

UC Davis

UC Davis Electronic Theses and Dissertations

Title

The role of human-cat interactions in shaping domestic cat (*Felis catus*) hunting behavior

Permalink

<https://escholarship.org/uc/item/5fp6x023>

ISBN

9798189225574

Author

Chung, Hee Jin

Publication Date

2026-06-25

Peer reviewed|Thesis/dissertation

The Role of Human-Cat Interactions in Shaping Domestic Cat (*Felis catus*) Hunting Behavior

By

HEE JIN CHUNG
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Animal Behavior

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

Andrew Sih, Chair

Carly Moody, Co-Chair

Benjamin N. Sacks

Committee in Charge

2026

Table of Contents

<i>Abstract</i>	<i>III</i>
<i>Acknowledgements</i>	<i>VI</i>
<i>Dedication</i>	<i>IX</i>
<i>Chapter 1: Safe or sound? Factors influencing outdoor access, cat behavior, and hunting history with implications for conservation and welfare</i>	<i>1</i>
ABSTRACT	1
INTRODUCTION	3
METHODS	6
RESULTS	11
DISCUSSION	21
CONCLUSION	27
ACKNOWLEDGEMENTS	28
REFERENCES	28
<i>Chapter 2: Utilizing citizen science to assess consistent individual variation in play behavior of the domestic cat, Felis catus</i>	<i>33</i>
ABSTRACT	33
INTRODUCTION	34
METHODS	36
RESULTS	44
DISCUSSION	51
CONCLUSION	55
ACKNOWLEDGEMENTS	56
REFERENCES	57
<i>Chapter 3: Using DNA metabarcoding and citizen science to assess the diet of owned domestic cats</i>	<i>60</i>
ABSTRACT	60
INTRODUCTION	61
METHODS	64
RESULTS	69
DISCUSSION	76
CONCLUSION	80
REFERENCES	81

ABSTRACT

Domestic cats, *Felis catus*, play an interesting role in human society, both as companion animals and as predators of local wildlife. Cats are one of the most commonly kept companion animals, surpassing dogs in many areas around the world, and are frequently directly managed by caretakers. However, cats are also one of the most widespread mesopredators with the ability to impact vulnerable prey species, and when outdoors (both owned and unowned) can cause prey population decline or even extinction. This has led to much discussion among scientists and the public on the proper management of owned cats, and whether possible benefits for their welfare warrant the provisioning of free-roaming outdoor access. The objective of this dissertation was to assess the role of management decisions and human-cat interactions on cats' hunting behavior, bringing together the perspectives of behavioral ecology, conservation ecology, animal welfare, and anthrozoology.

Many efforts have been made to reduce cats' hunting of wildlife, spanning from population control measures such as lethal population control and Trap-Neuter-Release, to targeting caretaker-led interventions such as reduced outdoor access and increased interactive play in the home. Some methods designed to reduce cat impact on wildlife can raise concern among caretakers, particularly if they are perceived to negatively impact their cat's welfare. This research focused on understanding the role of caretakers, not only as a mediator of cat-wildlife interactions, but also as partners of data collection on their cats' hunting and hunting-related behaviors. We relied on citizen science to better understand the perspectives and motivations of cat caretakers regarding outdoor access and hunting (Chapter 1), quantify individual variation in cats' hunting-related behaviors (Chapter 2), and test molecular methods of scat analysis for detection of prey consumption (Chapter 3).

In Chapter 1, we aimed to explore attitudes towards providing or reducing outdoor access, identify specific predictors of free-roaming outdoor access, and identify predictors of hunting behavior among cats that are allowed to freely roam. Using a quantitative questionnaire (N=361), we found that more than half of outdoor cat caretakers were likely or somewhat likely to reduce outdoor access if they lived near busier streets (59%), if more dangerous animals were around (59%), if their cat stopped coming home consistently (56%), or if they knew their cat was negatively impacting wildlife (53%). We found that cats had higher odds of freely roaming if they were older, obtained from the street, living close to traffic, or if they showed aggressive behaviors in the home. Among those allowed outside, cats were more likely to have hunted prey in the last year if they were allowed to freely roam instead of being supervised, or were allowed outdoors between in the morning hours.

In Chapter 2, we recruited participants in the United States (N=87) to expose their cat to standardised behavioral assay trials using a wand toy, to measure individual variation in response to the toy and the consistency of their responses over time. We found high repeatability of all behaviors indicative of cat interest measured across trials (mean $R = 0.467$). We then calculated a composite interest score consisting of all behavior variables, and found significant consistent individual variation in the cats' interest in the toy (ICC = 0.717). Interestingly, we did not find any significant connections between the cats' interest score and other factors of interest relating to their management in the home. This study was the first recorded use of citizen science to quantify consistent individual differences in play behavior of cats, and highlights the importance of assessing individual variation.

In Chapter 3, we relied on owner-collected scats ($n = 32$) from litterboxes and backyards to test whether DNA metabarcoding could be used to detect consumption of wild prey by owned,

indoor-outdoor domestic cats. We found only one instance of wild prey consumption, a California vole (*Microtus californicus*), despite the caretaker not witnessing any prey returns during the 24-hour period prior to scat sampling. Conversely, in the instances where the caretaker reported returns of intact prey by their cats, prey DNA was not detected in their scat. This study presents the first recorded use of DNA metabarcoding to detect consumption of wild prey by owned domestic cats, and suggests that caretaker reports and scat analysis should be used in combination to obtain a more thorough understanding of their hunting of wild prey. Together, this dissertation research provides much needed insight into the role of cat caretakers in mediating cat-wildlife dynamics, such as through their influence on their cats' outdoor access and behavior, and highlights their potential and importance as partners of data collection when studying companion animal behavior.

Acknowledgements

Words are not sufficient to show my appreciation for the wonderful mentors who have supported me throughout my dissertation. I would first like to thank Andy for believing in me coming into graduate school. Andy has taught me how to be a broad thinker, to host karaoke parties, and how to cultivate an intellectual community that fosters curiosity and critical thinking. Thank you for your generosity and patience as I navigated many challenges and milestones throughout this degree.

I would like to thank Carly for taking me on as a graduate student even though I knew next to nothing about cats. Carly has taught me how to not miss the details, to maintain academic rigor, and how to communicate my science to broad audiences. Thank you for your unwavering support, for your generosity with your time and resources, and always making sure my well-being came first.

I would like to thank Ben for welcoming me into his lab, which has been such a valuable community I have gotten to be a part of for the past few years. Thank you for your genuine care for your students, your honest and constructive feedback on ideas and methodology, and your patience as I tried to distinguish the baby from the bathwater.

I want to extend my gratitude to the labs that I have been a part of. Thank you to the Sih lab for the weekly lab meetings, and for willing to delve deep into some idea or project together, sometimes for 3 hours at a time. Thank you especially to Sonja Wild and Kaz Uyehara for dissecting my projects with me for hours, for lending an ear, and for feeding me high-protein snacks and meals. Thank you to all members of the Animal Welfare Epidemiology lab, for all your cat and welfare knowledge and expertise, for all the countless hours of feedback on surveys and study design, and helping me slowly establish myself as a cat scientist. Thank you especially

to Jen Link for being my lab friend and amazing colleague. I would like to thank all members of the Mammalian Ecology and Conservation Unit, for providing me a home where I felt welcomed, and for letting me listen in and ask basic questions about genetics and genomics when I know very little. Thank you especially to Stevi Vanderzwan for being our wonderful lab manager, for training me on new skills, and for all that you do for the lab.

I would like to thank all of the participants of my studies for making this research possible. Thank you for your dedication to learning more about these wonderful animals. Thank you to all the cats that participated (some more eagerly than others). I would like to express my gratitude towards all cat caretakers, cat behaviorists, and enthusiasts, for helping cats all over the world be better understood and cared for.

None of this would have been possible without the support of my peers, my fellow graduate students that I leaned on for support through various challenges. I want to thank all members of the Animal Behavior Graduate Group for being my community, particularly my cohort, Cassidy Cooper, Isabelle McDonald-Gilmartin, Jacob Johnson, Alice Michel, Sabrina Seaborn, and Kirsten Sheehy for starting graduate school with me in 2020 and going through this together. I feel grateful to have found an amazing group of friends who were there with me through good times and bad, especially Sofia Meléndez Cartagena, Deedee Chao, Francine DeCastro, Jordan Colby, Emmarie Purvis, the past and current Halsey crew, and the Crawlspace crew. Thank you to my best friend Natalie Schweizerhof for predicting that I will be a Dr. Chung someday. Thank you all for being part of my community, and for all the moments that we shared that kept me going.

I would like to thank all of my healthcare providers, who have supported me through multiple surgeries and health issues throughout graduate school and thus made this possible. I

especially would like to thank the UCD Student Health Physical Therapy Department, Jocelyn Lam, and Abby Hollandsworth for helping me through the toughest moments.

I would like to thank Harry Anselmo (Mr. A) for first igniting my passion for science back in 7th grade. I would not be here if it weren't for you and your dedication to raising the next generation of scientists and curious thinkers.

Finally, I would like to thank my wife, Elsie Platzer, for her unconditional support and love throughout graduate school, and in our lives together. Thank you for being the best stepmom to our cats, and for being my life partner and constant source of fun and inspiration. None of this would have been possible without you. To Memi and Zoe, for being yourselves, and for being willing to be my test subjects #0.

Dedication

For all animals around the world, big and small. For the cats in our lives, inspiring curiosity in us by being curious themselves. For Rocket, who made me decide to study cats – I hope you are in a better place now.



Chapter 1: Safe or Sound? Factors influencing outdoor access, cat behavior, and hunting history with implications for conservation and welfare

Published in *Applied Animal Behaviour Science* (2024,

<https://doi.org/10.1016/j.applanim.2024.106425>)

ABSTRACT

Outdoor access may provide mental stimulation and enrichment for companion cats (*Felis catus*); however, it also raises concerns regarding their safety and predation of vulnerable local wildlife. Understanding factors that shape cats' outdoor access and behavioral outcomes is therefore crucial for identifying nuanced solutions to the human-cat-wildlife conflict. In this exploratory study, we aimed to 1) explore owner attitudes towards providing or reducing outdoor access, 2) identify general predictors of free-roaming outdoor access, and 3) identify predictors of hunting behavior among cats that are allowed outside. Using a quantitative questionnaire (n=75 questions), we collected information on cat owners' demographics and attitudes, their cat's characteristics and behaviors in the home, home environment, outdoor lifestyle, and hunting history. Of 631 cat owners living near Davis, California, USA, recruited through convenience and snowball sampling, 36.7 % indicated they were keeping their cat indoor-only (n=232), 34.1 % providing supervised or controlled outdoor access (n=215), and 29.2 % providing uncontrolled, or free-roaming outdoor access (n=184). More than half of outdoor cat owners reported they were likely or somewhat likely to reduce outdoor access if they lived near busier streets (59 %, n=235), if more dangerous animals were around (59 %, n=235), if their cat stopped coming home consistently (56 %, n=223), or if they knew their cat was negatively impacting wildlife (53 %, n=211). We found that cats had higher odds of freely roaming if they were 7–10 years old compared to <1 year old ($p=0.030$), obtained from the street rather than a

shelter ($p=0.007$), living within 500 ft of traffic ($p=0.002$), living with a dog ($p=0.014$) or a child ($p=0.005$), or if they showed aggressive behaviors in the home ($p=0.008$). Cats had lower odds of freely roaming if they had scratching areas in the home ($p=0.017$), or were living with one ($p=0.020$) or two ($p=0.017$) other cats. Among those allowed outside, cats had higher odds of having hunted prey in the last year if they were allowed to freely roam without supervision ($p=0.016$), or were allowed outdoors between 6:00–11:59 am ($p=0.014$). These results generate directions for future research in the field of companion animal management, and emphasize the role of cat owners in the intersection of animal welfare and wildlife conservation.

INTRODUCTION

The domestic cat (*Felis catus*) is one of the most common household pets, present in approximately 37 million households in the United States (AVMA, 2022). Despite their long association with humans (Driscoll, Macdonald, and O'Brien 2009) and importance as pets in modern homes, cats retain several traits seen in their ancestor, *Felis silvestris lybica*. Cats exhibit obligate and hyper-carnivory, often supplementing their nutritional need for animal-based protein by predation (Eisert 2011; Cecchetti, Crowley, and McDonald 2021). Even when their nutritional needs are met, they exhibit surplus hunting (Yamaguchi et al. 2004; Bradshaw 2006; 2016), which have been observed in both owned (Lloyd et al. 2013) and unowned (McGregor et al. 2015) domestic cats. Such traits have and continue to make them excellent biocontrol agents against rodents in association with humans (Pongrácz and Lugosi 2024), but the impacts of their surplus hunting on local wildlife have sparked debate among conservation biologists, ecologists, and animal welfare experts regarding best practices for owned cat management (Machado et al. 2021; Foreman-Worsley et al. 2021; Rochlitz 2005).

On one hand, outdoor domestic cats have been implicated in approximately 26% of the world's bird, reptile, and mammal species extinctions (Doherty et al. 2016), and are well documented as effective non-native predators in both rural and urban environments (Baker et al. 2008; Balogh, Ryder, and Marra 2011; Lepczyk et al. 2023; Loss and Marra 2017). In addition, uncontrolled outdoor access can impact cat health and safety through transmission of communicable disease and parasites (Burling et al. 2017; Tan, Stellato, and Niel 2020), trauma (Olsen and Allen, 2001), predation (Timm et al., 2004), or injury or death through encounters with other animals or automobiles (Tan, Stellato, and Niel 2020). Lastly, while not a problem in the United States, in other regions of the world, the risk of hybridization between free-roaming

domestic cats and other related species (e.g., the European wildcat) is of serious conservation concern (Nussberger et al. 2023), especially given that cat owners have been shown to manage their cats irrespective of their knowledge of such risks (Pongrácz et al. 2024).

On the other hand, restriction to indoor-only living without the provision of appropriate resources may compromise cat welfare, potentially leading to behavioral problems such as house-soiling (periuria), excessive activity and vocalization, and aggression towards other animals and humans (Amat et al. 2009; Amat, Camps, and Manteca 2016; Strickler and Shull 2014; Heidenberger 1997). Outdoor access is also associated with physical activity and thus may decrease their risk of obesity, which may have implications for their overall health and well-being (Rowe et al. 2015). The American Association of Feline Practitioners (AAFP) suggests a series of environmental, social, and behavioral recommendations for enhancing indoor companion cat welfare (Ellis et al. 2013), such as hiding areas, separated areas with various resources, and opportunities for play and predatory behavior; however, providing these resources may not always be viable or desirable for owners, which may lead owners to rely on outdoor access as an alternative.

Much focus in the literature has been on quantifying the effect of both owned and unowned outdoor cats on wildlife. Recently, studies have identified the need to include cat-owners in the discourse (Crowley, Cecchetti, and McDonald 2019; 2020), with increased focus on understanding owner attitudes and perspectives in order to implement change in management decisions (McLeod, Hine, and Bengsen 2015; Linklater et al. 2019). Some studies have surveyed cat owners on their attitudes, cat's characteristics, and living environment to predict uncontrolled outdoor access (Clancy, Moore, and Bertone 2003; McLeod, Hine, and Bengsen 2015; Foreman-Worsley et al. 2021; Tan et al. 2021). However, few studies to date also include information

about the cat's hunting behavior (e.g. Escobar-Aguirre et al. 2019), distinguish between supervised and free-roaming outdoor access, or explicitly ask owners if certain hypothetical scenarios would make them reduce outdoor access. We attempt to fill these knowledge gaps and explore the connections between several welfare-focused factors (e.g. behavior problems, presence of necessary resources in the home) as well as conservation-oriented factors (e.g. hunting history, degree and type of outdoor access) in one sample. We examined: 1) factors that may predict provisioning of free-roaming outdoor access, 2) factors that may predict hunting history among cats that are allowed outside, and 3) owners' reported likelihood to alter outdoor access in response to hypothetical scenarios.

Although this was an exploratory study, we had *a priori* predictions about the relationship between some management factors and cat characteristics and the provisioning of outdoor access. Specifically, based on previous studies, we predicted that cats taken in from the street (Clancy, Moore, and Bertone 2003), living with other animals in the home (Clancy, Moore, and Bertone 2003; Tan et al. 2021), or living farther from traffic and closer to green space (Foreman-Worsley et al. 2021) would be more likely to be allowed outside. We also predicted that male cats (Tan et al. 2021; MacDonald, Milfont, and Gavin 2015), and older cats (Foreman-Worsley et al. 2021; Tan et al. 2021) would be more likely to be allowed outside to freely roam. Regarding hunting history, based on results found by Escobar-Aguirre et al. 2019, we predicted that cats would be more likely to have hunted in the last year if they were living closer to green space, or allowed outside to roam freely rather than be supervised. Additionally, given the activity patterns of potential prey species of cats, we predicted that the time of day the cat is allowed outside would be a significant factor influencing their hunting history (Woods, McDonald, and Harris 2003; McDonald et al. 2015; Linklater et al. 2019).

Lastly, given that owners have been shown to be motivated by their cat's safety (Woods, McDonald, and Harris 2003; McDonald et al. 2015; Linklater et al. 2019) but not their cat's effect on wildlife (Van Eeden et al. 2021; MacDonald, Milfont, and Gavin 2015), we predicted that they would report being likely to reduce outdoor access under hypothetical scenarios that threaten their cat's safety, but not scenarios that suggest their cat's increased impact on wildlife.

Given the disjointed and controversial rhetoric surrounding the topic of outdoor cats, especially between the fields of animal welfare and wildlife conservation, we recognize the importance of considering the interests of both fields when tackling this issue. While various elements of our study were individually explored in previous studies, we tackle these elements together in one sample for the first time and emphasize the need for further connection between different fields in the topic of human-cat-wildlife dynamics.

METHODS

Ethical Note

This study was reviewed and determined exempt (non-human subjects research) by the Institutional Review Board of the University of California, Davis (#1905569). A consent form including the inclusion criteria and a detailed description of the objectives of the study was presented to each participant at the beginning of the questionnaire. No identifiable information was asked of the participant, and each participant was given an option to click on a separate link at the end of the questionnaire to provide their email to enter a raffle for a chance to win one of three \$50 Amazon gift cards. Each participant was also given an option to click on a separate link to provide their email to be contacted for a follow-up study with their cat.

Study Design

A cross-sectional, quantitative questionnaire was created and distributed online using Qualtrics™ survey software (Provo, Utah, USA). To participate, respondents had to be at least 18 years old, the current primary caretaker of at least one cat, and reside in one of the following cities in Northern California: Davis, Woodland, Dixon, Winters, West Sacramento, or Sacramento. These cities were selected based on their proximity to the main campus of the University of California, Davis, since this questionnaire was partly distributed to recruit participants for a separate in-person study. Participants were not eligible for the study if they were caretakers of outdoor-only cats who never spent time indoors, given we were interested in assessing the interactions between the cats, the owners, and the home environment. Each participant who completed the questionnaire and provided their email was entered into a raffle to win one of three \$50 Amazon gift cards as an incentive to participate in the study.

This questionnaire (N=75 questions) contained questions on the owners' demographics (n=4), their opinions about outdoor cats (n=7), motivations for letting or not letting their cat outside (n=7), cat characteristics and behavior (n=25), cat-human interactions (n=5), the home environment (n=11), and the cat's outdoor lifestyle and hunting-related behaviors (n=17). If the participant had multiple cats, they were directed to select the cat whose name appears first in the alphabet and answer the rest of the questionnaire about that cat. We used the MacArthur Scale of Subjective Social Status (Adler et al. 2000) as a proxy for the respondents' perceived self-status in relation to their community, since subjective social status has been found to be more informative than objective measures of social status (e.g. Präg, Mills, and Wittek 2016); participants were asked to place themselves on a ladder from a scale of 1 to 10, with 1 being the best off in their community and 10 being the worst off in their community. These questions were chosen based on previous studies on this topic and factors for which we had *a priori* hypotheses.

The study minimum sample size ($n=360$) was calculated by using a standard sample size calculation using a 95% confidence interval and 5% margin of error, with the target population of cat owners estimated to be 25.4% (AVMA, 2022) of the source population of each city (Davis, Woodland, Dixon, Winters, West Sacramento, Sacramento, CA).

Data Collection

The questionnaire was distributed online from August 1st, 2022 to August 11th, 2022 using convenience and snowball sampling. Given our regional source population, an advertisement was distributed to local community and academic listservs and social media groups. Sampling was terminated when we no longer obtained any new responses, with a final number of 1230 responses.

Data Cleanup & Preparation

Following data collection, 97 incomplete responses were removed. 53 additional responses that did not meet the inclusion criteria were removed. Lastly, responses of outdoor-only cat owners, and responses with duplicate IP addresses were removed, thus leading to a final sample size of $N=631$. All ordered and binary categorical variables were converted into numerical values, and these codes were recorded on a separate spreadsheet. Open text box responses associated with each “Other” response selection were removed, and all variables were coded. The cleaned dataset was inputted into R Statistical Software (v4.2.2; R Core Team 2022). All statistical analyses and visualizations were performed in R. Descriptive statistics were run for all variables included in the survey, and frequency and percentage tables were generated for the variables to be included in the models in STATA™ statistical software (College Station, TX: StataCorp, LLC) prior to model building. The distribution of all biologically plausible predictor variable response categories for outdoor access and hunting history were visually assessed, and

sparsely distributed categories ($n < 20$) were collapsed when appropriate in order to increase accuracy of estimation parameters.

