused by rats (Campbell and Atkinson 2002; Kurlle et al. 2021). For example, on some islands, invasive rats help sustain populations of co-occurring invasive predator species

at higher trophic levels as an abundant prey source (e.g., cats, stoats, snakes; Savidge 1986, 1987; Murphy and Bradfield 1992). Such multitrophic invasive predator assemblages inflict compounded impacts to the islands they inhabit and make invasive species control more complicated and costly (Stobo-Wilson et al. 2021). Invasive rats can also serve as vectors for novel pathogens to highly susceptible insular faunas (Pickering and Norris 1996).

Trapping (Duron et al. 2020) and rodenticides (Keitt et al. 2015) can reduce rat densities, and both approaches have been employed in successful rodent eradications on hundreds of islands (Howald et al. 2007). However, cost-effective rodenticide treatments or other control efforts first require accurate target population density estimates to inform treatment timing and effort and to assess treatment efficacy (Kim et al. 2020). Capture-mark-recapture data are frequently used to produce robust density estimates (Otis et al. 1978; Williams et al. 2002; Wiewel et al. 2009a; Yackel Adams et al. 2011), but collecting these data can be expensive, time-consuming, and labor-intensive (Wiewel et al. 2009b). Developing cheaper and easier techniques for indexing density (i.e., count-based indices) is thus a priority.

Count-based indices are commonly used as relative measures of abundance or density (McKelvey and Pearson 2001). An effective count-based density index enumerates animal evidence, meets the assumption of constant detection (Anderson 2003), and strongly correlates with true density across all possible densities (Nichols 1992). Ideal indices should also be inexpensive, user-friendly, and applicable at large spatial scales (Williams et al. 2002; Engeman 2005; Engeman and Whisson 2006). However, indices have been criticized because assumptions are often ignored and untested (e.g., constant detection probability; Anderson 2001, 2003; Skalski et al. 2005), and they are frequently used to make inferences or inform management decisions without any preceding testing or calibration (Rosenstock et al. 2002). Nevertheless, indices can represent relative differences in abundance (Engeman 2003) if *in situ* calibration studies show a positive, monotonic relationship between the index and true density across the range of possible densities in a given region (Nichols 1992). Counts of animal observations (Fagerstone and Biggins 1986), automated-camera photos (Engeman et al. 2006), tracks (Brown et al. 1996), chew-marks (Sweetapple and Nugent 2011), bait-take rates (Byers 1975), hair deposition (Zielinski et al. 2006), physical captures (Village and Myhill 1990), and feces detections (Mills et al. 2005) have all been used to index diverse small mammal populations across the globe with varying successes and limitations.

Chew-track-cards, a tool for indexing rodents, are baited pieces of plastic that retain animal tooth impressions and footprints. Seminal work conducted in Australia and New Zealand determined that chew-track-cards are a cost-effective means of accurately indexing small mammal abundances across multiple species, including rats (Caughley et al. 1998; Sweetapple and Nugent 2011; Kavermann et al. 2013; Ruffell et al. 2015; Burge et al. 2017; Forsyth et al. 2018; Balls 2019). However, to our knowledge, no study since Caughley et al. (1998) has validated chew-track-card counts against measured (i.e., capture-mark-recapture) density estimates; latter stud-

ies simply document relationships among multiple small mammal abundance indices (Sweetapple and Nugent 2011; Kavermann et al. 2013; Ruffell et al. 2015; Forsyth et al. 2018; Balls 2019; Nottingham et al. 2021; Brown et al. 2022). Further, indices should be tested and calibrated in other ecosystems, regions, and climates before they are used to approximate population sizes beyond the range of existing studies. For example, rats on temperate islands (e.g., New Zealand) respond differently to baits than rats on tropical islands (Keitt et al. 2015); regionally based differences in foraging behavior may affect chew-track-card interaction frequencies and thus influence their effectiveness in the tropics. Consequently, evaluating chew-track-cards on tropical islands informs the ability to apply a detection tool developed in temperate environments to other ecosystems.

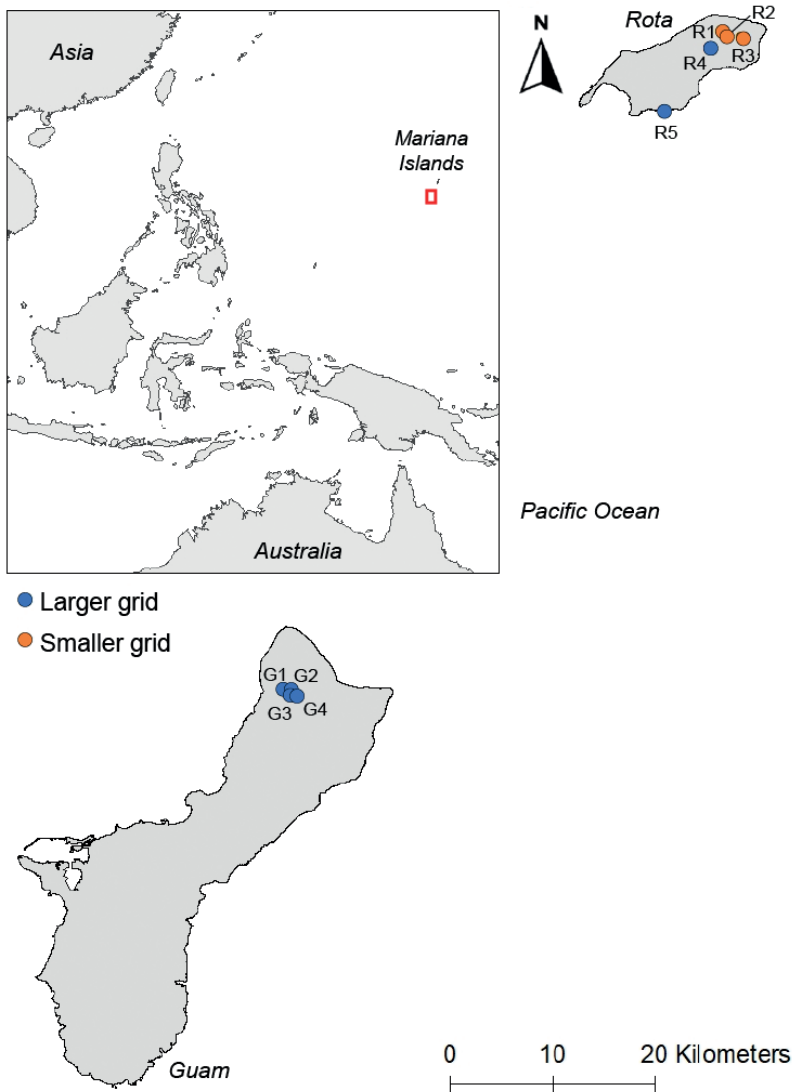
The Marianas are a chain of 15 volcanic islands in the western North Pacific Ocean (Fig. 1) that are optimal locations for field validating chew-track-cards in the tropics because they have a range of rat densities (Wiewel et al. 2009a) and are the focus of extensive conservation research and action (e.g., Faegre et al. 2019). We tested the accuracy of chew-track-cards as a count-based index of invasive rat density in forests on Guam and Rota, two Mariana Islands with low and high rat densities, by comparing chew-track-card counts to capture-mark-recapture density estimates and manipulated densities to test whether the relationship was retained. Our methods allowed us to identify prospects and caveats to the use of chew-track-cards on tropical islands, described herein. Our study informs future management efforts by testing a rat density index that, if effective, should reduce costs and improve efficiency for monitoring invasive rat populations in forests in the Marianas and, potentially, similar habitats on other tropical islands.

## Methods

### Study area

Guam and Rota are the southernmost and larger (Guam = 550 km<sup>2</sup>; Rota = 85 km<sup>2</sup>) of the Mariana Islands (Fig. 1). The southern Marianas are characterized as coralline limestone islands and are dominated by forest and grassland habitats. The climate is tropical with seasonal rains during July–October.

Pacific rats (*Rattus exulans*), brown rats (*Rattus norvegicus*), and black rats (*Rattus rattus*) have been established in the Mariana Islands—where bats are the only native mammals—for centuries (Baker 1946; Steadman 1999; Musser and Carleton 2005). Despite their proliferation, rats have had minimal direct impacts on native plants and animals in the Marianas compared to other oceanic islands (Fritts and Rodda 1998). In fact, rats did not become a major conservation concern in the Mariana Islands until they became key prey for an alien apex invader, the brown treesnake (*Boiga irregularis*; Fritts and Rodda 1998).



**Figure 1.** Nine forest grids sampled via chew-cards and live-trapping for rats (*Rattus* spp.) during June 2018–August 2019 on Guam (G1–4) and Rota (R1–5) in the Mariana Islands. In the inset map, the red rectangle indicates the location of the two islands in the western North Pacific Ocean. In the main map, the blue circles indicate  $11 \times 11$  grids with 12.5-m intervals between each station (grid area = 1.56 ha), and orange circles indicate  $10 \times 10$  grids with 10-m intervals between each station (grid area = 0.81 ha).

The brown treesnake was accidentally introduced from its native range in the South Pacific (Shine 1991) to the naturally snake-free island of Guam shortly after World War II (Rodda et al. 1999). By the 1980s, the snakes were widespread and abundant across Guam (Savidge 1987) and caused ecological destruction in their wake (Rodda

and Savidge 2007). Most notably, brown treesnakes extirpated most of Guam's forest birds (Savidge 1987; Fritts and Rodda 1998; Wiles et al. 2003). Decades of research and adaptive management have culminated in the potential for landscape-scale brown treesnake suppression in Guam forests (Clark et al. 2018; Siers et al. 2019, 2020). However, synchronous monitoring and control of rats is likely to be important because they are a key prey base for snakes on Guam and may affect the efficacy of some snake control tools (Gragg et al. 2010; Siers et al. 2018).

Our work on Guam occurred during 2018 within a 55-ha plot of homogenous disturbed limestone forest located on Andersen Air Force Base, termed the Habitat Management Unit. An extensive, interagency restoration plan including removal of non-native animals, constructing barriers, native plant recovery, and bird reintroductions exists for the Habitat Management Unit (Siers and Savidge 2017). A fence surrounding the entire site was erected in 2010 to prevent brown treesnake immigration and exclude non-native deer (*Rusa marianna*) and pigs (*Sus scrofa*; Siers and Savidge 2017). The Habitat Management Unit has undergone two major periods of experimental lethal snake treatments involving aerial deployment of toxic baits (dead neonatal mice laced with acetaminophen; Dorr et al. 2016; Siers et al. 2019). The first occurred during 2013 and 2014, and the second started during our study in 2018 and is ongoing. Without any rat treatments, we expected rat densities to increase following snake treatments via prey release (Ritchie and Johnson 2009), thereby providing a gradient of rat densities to test chew-track-cards on Guam. However, rat populations remained low. So, in 2019, we conducted additional fieldwork on Rota to test our index method on an island with higher rat densities (Savidge 1987; Wiles et al. 2003; Wiewel et al. 2009a, b). Rota lacks brown treesnakes and, consequently, has more ecologically intact forests with abundant native birds and fruit bats that represent what successfully restored forests may resemble on Guam.

## Grid selection and setup

We sampled nine forest grids on Guam ( $n = 4$  grids) and Rota ( $n = 5$  grids; Fig. 1). All four grids on Guam were located within the Habitat Management Unit, hereafter G1, G2, G3, and G4, with selection to maximize spatial coverage as well as avoid threatened and endangered plant species. Of the five grids sampled on Rota, three were part of a concurrent rat study where high populations were anticipated (Page 2020), hereafter R1, R2, and R3 (corresponding to grids 1, 2, and 5 in Page 2020). The other two grids had historically high rat densities, hereafter R4 and R5 (mixed and *Leucaena* forest habitats, respectively, in Wiewel et al. 2009a, b). After sampling each grid once, we manipulated rat densities in G2, G3, and R4 before resampling to increase our sample size without having to establish new grids. We resampled G3 and G2 three months after lethal snake treatments that we anticipated would increase rat density via predator reduction. At R4, we humanely euthanized rats to manually reduce the population size before resampling with cards. To denote this, we appended .1 and .2 to the codes of grids we sampled twice (e.g., first sampling period in G2 = G2.1, second sampling period in G2 = G2.2).