Logistic Regression

Binary logistic regression models were built to assess biologically relevant factors that predicted the following two outcomes: 1) being provided free-roaming outdoor access (yes, no), and 2) having a history of hunting within the past year (yes, no). A large number of predictor variables were involved in modeling the probability of being provided free-roaming outdoor access – thus, in order to avoid overfitting given the sample size, these variables were divided and assessed in two different models: cat characteristics and home environment predictor variables (Model 1), and owner demographics and opinion predictor variables (Model 2). For these models, we compared indoor-only to free-roaming outdoor cats and omitted supervised outdoor cats. This was due to a lack of a clear boundary between supervised outdoor and free-roaming outdoor cats; although owners were given examples of supervised and controlled outdoor access (e.g. catios, leash-walking), the method of supervision or control may heavily influence the cat's degree of interaction with local wildlife.

Cat characteristics and home environment variables of interest (Model 1) included: number of dogs in the household (0, 1, 2+), number of cats in the household (1, 2, 3, 4+), number of children in the household (0, 1, 2+), presence of vertical space (0/1), presence of scratching areas (0/1), number of litter areas (0, 1, 2, 3, 4, 5, 6+) minus the number of cats (1, 2, 3, 4, 5+), the method in which the cat was obtained (animal shelter, street, friend or family, breeder, bred at home, or other), declaw status (0/1), age of cat (<1 year, 1-6 years, 7-10 years, 11+ years), and the current presence or absence of each of the five categories of the most common behavior problems (house-soiling, aggressive behaviors, destructive behaviors, fearfulness, nuisance

behaviors, excessive vocalization, and gastrointestinal and ingestive disorders). We subtracted the number of cats in the home from the number of litter areas due to current guidelines (Ellis et al. 2013) recommending one more litter areas than the number of cats in the household. This calculation allowed us to assess the relationship between the owner's adherence to this recommendation and their likelihood to provide outdoor access.

Owner demographics and opinions variables of interest included: age of the owner (18-24, 25-34, 45-54, 55-64, 65-74, 75+ years old), gender of the owner (man, woman, non-binary, or prefer to self-describe), subjective social status of the owner (self-scoring from 1 to 10, with 1=worst off in the community, and 10=best off in the community), city (Davis, Sacramento, West Sacramento, Dixon, Winters, or Woodland), distance to green space and traffic (<500ft, 500ft – 0.25mi, 0.25mi-0.5mi, 0.5mi-1mi, 1mi-2mi, or 2+ miles), and Likert-scale responses (“strongly disagree”, “somewhat agree”, “neither agree nor disagree”, “somewhat agree”, “strongly agree”) to the following statements: “Some cats prefer to be indoors”; “Some cats prefer to be outdoors”; “All cats should be allowed outside”; “No cat should be allowed outside”; “Cats are positive for the environment”; “Cats are negative for the environment”; and “I am responsible for my cat's effect on wildlife”. Biologically plausible two-way interaction between distance to traffic and response to the statement “All cats should be allowed outside” was tested.

The third logistic regression model was built to examine factors that may plausibly predict the outcome of having a history of hunting within the past year (yes, no), given that the cat is allowed outdoors. The explanatory variables in this base model included: whether or not the cat goes outside during any of the following groups of hours (morning: 6am-11:59am, afternoon: 12pm-5:59pm, evening: 6pm-11:59pm, and overnight: 12am- 12am-5:59am), total number of groups of hours outside, type of outdoor status (free-roaming vs. supervised),

frequency of interactive object play (never, monthly, weekly, or daily), frequency of nail trims (never, once a year, every few months, once a month, every few weeks, or once a week), distance to green space and traffic, age of cat, and sex of cat.

For all models, each predictor variable was tested individually against the outcome variable, and only variables less than or equal to a liberal p-value of 0.2 were retained (Ferguson and Cox 1993). The final models were built following the stepwise selection method with Akaike Information Criterion (AIC) using the `stepAIC()` function in the MASS package in R (Venables and Ripley 2002). Non-significant variables were left in the model if model fit increased with those variables. Variance inflation factors were assessed using the `regclass` R package (v1.6; Petrie 2020). Tukey's adjustment was applied for the p-values and confidence intervals associated with post-hoc pairwise comparisons with four or more categories to reduce the chance of Type 1 errors.

RESULTS

Owner and Cat Demographics

Participating cat owners (N=631) were mostly women (76.6%) with no children (67.8%) residing in Davis (56.4%) and Sacramento (23.1%), California. The respondents were most frequently between 25 and 34 years old (39.0%). The reported mean Subjective Social Status score was 6.44 (min=1, max=10, 95% CI=3.28, 9.60). Thirty-seven percent of respondents indicated their cat was kept indoor-only, 34.1% indicated providing supervised or controlled outdoor access, and 29.2% indicated providing uncontrolled, or free-roaming outdoor access.

Fifty-one percent of the respondents' cats were male and 48.8% were female. The majority of the cats were neutered (90.2%), and not declawed (87.7%), and most frequently

adopted from an animal shelter (38.4%). The cats were most frequently reported to be the only cat in the household (48.7%), and owners most frequently indicated having been a caretaker of their cat for 1-3 years (39.6%).

Behavior Problems

A total of 465 respondents (73.7%) reported currently seeing at least one type of behavior problem in their cat (Table 1.1). Of those who were currently showing at least one behavior problem, 29.5% were being provided free-roaming outdoor access, 33.3% were provided supervised outdoor access, and the remaining 37.2% were kept indoor-only. Respondents most frequently reported the presence of nuisance behaviors (n=219) and excessive vocalizations (n=196) in their cats. A total of 32 indoor-only (5.09%), 31 supervised outdoor (4.93%), and 43 free-roaming (6.84%) cats were reported to currently exhibit aggressive behaviors in the home.

Table 1.1. Frequencies and percentages of participant cats' demographics and presence of behavioral problems (N=631).

		Indoor-only (n=232)	Supervised Outdoor (n=215)	Free-roaming (n=184)
Age of Cat	<1 year	25 (10.8%)	7 (3.3%)	4 (2.2%)
	1-6 years	129 (55.6%)	141 (65.6%)	90 (48.9%)
	7-10 years	38 (16.7%)	39 (18.1%)	50 (27.2%)
	11+ years	40 (17.2%)	28 (13.0%)	40 (21.7%)
Method Obtained	Animal shelter	106 (45.7%)	87 (40.5%)	49 (26.6%)
	From the street	38 (16.4%)	36 (16.7%)	53 (28.8%)
	From friend/family	41 (17.7%)	34 (15.8%)	37 (20.1%)
	Breeder or Bred at home	7 (3.0%)	30 (14.0%)	18 (9.8%)
	Other	40 (17.2%)	28 (13.0%)	27 (14.7%)
Behavior	House soiling	11 (4.7%)	23 (10.7%)	13 (7.1%)

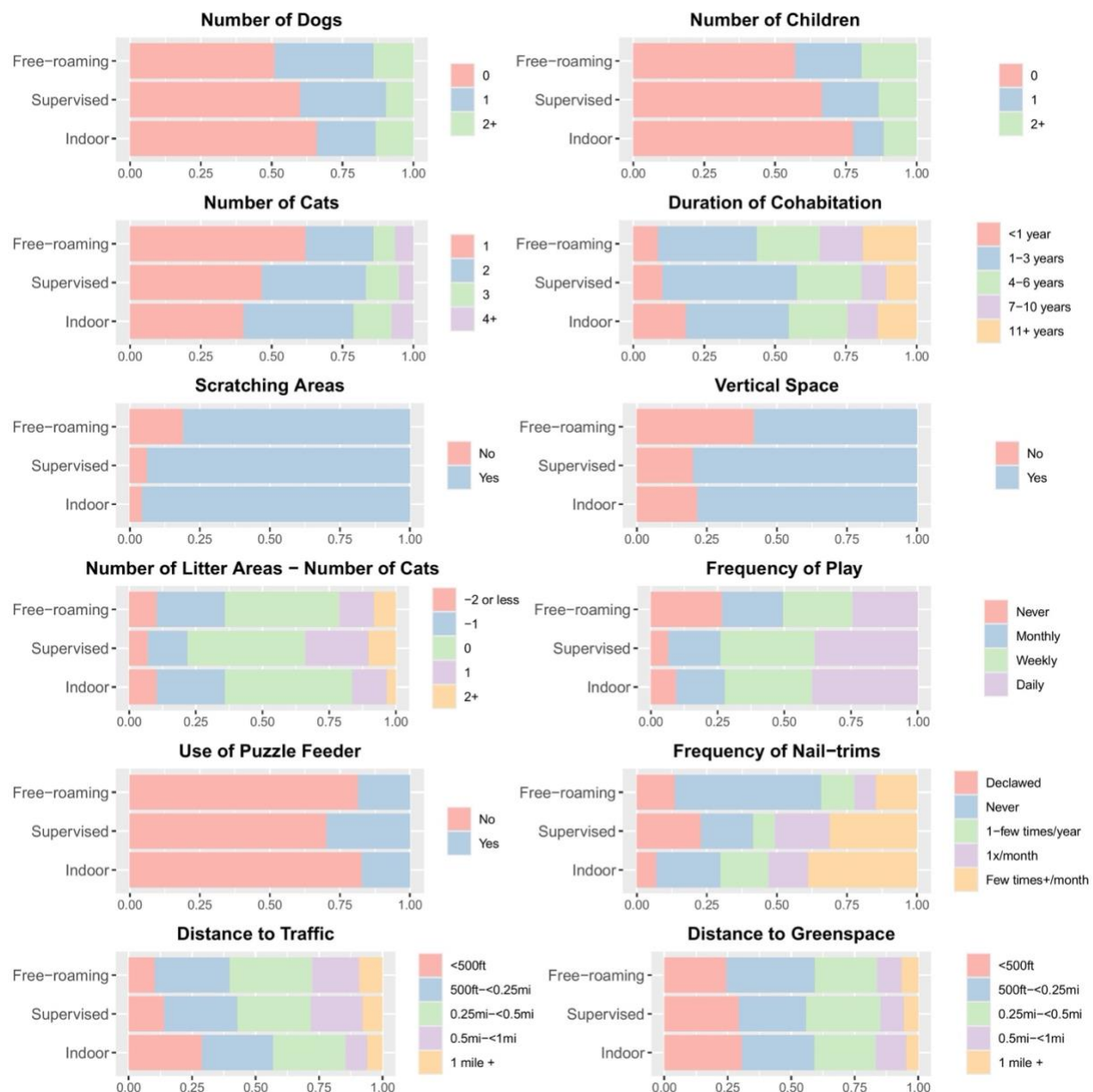
problems	Aggression	32 (13.8%)	31 (14.4%)	43 (23.4%)
	Gastrointestinal / Ingestive Disorder	17 (7.3%)	19 (8.8%)	10 (5.4%)
	Destructive behaviors	62 (26.7%)	51 (23.7%)	48 (26.1%)
	Excessive vocalization	67 (28.9%)	76 (35.3%)	53 (28.8%)
	Nuisance Behaviors	86 (37.1%)	73 (34.0%)	60 (32.6%)
	Fearfulness	66 (28.4%)	45 (20.9%)	34 (18.5%)
	Other	9 (3.9%)	5 (2.3%)	6 (3.3%)

Home Environment

Various home environment factors that were predicted to influence outdoor access are summarized in Figure 1.1. Overall, of 631 respondents, the majority reported they were providing scratching areas (90.8%) and vertical space (73.1%) for their cat. Respondents most frequently reported they were providing as many distinct litter areas as there were cats in the home (45.2%). The majority of respondents also reported they were either never trimming their cat's nails (30.0%) or trimming their nails at least a few times a month (29.2%).

Of the respondents who were providing any outdoor access for their cat (n=317), the majority were allowing them outside between 6:00 – 11:59 AM (74.8%), 12:00 – 5:59 PM (61.5%), and/or 6:00 – 11:59 PM (63.7%). Fewer respondents (22.1%) were allowing their cat outside overnight, between 12:00 – 5:59 AM.

Figure 1.1: Distribution of responses of free-roaming outdoor (n=184), supervised-outdoor (n=215), and indoor-only (n=232) cat owners in their a) Demographics information and b) Management factors, shown in proportions out of 1.00.

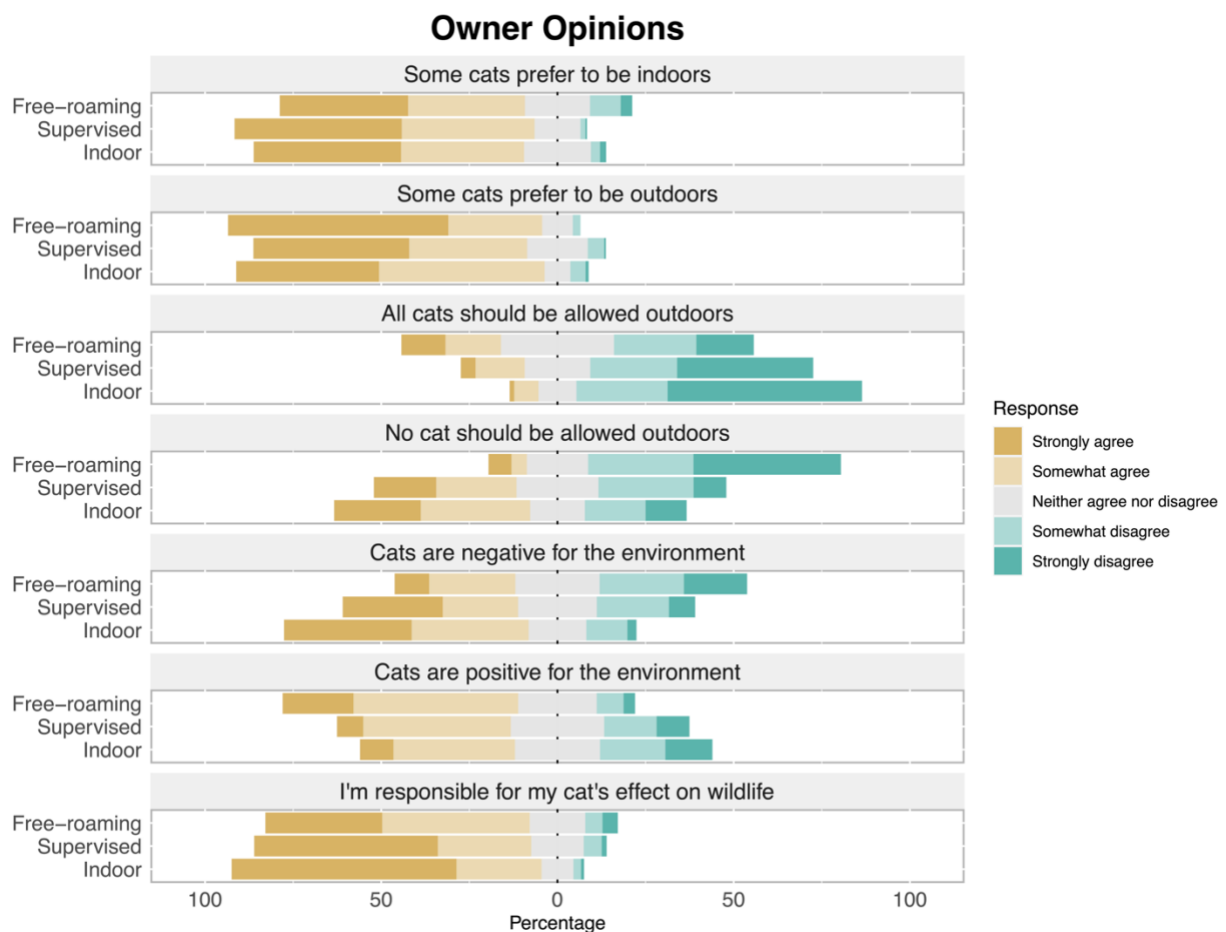


Cat Owner Attitudes

Cat owner ratings of how strongly they agree or disagree with various generalized statements about cats and outdoor access are summarized in Figure 1.2. The majority of indoor-only (81.0%) and supervised-outdoor (63.3%) cat owners, but fewer (39.7%) free-roaming cat owners, strongly or somewhat disagreed with the statement “All cats should be allowed outside”.

The majority of indoor-only (69.4%) and almost half of supervised outdoor (49.8%) cat owners, but fewer (34.2%) free-roaming cat owners, strongly or somewhat agreed with the statement “Cats are negative for the environment”. Notably, the majority of all three groups of respondents – indoor-only (87.9%), supervised outdoor (78.6%), and free-roaming outdoor (75.0%) – strongly or somewhat agreed with the statement “I’m responsible for my cat’s effect on wildlife”.

Figure 1.2. Participating cat owner ratings of how strongly they agree with given statements about cats (Free-roaming n=184; supervised n=215; indoor n=232). Likert-scale responses ranged from “strongly agree” to “strongly disagree”.



Predictors of Free-roaming Outdoor Access

Participants' cats had higher odds of being provided free-roaming outdoor access if they were: living with a dog ($p=0.014$; Table 1.2), living with one ($p=0.005$) or two ($p=0.025$) children, taken in from the street ($p=0.007$), between 7-10 years old ($p=0.030$), or if they showed aggressive behaviors in the home ($p=0.008$). In contrast, cats had lower odds of being provided free-roaming outdoor access if they were: living with one ($p=0.020$) or two ($p=0.017$) other cat(s), or were provided scratching areas in the home ($p=0.017$).

Table 1.2. Logistic regression model results showing cat characteristics and home environment factors associated with providing free-roaming outdoor access ($N=403$ participants). Only significant results are reported with odds ratios (ORs), 95% CI's, and p-values.

		p-value	Odds Ratio	95% CI [lower, upper]
(Intercept)		0.661	1.228	[0.490, 3.082]
Number of dogs	0 (ref.) ^a	-	-	-
	1	0.014	1.952	[1.145, 3.329]
Number of children	0 (ref.)	-	-	-
	1	0.005	2.516	[1.314, 4.820]
	2+	0.025	2.130	[1.099, 4.127]
Number of cats in the home	1 (ref.)	-	-	-
	2	0.020*	0.463	[0.274, 0.784]*
	3	0.017*	0.308	[0.139, 0.680]*
Scratching areas	No (ref.)	-	-	-
	Yes	0.017	0.343	[0.142, 0.828]
Method obtained	Animal shelter (ref.)	-	-	-
	Street	0.007*	2.920	[1.550, 5.501]*
Age of cat	<1 year old (ref.)	-	-	-
	7-10 years old	0.030*	8.839	[1.828, 42.744]*
Aggressive behaviors	No (ref.)	-	-	-
	Yes	0.008	2.197	[1.224, 3.943]

^a referent category.

*Tukey's adjusted p-values and confidence intervals used.

Participating cat owners also had higher odds of providing their cat free-roaming outdoor access if they lived 0.25-0.5 miles ($p=0.002$) or 0.5-1 mile ($p=0.002$) away from traffic, or somewhat ($p=0.029$) or strongly ($p=0.001$) agreed with the statement “All cats should be allowed outside” (Table 1.3). In contrast, respondents had lower odds of providing free-roaming outdoor access if they somewhat ($p<0.001$) or strongly ($p<0.001$) agreed with the statement “No cat should be allowed outside”, or strongly agreed with the statement “Cats are negative for the environment” ($p=0.028$).

Table 1.3. Logistic regression model results showing cat owner demographics and opinion factors associated with providing free-roaming outdoor access ($N=403$ participants). Only significant results are reported with odds ratios (ORs), 95% CI's, and p-values.

		p-value	Odds Ratio	95% CI [lower, upper]
(Intercept)		0.306	0.603	[0.228, 1.590]
Distance to Traffic	<500ft (ref.) ^a	-	-	-
	0.25-0.5 mile	0.002*	4.454	[1.996, 9.939]*
	0.5-1 mile	0.002*	6.102	[2.310, 16.118]*
“No cat should be allowed outside”	Strongly disagree (ref.)	-	-	-
	Neither agree nor disagree	0.016*	0.300	[0.140, 0.644]*
	Somewhat agree	<0.001*	0.054	[0.020, 0.147]*
	Strongly agree	<0.001*	0.105	[0.041, 0.268]*
	Somewhat disagree (ref.)	-	-	-
	Somewhat agree	<0.001*	0.095	[0.036, 0.253]*
	Strongly agree	0.005*	0.185	[0.070, 0.485]*
	Neither agree nor disagree (ref.)	-	-	-
	Somewhat agree	0.008*	0.180	[0.065, 0.499]*
“Cats are negative for the environment”	Strongly disagree (ref.)	-	-	-
	Strongly agree	0.028*	0.155	[0.044, 0.545]*
	Strongly disagree (ref.)	-	-	-

“All cats should be allowed outside”	Neither agree nor disagree	0.002*	4.386	[2.012, 9.579]*
	Somewhat agree	0.029*	3.987	[1.559, 10.199]*
	Strongly agree	0.001*	20.306	[4.258, 96.844]*
	Somewhat disagree (ref.)	-	-	-
	Strongly agree	0.024*	10.802	[2.222, 52.519]*

^a referent category.

*Tukey’s adjusted p-values and confidence intervals reported.