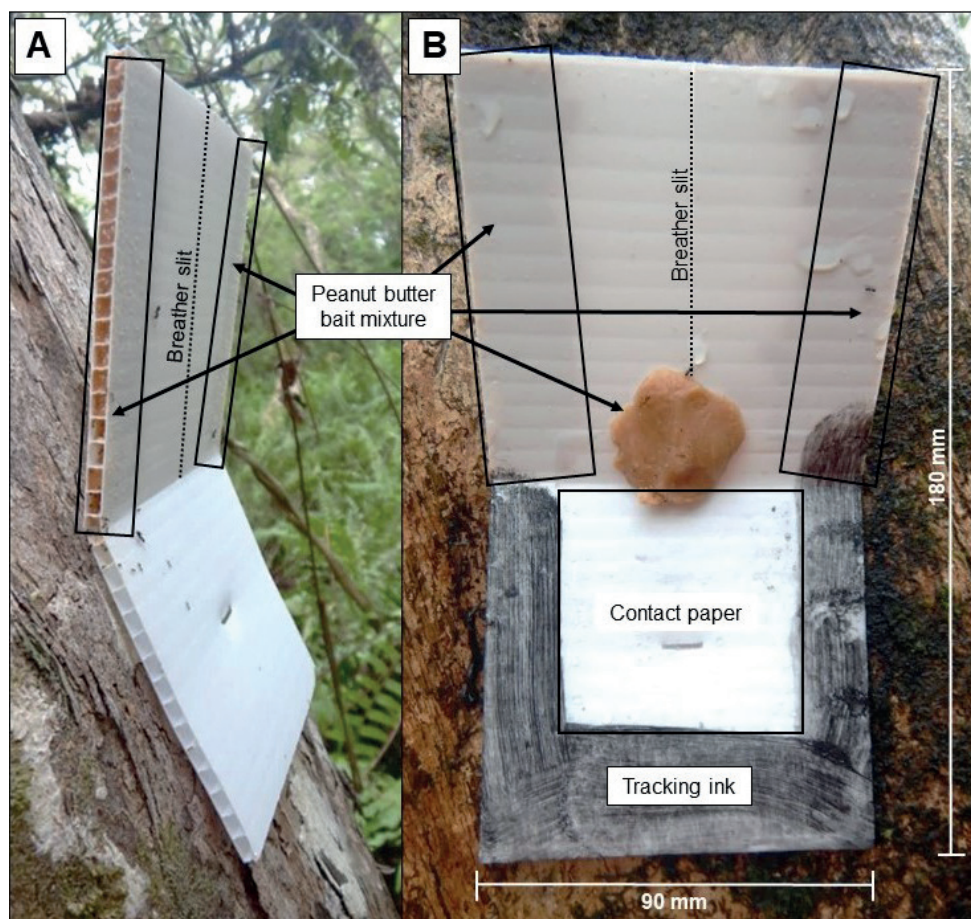
All Guam grids and Rota grids R4 and R5 consisted of  $11 \times 11$  trap stations with 12.5-m intervals between each station (grid area = 1.56 ha). The remaining three grids on Rota (R1–3) were part of a concurrent study (Page 2020) and consisted of  $10 \times 10$  trap stations with 10-m intervals between each station (grid area = 0.81 ha). For the larger grids, we placed one large folding Sherman live trap (H.B. Sherman Traps, Inc., Tallahassee, FL, USA) at each trap station ( $n = 121$  traps; spacing = 12.5 m) and one wire basket trap (Haguruma and Uni-King, Standard Trading Co., Honolulu, HI, USA) at every other station ( $n = 36$  traps; spacing = 25 m) for a total of 157 live traps per grid (every other station had two traps). We baited traps with a mixture of peanut butter, oats, and food-grade paraffin wax and live-trapped for 10 consecutive nights. For the smaller grids (R1–3), we placed one basket trap at every station ( $n = 100$  traps; spacing = 10 m) and baited traps with a combination of coconut and peanut butter. We live-trapped at these grids for four consecutive nights. Both grid sizes were at least four times the target species' home range estimates (Bondrup-Nielsen 1983), and spacing between stations was less than twice the target species' daily mean maximum distances moved (MMDM) in accordance with best practices (Otis et al. 1978; Wilson and Anderson 1985; Sun et al. 2014). Further, both trap and bait types are proven to be effective in this system (Baker 1946; Wiewel et al. 2009a, b; Page 2020). We accounted for trapping duration and all other sampling differences in our analyses.

## Data collection

We conducted capture-mark-recapture trapping of rats  $\leq 2$  days before (G1, G4, G2.2, G3.2, R1, R2, and R3) or after (G2.1, G3.1, and R5) a five-day card deployment so the cards would reflect the same rat densities estimated with capture-mark-recapture methods. We did not deploy live-traps and cards simultaneously to avoid competing baits on the landscape. We set baited, fixed-open traps two days prior to the start of live-trapping to allow the rats to acclimate to their presence (Wiewel et al. 2009a, b) and placed traps on flat ground beneath or adjacent to cover (e.g., vegetation, debris, rocks) to provide shelter from sun and rain. We checked traps every morning and recorded the trap station, the lowest possible taxonomic classification (e.g., *Rattus* spp.), and marked status (new or recaptured) for each captured individual. For newly captured rats, we determined sex and age via the external genitalia (imperforate vagina = juvenile female; perforated vagina = adult female; undescended testes = juvenile male; descended testes = adult male) and measured mass and head-body length. We double-marked individuals by inserting a numbered, metal ear tag (Style #1005-1, National Band and Tag Company, Newport, KY, USA) into each ear in the distal one-third of the pinna (Wang 2005) before releasing at the capture location. For recaptured individuals, we simply recorded both ear tag numbers before immediately releasing at the capture location (i.e., we did not collect additional mass and length measurements). We closed traps after the morning check to prevent mid-day captures when temperatures were highest to minimize heat-related trap mortalities. In the late afternoon/evening, we set and re-baited all traps and repaired and replaced them as necessary.



We constructed rat indexing cards by cutting 4-mm thick, twin-walled polypropylene sheeting into  $90 \times 180$ -mm rectangles and aligned the flutes parallel to the short sides of the cards (Fig. 2). We folded cards in half crosswise, cut a shallow slit lengthwise along the center of one half to prevent flutes from pressurizing when baited, and filled flutes with bait (peanut butter-paraffin mixture) to 2–3 cm from each edge (Fig. 2). On a subset of cards (chew-track-cards), we placed  $60 \times 75$  mm of contact paper in the center of the bottom halves of the cards and applied a 2–3-cm wide strip of black ink onto the plastic surrounding the contact paper (Fig. 2B). We placed additional bait ( $\sim 1$  oz) at the top of the contact paper (Fig. 2B). This design was intended to lure rats to walk through the ink and step on the contact paper, leaving visible tracks that could be identified to order (Rodentia [rats], Decapoda [crabs], Squamata [lizards], or Carnivora [cats]). However, we quickly



**Figure 2.** **A** chew-card and **B** chew-track-card designs used to index rat (*Rattus* spp.) density in forest habitats on Guam and Rota in the Mariana Islands during June 2018–August 2019. Designs were patterned after Sweetapple and Nugent (2011). Photographs taken by Emma B. Hanslowe during July 2018 in the Habitat Management Unit on Guam.



found this use of tracking ineffective due to the Marianas' wet climate (ink ran or faded) and the inability to distinguish rat tracks from those of non-target species. We therefore stopped alternating chew- (Fig. 2A) and chew-track-cards (Fig. 2B) within grids and, instead, deployed solely chew-cards after completing our fourth grid (G4) in August 2018.

At each station, we stapled the cards to trees approximately one meter off the ground with the baited half up (Fig. 2). We checked the cards each morning and recorded if a card had been chewed. To identify species chews, we cross-referenced our cards with published reference photos and guides (Sweetapple and Nugent 2011; Manaaki Whenua Landcare Research 2020) and cards we placed in captive rat enclosures on Guam. We did not replace, repair, or re-bait cards during the five-day deployment to simulate the cards being left in the field without maintenance, as they would likely be in practice (Sweetapple and Nugent 2011). We removed the cards and all associated materials at the conclusion of the five-day card deployment.

To confirm or refute rat-chew identification, we deployed a RECONYX PC900 HyperFire Professional Covert Camera Trap (RECONYX, Holmen, WI, USA) at six randomly selected cards from each grid, except R2 and R3, for the duration of the five-day card deployment. We initially programmed the cameras to trigger upon motion detection (for G1, G2.1, G3.1, G4, G3.2) but switched to a time-lapse setting after December 2018 (for grids G2.2, R1, R4.1, R4.2, and R5) to better capture species interactions with the cards. We reviewed all camera-trap photos and cross-referenced our field assessments of rat chews with the photos from the corresponding camera-trap night. We measured daily rainfall via rain gauges at all grids except R2 and R3.

## Data analyses

We calculated individual body condition indices by dividing mass by head-body length (Li et al. 2021). We evaluated differences between masses, head-body lengths, and body condition indices between rats trapped on Guam versus Rota with Wilcoxon rank-sum tests ( $\alpha = 0.05$ ).

## Density estimation

We used spatially explicit capture-recapture models (Efford 2004) executed in the R package *secr* 4.2.2 (Efford 2020; R Core Team 2020) to estimate rat density because these models produce unbiased density estimates for social species, like rats (Davis 1953), even when study animals' movements and home ranges may violate model assumptions (Efford et al. 2009; López-Bao et al. 2018). We used Akaike's Information Criterion corrected for small sample size ( $AIC_c$ ) to determine the best-supported models in our candidate sets (Burnham and Anderson 2002) and derived model-averaged density estimates and standard errors from the final model set (Burnham and Anderson 2002). Rationale for all models came from a combination of results from preceding studies, the biology and life history traits of small mammals, and knowledge of our system, described herein.

Capture probabilities can vary by time, behavior, and individual heterogeneity (Otis et al. 1978). Small mammals tend to be wary of new objects (Clapperton 2006; Yackel Adams et al. 2011) and, in the Marianas, have previously exhibited a two-day neophobic behavioral response where capture probabilities during the first two nights were lower than capture probabilities on the remaining occasions, even after a trap acclimation period (Wiewel et al. 2009a, b). Other hypothesized patterns of temporal variation included a time trend where rat capture probabilities changed linearly on the logit-scale over all capture occasions (Cusack 2011) or via daily changes in weather (e.g., rain; Stokes et al. 2001; Wiewel et al. 2009a, b). Behavioral responses are also well documented across taxa and systems and occur when animals become ‘trap-happy’ or ‘trap-shy’ (Hammond and Anthony 2006) and are associated with a positive (e.g., food) or negative (e.g., stress) trap experience resulting in unequal initial capture and recapture probabilities (Otis et al. 1978). We based our *a priori* hypotheses regarding individual heterogeneity largely on Wiewel et al. (2009a, b) who found higher capture probabilities for reproductively active (i.e., adult) female small mammals in the Marianas. Lastly, we tested a hypothesis that individuals in lower body condition may be more attracted to our baits, resulting in higher capture probabilities.

We analyzed data from each grid separately. At grids with sufficient data (R4–5), we used a two-step approach to model capture probabilities from which we derived density estimates. First (Step 1), we accounted for all available hypothesized sources of individual heterogeneity in capture probability by including sex, age, and body condition index as predictors. We fit models with additive combinations of temporal covariates, including a two-night neophobic response (neophobia2), a time trend (Time), daily rainfall amount (rain; when available), a behavioral response (behavior), and no temporal variation (.). We did not include neophobia2 with either rain or Time in the same model. We retained the best-supported temporal variation structure(s) to test all possible additive combinations of individual covariates, including sex, age, body condition index, and no individual heterogeneity (Step 2). We failed to collect individual covariate and rain data for Rota grids R1–3, and thus did not have sufficient data for the two-step approach. For these grids, we simply fit all other possible additive combinations of the remaining temporal covariates. We held the spatial parameter ( $\sigma$ ) constant (i.e., null) in all models.

Data from grids on Guam were too sparse (< 10 total captures per grid) to use spatially explicit models, so we used simpler closed-capture conditional likelihood models (Huggins 1989, 1991) from Program MARK 6.2 (White and Burnham 1999). We combined encounter histories from all Guam grids, differentiated grids by group, and—with the sparse data—were able to fit two simple models: constant capture probability (i.e., a null model) and a model with a behavioral effect (see Suppl. material 1: Table S1). We used the derived model-averaged abundance estimates to calculate density by dividing each estimate by an effective trapping area (ETA; Wilson and Anderson 1985; Efford 2004). We used results from the spatially explicit analysis to inform our choice of boundary strip (full MMDM) for our ETA calculations (see Suppl. material 2: Fig. S1). For grids with no movement metrics, we used the mean MMDM of all other grids from the same island and calculated standard errors using the delta method (Seber 2002).

## Chew-card density index calibration

We did not analyze tracking ink data because we deemed our tracking ink methods ineffective in this system and instead treated all cards as ‘chew-cards’ and limited our analysis to teeth impressions. We summed the cumulative number of cards with rat chews for each deployment day (1–5 days) for each grid and calculated the daily proportion of cards with rat chews. We used linear regression models and Pearson’s product-moment correlations, implemented in base R, to assess the relationship between card indices and capture-mark-recapture density estimates. We conducted these analyses five times, where the predictor variable in each regression analysis was the proportion of cards that detected rats after one, two, three, four, and five deployment nights, respectively, for each grid.

## Results

### Capture-mark-recapture of live-trapped rats

We captured 233 individual rats a total of 444 times in 10,090 corrected trap nights over the course of our study, where one corrected trap night equaled one active trap night corrected for sprung (via target and non-target captures and false trips) and non-functioning/missing traps by considering them to represent half of a night of trapping effort and no trapping effort, respectively (Table 1; Nelson and Clark 1973). We trapped almost 11 times as many rats on Rota ( $n = 213$  rats) as we did on Guam ( $n = 20$  rats) with approximately half the trapping effort (Table 1). We determined sex and age for 194 captured individuals. Of those, we captured more males than females and more adults than juveniles on both islands (Table 1). Collectively, rats were heavier (average Guam mass  $\pm$  SD =  $193.32 \pm 62.30$  g; average Rota mass  $\pm$  SD =  $95.75 \pm 42.81$  g; Wilcoxon rank-sum  $W = 2.812.5$ ;  $P = 6.09 \times 10^{-9}$ ) and had higher body condition indices (average Guam body condition index  $\pm$  SD =  $1.32 \pm 0.36$ ; average Rota body condition index  $\pm$  SD =  $0.68 \pm 0.24$ ; Wilcoxon rank-sum  $W = 2.942$ ;  $P = 1.46 \times 10^{-10}$ ) on Guam compared to Rota, but there was no difference in head-body lengths between the two islands (average Guam head-body length  $\pm$  SD =  $146.05 \pm 27.25$  mm; average Rota head-body length  $\pm$  SD =  $135.83 \pm 29.35$  mm; Wilcoxon rank-sum  $W = 1.798$ ;  $P = 0.25$ ; Fig. 3).