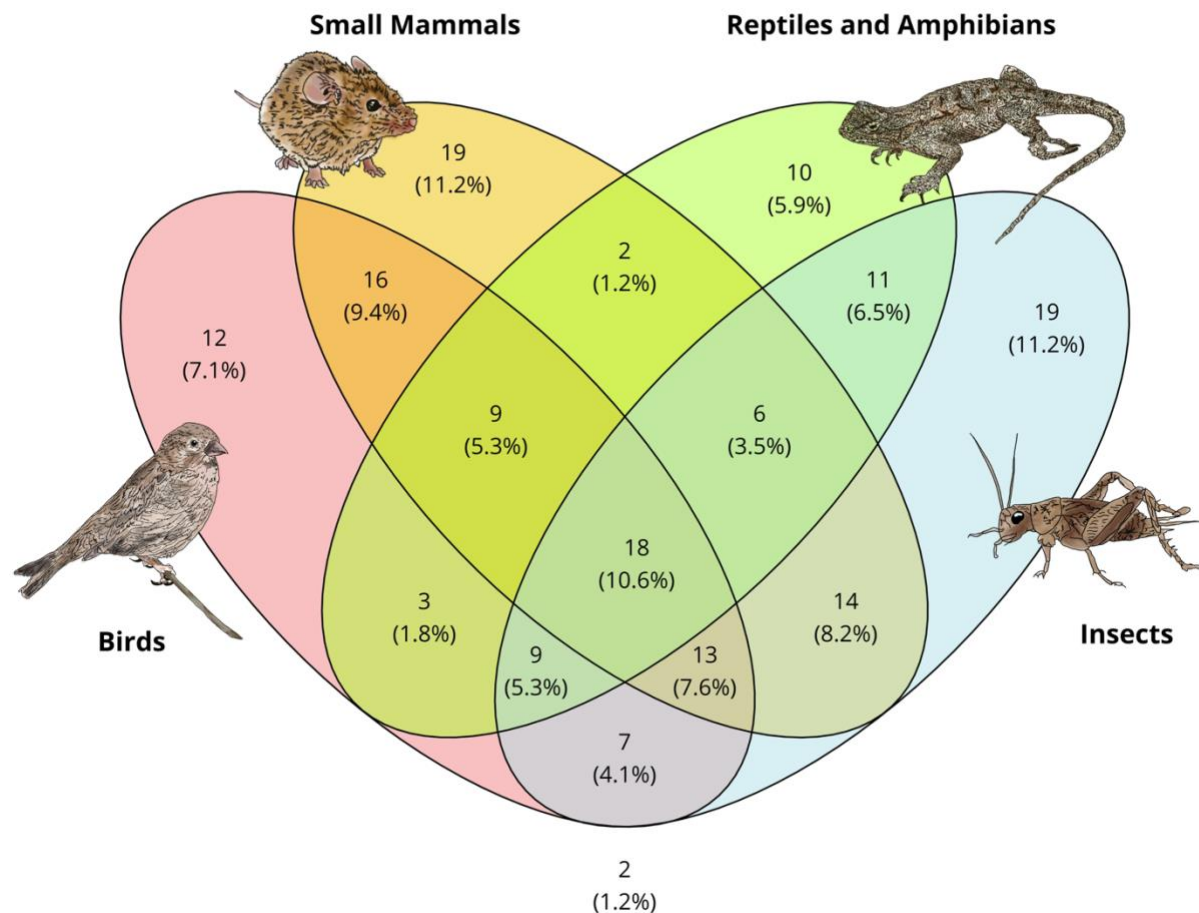
Predictors of Hunting in the Last Year

Respondents had higher odds of reporting their cat hunting in the last year if they were letting their cat outdoors in the morning hours, between 6am and 11:59am, compared to those who were not (OR=2.293; CI=1.179, 4.461). Respondents also had higher odds of reporting their cat hunting in the last year if they were providing free roaming outdoor access, rather than supervised (OR=2.039; CI=1.143, 3.637).

Types of Hunted Prey

Of 399 respondents providing their cats any outdoor access, 170 reported observing their cat hunting or bringing home any wild prey at least once in the past year. Individual cats and their reported prey types brought home or observed hunting are shown in Figure 1.3. Of the 170 cats who were reported to have hunted in the last year, 12 cats (7.1%) hunted birds only, 19 (11.2%) hunted small mammals only, 10 (5.9%) hunted reptiles and amphibians only, and 19 (11.2%) hunted insects only. Eighteen (10.6%) cats were observed hunting or bringing home all four groups of prey in the last year. Two of the cats who were reported to have hunted in the last year (1.2%) did not bring home any prey groups listed in our questionnaire.

Figure 1.3. Respondents’ individual cats (n=170) and their reported history of prey types returned home or observed hunting in the last year. Each ellipse indicates one of four prey types hunted (red = birds, orange = small mammals, green = reptiles and amphibians, blue = insects). Numbers indicate the number of cats in each category, and percentages are out of the total number of cats that the owner has observed with prey at least once in the last year.



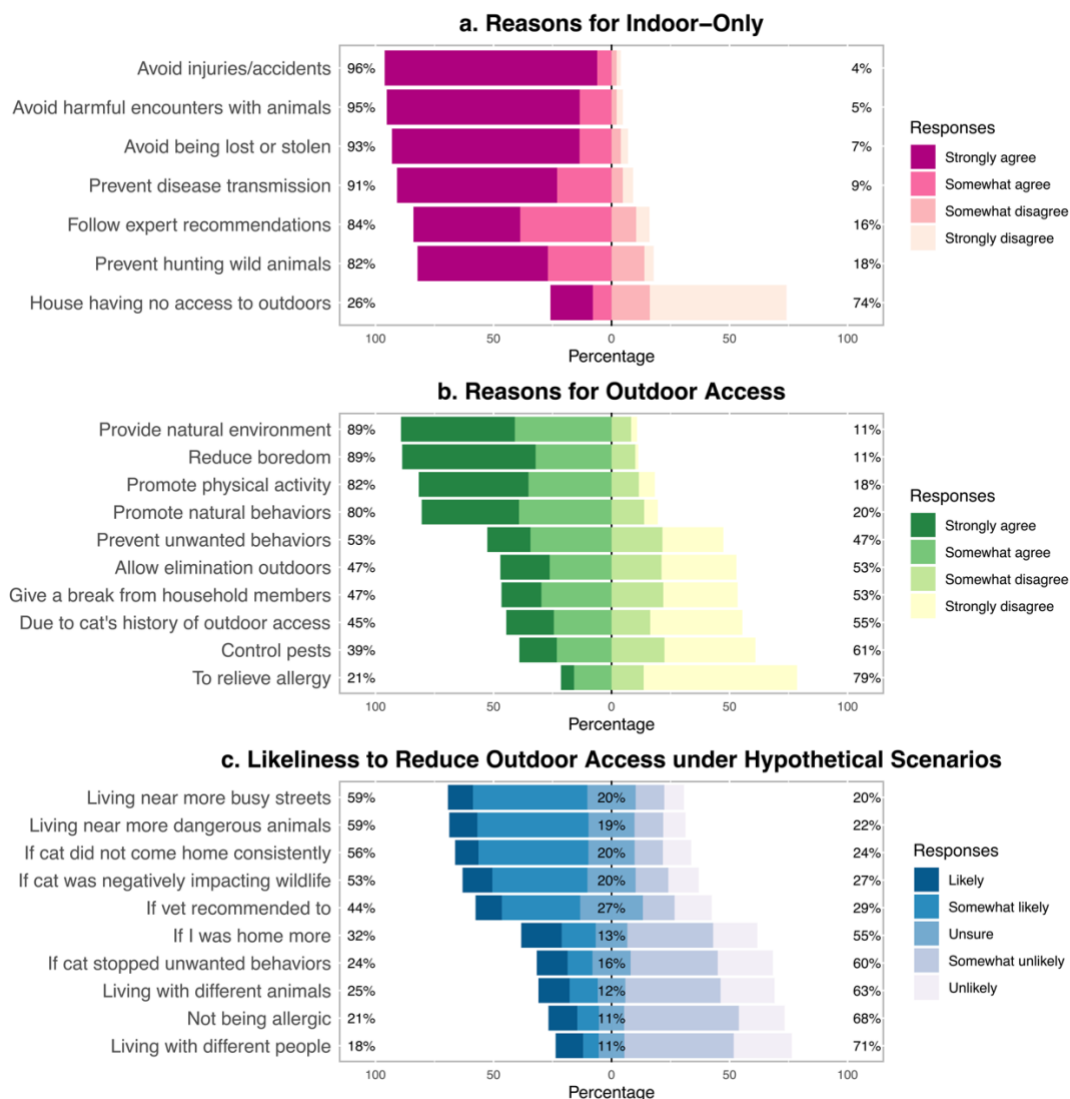
Owner Motivations

The majority of indoor-only cat owners (n=232) strongly or somewhat agreed that they keep their cat indoors in order to avoid: injuries or accidents (96%, Figure 1.4), harmful encounters with other animals (95%), being lost or stolen (93%), and/or disease transmission (91%). Expert recommendations (84%) and a desire to prevent cats from hurting wild animals (82%) were also indicated as reasons for keeping their cat indoors. In contrast, 28% of cat owners kept cats indoors due to their house not having access to the outdoors.

The majority of owners who provide their cat outdoor access (n=319) strongly or somewhat agreed they do so in order to: provide a natural environment (89%), reduce boredom (89%), promote physical activity (82%), and/or promote natural behaviors (80%). In addition,

the majority of these owners reported they were likely or somewhat likely to reduce outdoor access if: they lived near busier streets (59%), more dangerous animals were around (59%), their cat stopped coming home consistently (56%), and/or if they knew their cat was negatively impacting wildlife (53%).

Figure 1.4. Participating cat owner reported ratings of: a) reasons to not provide cat outdoor access (n=232), b) reasons to provide cat outdoor access (n=319), and c) likelihood of reducing outdoor access under given hypothetical scenarios (n=319). Percentages of participants who responded positively to the statements (strongly agree and somewhat agree) are shown on the left, and percentages of participants who responded negatively (somewhat disagree and strongly disagree) are shown on the right. Percentages of participants who responded neutrally to the statements (“unsure”) are shown in the middle.



DISCUSSION

Outdoor Access

The majority of owners who currently provide outdoor access – both supervised and free-roaming – reported they felt responsible for their cat's effect on wildlife, and that they were likely or somewhat likely to reduce outdoor access if they were to learn their cat was negatively impacting wildlife. These results suggest that while outdoor cat owners of this population are largely aware of the risks their cats pose on the environment, and believe they are responsible for their cat's effect on wildlife, they are most likely viewing their individual cat's effect on wildlife as insignificant. These patterns mirrored those of a previous study assessing owner attitudes (Crowley, Cecchetti, and McDonald 2019), and the contradiction between the owners' knowledge and their actual management styles is not a new phenomenon (e.g. Pongrácz et al. 2024). Nevertheless, given that prey returns to the home have been shown to be a gross underestimate of the actual number of captured prey (e.g. Loyd et al. 2013), and the potentially large total cumulative impact of dense populations of cats in the environment, we urge owners to consider the potentially unseen impacts of their cat and cats as a whole on local wildlife when making decisions about outdoor access.

The majority of owners in this population were motivated by their cats' safety and well-being, with over 80% reporting they agree they were providing or not providing outdoor access for reasons relating to safety and/or their cats' mental and physical well-being. These results are consistent with a previous study suggesting that traffic accidents are a major concern of indoor-only cat owners internationally, and the cats' mental well-being is a major reason outdoor cat owners provide outdoor access (Foreman-Worsley et al. 2021).

When presented with hypothetical scenarios that could compromise their cat's health and/or safety, over half of the owners who currently provide outdoor access (supervised or free-roaming) reported they were likely or somewhat likely to reduce outdoor access. This suggests that owners who provide outdoor access may view the potential risks of outdoor access as outweighed by the benefits for their cat's mental stimulation and physical activity, but that they are aware of and sensitive to the risks associated with outdoor access.

In this population, various factors about the home environment were significant predictors of free-roaming outdoor access. As seen in previous studies (Clancy, Moore, and Bertone 2003; Tan et al. 2021; Foreman-Worsley et al. 2021), living with a dog or children in the household increased the odds of the cat being provided free-roaming outdoor access, but living with one or two other cat(s) in the home decreased the odds. We conclude that despite potential challenges cats may face living with other cats (Trevorrow, 2019), participants may have viewed the presence of dogs or children as more stressful for the cat than other cats, thus opting for outdoor access to alleviate such challenges in the home. Alternatively, dog-owning homes may have already been providing outdoor access (e.g., a pet door), thus making it easier to provide outdoor access for their cats. However, due to our study design, it is not possible to elucidate causal relationships from our results.

Similarly, as found by Tan et al. (2021), we found that cats taken in from the street, as well as cats currently showing aggressive behaviors had higher odds of being allowed to freely roam outside. We suspect that owners who obtained their cat from the street may be more likely to view outdoor access as necessary for their cat, and that owners with aggressive cats may be more motivated to allow outdoor access to relieve tension in the home. Alternatively, owners of free-roaming outdoor cats may have reported the presence of aggressive behaviors their cat

exhibits towards other animals outside the home, since our questionnaire did not direct owners to only report behaviors seen in the home.

Additionally, mature adult cats (7-10 years old) had higher odds of being allowed to freely roam than kittens (<1 year old). It is interesting to note, however, that no other age group comparisons were significant; outdoor provision in this population seemed to only differ significantly between 7–10-year-olds and kittens, suggesting that cat age may not be linearly associated with the provisioning of outdoor access. However, we saw unusually large confidence intervals associated with this variable, suggesting there is some uncertainty in these results; more research is needed to further elucidate the relationship between cat age and outdoor access.

We also found that cats with designated scratching areas in the home had lower odds of being provided free-roaming outdoor access. While scratching areas may be viewed as unnecessary when cats have access to their natural counterparts (e.g. trees), not providing them may negatively impact cat welfare when they spend time indoors (Heidenberger 1997), which may happen more frequently under certain circumstances that are outside of the cat's control (e.g. inclement weather, or post-medical procedures).

Owner responses to statements “no cat should be allowed outside” and “all cats should be allowed outside” were significant predictors of outdoor access; however, responses to general statements about the effect of cats on wildlife (“cats are negative for the environment”, “cats are positive for the environment”) or their inherent preference for the outdoors (“some cats prefer to be indoors”, “some cats prefer to be outdoors”) were not predictors of outdoor access, with the exception of the comparison between those who strongly disagreed and those who strongly agreed with the statement “cats are negative for the environment”. This pattern has been mirrored by other research (Van Eeden et al. 2021; MacDonald, Milfont, and Gavin 2015). All

significant patterns found regarding owner attitudes were of logical directionality – those who tended to agree with the statement “no cat should be allowed outside” had higher odds of letting their cats outside, whereas those who tended to agree with the statement “all cats should be allowed outside” had lower odds of letting their cats outside. However, we found that the statement “all cats should be allowed outside” led to some large confidence intervals, suggesting there is uncertainty with these results. Interestingly, we did not find that strongly agreeing with any statement, compared to being neutral (“neither agree nor disagree”), was associated with providing free-roaming outdoor access; this may be due to social desirability bias, potentially leading to the respondents’ hesitancy to strongly agree with statements that may be perceived as extreme.

Additionally, while previous studies have found significant associations between cat owner respondent gender (Tan et al. 2021), age (Foreman-Worsley et al. 2021; Machado et al. 2021), and their provisioning of outdoor access, in this study, we did not find any owner demographics factors that significantly predicted outdoor access. We predict that this may be due to the limited geographic distribution of the participants of our study.

Hunting

As expected, cats provided free-roaming outdoor access compared to those provided supervised or controlled outdoor access, had higher odds of having hunted in the last year. Not being inhibited when outdoors, and spending more time outdoors, logically may have led to more exposure to wild prey when outdoors. Interestingly, we found that while the total number of groups of hours outside was not significantly associated with having hunted in the last year, cats who were allowed outside during the morning hours (between 6am – 11:59am) had higher odds of reported hunting in the last year compared to those not allowed outside in the morning. Being

outdoors in any other group of hours was not significantly associated with reported hunting in the last year. This was surprising given that confinement overnight is a common recommendation made to outdoor cat owners (Crowley, Cecchetti, and McDonald 2019) due to its potential to reduce their overlap with nocturnal prey animals (Woods, McDonald, and Harris 2003; Cecchetti, Crowley, and McDonald 2021; Crowley, Cecchetti, and McDonald 2020), and has been suggested to be the most suitable management strategy to reduce cats' hunting, given its likelihood of implementation by owners and conservation value (Linklater et al. 2019).

Cat characteristics such as age or sex were not associated with having hunted in the last year; this was consistent with previous studies that have shown that a cat's hunting frequency is not correlated with their sex (Tschanz et al. 2011; Dickman and Newsome 2015), but inconsistent with other studies that have shown that younger cats are more prolific hunters (Woods, McDonald, and Harris 2003; van Heezik et al. 2010).

Participants whose cats have a history of hunting reported much lower rates of hunting in the last year by their cats in this study than other studies on owner-reported prey returns; for example, in Cecchetti et al. 2021, UK owners of cats with a history of hunting reported approximately 8 prey items returned per cat in a 49-day pre-treatment period. In another study, 84.1% of owners reported their cats brought home prey (Escobar-Aguirre et al. 2019). In comparison, only 42.6% of cat owners of this population who allowed their cat outdoors reported their cat brought home any prey in the last year. We suspect that there may be regional differences in attitudes towards uncontrolled outdoor access. It is also possible that our survey participants may have been underestimating or underreporting the number of prey returns, potentially due to recall bias associated with asking owners to recall their cat's hunting behavior across a long time period (1 year). In addition, social desirability bias may have resulted in

hesitancy to report accurate prey return numbers of birds, though cat owners have been found to vary widely in their degree of acceptance of hunting behavior in cats (Crowley, Cecchetti, and McDonald 2020).

In this study, many cats were reported to hunt and/or bring home prey items of various taxa, and the degree of specialization generally varied across the responses. Respondents frequently reported their cat hunted small mammals only (11.2%), insects only (11.2%), or all four prey types (10.6%). The variation in the degree of prey specialization has been observed in *Felis catus* (Dickman and Newsome 2015); however, given the small sample size and the questionnaire-based nature of our study, these results should not be used to extrapolate beyond this study.

Limitations

As with all questionnaires, responses to our questionnaire are vulnerable to selection, recall, and social desirability biases. We suspect that our sample may have been more knowledgeable and/or more interested in the impact of cats on wildlife than the general public. This may have led to a bias in responses, and owners whose cats already do not return many prey items or owners who already provide necessary resources in the home may have been unintentionally selected. We suspect that social desirability bias may have influenced responses to various owner-attitude questions and hypothetical scenarios, and recall bias may have impacted the number of prey returns reported. Lastly, given the relative lack of outdoor-only cats in this population, we did not recruit outdoor-only cat owners in this study; however, we recognize the importance of their unique management styles and urge future studies to consider their perspectives.

CONCLUSION

This study explored the topics of cat welfare, conservation, and human behavior together to garner a holistic approach to the human-cat-wildlife dynamics. Cat owners of this population were largely motivated by and sensitive to their cats' safety and well-being, but also their cats' potential impact on local wildlife. The majority of owners who provide outdoor access reported they felt responsible for their cat's effect on wildlife, and that they were likely or somewhat likely to consider reducing outdoor access if they knew that their cat was negatively impacting wildlife. Responses showed an interesting variation of prey specialization by the cats, but hunting rates reported by owners in this study were much lower compared to research conducted in other regions, which may reflect differing regional attitudes on outdoor cat access. We suggest that increased owner awareness of the unseen impact of cats on wildlife may encourage more owners to reduce uncontrolled outdoor access, and that future conservation strategies should further incorporate owners' motivations for their cats' safety and well-being. Providing controlled, supervised outdoor access for cats may be an effective strategy to reduce the consequences associated with uncontrolled outdoor access while maintaining its benefits. Finally, we emphasize the importance of addressing both cat welfare as well as wildlife conservation together when targeting the complex topic of outdoor cats.

CRedit Author Statement

Hee Jin Chung: Conceptualization, Methodology, Software, Formal Analysis, Investigation, Data Curation, Visualization, Writing – Original Draft. **Carly Moody:** Conceptualization, Methodology, Writing – Review & Editing, Supervision. **Jasmyn Aulakh:** Data Curation, Software, Formal analysis, Visualization. **Jennifer Link:** Methodology, Writing

– Review & Editing. **Andrew Sih:** Conceptualization, Methodology, Writing – Review & Editing.

ACKNOWLEDGEMENTS

We acknowledge the Sih lab and the Animal Welfare Epidemiology lab for assisting with study design and conceptualization. We declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

- Adler, Nancy E., Elissa S. Epel, Grace Castellazzo, and Jeannette R. Ickovics. 2000. “Relationship of Subjective and Objective Social Status with Psychological and Physiological Functioning: Preliminary Data in Healthy, White Women.” *Health Psychology* 19 (6): 586–92. <https://doi.org/10.1037/0278-6133.19.6.586>.
- Amat, Marta, Tomàs Camps, and Xavier Manteca. 2016. “Stress in Owned Cats: Behavioural Changes and Welfare Implications.” *Journal of Feline Medicine and Surgery* 18 (8): 577–86. <https://doi.org/10.1177/1098612X15590867>.
- Amat, Marta, José Luís Ruiz De La Torre, Jaume Fatjó, Valentina M. Mariotti, Sophie Van Wijk, and Xavier Manteca. 2009. “Potential Risk Factors Associated with Feline Behaviour Problems.” *Applied Animal Behaviour Science* 121 (2): 134–39. <https://doi.org/10.1016/j.applanim.2009.09.012>.
- AVMA. 2022. “U.S. Pet Ownership Statistics.” *American Veterinary Medical Association*. ISBN: 978-1-882691-52-4.
- Baker, Philip J., Susie E. Molony, Emma Stone, Innes C. Cuthill, and Stephen Harris. 2008. “Cats about Town: Is Predation by Free-Ranging Pet Cats *Felis Catus* Likely to Affect Urban Bird Populations?: Predation by Pet Cats and Urban Bird Populations.” *Ibis* 150 (August):86–99. <https://doi.org/10.1111/j.1474-919X.2008.00836.x>.
- Balogh, Anne L., Thomas B. Ryder, and Peter P. Marra. 2011. “Population Demography of Gray Catbirds in the Suburban Matrix: Sources, Sinks and Domestic Cats.” *Journal of Ornithology* 152 (3): 717–26. <https://doi.org/10.1007/s10336-011-0648-7>.
- Bradshaw, John W. S. 2006. “The Evolutionary Basis for the Feeding Behavior of Domestic Dogs (*Canis Familiaris*) and Cats (*Felis Catus*).” *The Journal of Nutrition* 136 (7): 1927S–1931S. <https://doi.org/10.1093/jn/136.7.1927S>.
- Bradshaw, John W.S. 2016. “Sociality in Cats: A Comparative Review.” *Journal of Veterinary Behavior* 11 (January): 113–24. <https://doi.org/10.1016/j.jveb.2015.09.004>.
- Burling, Amie N., Julie K. Levy, H. Morgan Scott, Michael M. Crandall, Sylvia J. Tucker, Erin G. Wood, and Jessie D. Foster. 2017. “Seroprevalences of Feline Leukemia Virus and Feline Immunodeficiency Virus Infection in Cats in the United States and Canada and Risk Factors for Seropositivity.” *Journal of the American Veterinary Medical Association* 251 (2): 187–94. <https://doi.org/10.2460/javma.251.2.187>.