We found that rat capture probability on both islands exhibited a behavioral effect (Fig. 4; see Suppl. material 1: Tables S1–S4). There was little evidence of additional temporal variation in capture probability; a model with a two-night neophobic effect was the best-supported model for one grid on Rota ( $R5$ ;  $\hat{\beta} = 0.07$ ;  $\widehat{SE}[\hat{\beta}] = 0.24$ ). We found no evidence of variation in capture probability among individuals (associated with body condition, age, or sex) and no evidence that capture probability varied as a function of rain (see Suppl. material 1: Tables S3, 4).

Our grids represented a range of rat density estimates ( $\widehat{D}$  range = 0.00–34.73 rats/ha) to test card indices. Rat densities on Rota ( $\widehat{D}$  range = 7.09–34.73 rats/ha) were

**Table 1.** Corrected trap nights†, number of individual rats (*Rattus* spp.) captured (# indiv. rats), total number of rat captures (including recaptures; total rat caps.), sex (M = male; F = female; U = undetermined sex), age (A = adult; J = juvenile; U = undetermined age), density estimate plus/minus standard error ( $\widehat{D} \pm SE$ ), and proportion of chew-cards with rat chews after nights 1–5 for each sampling grid in forest habitats on Guam and Rota in the Mariana Islands during June 2018–August 2019.

Grid	Live-trap dates	Corrected trap nights†	# indiv. rats	Total rat caps.	Sex			Age			$\widehat{D} \pm SE$	Chew-card proportions				
					M	F	U	A	J	U		1	2	3	4	5
Guam																
G1	11–20 Jun 2018	1,296.5	1	1	1	0	0	1	0	0	0.26 ± 0.78	.00	.00	.00	.01	.01
G2.1	19–28 Jul 2018	1,153.0	4	5	2	2	0	4	0	0	1.37 ± 0.14	.00	.00	.02	.05	.10
G3.1	19–28 Jul 2018	879.0	3	3	2	1	0	3	0	0	0.79 ± 2.35	.00	.01	.03	.05	.11
G4	04–13 Aug 2018	1,009.5	0	0	0	0	0	0	0	0	0.00 ± 0.00	.00	.02	.04	.05	.10
G3.2	29 Nov–08 Dec 2018	1,155.5	6	11	4	2	0	5	1	0	1.01 ± 9.65	.02	.05	.06	.07	.10
G2.2	02–11 Feb 2019	1,243.5	6	7	5	1	0	5	1	0	1.93 ± 0.18	.03	.09	.14	.18	.29
	Guam total	6,737.0	20	27	14	6	0	18	2	0						
Rota																
R1	04–07 Jun 2019	286.0	20	35	9	5	6	5	9	6	7.09 ± 1.71	.28	.47	.61	.67	.73
R2	11–14 Jun 2019	334.0	12	14	4	4	4	2	6	4	20.83 ± 12.65	.02	.07	.14	.25	.39
R3	11–14 Jun 2019	311.0	17	27	3	5	9	7	1	9	9.37 ± 1.79	.27	.76	.84	.87	.87
R4.1	28 Jun–07 Jul 2019	1,285.5	92	196	48	44	0	51	41	0	34.73 ± 4.52	.12	.59	.83	.91	.94
R4.2‡											19.99 ± 4.52	.69	.89	.96	.96	.96
R5	27 Jul–05 Aug 2019	1,136.5	72	145	40	32	0	56	16	0	21.86 ± 3.21	.02	.10	.21	.36	.48
	Rota total	3,353.0	213	417	104	90	19	121	73	19						
Total		10,090.0	233	444	118	96	19	139	75	19						

† One corrected trap night equals one active trap night corrected for sprung (via target and non-target captures and false trips) and non-functioning/missing traps by considering them to represent half of a night of trapping effort and no trapping effort, respectively:

$$corrected\ trap\ nights = traps \times nights - \left( \frac{targets + nontargets + false\ trips}{2} \right) - (nonfunctioning + missing)$$

(Nelson and Clark 1973).

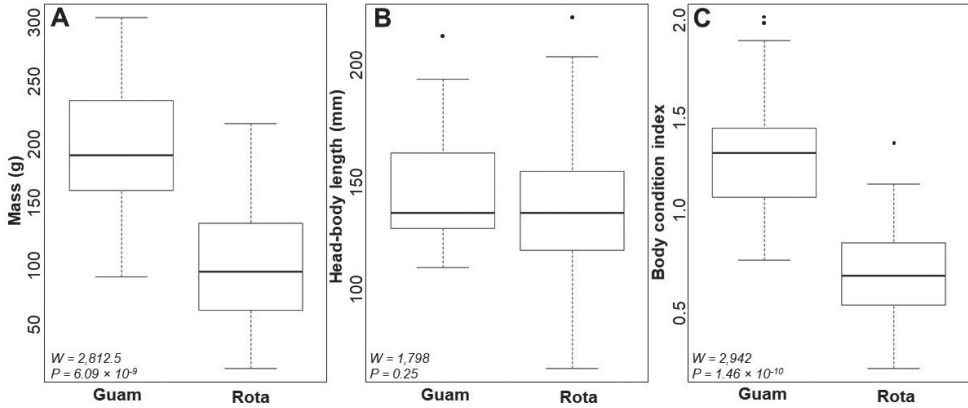
‡ We did not live-trap in Rota grid R4 twice. Instead, we euthanized all individuals that were live-trapped on 07 July 2019, the fifth/ final day of trapping, and used the total number of rats removed—including any that died incidentally during trapping—to calculate a reduced density estimate for the second chew-card session during 09–13 July 2019 (R4.2).

higher than those on Guam ( $\widehat{D}$  range = 0.00–1.93 rats/ha). At the two grids we re-sampled after lethal snake treatments on Guam, G3.2 and G2.2, rat density increased by 28% and 41%, respectively, but remained comparatively low even three months after snake control was applied ( $\widehat{D} = 1.01$ ;  $\widehat{SE}[\widehat{D}] = 9.65$  and  $\widehat{D} = 1.93$ ;  $\widehat{SE}[\widehat{D}] = 0.18$ , respectively; Table 1; see Suppl. material 2: Fig. S1).

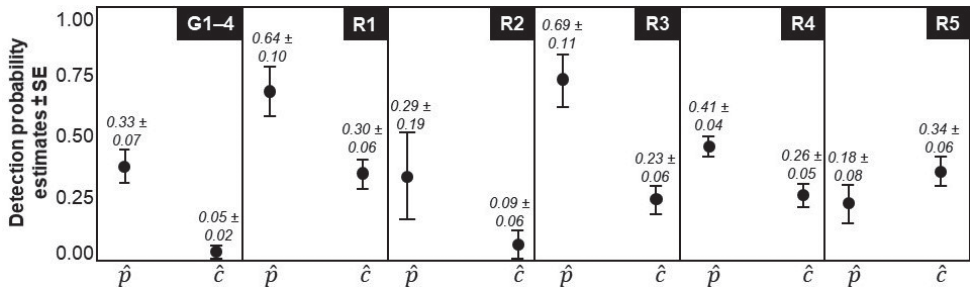
Chew-cards

We deployed 1,389 chew-cards during 60 days of sampling on Guam ( $n = 6$  deployments) and Rota ( $n = 6$  deployments). The mean proportion of cards chewed after five days was 0.12 (SD = 0.09) on Guam and 0.73 (SD = 0.24) on Rota. On average, the proportion of cards with chews increased by 0.03 (SD = 0.03) a day on Guam and 0.10 (SD = 0.10) a day on Rota.

The proportion of cards chewed by rats was correlated with density estimates when cards were left in the field for at least three nights (Fig. 5). The correlation increased daily and was highest after five nights ( $R^2 = 0.74$ ). When chew-cards were deployed for



**Figure 3.** Boxplots depicting the medians (bold lines), interquartile ranges (IQRs; 25th–75th percentiles; rectangles), minimums (first quartile–1.5\*IQR) and maximums (third quartile+1.5\*IQR; dashed lines), and any outliers (black dots) for **A** mass, **B** head-body length, and **C** body condition index for live-trapped rats (*Rattus* spp.) in forest habitats on Guam ( $n = 19$  rats) and Rota ( $n = 163$  rats) in the Mariana Islands during June 2018–August 2019. Statistics shown in the bottom-left corners are for Wilcoxon rank-sum tests ( $\alpha = 0.05$ ). Rats were **A** heavier and had **C** higher body condition indices on Guam compared to Rota, but there was no difference in **B** head-body lengths between the two islands.



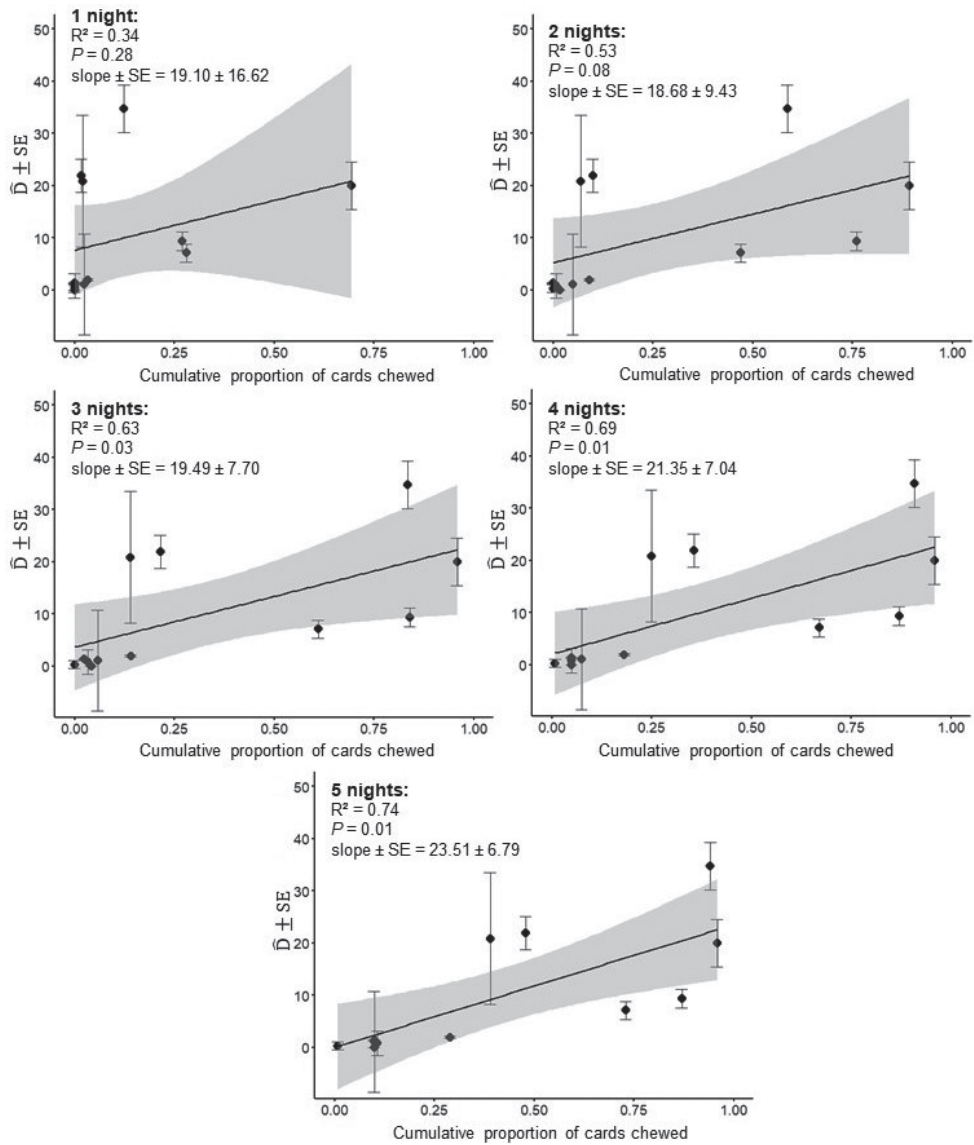
**Figure 4.** Capture ( $\hat{p}$ ) and recapture ( $\hat{z}$ ) probability estimates from closed-capture conditional likelihood models for rats (*Rattus* spp.) in forest habitats on Guam (G1–4) and Rota (R1–5) in the Mariana Islands during June 2018–August 2019.

five nights, a 10% increase in the proportion of cards chewed equated to an estimated increase in rat density of approximately 2.4 individuals per ha:

$$\widehat{\text{rat density}} = 23.51 \text{ (cumulative proportion of cards with rat chews)}$$

Note that an intercept ( $B_0$ ) was not included in this equation because it rounded to zero.