- Cecchetti, Martina, Sarah L. Crowley, Cecily E.D. Goodwin, and Robbie A. McDonald. 2021. "Provision of High Meat Content Food and Object Play Reduce Predation of Wild Animals by Domestic Cats *Felis Catus*." *Current Biology* 31 (5): 1107-1111.e5. <https://doi.org/10.1016/j.cub.2020.12.044>.
- Cecchetti, Martina, Sarah L. Crowley, and Robbie A. McDonald. 2021. "Drivers and Facilitators of Hunting Behaviour in Domestic Cats and Options for Management." *Mammal Review* 51 (3): 307–22. <https://doi.org/10.1111/mam.12230>.
- Churcher, P. B., and J. H. Lawton. 1987. "Predation by Domestic Cats in an English Village." *Journal of Zoology* 212 (3): 439–55. <https://doi.org/10.1111/j.1469-7998.1987.tb02915.x>.
- Clancy, Elizabeth A., Antony S. Moore, and Elizabeth R. Bertone. 2003. "Evaluation of Cat and Owner Characteristics and Their Relationships to Outdoor Access of Owned Cats." *Journal of the American Veterinary Medical Association* 222 (11): 1541–45. <https://doi.org/10.2460/javma.2003.222.1541>.
- Crooks, Kevin R., and Michael E. Soulé. 1999. "Mesopredator Release and Avifaunal Extinctions in a Fragmented System." *Nature* 400 (6744): 563–66. <https://doi.org/10.1038/23028>.
- Crowley, Sarah L., Martina Cecchetti, and Robbie A. McDonald. 2019. "Hunting Behaviour in Domestic Cats: An Exploratory Study of Risk and Responsibility among Cat Owners." *People and Nature* 1 (1): 18–30. <https://doi.org/10.1002/pan3.6>.
- Crowley, Sarah L., Martina Cecchetti, and Robbie A McDonald. 2020. "Diverse Perspectives of Cat Owners Indicate Barriers to and Opportunities for Managing Cat Predation of Wildlife." *Frontiers in Ecology and the Environment* 18 (10): 544–49. <https://doi.org/10.1002/fee.2254>.
- Dickman, Christopher R., and Thomas M. Newsome. 2015. "Individual Hunting Behaviour and Prey Specialisation in the House Cat *Felis Catus*: Implications for Conservation and Management." *Applied Animal Behaviour Science* 173 (December): 76–87. <https://doi.org/10.1016/j.applanim.2014.09.021>.
- Doherty, Tim S., Alistair S. Glen, Dale G. Nimmo, Euan G. Ritchie, and Chris R. Dickman. 2016. "Invasive Predators and Global Biodiversity Loss." *Proceedings of the National Academy of Sciences* 113 (40): 11261–65. <https://doi.org/10.1073/pnas.1602480113>.
- Driscoll, Carlos A., David W. Macdonald, and Stephen J. O'Brien. 2009. "From Wild Animals to Domestic Pets, an Evolutionary View of Domestication." *Proceedings of the National Academy of Sciences* 106 (supplement_1): 9971–78. <https://doi.org/10.1073/pnas.0901586106>.
- Eisert, Regina. 2011. "Hypercarnivory and the Brain: Protein Requirements of Cats Reconsidered." *Journal of Comparative Physiology B* 181 (1): 1–17. <https://doi.org/10.1007/s00360-010-0528-0>.
- Ellis, Sarah L H, Ilona Rodan, Hazel C Carney, Sarah Heath, Irene Rochlitz, Lorinda D Shearburn, Eliza Sundahl, and Jodi L Westropp. 2013. "AAFP and ISFM Feline Environmental Needs Guidelines." *Journal of Feline Medicine and Surgery* 15 (3): 219–30. <https://doi.org/10.1177/1098612X13477537>.
- Escobar-Aguirre, Sebastián, Raúl A. Alegría-Morán, Javiera Calderón-Amor, and Tamara A. Tadich. 2019. "Can Responsible Ownership Practices Influence Hunting Behavior of Owned Cats?: Results from a Survey of Cat Owners in Chile." *Animals* 9 (10): 745. <https://doi.org/10.3390/ani9100745>.

- Ferguson, Eamonn, and Tom Cox. 1993. "Exploratory Factor Analysis: A Users' Guide." *International Journal of Selection and Assessment* 1 (2): 84–94. <https://doi.org/10.1111/j.1468-2389.1993.tb00092.x>.
- Foreman-Worsley, Rachel, Lauren R. Finka, Samantha J. Ward, and Mark J. Farnworth. 2021. "Indoors or Outdoors? An International Exploration of Owner Demographics and Decision Making Associated with Lifestyle of Pet Cats." *Animals* 11 (2): 253. <https://doi.org/10.3390/ani11020253>.
- Heezik, Yolanda van, Amber Smyth, Amy Adams, and Joanna Gordon. 2010. "Do Domestic Cats Impose an Unsustainable Harvest on Urban Bird Populations?" *Biological Conservation* 143 (1): 121–30. <https://doi.org/10.1016/j.biocon.2009.09.013>.
- Heidenberger, Eva. 1997. "Housing Conditions and Behavioural Problems of Indoor Cats as Assessed by Their Owners." *Applied Animal Behaviour Science* 52 (3–4): 345–64. [https://doi.org/10.1016/S0168-1591\(96\)01134-3](https://doi.org/10.1016/S0168-1591(96)01134-3).
- Henning, Julia, Torben Nielsen, Eduardo Fernandez, and Susan Hazel. 2023. "Cats Just Want to Have Fun: Associations between Play and Welfare in Domestic Cats." *Animal Welfare* 32: e9. <https://doi.org/10.1017/awf.2023.2>.
- Hernandez, Sonia M., Kerrie Anne T. Loyd, Alexandra N. Newton, Benjamin L. Carswell, and Kyler J. Abernathy. 2018. "The Use of Point-of-View Cameras (Kittycams) to Quantify Predation by Colony Cats (*Felis Catus*) on Wildlife." *Wildlife Research* 45 (4): 357. <https://doi.org/10.1071/WR17155>.
- Jongman, Ellen C. 2007. "Adaptation of Domestic Cats to Confinement." *Journal of Veterinary Behavior* 2 (6): 193–96. <https://doi.org/10.1016/j.jveb.2007.09.003>.
- Lepczyk, Christopher A., Jean E. Fantle-Lepczyk, Kylee D. Dunham, Elsa Bonnaud, Jocelyn Lindner, Tim S. Doherty, and John C. Z. Woinarski. 2023. "A Global Synthesis and Assessment of Free-Ranging Domestic Cat Diet." *Nature Communications* 14 (1): 7809. <https://doi.org/10.1038/s41467-023-42766-6>.
- Linklater, Wayne L., Mark J. Farnworth, Yolanda Heezik, Kevin J. Stafford, and Edith A. MacDonald. 2019. "Prioritizing Cat-owner Behaviors for a Campaign to Reduce Wildlife Depredation." *Conservation Science and Practice* 1 (5): e29. <https://doi.org/10.1111/csp2.29>.
- Loss, Scott R., and Peter P. Marra. 2017. "Population Impacts of Free-Ranging Domestic Cats on Mainland Vertebrates." *Frontiers in Ecology and the Environment* 15 (9): 502–9. <https://doi.org/10.1002/fee.1633>.
- Loss, Scott R., Tom Will, and Peter P. Marra. 2013. "The Impact of Free-Ranging Domestic Cats on Wildlife of the United States." *Nature Communications* 4 (1): 1396. <https://doi.org/10.1038/ncomms2380>.
- Loyd, Kerrie Anne T., Sonia M. Hernandez, John P. Carroll, Kyler J. Abernathy, and Greg J. Marshall. 2013. "Quantifying Free-Roaming Domestic Cat Predation Using Animal-Borne Video Cameras." *Biological Conservation* 160 (April): 183–89. <https://doi.org/10.1016/j.biocon.2013.01.008>.
- MacDonald, Edith, Taciano Milfont, and Michael Gavin. 2015. "What Drives Cat-Owner Behaviour? First Steps towards Limiting Domestic-Cat Impacts on Native Wildlife." *Wildlife Research* 42 (3): 257. <https://doi.org/10.1071/WR14164>.
- Machado, Ds, Aff Bragança, Ic Travník, Ap Rossi, and Ac Sant'Anna. 2021. "Should Cats Be Allowed Outdoors? A Research Survey on Animal Welfare Risks for Free-Ranging Cats in Brazil." *Animal Welfare* 30 (3): 331–39. <https://doi.org/10.7120/09627286.30.3.011>.

- McDonald, Jennifer L., Mairead Maclean, Matthew R. Evans, and Dave J. Hodgson. 2015. "Reconciling Actual and Perceived Rates of Predation by Domestic Cats." *Ecology and Evolution* 5 (14): 2745–53. <https://doi.org/10.1002/ece3.1553>.
- McGregor, Hugh, Sarah Legge, Menna E. Jones, and Christopher N. Johnson. 2015. "Feral Cats Are Better Killers in Open Habitats, Revealed by Animal-Borne Video." Edited by Benjamin Lee Allen. *PLOS ONE* 10 (8): e0133915. <https://doi.org/10.1371/journal.pone.0133915>.
- McLeod, Lynette J., Donald W. Hine, and Andrew J. Bengsen. 2015. "Born to Roam? Surveying Cat Owners in Tasmania, Australia, to Identify the Drivers and Barriers to Cat Containment." *Preventive Veterinary Medicine* 122 (3): 339–44. <https://doi.org/10.1016/j.prevetmed.2015.11.007>.
- Medina, Félix M., Elsa Bonnaud, Eric Vidal, Bernie R. Tershy, Erika S. Zavaleta, C. Josh Donlan, Bradford S. Keitt, Matthieu Corre, Sarah V. Horwath, and Manuel Nogales. 2011. "A Global Review of the Impacts of Invasive Cats on Island Endangered Vertebrates." *Global Change Biology* 17 (11): 3503–10. <https://doi.org/10.1111/j.1365-2486.2011.02464.x>.
- Nussberger, Beatrice, Soraia Barbosa, Mark Beaumont, Mathias Currat, Sébastien Devillard, Marco Heurich, Jo Howard-McCombe, et al. 2023. "A Common Statement on Anthropogenic Hybridization of the European Wildcat (*Felis silvestris*)." *Frontiers in Ecology and Evolution* 11 (July):1156387. <https://doi.org/10.3389/fevo.2023.1156387>.
- Olsen, Tammy F, and Andrew L Allen. n.d. "Causes of Sudden and Unexpected Death in Cats: A 10-Year Retrospective Study."
- Petrie A (2020). `_regclass`: Tools for an Introductory Class in Regression and Modeling. R package version 1.6, <https://CRAN.R-project.org/package=_regclass>
- Präg, Patrick, Melinda C. Mills, and Rafael Wittek. 2016. "Subjective Socioeconomic Status and Health in Cross-National Comparison." *Social Science & Medicine* 149 (January): 84–92. <https://doi.org/10.1016/j.socscimed.2015.11.044>.
- Pongrácz, Péter, and Csenge Anna Lugosi. 2024. "Predator for Hire: The Curious Case of Man's Best Independent Friend, the Cat." *Applied Animal Behaviour Science* 271 (February):106168. <https://doi.org/10.1016/j.applanim.2024.106168>.
- Pongrácz, Péter, Luca Turi, Petra Dobos, Virág Burányi, Gergely Schally, and Zsolt Biró. 2024. "Do as I Say, Not as I Do – The Paradoxical Relationship between Actual Cat Keeping Practices and Knowledge about Wildcat Conservation among Hungarian Cat Owners." *Biological Conservation* 293 (May):110586. <https://doi.org/10.1016/j.biocon.2024.110586>.
- Rochlitz, Irene. 2005. "A Review of the Housing Requirements of Domestic Cats (*Felis silvestris catus*) Kept in the Home." *Applied Animal Behaviour Science* 93 (1–2): 97–109. <https://doi.org/10.1016/j.applanim.2005.01.002>.
- Rowe, Elizabeth, William Browne, Rachel Casey, Tim Gruffydd-Jones, and Jane Murray. 2015. "Risk Factors Identified for Owner-Reported Feline Obesity at around One Year of Age: Dry Diet and Indoor Lifestyle." *Preventive Veterinary Medicine* 121 (3–4): 273–81. <https://doi.org/10.1016/j.prevetmed.2015.07.011>.
- Stella, Judi, Candace Croney, and Tony Buffington. 2014. "Environmental Factors That Affect the Behavior and Welfare of Domestic Cats (*Felis silvestris catus*) Housed in Cages." *Applied Animal Behaviour Science* 160 (November): 94–105. <https://doi.org/10.1016/j.applanim.2014.08.006>.

- Strickler, Beth L., and Elizabeth A. Shull. 2014. "An Owner Survey of Toys, Activities, and Behavior Problems in Indoor Cats." *Journal of Veterinary Behavior* 9 (5): 207–14. <https://doi.org/10.1016/j.jveb.2014.06.005>.
- Tan, Sarah M. L., Sarah Jajou, Anastasia C. Stellato, and Lee Niel. 2021. "Perspectives of Canadian and American Cat Owners on Provision of Uncontrolled Outdoor Access for Owned Domestic Cats." *Frontiers in Veterinary Science* 8 (October):742245. <https://doi.org/10.3389/fvets.2021.742245>.
- Tan, Sarah M.L., Anastasia C. Stellato, and Lee Niel. 2020. "Uncontrolled Outdoor Access for Cats: An Assessment of Risks and Benefits." *Animals* 10 (2): 258. <https://doi.org/10.3390/ani10020258>.
- Timm, Robert. n.d. "COYOTE ATTACKS: AN INCREASING SUBURBAN PROBLEM."
- Trevorrow, Nicky. 2019. "The implications of social living in cats." *Companion Animal*, 24(5): 257-261.
- Tschanz, Britta, Daniel Hegglin, Sandra Gloor, and Fabio Bontadina. 2011. "Hunters and Non-Hunters: Skewed Predation Rate by Domestic Cats in a Rural Village." *European Journal of Wildlife Research* 57 (3): 597–602. <https://doi.org/10.1007/s10344-010-0470-1>.
- Van Eeden, Lily M., Fern Hames, Richard Faulkner, Andrew Geschke, Zoe E. Squires, and Emily M. McLeod. 2021. "Putting the Cat before the Wildlife: Exploring Cat Owners' Beliefs about Cat Containment as Predictors of Owner Behavior." *Conservation Science and Practice* 3 (10): e502. <https://doi.org/10.1111/csp2.502>.
- Venables, W. N. & Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0
- Wilson, Colleen, Melissa Bain, Theresa DePorter, Alexandra Beck, Vanessa Grassi, and Gary Landsberg. 2016. "Owner Observations Regarding Cat Scratching Behavior: An Internet-Based Survey." *Journal of Feline Medicine and Surgery* 18 (10): 791–97. <https://doi.org/10.1177/1098612X15594414>.
- Woods, Michael, Robbie A. McDonald, and Stephen Harris. 2003. "Predation of Wildlife by Domestic Cats *Felis Catus* in Great Britain." *Mammal Review* 33 (2): 174–88. <https://doi.org/10.1046/j.1365-2907.2003.00017.x>.
- Yamaguchi, Nobuyuki, Carlos A. Driscoll, Andrew C. Kitchener, Jennifer M. Ward, and David W. Macdonald. 2004. "Craniological Differentiation between European Wildcats (*Felis Silvestris Silvestris*), African Wildcats (*F. s. Lybica*) and Asian Wildcats (*F. s. Ornata*): Implications for Their Evolution and Conservation: WILDCAT EVOLUTION AND CONSERVATION." *Biological Journal of the Linnean Society* 83 (1): 47–63. <https://doi.org/10.1111/j.1095-8312.2004.00372.x>.

Chapter 2: Utilizing citizen science to assess consistent individual variation in play behavior of the domestic cat, *Felis catus*

Under review at *Behavioural Processes* (2026)

ABSTRACT

It is well known that domestic cats (*Felis catus*) have the potential to impact local vulnerable wildlife, and that individual cats differ in their ecological impact; however, less is known about how individual cats may differ in their behavior in simulated hunting contexts, such as in response to a moving, feathered object toy. Using an online questionnaire, we recruited participants in the United States to expose their cat (N=87) to standardised behavioral assay trials using a feather wand toy, aiming to capture individual variation in response to the toy and the consistency of the individuals' responses over time. We collected behavioral data indicative of cat interest in the toy, and found high repeatability of all behaviors measured across trials (mean $R = 0.467$). We then consolidated the data using Principal Component Analysis to obtain a composite interest score for further analysis. Overall, we found significant trial effects ($p = 0.002$), and consistent individual variation in the cats' composite interest score ($ICC = 0.717$). Interestingly, we did not find any significant connections between the cats' interest score and factors such as their outdoor access, history of hunting, frequency of play in the home, or rated playfulness by their caretakers. Our study presents the first recorded use of citizen science as a method of quantifying consistent individual differences in play behavior of cats, and highlights the importance of assessing individual variation when studying cat behavior.

INTRODUCTION

Domestic cats are one of the most common companion animals in the world, present in 49 million homes in the United States alone (APPA, 2025). Despite their ubiquity as household pets, cats have the potential to impact local vulnerable wildlife when outside, thus garnering much attention from scientists across disciplines (Cecchetti et al., 2021; Crowley et al., 2020; Linklater et al., 2019; Loss et al., 2013) and instigating much debate on best practices for managing owned cats (Linklater et al., 2019; Ma & McLeod, 2023; Pongrácz & Lugosi, 2024).

One aspect of cat management that may have direct implications for hunting behavior is the provision of prey-like toys in the home, often used by caretakers to engage their cat in interactive object play (Leyhausen, 1979). Provision of toys is recommended to promote positive welfare in cats, by strengthening human-animal bond and by encouraging expression of natural behaviors such as hunting (Ellis et al., 2013; Henning et al., 2022). Previous research on behavioral development of cats has indeed shown connections between a cat's responses to an object toy and their response to real, live prey (Caro, 1980; Hall & Bradshaw, 1998); recent studies suggest connections between a cat's response to a toy and their outdoor access (Pyari et al., 2021), and suggests that daily interactive object play may reduce prey returns in cats (Cecchetti et al., 2021). Interactive object play may therefore serve as a bridge between welfare and conservation scientists in generating solutions that benefit both cats and surrounding wildlife.

Most empirical studies indicate that cats' impact on wildlife is often attributable to a small percentage of the cats studied (Hernandez et al., 2018; Moseby et al., 2015; Seymour et al., 2020). Indeed, studies using direct measures of hunting suggest that some owned cats may hunt 1-2 prey items in a given week, while others may never (Loyd et al., 2013). Some studies have

thus explicitly highlighted the importance of assessing individual variation when studying cats' outdoor behaviors and hunting, suggesting that identifying "high-risk" individuals and targeting intervention strategies to those cats and their households may be key to generating sustainable and effective conservation solutions (Dickman & Newsome, 2015; Moseby et al., 2015). Given the connection between play and hunting behaviors, assessing consistent individual differences (CIDs) in play, and identifying factors that may explain these differences (e.g. age, outdoor access) may be crucial for identifying target individuals and informing conservation efforts.

CIDs in animal behavior (often referred to as animal personalities) are traditionally assessed using standardised behavioral assays, during which individual animals are exposed to a controlled arena, maze, or other experimental conditions over several trials (Bell et al., 2009; Sih & Bell, 2008). However, studying domestic cat hunting behavior in a controlled manner in a home environment can be challenging, as many cats show aversion to novel people, objects, or cues in their environment (Amat et al., 2016; Takagi et al., 2019). Therefore, utilizing familiar contexts within the household, such as having familiar people administer behavioral assay trials, may facilitate accurate capture of natural behaviors.