We deployed cameras on 60 cards and processed > 24,000 photos with animals on the cards. Twenty-eight of these cards had field recordings of rat chews, and we confirmed rat identification via photos at 27 of 28 (96%) of the card/camera nights (e.g., Fig. 6).

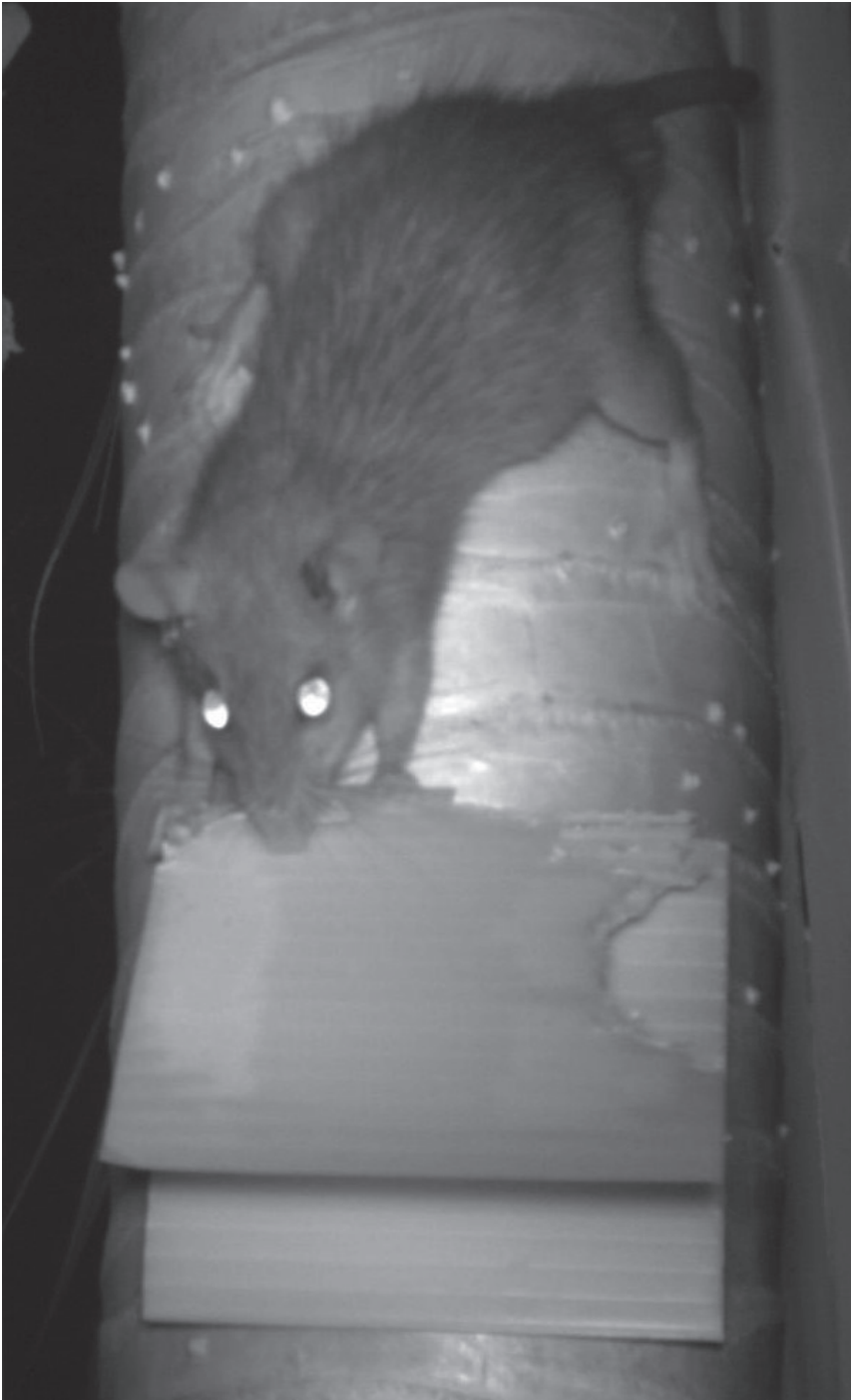


**Figure 5.** Linear regressions and Pearson's product-moment correlations to assess the relationship between the cumulative proportion of cards with rat (*Rattus* spp.) chews after one, two, three, four, and five nights (x-axis) and capture-mark-recapture density estimates plus/minus standard error ( $\hat{D} \pm SE$ ; y-axis) in forest habitats on Guam and Rota in the Mariana Islands during June 2018–August 2019.

## Discussion

Our study demonstrated a positive, monotonic relationship between chew-card counts and rat density estimates across a range of densities in Guam and Rota forests, and we thus conclude that chew-cards provided a valid index of rat densities and may be effective on similar tropical islands. Specifically, counts from chew-cards deployed for 3–5





**Figure 6.** Trail-camera photo of a rat (*Rattus* spp.) leaving visible chews on a chew-card. We used trail cameras to confirm or refute rat-chew identification at randomly selected cards from each grid. Emma B. Hanslowe photograph captured by an automated camera trap on 10 July 2019 in forest habitat on Rota in the Mariana Islands.

nights correlated with rat capture-mark-recapture density estimates. This relationship was retained across rat density estimates ranging from 0–35 rats/ha and after management. The correlation between the proportion of cards with rat chews and capture-mark-recapture density estimates increased daily and was highest after five nights, when nearly three quarters of the variance in capture-mark-recapture density estimates was predicted by variation in chew-card proportions ( $R^2 = 0.74$ ). Accordingly, chew-cards should be deployed for a minimum of three nights, but five nights is optimal as this duration provided the smallest standard error around the regression line. Evaluating longer chew-card deployment periods ( $\geq 6$  nights) may be advantageous, as additional nights might have even stronger correlations with rat density. However, the proportion of cards chewed will eventually stabilize or become 1.0 when all the cards are chewed, and this may occur more quickly at high rat densities (Burge et al 2017; Forsyth et al. 2018).

While chew-cards have been widely used to assess invasive small mammal populations (Oberge et al. 2014; Rouco et al. 2017; Gormley et al. 2018; Van Vianen et al. 2018; Nottingham et al. 2019; Robinson and Dick 2020; Ross et al. 2020; Nichols et al. 2021; Campos et al. 2022), our study is one of the few to validate this index using measured (capture-mark-recapture) density estimates (but see Caughley et al. 1998). Most studies simply compare chew-cards to other relative abundance indices (Sweetapple and Nugent 2011; Kavermann et al. 2013; Ruffell et al. 2015; Forsyth et al. 2018; Balls 2019; Nottingham et al. 2021; Brown et al. 2022). Different methodologies make it challenging to compare our results with those of previous chew-card studies. However, general conclusions across successful validation studies, including ours, as well as studies that compared multiple indices were consistent: chew-cards can represent relative differences in small mammal abundances or densities (Caughley et al. 1998; Sweetapple and Nugent 2011; Kavermann et al. 2013; Ruffell et al. 2015; Forsyth et al. 2018; Balls 2019; Nottingham et al. 2021; Brown et al. 2022).

Rat chews were easily distinguished from non-target chews (e.g., feral cats [*Felis catus*] and crab [*Coenobita brevimanus*; *Birgus latro*] pinches) and correctly identified in our study, as confirmed by our camera-trap data. Specifically, rats were photographed chewing cards at nearly all cards positive for rat chews (27 of 28 [96%] card/camera nights). The single unconfirmed chew was likely not misidentified but was more likely not captured because the camera's motion detection did not trigger. We switched camera settings from motion detection to time-lapse after this occurrence to improve rat detection on cameras, and all rat chews corresponding to a camera-trap night were photographed thereafter. Our study was the first to confirm chew-card species identification with cameras, as recommended by Forsyth et al. (2018).

We encountered significant issues with tracking ink during our study. First, the Marianas' tropical climate caused the ink to run and fade. Second, a multitude of non-target species (e.g., geckos, skinks, crabs, snails/slugs, ants, worms) left unidentifiable tracks that made distinguishing any rat tracks difficult, time-consuming, and erroneous. Similar to other studies, we found that tracking ink provided little additional information relative to chew marks alone (P. J. Sweetapple, Manaaki Whenua Landcare Research, written comm, 08 Sep 2018), and recent studies have discontinued its use in New Zealand (Ruffell et al. 2015; Burge et al. 2017; Forsyth et al. 2018; Nottingham et

al. 2021). Protecting the tracking ink and contact paper from the elements (e.g., placing them in tunnels) may reduce these issues in the tropics. A ‘tracking tunnel’ method was successful on tropical islands in the Caribbean with a similar non-target assemblage consisting of small lizards and invertebrates (Shiels et al. 2020). However, additional work with tracking ink may not be pressing, given our promising findings with chew-cards.

Non-targets may further hinder chew-card efficacy in Mariana Island forests via bait consumption and interspecific interference. We observed bait consumption by ants in the field, and reduced bait availability likely reduces chew-card attraction/effectiveness. In forests with abundant ants, chew-cards may be ineffective (pers. obs.). Use by non-targets may also affect rat chew-card detection (i.e., interspecific interference); for example, two studies in New Zealand found that individuals of one species were less likely to chew cards if they had already been chewed by another species (Sweetapple and Nugent 2009; Burge et al. 2017). Rats may be deterred from chew-cards if other species, especially stinging ants (e.g., little fire ants [*Wasmannia auropunctata*]) or coconut crabs, known predators of rats, are present. Further investigations of the relationships among rat chew-card detection rates, bait availability, and non-target species are warranted. Non-target exclusion methods could also be explored.

Our study results suggest that chew-cards can be appropriate for monitoring changes in rat distribution or relative density over space or time in association with invasive predator (e.g., brown treesnake) occurrence or suppression efforts in Mariana Island forests. Chew-cards have several advantages over capture-mark-recapture density estimation, at the forefront of which is cost. Extrapolating from cost analyses conducted by Wiewel et al. (2009b) and Sweetapple and Nugent (2011), we calculated that a single  $11 \times 11$  grid with 12.5-m spacing costs roughly 10 times more to employ capture-mark-recapture methods (~ U.S.\$3,000) than chew-cards (~ U.S.\$300). Like many indices, the reduced cost and simplified logistics of chew-cards make them more feasible for application at larger scales. Chew-cards also require less training and impose less risk than capture-mark-recapture sampling (e.g., no animal handling) and require minimal quantitative skills to use and interpret. However, capture-mark-recapture density estimation remains vital to scientists and managers by providing measures of precision and demographic and morphological data necessary for many studies and management decisions.

## Conclusion

Controlling invasive species on islands is a global conservation priority (Doherty et al. 2016), and cost-effective monitoring tools can stretch limited resources and enhance our understanding and management of islands with invasive species. Chew-cards can provide accurate indices of differences in rat densities in Mariana Island forests and, potentially, similar habitats when deployed for 3–5 nights. Chew-cards are a cheaper alternative to capture-mark-recapture sampling when relative differences in density are of interest and measures of precision or ancillary data are unnecessary. This is likely to be the case for many situations in the Marianas because rats are, foremost, prey for a more damaging invasive predator, the brown treesnake; chew-card based indices will

likely detect the major fluctuations in prey density that we expect following effective management of invasive predators (Howald et al. 2007). Further, with reduced costs and simplified logistics, chew-cards can be deployed more often or in more areas to gather estimates of relative rat densities and precision over time and space. These data could be used to inform invasive species control efforts, assess treatment efficacy, and investigate invasive predator-prey dynamics, all of which improve success of invasive species management on islands to preserve global biodiversity.

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## Supplementary material I

### Appendix 1, Tables S1–S4

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Data type: pdf file

Explanation note: Model selection results. **Table S1.** Guam: Huggins' closed-capture conditional likelihood model selection results for combined Guam grids sampled during June 2018–February 2019. **Table S2.** R1–3: Model selection results for spatially explicit models fit to data collected during June 2019 from grids for which we did not collect individual covariates. Results from the temporal models only (Step 1) are provided by grid. **Table S3.** R4: Spatially explicit model selection for rats sampled during June–July 2019. Step 1 models include all hypothesized sources of individual variation in capture probability (sex + age + BCI + temporal structures) listed below. We retained the best-supported temporal structure (behavior) when testing all possible additive combinations of individual covariates in Step 2 (sex, BCI, age, and no individual heterogeneity). **Table S4.** R5: Spatially explicit model selection for rats sampled during July–August 2019. Step 1 models include all hypothesized sources of individual variation in capture probability (sex + age + BCI + temporal structures) listed below. We retained the best-supported temporal structure (behavior + neophobia2) when testing all possible additive combinations of individual covariates in Step 2 (sex, BCI, age, and no individual heterogeneity).

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## Supplementary material 2

### Figure S1

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Data type: pdf file

Explanation note: Density estimator comparison. **Fig. S1.** Comparison of three density estimation approaches for rats (*Rattus* spp.) using capture-mark-recapture data from Guam (G1–4) and Rota (R1–5) forest grids during June 2018–August 2019. Black and dark gray bars represent density estimates ( $\hat{D}$ s) calculated from model-averaged abundance estimates ( $\hat{N}$ s) divided by effective trapping areas (ETAs) calculated by adding boundary strips equaling half of the mean maximum distances moved by rats captured more than once (0.5MMDM) and the full MMDM, respectively. Light gray bars represent  $\hat{D}$ s from spatially explicit capture-recapture (SECR) models for sites on Rota only.

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