Many studies have empirically assessed consistent individual differences in various behaviors of cats (Bradshaw & Cook, 1996; Durr & Smith, n.d.; Gartner & Weiss, 2013; Martínez-Byer et al., 2020). Some studies have repeatedly exposed cats to toys to capture changes in their behavior overtime (Hall et al., 2002), but to our knowledge no study has assessed consistent individual differences in their play behavior using caretaker-administered behavioral assays. In this study, we aimed to utilize citizen science to 1) quantify among-individual differences in cats' response to a moving, feathered object toy, 2) assess the consistency of their responses across trials over time, and 3) explore potential factors that may

predict an individual's response, such as their outdoor access, hunting history, frequency of normal interactive play in the home, and caretaker ratings of their cat's playfulness. Consistent with other studies on individual differences in cat behavior we predicted that cats would show among-individual variation in their response to a moving toy, and that they would show high consistency within themselves in these behaviors. We also predicted that similarly to previous studies, those who do not go outside (i.e., indoor-only cats) would show more interest in the moving toy compared to cats with outdoor access (Pyari et al., 2021). Additionally, given the connection between play and hunting behavior, we predicted that those who have hunted in the past would show increased interest in the moving toy, especially when they are not currently frequently played with. Finally, we predicted that caretaker ratings of their cat's playfulness would correspond with their cat's interest in the toy.

METHODS

Ethical Note

This study was reviewed and determined exempt (non-human subjects research) by the Institutional Review Board of the University of California, Davis (#2194091). This study was also reviewed and determined exempt by the Institutional Animal Care and Use Committee (IACUC) of University of California, Davis (#23321), though ethical endorsement was provided. A consent form including the inclusion criteria and a detailed description of the objectives of the study was presented to each participant at the beginning of the questionnaire.

Recruitment Questionnaire

We created and distributed a cross-sectional, quantitative recruitment questionnaire using Qualtrics™ survey software (Provo, Utah, USA). Participants had to be at least 18 years old

living in the United States, and a current caretaker of at least one adult (1+ year old) cat. For multi-cat households, participants were directed to report on the cat whose name appears first in the alphabet, as the household was the experimental unit and only one cat per household could participate. Each participant who completed the questionnaire and provided a valid email address was entered into a raffle to win one of three \$50 Amazon gift cards as an incentive to participate in the study. Participants who completed all three trials of the main study were provided a \$25 pet store gift card (Chewy, Inc., Plantation, FL, USA) as a reward for completing the study.

The recruitment questionnaire (N=43 questions) began with two consent forms (n=2), an attention check (n=1), and a commitment request (n=1) to ensure participants would provide thoughtful answers to the survey. The questionnaire contained questions on the participant demographics (n=10), their cat's demographics and health (n=8), outdoor access and hunting (n=10), and toy availability and play behavior at home (n=11). These questions were selected based on previous research on this topic, and factors for which we had *a priori* hypotheses. An advertisement to participate in the questionnaire was distributed online via social media groups from August 5, 2024 until October 15, 2024 using convenience and snowball sampling. Participants who completed the recruitment survey (N=366) and fit the inclusion criteria were automatically invited to participate in the main study and asked to provide a mailing address for shipment of the study materials.

Behavioral Assay Trials

Of those who completed the initial recruitment questionnaire, 179 participants provided a mailing address and therefore received a standard package containing an individually wrapped wand toy (Figure 2.1) with printed instructions for filming and submission of videos. The standard object toy consisted of a pink plastic handheld wand connected to a thin flexible metal

rod with a feather and bell affixed to the end (total toy length: approximately 95cm). This toy was chosen based on pilot trials that revealed participants were able to move this toy more consistently compared to other types of toys due to its rigidity, while maintaining movement that resembled prey, such as a flying bird. Participants were directed to keep the toy in its original packaging and to prevent visual and physical access by their cat until the trials commenced.

Figure 2.1. Still images from participant videos showing a cat attacking the toy (left) and a cat looking away (right), while the toy was moved by the participant caretakers.



We provided step-by-step, detailed video and written instructions for the behavioral assay trials. We directed participants to bring their cat into a room that their cat feels comfortable in, and in the case of multi-animal households, to lock other animals out of the testing room to avoid any interactions with the focal cat for the duration of each trial. Participants then recorded

themselves using their smartphone, either by holding the camera themselves, propping the camera on furniture, or asking another person in the household, ensuring that both the toy and the cat would be in full view of the camera at all times. We directed participants to then open the packaging and unravel the wire, and begin recording as they started to move the toy in a sideways figure-8 motion at a moderate speed (approximately 1 second per figure 8) in front of their cat, a few inches above their head. Participants moved the toy in this manner consistently and continuously for 2 minutes, after which they stopped recording and placed the toy back in the packaging and stowed away until the next trial. The 2-min duration of each trial was determined based on pilot trials that tested the minimum length of time necessary to observe behavioral changes in the cat while ensuring the retention of participants, and is consistent with previous research (Hall et al., 2002). Each participant then uploaded the video to Box Drive (Box Inc, Redwood City, CA, USA) via a file share link. We asked participants to submit three independent trials, so we could assess consistency of their cat's behavior over time. We requested that these videos are taken at least 12 hours apart, and later confirmed the gaps between trials using the timestamps provided by the smartphone during the upload. The 12-hour gap was chosen in order to ensure the independence of subsequent trials while minimizing participant dropout. We directed participants to keep the packaged toy away from the cat in between the recorded trials.

Video Coding

All videos were collected via Box File Share, and were downloaded, and immediately relabeled with a linking key containing a randomly generated number, the first letter of the cat's name, and the trial number (e.g., "107C2"). To ensure standardisation across video recordings, videos longer than 120 s were limited to the first 120 s. All coders were blind to this

nomenclature, and all identifying information such as email address, mailing address, participant's name, and linking key were stored in a password-protected cloud storage and only accessed for the purpose of contacting the participants.

We developed a study ethogram using the standard ethogram for Felidae by Stanton and colleagues, which can be found in Table 2.1 (Stanton et al., 2015) We aimed to collect relevant behavioral data indicative of the degree of the cat's passive and active engagement with the toy, including: visual orientation towards the toy, physical contact with the toy, walking away from the toy, swipes, captures, and jumps towards the toy. Additionally, to account for variation in the speed of the figure-8 motion during the trials, the total number of figure-8 movements during each 120-s trial was counted as a measure of the speed at which the participant moved the toy. We also noted the duration of time the cat spent out of view, denoted by both the cat's head and forepaws being out of the frame.

Table 2.1. Ethogram used to score domestic cat play behavior from caretaker submitted videos of play sessions using a standardised wand toy. Each measured behavior (“Behavior”) is listed with its corresponding descriptions (“Description”) and the type (duration or frequency).

Behavior	Description	Type
Walk away	Cat locomotes away from the toy, with the head and the body facing away from the toy.	Duration
Look away	Cat is static, and the cat’s head is not moving along with the movement of the toy and/or is facing elsewhere.	Duration
Visual orientation	Cat’s head is facing the toy and is moving along with the movement of the toy. Ears are generally up and pointed towards the toy.	Duration
Contact with toy	Cat holds the toy (makes contact) either with their mouth or forepaw(s) for 2 seconds or more.	Duration

Out of view	Cat's head and forelegs are out of view.	Duration
Swipe attack	Cat extends one forepaw after the moving toy.	Frequency
Two-paw attack (Capture attempts)	Cat extends both forepaws simultaneously after the toy. Cat is generally on their hindlegs.	Frequency
Jump	Cat locomotes vertically after the toy off the ground. All four legs are off the ground, with forepaws extended after the toy.	Frequency

All behaviors were coded using The Observer XT™ software (Noldus, Wageningen, The Netherlands). To assess observer reliability, we trained three research assistants on a test subset of seven, 120-s pilot videos derived from volunteers and participants not included in the main study. State variables with duration and/or latency (walk away, look away, visual orientation, contact with toy) were continuously coded, and discrete variables (swipe, capture attempts, jump) were assessed using a 25-question video test (13 yes, 12 no) testing their ability to accurately identify both the presence as well as the absence of each behavior. We trained all three observers to an interrater reliability score of $\geq 80\%$ (Cohen's kappa, irr package version 0.84.1, Gamer et al., 2019) between all pairs including the expert observer (HC), except ‘capture attempts’, which were trained to reliability of $\geq 75\%$ due to difficulty of identification. Once reaching the minimum inter-rater reliability, observers coded all videos in the main dataset from Jan - Mar 2025.

Data Cleaning and Preparation

All coded behavioral data (duration, latency, frequency) from the videos were downloaded from The Observer XT™ as an xlsx file and converted into seconds. All duration behaviors were divided by the total amount of time the cat was visible in the video to account for the time the cat was out of frame and avoid penalizing cats during the time they were out of

view. The cat needed to be within view for at least half of the trial, or 60 seconds, to be included in the dataset. All cleaned behavioral data were then inputted into the statistical software program R as a csv file (v4.4.3; R Core Team, 2022).

All questionnaire data were coded and open-text box responses associated with "Other" response options were reviewed, and either reclassified or removed to reduce misclassification bias. We extracted only the questionnaire data of the 87 caretaker-cat dyads who completed all three trials of the main study. We created a comprehensive dataset linking the behavioral data and the questionnaire data, and descriptive statistics were run for all variables of interest. All data visualization and statistical analyses were performed in R.

Repeatability

We estimated the repeatability of each behavior across trials by calculating the intraclass correlation (ICC), which compares within-individual variance (across trials) and among-individual variance (across individuals), using the rptR package (v0.9.23, Stoffel, Nakagawa, & Schielzeth, 2017). We included parametric bootstrapping (nboot=2000) to generate reliable confidence intervals, and fitted a Poisson distribution for count data (Swipe, Capture attempts, Jumps). For non-Gaussian latency data, we applied a log1p (log of 1+value) transformation prior to calculation of repeatability estimates, to avoid taking the log of zero. In the case of Poisson distributions, the original scale approximations (rather than latent scale approximations) were reported.

Principal Component Analysis

Rather than individually assessing each behavior variable, we created composite scores for cat interest using principal component analysis. The behavior variables included in the principal component analysis were: latency to look away from toy (Lose Interest), latency to

attack, latency to walk away from toy, total number of swipes, total number of capture attempts, total number of jumps, proportion of visible time spent visually oriented towards toy (Visual Orientation), and proportion of visible time spent in contact with toy (Contact with Toy). We first standardized and centered all variables to be inputted into the PCA using the `scale()` function in R, which was necessary to visualize all behavioral data on the same scale. We then computed a covariance matrix between variables using the `cor()` and `corrplot()` functions to visually assess correlations between variables and ensure no variables were highly correlated (>0.90). We then applied the PCA using the `princomp()` function in the `factoextra` package (v1.0.7; Kassambara, 2020), and extracted eigenvectors and eigenvalues. We visualized the quality of representation of variables, variable correlations, and proportion of variance explained by each component to guide our selection of components.

Model Building

We built linear mixed-effects models with PC1 and PC2 as the outcome variables using the `lme()` function in R. We included the following predictor variables as fixed effects: age of the cat (1-2 years, 3-6 years, 7-10 years, 11+ years), method obtained (street, shelter or rescue, rehomed directly from previous caretaker, or other), outdoor access (y, n), hunting history (y,n), frequency of play with caretaker (more than once a day, once a day, a few times a week, a few times a month or less), rated playfulness of the cat by the caretaker (extremely playful, somewhat playful, neither playful nor unplayful, somewhat unplayful, extremely unplayful), presence of a similar feather wand toy at home (y,n), and frequency of receiving a new toy in the home (once a year or less, a few times a year, monthly, weekly or more). We also included time since last trial (continuous) and trial number (1, 2, or 3) as fixed effects, with cat identity as a random effect, and tested biologically feasible interaction terms (e.g., hunting history x play frequency). Aside

from random intercepts, we also tested cat identity as a random slope, to assess if the rate of change in cat behavior across repeated trials differed between individuals. We conducted a power analysis using the *pwr* package in R (v1.3-0, Champely, 2020) to calculate the sample size needed for our model ($k=13$) to explain at least 30% of the variance of our data at 80% power, with $\alpha=0.05$, following Cohen (1988), which resulted in a minimum sample size of $n=79$. The target R^2 of 0.30 was chosen based on the conventional values found in ecology and evolutionary sciences (Lin & Wiegand, 2023; Møller & Jennions, 2002) and the baseline range of acceptable R^2 values between 0.1-0.5 (Ozili, 2022). Upon building the full model with all predictor variables of interest including interactions, we performed model selection by comparing the full model against a version of the model with one variable removed at a time, beginning with the interaction terms. We quantified the model fit using the Akaike Information Criterion and Bayes Inference Criterion, and compared the models using the log-likelihood ratio (LLR) and p-values obtained from the *anova()* function in R. Once the model was finalized, we tested the model fit by calculating adjusted R^2 values, and visually inspected several model diagnostics such as homoscedasticity and normality of residuals. Finally, we applied Tukey's adjustment to p-values and confidence intervals of post-hoc comparisons for variables with four or more categories in order to reduce the chance of Type I error.

RESULTS

Of those who were mailed a toy ($n=179$), 76.0% ($n=134$) participated in at least one trial. Four participants dropped out of the study due to their cat being too afraid of the new toy to participate, and five participants submitted videos that did not meet quality standards for analysis (e.g. angle of the camera, video quality). Four additional participants were dropped from the study due to having the study toy in the home, to ensure the toy was equally novel to all study

cats. In total, 121 caretaker-cat dyads followed instructions and completed at least one trial, and 87 completed all three independent trials. Only those who completed all three trials were retained for further analysis (N=87). Participants on average submitted trials that were about two days apart (mean = 50.31h, SE = 5.96h).

Demographics

Participating cat caretakers (N=87) were mainly women (79.31%) with no children (79.31%), between 25 and 44 years old (51.72%), living in suburban areas (62.07%). Participants were most frequently residing in California (18.39%), though 31 states were represented in total. Of the participating cats, 50.57% were female, 49.43% male, and 96.55% were neutered or spayed. The majority of the cats were mixed-breed (89.66%), between 1 and 6 years old (72.41%), and obtained from a shelter or rescue (54.02%). Most were living either as the only cat in the household (37.93%) or with one other cat (40.23%), and without any dogs in the home (86.21%). The majority of the cats had been living with their caretaker for 1-5 years at the time of the study (67.82%).

Survey data

A summary of variables that were predicted to influence cat interest in the toy are presented in Table 2.2. Overall, the majority of the cats were kept indoor-only (59.77%), while the remaining 40.23% were being provided some form of outdoor access, with 29.89% provided controlled outdoor access (e.g. catio, fencing, leash walking), and 10.34% provided uncontrolled outdoor access (free-roam). Most cats were rated by their caretakers as either extremely (33.33%) or somewhat (55.17%) playful. The majority of caretakers reported currently playing with their cat at least once a day (68.96%). Forty-five percent (44.83%) of the cats were reported to have been seen hunting wild animals either inside or outside of the home.

Table 2.2. Frequency and percentages of recruitment questionnaire variables included in the linear regression model (N=87).

Variable	Category	Freq (%)
Cat age	1-2 years old	26 (29.89%)
	3-6 years old	37 (42.53%)
	7-10 years old	12 (13.79%)
	11+ years old	12 (13.79%)
Method obtained	Street / Stray	19 (21.84%)
	Shelter or rescue	47 (54.02%)
	Rehomed directly	15 (17.24%)
	Other	6 (6.90%)
Outdoor access	Indoor-only	52 (59.77%)
	Controlled outdoor	26 (29.89%)
	Uncontrolled outdoor	9 (10.34%)
History of hunting wild prey	No	48 (55.17%)
	Yes	39 (44.83%)
Frequency of play	More than once a day	32 (36.78%)
	Once a day	28 (32.18%)
	A few times a week	20 (22.99%)
	A few times a month or less	7 (8.05%)
Rated playfulness	Extremely playful	29 (33.33%)
	Somewhat playful	48 (55.17%)
	Neither playful nor unplayful	5 (5.75%)
	Somewhat unplayful	5 (5.75%)
	Extremely unplayful	0 (0%)
Feather wand toy at home	No	14 (16.09%)
	Yes	73 (83.91%)
Frequency of receiving new toy	Once a year or less	5 (5.75%)
	A few times a year	51 (58.62%)
	Monthly	23 (26.44%)
	Weekly or more	8 (9.20%)

Behavior data

The ranges, means, standard deviations, as well as repeatability scores of all behavior data collected from the videos are summarized in Table 2.3. All behavior variables were highly repeatable within individuals, with scores ranging from 0.305 to 0.734.

Table 2.3. Metadata of behavior variables collected from caretaker submitted videos of cat responses to a standard toy. Ranges, means, and standard deviations of behavior variables, and their repeatability (ICC) scores along with their standard error and 95% confidence intervals. Ranges, means, and standard deviations are reported in seconds as raw data, prior to data transformation. R = repeatability (ICC), SE = standard error, and CI = 95% confidence intervals. Log1p transformation was applied to latency variables. Duration variables (visual orientation, latency to lose interest, latency to walk away from toy, latency to attack, contact with toy) are reported in seconds with a minimum of 0 and maximum of 120 s, and the remaining variables (swipes, capture attempts, jumps) are reported in frequencies.

	Range (mean)	SD	R	SE	CI
Visual orientation	0 – 120 (62.20)	34.64	0.734	0.042	[0.634, 0.804]
Latency to lose interest ^a	0 – 120 (20.29)	31.01	0.430	0.067	[0.287, 0.554]
Latency to walk away from toy ^a	0 – 120 (73.31)	47.73	0.305	0.070	[0.164, 0.438]
Latency to attack ^a	0 – 120 (29.85)	37.22	0.341	0.067	[0.204, 0.467]
Contact with toy	0 – 117.4 (4.07)	11.74	0.485	0.064	[0.344, 0.597]
Swipes ^b	0 – 45 (11.65)	9.79	0.558	0.056	[0.439, 0.661]
Capture attempts ^b	0 – 37 (7.10)	8.05	0.492	0.058	[0.314, 0.609]
Jumps ^b	0 – 23 (1.58)	3.39	0.391	0.104	[0.192, 0.590]

^aLog1p transformed (log transform after adding 1)

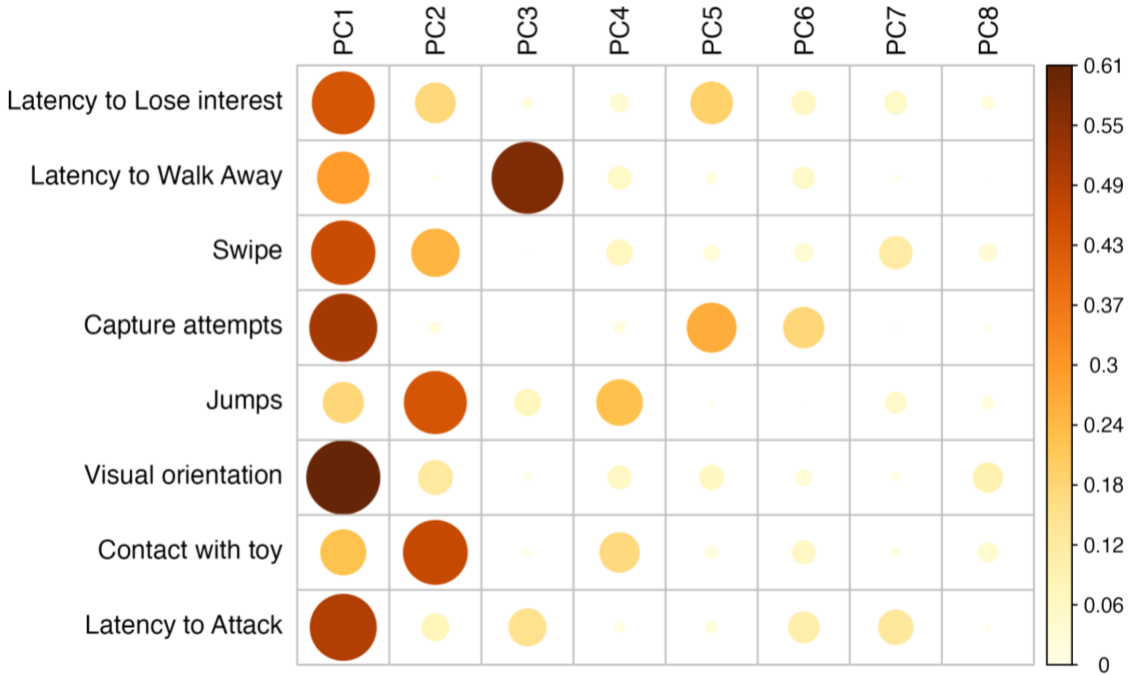
^bCount data fitted to Poisson distribution

The first two components (PC1, PC2) cumulatively captured 60.0% of our data. The loading values indicating correlation between each principal component and the variables are shown in Table 2.4. The quality of representation of each variable in each principal component, or the proportion of variance explained by each principal component, is measured by the squared cosine values and can be found in Figure 2.2.

Table 2.4. PC loadings of each behavior variable included in the principal component analysis for cat interest level in toy. Positive values indicate positive association, and negative values indicate negative association with the PC, with larger values indicating stronger association.

	PC1	PC2
Latency to lose interest	0.368	-0.338
Latency to walk away	0.305	-0.029
Latency to attack	-0.392	0.404
Swipe	0.377	0.096
Capture attempts	0.399	-0.530
Jumps	0.238	0.288
Visual orientation	0.435	-0.545
Contact with toy	0.267	-0.228

Figure 2.2. Plot of quality of representation of each variable in each dimension. Squared cosine values represent the proportion of variance explained by each principal component, with a minimum value of 0 and maximum value of 1. Larger circles and darker colors indicate larger square cosine values, or better representation in the principal component.



The majority of variables were well represented by PC1 (Figure 2.2); i.e., PC1 is an indicator of higher general interest in the toy. The total number of jumps (Jumps) and the proportion of visible time spent in contact with the toy (Contact with Toy) were best represented in PC2, and latency to walk away was the only variable well represented by PC3. Given the relatively poor representation of most variables in PC3, we excluded PC3 from further analysis and only retained the first two components.

Predictors of cat interest

Table 2.5. Summary of linear regression model showing significant predictors of PC1, or cat interest in the moving toy (N=87). Estimates, standard error, p-values, and 95% confidence intervals are shown, and only significant pairwise comparisons are included. Conditional R^2 indicates proportion of variance explained by both fixed and random effects, whereas the marginal R^2 indicates the proportion of variance explained just by the fixed effects alone. The intraclass correlation (ICC) is listed as a measure of effect size of the random effect.

	Estimate	SE	p	95% CI [lwr, upr]
Fixed Effects				
(Intercept)	1.081	0.337	0.002	[0.422, 1.740]

Trial		-0.219	0.070	0.002	[-0.356, -0.082]
Cat age	1-2 y (ref.)	-	-	-	-
	11+ y	-1.439	0.541	0.038 ⁺	[-2.514, -0.364] ⁺
Random Effect					
		σ(intercept)	Residual	p	95% CI [lwr, upr]
Cat Identity		1.463	0.904	<0.001	[1.230, 1.724]
Model Performance					
		Conditional R²	Marginal R²	ICC	
		0.740	0.082	0.717	

⁺ Tukey adjusted p-values and confidence intervals.

There were significant trial effects, with cats generally losing interest in the moving toy with subsequent trials ($\beta = -0.219$; CI=-0.356, -0.082). Cats who were 11 years old or older showed significantly less interest in the toy than young, 1-2 year old cats ($\beta = -1.439$; CI=-2.514, -0.364). No other comparison between age groups was significant. Additionally, no other explanatory variable significantly predicted the cats' interest in the toy. Despite some differences in the gap between trials (mean=50.3h, max = 838h), we did not see any significant effect of time since last trial on the cats' interest in the toy. Cat identity was a significant random effect (ICC=0.717), as random intercepts but not as random slopes across trials, indicating that while individual cats were different from each other in their interest level in the toy, the rate or the pattern in which they lost interest over time did not differ between individuals. Cat identity was the single most important variable in the model, explaining approximately 65.8% of variance in our data, as evidenced by the difference between the marginal and conditional R² values seen in Table 2.5. No explanatory variable contributed to the model with PC2 as the outcome variable, except cat identity as a random effect (ICC = 0.528).

DISCUSSION

Individual Variation

Domestic cats have been known to exhibit individual variation in their hunting tendencies (Dickman & Newsome, 2015; Loyd et al., 2013; Moseby et al., 2015), but very few studies have quantified consistent individual variation in cats' response to a simulated hunting context (Hall et al., 2002; Martínez-Byer et al., 2020), and to our knowledge, none have utilized a cat's home environment and caretakers. By training cat caretakers to administer standardized behavioral assay trials on their cats, we quantified individual cat responses to a consistent, moving toy over time across three independent trials. We found significant among-individual variation in cat interest in this toy, with cat identity as a random effect associated with 65.8% of the variance in our data (Conditional R^2 - Marginal R^2 in Table 2.5). Additionally, we found that all individual behaviors that we measured as proxies of interest in the toy were highly repeatable within individuals across trials, with Visual Orientation (proportion of visible time spent visually oriented towards the toy) showing the highest repeatability of 0.734. Given that the average repeatability of behaviors as calculated in a meta-analysis by Bell et al. is 0.37 (Bell et al., 2009), our repeatability range of 0.305 to 0.734 showed a relatively high estimate of repeatability across all behaviors. This suggests that cat playfulness, or interest level in a toy, may be a consistent trait inherent to each individual and therefore provide further support for the existence of cat personality (Gartner et al., 2013; Cecchetti et al., 2022). We suggest that future research explore cats' responses to multiple types of toy movements to increase external validity of this study, and to explore connections between individual differences in cat object-play behavior and other behaviors relevant to Felid personality, such as exploratory tendencies (Hinde, 1970; Hall et al., 2002) or sociability (Bradshaw, 2016; Durr & Smith, 1997). We highlight that more research on

individual differences in cat behaviors is needed, particularly involving multiple domains or axes of behavior at once.

Predictors of Interest Level in Toy

Participating cats in our study generally lost interest in the toy over time with subsequent trials. The presence of a significant trial effect suggests that cats are prone to losing interest in a novel object quickly, which is consistent with previous research (Hall et al., 2002), and we suggest future similar studies to implement gaps larger than 24 hours if possible, to minimize similar trial effects.

As expected, older cats in our study showed less interest in the toy than younger cats. Interestingly, the only statistically significant pairwise comparison was between 1-2 year olds and 11+ year olds, the most extreme group comparisons in age. Since the majority of cats in our study were obtained from a shelter or rescue, it is possible that their age estimates were not precise and thus only the differences between the most extreme age groups showed statistical significance. Previous research on a similar test showed no significant differences between age groups in their response (Martínez-Byer et al., 2020); we suggest that future studies further investigate this connection between age and object play behavior. Further, we suggest that longitudinal studies that track changes in behavior as animals age are much needed, particularly to assess how these changes may be influenced by the animals' experiences in and outside the home.

Contrary to previous research (Pyari et al., 2021), we found that outdoor access was not a significant predictor of the cats' response to the toy. We suggest that this may be due to the overrepresentation of controlled-outdoor cats and relative lack of free-roaming cats in the outdoor cat group of our sample, and expect more differences to arise in samples with more free-

roaming cats. Additionally, neither the cats' hunting history, their background (method obtained), nor the current frequency of interactive play with their caretaker significantly predicted their response to the toy. Based on these results, we conclude that since the variance in our data was largely associated with cat identity, the inherent behavioral differences between individual cats overwhelmed any potential effects of their hunting tendencies, background, or current frequency of interactive play. We suspect that in our study, the reported history of hunting (0,1) may not have been meaningful, and suggest that more precise measurements of hunting such as through animal-borne cameras or fecal analysis may lead to more conclusive data exploring connections between hunting history and play behavior. Further, given that cat hunting behavior is influenced by their early life history such as exposure to hunting through their mothers (Cecchetti et al., 2020), we suggest that a more complete history of the cat's background, and particularly about their early kittenhood, may provide more insight into the relationship between cat background and playfulness. Finally, as seen in other studies (Henning et al., 2023), measures of the bond between cats and their caretakers, such as the Cat Owner Relationship Scale (Howell et al., 2017), may shed more light on the connections between the cat-caretaker relationship and the cats' response to simulated play sessions. We therefore urge future studies to consider cat-caretaker relationships and even individual differences in caretaker behaviors (Finka et al., 2022) when assessing cat behaviors in the home.

Surprisingly, we found no significant connections between the cats' playfulness as rated by their caretakers and their actual response to a moving toy. Given that the majority of the cats in our sample were already rated as extremely (33.33%) or somewhat (55.16%) playful, it is possible that caretakers who perceived their cat as more playful (regardless of the cat's 'true' playfulness) may have been more interested in the study topic and thus more motivated to

participate. This indicates potential sampling bias resulting in an overrepresentation of playful cats in our study. Given the lack of representation of cats that were rated as somewhat or extremely unplayful, we lacked statistical power to assess meaningful differences between cats that were rated as more versus less playful. On the other hand, it is also possible that caretaker-reported questionnaire data may generally not be reliable measures of cat behavior in the home; for example, one study found discrepancies between caretaker reports of hunting and their actual hunting (Loyd et al., 2013). Captive animals, and particularly companion animals, pose a unique system to compare ratings of personality with objective assessments of behavioral responses to standardized assay trials. We therefore emphasize the need for more studies to systematically compare caretaker responses in a questionnaire against observation-based data, to better understand the reliability of questionnaire-based versus observation data in different contexts and obtain a more comprehensive understanding of individual differences in animal behavior.

Play behavior, particularly as it involves interaction between animals, has been shown to play a key role in behavioral development, including emotional, cognitive and sensorimotor skills (Vanderschuren & Trezza, 2014). The adaptive significance of play has long been highlighted and explored (Caro 1988; Smith 1982), with some of the seminal research on animal play behavior occurring in domestic animals such as cats and dogs (Thomas and Schaller, 1954; Caro 1988). Despite their accessibility as study subjects and direct interactions with humans, companion animals, and particularly domestic cats, have been underutilised as a model for investigating the mechanisms of play behavior and its development in animals (Henning et al., 2022). Our study highlights the potential for domestic cats to serve as a model system to assess the impact of humans on animal play behavior, with particular implications for the animals' welfare and ecology in the broader environment.

Limitations

As with all studies involving questionnaires and recruitment of people and their animals, our study likely experienced sampling bias, social desirability bias, and recall bias. We suspect that given our study design and recruitment methods, those with cats who are more playful were more likely to agree to participate in the study, and those who are more likely to view their cats as more playful were more likely to participate in our study. Additionally, age estimates of our sample may not be precise, given that the majority of cats were obtained from a shelter or rescue. We acknowledge that all of our survey data were prone to social desirability bias, in that respondents may have altered or selectively shared responses that they perceive to be socially acceptable or be most consistent with our study goals. Additionally, while citizen science approach used in this study facilitated the collection of behavioral data and engaged caretakers, the variability in submitted videos (e.g., camera angles, resolution, and environmental conditions) made complete standardisation of our behavioral assay trials difficult. Finally, restricting analyses to cats who completed all three trials reduced the sample size and may have introduced attrition bias, which may have led to an overrepresentation of more engaged caretakers with more compliant or playful cats. Thus, we caution readers when extrapolating from our questionnaire data in their ability to explain patterns in the cats' response to the standardized assays.

CONCLUSION

Domestic cats are an emerging study system in predator-prey ecology due to their uniquely accessible nature as companion animals despite their potential as predators of local vulnerable wildlife. Our study utilized their accessibility to quantify consistent individual variation in the predator's response to a hunting-related context, using citizen science. We found

that domestic cats showed significant consistent individual variation in their responses to a standardised, moving toy, and that their inherent individual variation overwhelmingly explained variance in the behavioral data. We suggest that more studies should involve assessment of consistent individual variation in behavior across multiple contexts, and that studies of companion or domestic animals should compare caretaker-reported assessments of behavior with observational assessments conducted in standardized assay trials. Overall, our findings indicate that citizen science is a viable method of engaging caretakers in submitting longitudinal in-home behavioral and human-animal interaction data, and highlights the importance of accounting for inherent individual differences in studies of cat behavior.

ACKNOWLEDGEMENTS

We acknowledge research assistants Michelle Jiang, Elena Aquino, and Hannah Kim for their help with coding the videos collected in this study. This study was funded by the Christine Stevens Award from the Animal Welfare Institute.

REFERENCES

- Amat, M., Camps, T., & Manteca, X. (2016). Stress in owned cats: Behavioural changes and welfare implications. *Journal of Feline Medicine and Surgery*, 18(8), 577–586. <https://doi.org/10.1177/1098612X15590867>
- Bell, A. M., Hankison, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: A meta-analysis. *Animal Behaviour*, 77(4), 771–783. <https://doi.org/10.1016/j.anbehav.2008.12.022>
- Bradshaw, J. W. S. (2016). Sociality in cats: A comparative review. *Journal of Veterinary Behavior*, 11, 113–124. <https://doi.org/10.1016/j.jveb.2015.09.004>
- Bradshaw, J. W. S., & Cook, S. E. (1996). Patterns of pet cat behaviour at feeding occasions. *Applied Animal Behaviour Science*, 47(1–2), 61–74. [https://doi.org/10.1016/0168-1591\(95\)01011-4](https://doi.org/10.1016/0168-1591(95)01011-4)
- Caro, T. M. (1980). Effects of the mother, object play, and adult experience on predation in cats. *Behavioral and Neural Biology*, 29(1), 29–51. [https://doi.org/10.1016/S0163-1047\(80\)92456-5](https://doi.org/10.1016/S0163-1047(80)92456-5)
- Caro, T.M. (1988). Adaptive Significance of Play: Are We Getting Closer? *Trends in Ecology and Evolution*, 3(2), 50–54.
- Cecchetti, M., Crowley, S. L., Goodwin, C. E. D., & McDonald, R. A. (2021). Provision of High Meat Content Food and Object Play Reduce Predation of Wild Animals by Domestic Cats *Felis catus*. *Current Biology*, 31(5), 1107–1111.e5. <https://doi.org/10.1016/j.cub.2020.12.044>
- Cecchetti, M., Crowley, S. L., McDonald, J., & McDonald, R. A. (2022). Owner-ascribed personality profiles distinguish domestic cats that capture and bring home wild animal prey. *Applied Animal Behaviour Science*, 105774. <https://doi.org/10.1016/j.applanim.2022.105774>
- Cecchetti, M., Crowley, S. L., & McDonald, R. A. (2021). Drivers and facilitators of hunting behaviour in domestic cats and options for management. *Mammal Review*, 51(3), 307–322. <https://doi.org/10.1111/mam.12230>
- Champely, S (2020). *_pwr: Basic Functions for Power Analysis_*. R package version 1.3-0, <<https://CRAN.R-project.org/package=pwr>>.
- Crowley, S. L., Cecchetti, M., & McDonald, R. A. (2020). Diverse perspectives of cat owners indicate barriers to and opportunities for managing cat predation of wildlife. *Frontiers in Ecology and the Environment*, 18(10), 544–549. <https://doi.org/10.1002/fee.2254>
- Dickman, C. R., & Newsome, T. M. (2015). Individual hunting behaviour and prey specialisation in the house cat *Felis catus*: Implications for conservation and management. *Applied Animal Behaviour Science*, 173, 76–87. <https://doi.org/10.1016/j.applanim.2014.09.021>
- Durr, R., & Smith, C. (1997). *Individual Differences and Their Relation to Social Structure in Domestic Cats*. 7.
- Ellis, S. L. H., Rodan, I., Carney, H. C., Heath, S., Rochlitz, I., Shearburn, L. D., Sundahl, E., & Westropp, J. L. (2013). AAFP and ISFM Feline Environmental Needs Guidelines. *Journal of Feline Medicine and Surgery*, 15(3), 219–230. <https://doi.org/10.1177/1098612X13477537>
- Finka, L. R., Ripari, L., Quinlan, L., Haywood, C., Puzzo, J., Jordan, A., Tsui, J., Foreman-Worsley, R., Dixon, L., & Brennan, M. L. (2022). Investigation of humans individual

- differences as predictors of their animal interaction styles, focused on the domestic cat. *Scientific Reports*, 12(1), 12128. <https://doi.org/10.1038/s41598-022-15194-7>
- Gartner, M. C., Powell, D. M., & Weiss, A. (2014). Personality structure in the domestic cat (*Felis silvestris catus*), Scottish wildcat (*Felis silvestris grampia*), clouded leopard (*Neofelis nebulosa*), snow leopard (*Panthera uncia*), and African lion (*Panthera leo*): A comparative study. *Journal of Comparative Psychology*, 128(4), 414–426. <https://doi.org/10.1037/a0037104>
- Gartner, M. C., & Weiss, A. (2013). Personality in felids: A review. *Applied Animal Behaviour Science*, 144(1–2), 1–13. <https://doi.org/10.1016/j.applanim.2012.11.010>
- Hall, S. L., & Bradshaw, J. W. S. (1998). The influence of hunger on object play by adult domestic cats. *Applied Animal Behaviour Science*, 58(1–2), 143–150. [https://doi.org/10.1016/S0168-1591\(97\)00136-6](https://doi.org/10.1016/S0168-1591(97)00136-6)
- Hall, S. L., Bradshaw, J. W. S., & Robinson, I. H. (2002). Object play in adult domestic cats: The roles of habituation and disinhibition. *Applied Animal Behaviour Science*, 79(3), 263–271. [https://doi.org/10.1016/S0168-1591\(02\)00153-3](https://doi.org/10.1016/S0168-1591(02)00153-3)
- Henning, J., Fernandez, E., Nielsen, T., & Hazel, S. (2022). Play and welfare in domestic cats: Current knowledge and future directions. *Animal Welfare*, 31(4), 407–421. <https://doi.org/10.7120/09627286.31.4.005>
- Henning, J., Nielsen, T., Fernandez, E., & Hazel, S. (2023). Cats just want to have fun: Associations between play and welfare in domestic cats. *Animal Welfare*, 32, e9. <https://doi.org/10.1017/awf.2023.2>
- Hernandez, S. M., Loyd, K. A. T., Newton, A. N., Carswell, B. L., & Abernathy, K. J. (2018). The use of point-of-view cameras (Kittycams) to quantify predation by colony cats (*Felis catus*) on wildlife. *Wildlife Research*, 45(4), 357. <https://doi.org/10.1071/WR17155>
- Hinde, R. A., 1970. *Animal Behaviour: A Synthesis of Ethology and Comparative Psychology*, 2nd ed. McGrawHill, New York, pp. 351±356.
- Howell, T. J., Bowen, J., Fatjó, J., Calvo, P., Holloway, A., & Bennett, P. C. (2017). Development of the cat-owner relationship scale (CORS). *Behavioural Processes*, 141, 305–315. <https://doi.org/10.1016/j.beproc.2017.02.024>
- Leyhausen, P., 1979. *Cat Behaviour: The Predatory and Social Behaviour of Domestic and Wild Cats*. Garland STPM Press, New York.
- Lin Y., Wiegand K. (2023). Low R2 in ecology: Bitter, or B-side? *Ecological Indicators*, 153(110406). <https://doi.org/10.1016/j.ecolind.2023.110406>.
- Linklater, W. L., Farnworth, M. J., Heezik, Y., Stafford, K. J., & MacDonald, E. A. (2019). Prioritizing cat-owner behaviors for a campaign to reduce wildlife depredation. *Conservation Science and Practice*, 1(5), e29. <https://doi.org/10.1111/csp2.29>
- Loss, S. R., Will, T., & Marra, P. P. (2013). The impact of free-ranging domestic cats on wildlife of the United States. *Nature Communications*, 4(1), 1396. <https://doi.org/10.1038/ncomms2380>
- Loyd, K. A. T., Hernandez, S. M., Carroll, J. P., Abernathy, K. J., & Marshall, G. J. (2013). Quantifying free-roaming domestic cat predation using animal-borne video cameras. *Biological Conservation*, 160, 183–189. <https://doi.org/10.1016/j.biocon.2013.01.008>
- Ma, G. C., & McLeod, L. J. (2023). Understanding the Factors Influencing Cat Containment: Identifying Opportunities for Behaviour Change. *Animals*, 13(10), 1630. <https://doi.org/10.3390/ani13101630>

- Martínez-Byer, S., Urrutia, A., Szenczi, P., Hudson, R., & Bánszegi, O. (2020). Evidence for Individual Differences in Behaviour and for Behavioural Syndromes in Adult Shelter Cats. *Animals*, 10(6), 962. <https://doi.org/10.3390/ani10060962>
- Moseby, K. E., Peacock, D. E., & Read, J. L. (2015). Catastrophic cat predation: A call for predator profiling in wildlife protection programs. *Biological Conservation*, 191, 331–340. <https://doi.org/10.1016/j.biocon.2015.07.026>
- Møller, A., Jennions, M.D. How much variance can be explained by ecologists and evolutionary biologists? *Oecologia*, 132(1), 492–500. <https://doi.org/10.1007/s00442-002-0952-2>
- Ozili, Peterson. (2022). The Acceptable R-Square in Empirical Modelling for Social Science Research. *SSRN Electronic Journal*. 10.2139/ssrn.4128165.
- Pongrácz, P., & Lugosi, C. A. (2024). Predator for hire: The curious case of man's best independent friend, the cat. *Applied Animal Behaviour Science*, 271, 106168. <https://doi.org/10.1016/j.applanim.2024.106168>
- Seymour, C. L., Simmons, R. E., Morling, F., George, S. T., Peters, K., & O'Riain, M. J. (2020). Caught on camera: The impacts of urban domestic cats on wild prey in an African city and neighbouring protected areas. *Global Ecology and Conservation*, 23, e01198. <https://doi.org/10.1016/j.gecco.2020.e01198>
- Shajid Pyari, M., Ucheddu, S., Lenkei, R., & Pongrácz, P. (2021). Inexperienced but still interested – Indoor-only cats are more inclined for predatory play than cats with outdoor access. *Applied Animal Behaviour Science*, 241, 105373. <https://doi.org/10.1016/j.applanim.2021.105373>
- Sih, A., & Bell, A. M. (2008). Chapter 5 Insights for Behavioral Ecology from Behavioral Syndromes. In *Advances in the Study of Behavior* (Vol. 38, pp. 227–281). Elsevier. [https://doi.org/10.1016/S0065-3454\(08\)00005-3](https://doi.org/10.1016/S0065-3454(08)00005-3)
- Smith, P. (1982). Does play matter? Functional and evolutionary aspects of animal and human play. *The Behavioral and Brain Sciences*, 5(1), 139–184. <https://doi.org/10.1017/S0140525X0001092X>
- Stanton, L., Sullivan, M., & Fazio, J. (2015). A standardized ethogram for the felidae: A tool for behavioral researchers. *Applied Animal Behaviour Science*, 173(1), 3–16. <https://doi.org/10.1016/j.applanim.2015.04.001>
- Takagi, S., Arahori, M., Chijiwa, H., Saito, A., Kuroshima, H., & Fujita, K. (2019). Cats match voice and face: Cross-modal representation of humans in cats (*Felis catus*). *Animal Cognition*, 22(5), 901–906. <https://doi.org/10.1007/s10071-019-01265-2>
- Thomas, E., Schaller, F. (1954). Das Spiel der optisch isolierten, jungen Kaspar-Hauser-Katze. *Naturwissenschaften*, 41(1), 557–558.
- Vanderschuren, L. J. M. J., & Trezza, V. (2014). What the laboratory rat has taught us about social play behavior: Role in behavioral development and neural mechanisms. In S. L. Andersen & D. S. Pine (Eds.), *The neurobiology of childhood* (pp. 189–212). Springer-Verlag Publishing/Springer Nature.

Chapter 3: Using DNA metabarcoding and citizen science to assess the diet of owned domestic cats

Submitted to *Ecology and Evolution* (2026)

ABSTRACT

Various methods have been attempted to quantify hunting and consumption of wild prey by domestic cats, but molecular analysis of their scat using DNA metabarcoding has rarely been attempted. In this study, we relied on owner-collected scats ($n = 32$) from litterboxes and backyards to test whether this methodology could be used to detect consumption of wild prey by owned, indoor-outdoor domestic cats, and whether pooling scat samples could enhance efficiency or would result in a loss of resolution. We monitored 8 cats for an average of 12.1 days, collecting an average of 4 scats from each. Metabarcoding revealed only one instance of wild prey consumption, a California vole (*Microtus californicus*), despite the owner not witnessing any prey returns during the 24-hour period prior to scat sampling. Conversely, in three instances where the owner reported returns of intact prey by their cats, prey DNA was not recovered, presumably because no prey parts were consumed. Thus, consumption and owner-reported capture of prey may be poorly correlated. Pooling scats did not influence detection of ubiquitous, abundant taxa such as chicken contained in their owner-provided food, but consumption of a vole by one cat was only detectable in the scat tested alone. Pooling scats for analysis may sacrifice sensitivity to detection of wild prey. Our study suggests that DNA metabarcoding may be a viable method to detect consumption of wild prey by owned domestic cats, however a larger sample size is needed to investigate the biodiversity of taxa in the diet, as the frequency of prey consumption by cats may be lower than that of prey capture.

INTRODUCTION

Non-native predators have the potential to impact naïve local wildlife (Carthey and Banks, 2014; Jakubavičiūtė and Candolin, 2021; Sih et al., 2010), especially when in increased densities or abundance due to human activity (Early et al., 2016; Pyšek et al., 2010). One such predator of concern is the domestic cat, for which hunting tendencies have been thought to cause both consumptive and non-consumptive effects on vulnerable prey species, such as changing prey physiology and behavior (Beckerman et al., 2007; Bedoya-Pérez et al., 2021; Bonnington et al., 2013), population decline (Loss et al., 2013; Loss and Marra, 2017; van Heezik et al., 2010), and even species extinction (Crooks and Soulé, 1999; Doherty et al., 2016; Medina et al., 2011). Unlike most other predators, however, domestic cats also play a significant role in human society as companion animals (Crowley et al., 2020; Jaroš, 2016), and unique challenges exist when monitoring their impact and planning sustainable conservation solutions (Cecchetti et al., 2021c; Crowley et al., 2020; MacDonald et al., 2015).

One major goal in mitigating the impact of domestic cats (“cats” hereafter) on wildlife is monitoring and quantifying their hunting behavior. Various methods traditionally used in wildlife monitoring have been attempted in domestic cats (Lockwood and Huck, 2025), such as camera traps (Herrera et al., 2022), cat-borne cameras (Hernandez et al., 2018; Loyd et al., 2013), manual assessment of stomach contents or scats (Krauze-Gryz et al., 2012; Piontek et al., 2021), and stable isotope analyses (Cecchetti et al., 2021a). In addition to the typical challenges associated with these methods (e.g. selection bias, limited battery power, and lack of resolution), some of these methods may also face pushback from the public when implemented for cats, particularly when it is viewed that cat welfare may be compromised (e.g. Crowley et al., 2020; Linklater et al., 2019). Cat owner-reported observations of hunting may mitigate some of these

concerns (e.g. Cecchetti et al., 2021b; Chung et al., 2024; Kauhala et al., 2015), but the reliability of such data may be questionable (Pongrácz et al., 2024). It is therefore crucial to test alternative, non-invasive methods of monitoring cat impact on wildlife that involve active participation from caretakers of domestic cats.

One method that has been underutilized in research is dietary analysis of cat scats. Scats provide a non-invasive method of monitoring diet, and are regularly produced by animals, ensuring sample availability. DNA Metabarcoding is a widely used method of monitoring DNA composition of samples such as scats, and can provide high resolution data up to the species level (De Barba et al., 2014; Pompanon et al., 2012; Shehzad et al., 2012; Thuo et al., 2019; Van Der Heyde et al., 2021). Diet metabarcoding may thus provide a reliable, high-resolution, non-invasive alternative to existing methods of quantifying cat hunting.

While DNA metabarcoding has been used to assess the diet of feral cats (Galão et al., 2025; Plimpton et al., 2021), no study to our knowledge has tested this methodology on owned cats. Owned cats are an important population to be studied, given that those allowed outside continue to exhibit hunting behavior (Bradshaw and Cook, 1996; Driscoll et al., 2009), and sterilization and regular feeding have not been shown to reduce their hunting behavior (Cecchetti et al., 2021c; Guttilla and Stapp, 2010). Targeting owned cats allows collection of detailed individual-level data, and can utilize citizen science to obtain scat samples, directly reducing the substantial effort and costs associated with sample collection. Given that they are fed by their caretakers, owned cats can also allow a direct comparison between what the cat consumes (e.g. their provisioned food) and what is found in their scats. We therefore sought to investigate whether owned cats that regularly produce scats in the home environment, either in a litter box or

an outdoor yard, could provide a useful study system to assess the impact of this predator on local wildlife.

One potential barrier to molecular methods of diet analysis is the cost associated with DNA extraction and sequencing. Pooling independently-collected scat samples within an individual animal prior to DNA extraction may reduce the total number of extractions to be performed. While studies have assessed some optimization techniques for DNA metabarcoding (McInnes et al., 2017), no study to our knowledge has systematically compared the overall resolution of data obtained when extracting and amplifying DNA separately from individual samples versus from pooled combinations of those samples. We tested whether pooling independent samples from an individual cat prior to extraction would maintain similar representation of the animals' diet.

Here, we use DNA metabarcoding to assess the diet of owned domestic cats in the Davis and Sacramento regions of California, USA. We designed this study to pilot test this method's viability as a tool to capture consumption of wild prey by owned domestic cats despite their reliance on humans and consumption of human-provisioned cat food. We had three main aims for this study: 1) To assess if DNA metabarcoding could be used to detect wild prey DNA in the scat of owned, indoor-outdoor domestic cats; 2) to collect cat food samples that were being fed to our study cats and compare DNA found in their foods vs. in their scats; and 3) to test a modified sampling technique where extractions from individual fecal samples are compared to extractions from pooled samples. We predicted that scats of owned indoor-outdoor cats would show the presence of wild prey species, especially if the cat was seen hunting wild prey by their owner. Alternatively, if cats that hunt prey for food are more likely to consume prey out of the

owner's vision than cats that capture prey for play, it is possible that the two types of hunting are decoupled.

METHODS

Ethical Note

Cat owners who participated in another study (Chung et al., 2024) were invited to provide scat samples of their cats at the end of the study. A consent form was provided at the beginning of the questionnaire for the previous study, and consenting participants were asked to provide an email address for coordination of pickup of the scat samples.

This study was reviewed and determined exempt (non-human subjects research) by the Institutional Review Board of the University of California, Davis (#1905569). This study did not require approval from the Institutional Animal Care and Use Committee of University of California, Davis, as it did not involve direct interaction with, handling, or manipulation of any animals.

Sample Collection

In total, 65 owners were invited to participate in this study, 49 of which responded to the initial contact, and 17 of which received the sample collection kit. We successfully received scat samples from 9 of the participating owners. For sample collection, we provided a small collection kit to each participating owner with the following: 1) five 50-mL falcon tubes including approximately 25mL of 95% ethanol, 2) five pairs of nitrile gloves to aid in sample collection, and 3) printed instructions including a study log to record the date, time, and place the sample(s) were collected, and whether their cat was seen with a caught wild animal within the past 24 hours of each collection. The collection kit was dropped off at the location the participant

provided, and later picked up upon the owner's completion of sample collection. We directed each owner to provide five scat samples from their cat and to store them in the falcon tubes provided. Along with scat samples, we also requested a sample of their cat's food that was currently being provisioned in the home. In total, we collected 39 scats from 9 different cats in Davis and Sacramento, CA, USA, from 8 November to 21 December 2023.

Laboratory Procedure

We conducted all laboratory analyses at the Mammalian Ecology and Conservation Unit of the Veterinary Genetics Laboratory at University of California, Davis from December 2023 to July 2024. We extracted DNA from all samples using the EurX Stool DNA Purification Kit (EurX, Gdansk, Poland) following manufacturer's instructions, except that DNA was eluted in a 55 µl volume to increase DNA concentration.

During the scat teasing process, we created pooled samples of each cat's scats by homogenizing small subsamples of each of the 3-5 individual scats into a combined, pooled scat sample. We avoided subsampling the external layer of each scat and the hair, as these were most likely to contain host DNA rather than their diet. A sample of the pooled scat was then collected twice and treated as two additional samples during extraction.

We prepared two plates, one for each primer set (12S and 16S), and included three concentrations (1x, 10x, 100x) of the positive controls, along with five PCR-negative controls and seven extraction blanks, one from each extraction event. For 12S, we included a mixture of positive controls containing equal concentrations (0.002 ng/ul) of DNA of each of the following vertebrate species, which represented a variety of taxa both local and not local to the study area: Desert cottontail (*Silvilagus audubonii*), Laysan albatross (*Phoebastria immutabilis*), Northern elephant seal (*Mirounga angustirostris*), yellow-backed spiny lizard (*Sceloporus uniformis*),

western whiptail lizard (*Aspidoscelis tigris*), western harvest mouse (*Reithrodontomys megalotis*), western fence lizard (*Sceloporus occidentalis*), alligator lizard (*Elgaria multicarinata*), and blunt nosed leopard lizard (*Gambelia sila*). Several species of lizards were included, as we predicted reduced amplification of herptile DNA using 12SV5. For 16S, we included a mixture of positive controls containing equal concentrations (0.002 ng/ul) of DNA of each of the following invertebrates: moth (Lepidoptera spp.), stiletto fly (*Thereva spp.*), pholcid spider (Pholcidae spp.), and whiteleg shrimp (*Penaeus vannamei*). We included several PCR-negative controls and extraction blanks to assess background contamination. For the remaining 23 wells, we selected an arbitrary set of samples to replicate as PCR replicates to further ensure repeatability of our results.

We prepared libraries in three steps, following protocols outlined by Caspi et al. (2025). First, we performed an amplicon PCR targeting the V5 loop of the mitochondrial 12S gene and included sequence overhangs to 5' ends to allow annealing to Illumina index sequences in the index PCR. To reduce amplification of host DNA, we designed a blocking oligonucleotide specific to domestic cats using previously described methods (Shehzad et al., 2012; Vestheim and Jarman, 2008). The blocker sequence was

CTATGCTTAGCCCTAACTTAGATAGTTACCCTAAACAAAACACTATC/3SpC3/, where 3SpC3 is a 3' spacer of 3 carbons that prevents further synthesis along the template strand, and therefore prevents amplification. For 16S, we amplified the mitochondrial 16S rRNA gene using universal primers, IN16STK-1F: TGAAGTCAGATCATGTAA and IN16STK-1R:

TTAGGGATAACAGCGTAA (Kartzin et al., 2015), except that we included 5' tags on each primer enabling annealing to Illumina adapters during a second PCR. Due to limited resources, we prioritized maximizing inclusion of biological replicates (different scats of the same

individual) rather than technical replicates (PCR replicates), given that biological replicates have been shown to yield more variation in diet compared to technical replicates (Mata et al., 2019). We performed the amplicon PCR with a thermal profile of 95deg for 15 min, then 40 cycles of 30s at 94deg and 90s at 55deg, and then held at 15deg.

Second, we purified the PCR product using an Exo-SAP-IT reaction (Caspi et al., 2025). The reaction consisted of 3 ul of amplicon PCR product, 2.78 ul of 1X TE (10mM Tris-HCl + 1mM EDTA), 0.075ul of 20 U/ul Exonuclease I and 0.15ul of 1 U/ul shrimp alkaline phosphatase (New England Biolabs, MA, USA). The reaction was then incubated for 1 cycle of 37 deg for 30min and 1 cycle of 80deg for 15min. Finally, we performed an index PCR to anneal Illumina sequencing-compatible adapters and assign unique index pairs to each sample. The index pairs included one 8-bp i5 index sequence assigned to the plate (96 samples including controls), and one 6-bp i7 index sequence assigned to each sample within the plate. The index PCR was run for 1 cycle of 98deg for 30sec, 8 cycles at 98deg for 10sec and 65deg for 75sec, and 1 cycle of 65deg for 5min, then held at 4deg.

Bioinformatic Processing

Upon recovery of sequences, we trimmed and filtered out low-quality sequences using cutadapt (Martin, 2011). We identified Amplicon Sequence Variants (ASVs) using DADA2 (Callahan et al., 2016), and used the blastn feature of BLAST+, as well as the assignTaxonomy feature of DADA2 to assign ASVs to the custom reference libraries. We then used the basic local alignment search tool (BLAST) in NCBI to confirm assignments, or to assign to species not included in the reference libraries (Zhang et al., 2000). We assigned ASVs at the species or genus level whenever possible, though most invertebrate taxa could not be identified beyond the Order. The majority of our ASVs showed 100% query coverage and matched at 98–100%

identity. In the case where the ASV matched with more than one species, or were matched with species not found in northern California, we used iNaturalist (www.inaturalist.org) to identify the species found in the local areas, with the exception of species likely to be found in commercially available pet foods.

Upon assigning taxonomy, we removed ASVs that comprised <0.001% of the total sequence reads in a lane, and ASVs with low percent identity (<97% for 12S, <94% for 16S) and/or query coverage (<98%). We then concatenated by taxa to combine ASVs assigned to the same species. We used positive and negative controls to determine filtering thresholds (minimum numbers of reads), and discarded samples failing to exceed them (848 reads for 12S and 16,582 reads for 16S). Upon filtering for successful amplifications, we removed non-diet items, including host (cat) DNA, human, and domestic dog. After removing non-diet items, we then removed diet items representing <0.5% of reads within a scat. Diet items were grouped into anthropogenic (items expected in provisioned pet food), wild prey, and arthropods (16S).

Domestic Cat Diet Analysis

We performed all analysis and visualization in the statistical software program R (v4.4.3; R Core Team, 2022). We turned raw sequence reads into frequency of occurrence, calculated by the number of occurrences of an item divided by the total number of samples. We compared the presence/absence of each diet item across individual cats, and for those whose provisioned pet food samples were available, compared the presence/absence of the diet items found in the provisioned food against the scat samples that were collected. We then compared the presence/absence of diet items found in individual scat samples against the pooled samples, to assess the consistency of recovered diet items between pooled and un-pooled samples.

RESULTS

Of the surveyed cats, 3 were male and 5 were female, and all were neutered or spayed. Scats were collected for the duration of 12.1 days per cat on average (Range = 1 – 25 days, SD = 9.5 days). All scat samples were relatively fresh, collected within an average of 5.5 hours post defecation (Range = 1 – 960 min, SD = 289.02 min). The majority (94.7%) were collected from a litterbox, with only 2 samples (both from Cat C) collected from the backyard.

We retained a total of 32 individual samples from 8 cats after filtering for amplification success, along with two replicate pooled samples for each cat. One individual scat (from Cat C) was identified as an opossum (*Didelphis virginianis*) scat misidentified by the owner as a cat scat, and was subsequently removed from the dataset, along with the two pooled samples it was associated with (Cat C). While 8 other scats contained opossum DNA, we suspected possible contamination from the opossum scat and excluded opossum from further analysis. Although we designed a blocking oligonucleotide to reduce amplification of cat DNA in our samples, the majority of DNA found in each scat sample still remained cat DNA, which is not unusual (Shehzad et al., 2012).

Note on Invertebrate DNA

Invertebrate diet items recovered with 16S primers suffered from significant contamination (>8000 raw sequence reads) in negative controls and low amplification across samples. Overall, we retained 3 cat food samples, 6 individual scat samples from 2 cats, and 3 pooled samples after filtering for amplification success. All scat samples that were retained contained Orthoptera. Diptera (flies), Hemiptera (true bugs), Salticidae (jumping spider), and Noctuidae (noctuid moths) were also found in cat scats. From cat food samples, we identified Orthoptera (grasshoppers and crickets), Coleoptera (beetles), Hemiptera, Noctuidae,

Hymenoptera (wasps, bees, and ants), Cephalopoda (cephalopods), and Diptera. Given the low resolution of data, we excluded 16S data from further examination.

Presence of wild prey

Despite our study cats being fed animal-based pet foods, about a third (35.5%) of our scat samples contained no vertebrate DNA except cat DNA. Throughout the study period, there were three instances where the cat was observed by the owner carrying a wild prey item within 24 hours prior to each scat collection: a house mouse (*Mus musculus*) caught in the home, a small rodent of unknown species brought to the door, and a lizard of unknown species caught in front of the home. In all three instances, the owner reported that the prey remained alive and intact, and the scats that were produced by the cat within 24 hours of the hunting event showed no presence of wild prey. Of all 32 scat samples, only one scat (from Cat H) showed presence of wild vertebrate prey DNA, which was California vole (*Microtus californicus*). The cat that produced this scat was not seen by their owner with any wild prey within 24 hours prior to the collection of this scat. Vole was only detected in one scat sample of this cat, and was not seen in any subsequent scat samples produced by this cat.

Provisioned foods vs. Scats

We were able to collect samples of the foods that were being provisioned to four of our study cats (Cats A, B, D, E). Overall, six taxa of anthropogenic origin were identified in these four cats' diets: chicken, pig, turkey (*Meleagris gallopavo*), and fish in the drum and croaker family (Sciaenidae), hake family (Merluccius), and herring family (Clupeidae) (Table 3.1). Chicken and pig (or pork) were found in all four food samples. Chicken had the highest rate of recovery after digestion, with 14 out of the 15 expected scats showing its presence. Fish (specifically, Sciaenidae) was the only taxon of anthropogenic origin in the scat that was not also

found in the food sample; we presume that this may be due to a supplemental food source containing fish, such as a treat. Several scat samples showed no animal DNA other than cat DNA (Table 3.1).

Table 3.1. Comparisons between vertebrate (12S) diet items recovered from scat samples (n = 15) produced by a subset of cats (n = 4) vs. diet items found in the pet food provisioned to those cats in Davis and Sacramento, CA, USA.

	Chicken	Pig	Turkey	Fish (Clupeidae)	Fish (Sciaenidae*)	Fish (Merluccius)
Cat A – Food (HP ^a , dry)	✓	✓		✓		
Cat A – Scat Sample 1						
Cat B – Food (kidney care ^b , wet)	✓	✓				
Cat B – Scat Sample 1						
Cat B – Scat Sample 2						
Cat B – Scat Sample 3						
Cat B – Scat Sample 4						
Cat B – Scat Sample 5	✓					
Cat D – Food (12+ ^c , wet)	✓	✓				
Cat D – Scat Sample 1	✓					
Cat D – Scat Sample 2	✓					
Cat D – Scat Sample 3	✓					
Cat D – Scat Sample 4	✓					

Cat D – Scat Sample 5	✓	✓				
Cat E – Food (chicken & turkey, dry)	✓	✓	✓			✓
Cat E – Scat Sample 1	✓	✓			✓	
Cat E – Scat Sample 2	✓	✓				
Cat E – Scat Sample 3	✓					
Cat E – Scat Sample 4	✓	✓	✓			
Total	14/15	4/15	1/5	0/1	N/A	0/5

*All samples shown above successfully amplified cat DNA. ✓ indicates presence of the taxon in the food or scat sample. “Total” indicates the following: (total number of observed presences of the taxon in the scat) / (total number of expected presences of the taxon in the scat based on its presence in the food). Samples listed above that are blank indicate that no vertebrate taxa were found in the sample except cat DNA. Some data are excluded (cats C, F, G, and H) due to lack of availability of their provisioned food samples. ^aHP indicates prescription hydrolyzed protein. ^bKidney care indicates prescription kidney care food. ^c12+ indicates food formulated for senior cats 12 years or older. *Sciaenidae was found in one of the scat samples of Cat E, but was not present in the provisioned food sample.*

Vertebrate diet items found in food samples A, B, D, and E were recovered in scat samples of the corresponding cats to varying degrees (Table 3.2). Only two samples showed presence of identical taxa between scats and the food (Cat D - scat sample 5, and Cat E - scat sample 4, Table 3.1). Given that the food samples were collected from the owners, the food types were outside of our control and naturally differed in their type (wet/dry), formulation and composition (Table 3.2). Food A was a prescription hydrolyzed protein cat dry food, designed for cats intolerant of or allergic to common proteins such as chicken and beef; food B was a prescription kidney care wet food; food D was wet food formulated for senior cats 12 years and older; and food E was a chicken and turkey based dry food.

Table 3.2. Proportions of vertebrate diet items in provisioned foods A, B, D, and E that were recovered in the scats (n = 15) produced by corresponding cats (n = 4) living in Davis and Sacramento, CA, USA.

	Proportion of diet items in food that were recovered in scat
Food A: Prescription Hydrolyzed protein dry food	0/3
Food B: Kidney care, wet food	1/2
Food D: Senior dry food	2/2
Food E: Chicken & turkey, dry food	3/4
Total	Range= 0-1, Mean= 0.563, SD=0.370

Pooling scats

We found varying levels of similarity between individually-extracted scat samples and pooled-extracted samples (Table 3.3). Overall, individually extracted scat samples yielded more diet item detections than did pooled-extracted scat samples, with the exception of one cat (Cat D); Cat D showed more diet items in pooled samples than individually extracted samples (Sciaenidae, turkey, and opossum). While it is possible this occurred due to pooled samples containing parts of the scat not represented by individually-extracted samples, we suspect that contamination may have occurred from samples from Cat E, due to their proximity to each other on the PCR plate. Seven individually extracted samples and 3 pooled-extracted samples showed no animal DNA except cat DNA.

Chicken was always recovered from pooled samples when it was present in individually-extracted samples (Table 3.3). In contrast, vole, a wild prey species, was found in an individually extracted scat sample (Cat H) but not recovered when the sample was pooled with other scats.

Table 3.3. Comparisons between diet items recovered in individually extracted scat samples of each cat (n = 28) vs. diet items recovered in pooled-extracted samples (n = 13) within each cat of our sample in Davis and Sacramento, CA, USA. “Individual” indicates all diet items recovered

from individual scat samples of each cat. “Pooled” indicates all diet items recovered from extractions performed on homogenized pooled samples from the corresponding cat.

Individually extracted vs. pooled									
	Chick en	Pig	Turkey	Fish (Merlucci us)	Fish (Sciaenidae)	Fish (Salmonida e)	Mackerel	Californi a vole	Opossum
Cat A: Individual (n=1)									
Cat A: Pooled (n=2)									
Cat B: Individual (n=5)	✓								✓
Cat B: Pooled (n=2)	✓								✓
Cat D: Individual (n=5)	✓	✓							
Cat D: Pooled (n=2)	✓		✓		✓				✓
Cat E: Individual (n=4)	✓	✓	✓		✓				✓
Cat E: Pooled (n=2)	✓	✓	✓		✓				✓
Cat F: Individual (n=5)	✓	✓		✓		✓			✓
Cat F: Pooled (n=1)	✓					✓			
Cat G: Individual (n=4)	✓	✓	✓		✓		✓		✓

Cat G: Pooled (n=2)	✓	✓							✓
Cat H: Individual (n=4)	✓		✓					✓	✓
Cat H: Pooled (n=2)	✓								
Total Ratio	6/6	2/4	1/3	-	1/2	1/1	0/1	-	3/5

✓ indicates the presence of the taxon in the sample. A greater match between Individual and Pooled data suggests that pooling samples prior to DNA extraction did not reduce resolution of the data. Total Ratio = (number of times the diet item was observed in individually-extracted samples) / (number of times the diet item was observed in pooled-extracted samples). Any deviation from n=5 for “Individual” and n=2 for “Pooled” samples is due to failed amplification of DNA. Cat C was excluded from this table due to one of its samples being identified as opossum scat that contaminated all of its pooled samples.

Overall, pooled extractions had varying degrees of success in amplifying diet items that were recovered in individually extracted samples (Table 3.4). Samples from two cats (cats B, E) showed a perfect match between diet items recovered from individually-extracted versus pooled-extracted samples.

Table 3.4. Proportions of diet items found in individually extracted samples that were recovered in pooled-extracted samples of scats produced by 7 cats in Davis and Sacramento, CA, USA. Diet items that were found in pooled samples but not found in individually-extracted samples were excluded, to assess only the effect of pooling methods on the resolution of data obtained.

	Proportion of diet items in individually extracted samples that were recovered in pooled samples
Cat A	N/A
Cat B	2/2 = 1.0
Cat D	1/2 = 0.50
Cat E	5/5 = 1.0
Cat F	2/5 = 0.40
Cat G	3/6 = 0.50
Cat H	1/4 = 0.25
Total	Range (0.10 – 1.0), Mean= 0.558, SD = 0.342

DISCUSSION

Presence of wild prey in diet

Overall, we found that in this sample, owner reports of catching and handling wild prey by cats did not coincide with DNA found in their scats. We detected no wild prey DNA in the scats of cats that were seen with a prey item within 24 hours prior to scat collection. This finding is likely attributable to the prey having been unconsumed, as observations showed prey intact and alive. Although possible that trace amounts of prey DNA could be ingested without the need to consume prey (e.g., hairs that become dislodged and swallowed when mouthing prey), our findings suggest that consumption of significant prey parts by cats is necessary for detection of prey DNA in their scats. Interestingly, of the 32 scats collected, only one scat showed any presence of wild prey in their diet, and this cat was not seen with any wild prey by their owner within 24 hours prior to the collection of this scat. Cats often hunt without consuming prey (Loyd et al., 2013; McGregor et al., 2015), and tend to consume certain types of prey and return others to the home (Lockwood and Huck, 2025); our data show preliminary support for using DNA metabarcoding to capture instances of hunting where the cat has consumed the prey upon capture instead of returning home with intact prey. We suggest that owner reports and molecular analysis of scats should be used in combination by future studies in order to capture multiple types of hunting done by owned, indoor-outdoor cats.

Aside from opossum, which we suspect to be contamination from the one scat processed with them and later identified as an opossum scat, the only wild prey detected was vole. This was not surprising given that voles are commonly found in this region, specifically *Microtus californicus* (inaturalist.org), and cats have been seen to consume other rodents of this size (Galão et al., 2025; Hernandez et al., 2018; Plimpton et al., 2021). *Microtus californicus* is a

species of meadow vole native to California; though the species found in our study region is not of conservation concern, other regions contain endangered subspecies such as *M. californicus scirpensis* (Cudworth and Koprowski, 2010) and more research may be needed on the relationship between cats and this species.

Interestingly, we found that vole DNA did not remain in the gut of the predator for several consecutive samples over time. The scat sample that contained vole DNA was reported to be produced by the animal (Cat H) around 14:55 on 11/21/23; the next sample, which was reported to be produced by the animal around 21:00 on 11/22/23 (approximately 30 hours later) no longer contained any vole DNA. As reference, cats have a gastrointestinal transit time of 12-24 hours and produce 1-2 scats per day, though there are inter-individual differences (Telles et al., 2022); this suggests that prey DNA may only be retained for one digestion cycle upon consumption. However, it is possible that these time estimates reported by owners were not accurate. More research is needed on the retention times of various amounts of prey in the cat digestive tract.

Domestic cats have been shown to exhibit significant individual variation in hunting behavior (Dickman and Newsome, 2015; Moseby et al., 2015). Studies quantifying hunting behavior of indoor-outdoor cats using animal-borne cameras have found that of cats that are studied, only a subset of cats ever exhibit hunting behavior, and an even smaller percentage consume the prey they captured (Hernandez et al., 2018; Loyd et al., 2013; Seymour et al., 2020). In fact, some studies even suggest that studying the impact of cats on wildlife on the population level can be misleading, and highlight the importance of focusing conservation efforts on the select number of individuals that hunt significant amounts of prey (Moseby et al., 2015; Thomas et al., 2012). Our data show that even among a small select number of indoor-outdoor

cats surveyed for a short period of time (mean = 12.1 days), wild prey DNA was detected by diet metabarcoding. A study conducted on a larger sample of cats for longer periods of time could illuminate the individual variation of hunting behavior of domestic cats.

Provisioned foods vs. scats

Metabarcoding of cat food samples revealed chicken, pig, turkey, and a variety of fish. Consistent with our predictions, we detected chicken and pig DNA in all food samples, which was not surprising given that chicken, pig, and cattle are the most common animals to be found in pet foods (“AAFCO DOG AND CAT FOOD,” n.d.). While our study lacked the sample size and representation needed to systematically compare between types of pet foods, we found some differences in success of recovery of diet items post digestion across different types of foods that warrant mention. Interestingly, the two prescription foods (Hydrolyzed Protein and Kidney Care) showed the lowest rates of recovery of animal DNA in the scats; it is possible that due to the processing required for these formulae, such as protein hydrolyzation, the animal DNA found in these foods are already highly degraded and post-digestion detection of these DNA was less likely. However, we caution readers from extrapolating this result given that we did not aim to systematically test the effect of different formulae on post-digestion recovery rates. Regardless, despite being fed animal-based cat foods, several of our cat scat samples showed no vertebrate DNA except cat; we suspect that this may either be due to the degradation of animal DNA in commercially available pet foods. Many studies assessing the diet of urban wildlife frequently encounter pet foods that are left outside as sources of anthropogenic food (Bateman and Fleming, 2012; Caspi et al., 2025); it is therefore important to understand how commercially available pet foods are digested by animals, and if or how well prey DNA from pet foods are retained in scat

samples. Studies involving feeding trials of different types of pet foods would enable a more systematic test of the detectability of these animal DNA pre- and post-digestion.

Pooling scats

Overall, we found that extracting DNA from individual samples led to a larger number of diet items recovered from scats than when these samples were pooled prior to extraction (Table 3.3). However, for some cats (cats B and E), there were no differences between these two protocols. Importantly, however, different diet items seemed to be differentially affected by pooling: common, anthropogenic food item such as chicken showed up regardless of pooling, but the only instance of a wild prey species in our study showed up only from the individually extracted sample. Thus, when research aims emphasize the detection of small, rarely consumed prey items, pooling multiple samples together prior to DNA extraction may reduce the likelihood of detecting these species.

Limitations

As with most molecular analyses of diet, we recognize the limitations associated with this methodology, such as primer biases in amplification of certain taxa (Liu et al., 2023; Nichols et al., 2018); in particular, the 12S primers we used here perform very poorly on herptiles (Caspi et al., 2025). Given that cats have been seen hunting lizards by previous studies as well as in our study, we suspect that the lack of lizard DNA in our scat samples may be due to biased amplification of taxa due to our primers, rather than a true absence of lizard DNA. Finally, our study had a small sample size. Given that we recruited participants of this study from a previous study (Chung et al., 2024), our participants were not recruited specifically for this study and therefore may not have been incentivized to participate through the duration of the study. The parts of our data obtained directly from the owners, such as the date/time of scat collection,

location of scat, its freshness, and whether/how the cat interacted with wild prey may have suffered from lack of reliability, social desirability bias, and recall bias (Bell et al., 2019; Larson, 2019).

CONCLUSION

The majority of the scats in our study contained only anthropogenic sources of vertebrate DNA, but 8 scats contained no vertebrate DNA at all other than cat DNA despite being fed animal-based cat foods, suggesting that commercially available pet foods may contain mainly highly degraded DNA that is not detectable in scat post-digestion using this methodology. Of 32 samples, only one scat showed wild prey DNA, which was not detected by the owner-reported observations of hunting behavior and, in the cases where owners reported their cat handling wild, intact prey we detected no prey DNA in their scat, suggesting that consumption of significant prey parts is needed for detection in scat analysis. Lastly, pooling was not successful in detecting the one wild prey consumption event in our study, warranting caution in use of this method. Overall, we suggest that DNA metabarcoding may be a viable method of detecting hunting behavior by cats when consumption of prey has occurred, and that it may be best utilized in conjunction with other methods designed to capture instances of hunting without consumption, such as owner reports in questionnaires.

ACKNOWLEDGEMENTS

We would like to acknowledge Karolyn Diao, our undergraduate intern, for help with DNA extraction on this project. This research was funded by the Animal Welfare Institute's Christine Stevens Award.

REFERENCES

- AAFCO DOG AND CAT FOOD, n.d.
- Bateman, P.W., Fleming, P.A., 2012. Big city life: carnivores in urban environments. *J. Zool.* 287, 1–23. <https://doi.org/10.1111/j.1469-7998.2011.00887.x>
- Beckerman, A.P., Boots, M., Gaston, K.J., 2007. Urban bird declines and the fear of cats. *Anim. Conserv.* 10, 320–325. <https://doi.org/10.1111/j.1469-1795.2007.00115.x>
- Bedoya-Pérez, M.A., Le, A., McGregor, I.S., Crowther, M.S., 2021. Antipredator responses toward cat fur in wild brown rats tested in a semi-natural environment. *Behav. Ecol.* 32, 835–844. <https://doi.org/10.1093/beheco/arab038>
- Bell, A., Ward, P., Tamal, Md.E.H., Killilea, M., 2019. Assessing recall bias and measurement error in high-frequency social data collection for human-environment research. *Popul. Environ.* 40, 325–345. <https://doi.org/10.1007/s11111-019-0314-1>
- Bonnington, C., Gaston, K.J., Evans, K.L., 2013. Fearing the feline: domestic cats reduce avian fecundity through trait-mediated indirect effects that increase nest predation by other species. *J. Appl. Ecol.* 50, 15–24. <https://doi.org/10.1111/1365-2664.12025>
- Bradshaw, J.W.S., Cook, S.E., 1996. Patterns of pet cat behaviour at feeding occasions. *Appl. Anim. Behav. Sci.* 47, 61–74. [https://doi.org/10.1016/0168-1591\(95\)01011-4](https://doi.org/10.1016/0168-1591(95)01011-4)
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583. <https://doi.org/10.1038/nmeth.3869>
- Carthey, A.J.R., Banks, P.B., 2014. Naïveté in novel ecological interactions: lessons from theory and experimental evidence. *Biol. Rev.* 89, 932–949. <https://doi.org/10.1111/brv.12087>
- Caspi, T., Serrano, M.G., Vanderzwan, S.L., Kessler, J., Schell, C.J., Sacks, B.N., 2025. Impervious surface cover and number of restaurants shape diet variation in an urban carnivore. *Ecosphere* 16, e70152. <https://doi.org/10.1002/ecs2.70152>
- Cecchetti, M., Crowley, S.L., Goodwin, C.E.D., Cole, H., McDonald, J., Bearhop, S., McDonald, R.A., 2021a. Contributions of wild and provisioned foods to the diets of domestic cats that depredate wild animals. *Ecosphere* 12. <https://doi.org/10.1002/ecs2.3737>
- Cecchetti, M., Crowley, S.L., Goodwin, C.E.D., McDonald, R.A., 2021b. Provision of High Meat Content Food and Object Play Reduce Predation of Wild Animals by Domestic Cats *Felis catus*. *Curr. Biol.* 31, 1107–1111.e5. <https://doi.org/10.1016/j.cub.2020.12.044>
- Cecchetti, M., Crowley, S.L., McDonald, R.A., 2021c. Drivers and facilitators of hunting behaviour in domestic cats and options for management. *Mammal Rev.* 51, 307–322. <https://doi.org/10.1111/mam.12230>
- Chung, H.J., Aulakh, J., Link, J., Sih, A., Moody, C., 2024. Safe or sound? Factors influencing outdoor access, cat behavior, and hunting history with implications for conservation and welfare. *Appl. Anim. Behav. Sci.* 280, 106425. <https://doi.org/10.1016/j.applanim.2024.106425>
- Crooks, K.R., Soulé, M.E., 1999. Mesopredator release and avifaunal extinctions in a fragmented system. *Nature* 400, 563–566. <https://doi.org/10.1038/23028>
- Crowley, Sarah L., Cecchetti, M., McDonald, R.A., 2020. Our Wild Companions: Domestic cats in the Anthropocene. *Trends Ecol. Evol.* 35, 477–483. <https://doi.org/10.1016/j.tree.2020.01.008>

- Crowley, Sarah L, Cecchetti, M., McDonald, R.A., 2020. Diverse perspectives of cat owners indicate barriers to and opportunities for managing cat predation of wildlife. *Front. Ecol. Environ.* 18, 544–549. <https://doi.org/10.1002/fee.2254>
- Cudworth, N.L., Koprowski, J.L., 2010. *Microtus californicus* (Rodentia: Cricetidae). *Mamm. Species* 42, 230–243. <https://doi.org/10.1644/868.1>
- De Barba, M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E., Taberlet, P., 2014. DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: application to omnivorous diet. *Mol. Ecol. Resour.* 14, 306–323. <https://doi.org/10.1111/1755-0998.12188>
- Dickman, C.R., Newsome, T.M., 2015. Individual hunting behaviour and prey specialisation in the house cat *Felis catus*: Implications for conservation and management. *Appl. Anim. Behav. Sci.* 173, 76–87. <https://doi.org/10.1016/j.applanim.2014.09.021>
- Doherty, T.S., Glen, A.S., Nimmo, D.G., Ritchie, E.G., Dickman, C.R., 2016. Invasive predators and global biodiversity loss. *Proc. Natl. Acad. Sci.* 113, 11261–11265. <https://doi.org/10.1073/pnas.1602480113>
- Driscoll, C.A., Macdonald, D.W., O'Brien, S.J., 2009. From wild animals to domestic pets, an evolutionary view of domestication. *Proc. Natl. Acad. Sci.* 106, 9971–9978. <https://doi.org/10.1073/pnas.0901586106>
- Early, R., Bradley, B.A., Dukes, J.S., Lawler, J.J., Olden, J.D., Blumenthal, D.M., Gonzalez, P., Grosholz, E.D., Ibañez, I., Miller, L.P., Sorte, C.J.B., Tatem, A.J., 2016. Global threats from invasive alien species in the twenty-first century and national response capacities. *Nat. Commun.* 7, 12485. <https://doi.org/10.1038/ncomms12485>
- Galão, A., Soto, E.J., Nunes, J., Pedroso, N.M., Rocha, R., Rato, C., 2025. When pets go wild: Integrating DNA metabarcoding and morphological analyses to investigate the impacts of free-ranging cats (*Felis catus*) on oceanic islands. *Biol. Conserv.* 305, 111089. <https://doi.org/10.1016/j.biocon.2025.111089>
- Gutilla, D.A., Stapp, P., 2010. Effects of sterilization on movements of feral cats at a wildland–urban interface. *J. Mammal.* 91, 482–489. <https://doi.org/10.1644/09-MAMM-A-111.1>
- Hernandez, S.M., Loyd, K.A.T., Newton, A.N., Carswell, B.L., Abernathy, K.J., 2018. The use of point-of-view cameras (Kittycams) to quantify predation by colony cats (*Felis catus*) on wildlife. *Wildl. Res.* 45, 357. <https://doi.org/10.1071/WR17155>
- Herrera, D.J., Cove, M.V., McShea, W.J., Flockhart, D.T., Decker, S., Moore, S.M., Gallo, T., 2022. Prey selection and predation behavior of free-roaming domestic cats (*Felis catus*) in an urban ecosystem: Implications for urban cat management. *Biol. Conserv.* 268, 109503. <https://doi.org/10.1016/j.biocon.2022.109503>
- Jakubavičiūtė, E., Candolin, U., 2021. Density-dependent behavioural interactions influence coexistence between a native and a non-native mesopredator. *Biol. Invasions* 23, 3427–3434. <https://doi.org/10.1007/s10530-021-02585-6>
- Jaroš, F., 2016. Cats and Human Societies: a World of Interspecific Interaction and Interpretation. *Biosemiotics* 9, 287–306. <https://doi.org/10.1007/s12304-016-9253-8>
- Kartzinel, T.R., Chen, P.A., Coverdale, T.C., Erickson, D.L., Kress, W.J., Kuzmina, M.L., Rubenstein, D.I., Wang, W., Pringle, R.M., 2015. DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proc. Natl. Acad. Sci.* 112, 8019–8024. <https://doi.org/10.1073/pnas.1503283112>

- Kauhala, K., Talvitie, K., Vuorisalo, T., 2015. Free-ranging house cats in urban and rural areas in the north: useful rodent killers or harmful bird predators? *Folia Zool.* 64, 45–55.
<https://doi.org/10.25225/fozo.v64.i1.a6.2015>
- Krauze-Gryz, D., Gryz, J., Goszczyński, J., 2012. Predation by domestic cats in rural areas of central Poland: an assessment based on two methods. *J. Zool.* 288, 260–266.
<https://doi.org/10.1111/j.1469-7998.2012.00950.x>
- Larson, R.B., 2019. Controlling social desirability bias. *Int. J. Mark. Res.* 61, 534–547.
<https://doi.org/10.1177/1470785318805305>
- Linklater, W.L., Farnworth, M.J., Heezik, Y., Stafford, K.J., MacDonald, E.A., 2019. Prioritizing cat-owner behaviors for a campaign to reduce wildlife depredation. *Conserv. Sci. Pract.* 1, e29. <https://doi.org/10.1111/csp2.29>
- Liu, M., BurrIDGE, C.P., Clarke, L.J., Baker, S.C., Jordan, G.J., 2023. Does phylogeny explain bias in quantitative DNA metabarcoding? *Metabarcoding Metagenomics* 7, e101266.
<https://doi.org/10.3897/mbmg.7.101266>
- Lockwood, H.L., Huck, M., 2025. A Global Systematic Review and Meta-Analysis of Methods Used to Evaluate Predation and Diet of Domestic Cats (*Felis catus*). *Ecol. Evol.* 15, e71349. <https://doi.org/10.1002/ece3.71349>
- Loss, S.R., Marra, P.P., 2017. Population impacts of free-ranging domestic cats on mainland vertebrates. *Front. Ecol. Environ.* 15, 502–509. <https://doi.org/10.1002/fee.1633>
- Loss, S.R., Will, T., Marra, P.P., 2013. The impact of free-ranging domestic cats on wildlife of the United States. *Nat. Commun.* 4, 1396. <https://doi.org/10.1038/ncomms2380>
- Loyd, K.A.T., Hernandez, S.M., Carroll, J.P., Abernathy, K.J., Marshall, G.J., 2013. Quantifying free-roaming domestic cat predation using animal-borne video cameras. *Biol. Conserv.* 160, 183–189. <https://doi.org/10.1016/j.biocon.2013.01.008>
- MacDonald, E., Milfont, T., Gavin, M., 2015. What drives cat-owner behaviour? First steps towards limiting domestic-cat impacts on native wildlife. *Wildl. Res.* 42, 257.
<https://doi.org/10.1071/WR14164>
- Martin, M., n.d. &XWDGDSW UHPRYHV DGDSWHU VHTXHQFHV IURP KLJK WKURXJKSXW VHTXHQFLQJ UHDGV.
- Mata, V.A., Rebelo, H., Amorim, F., McCracken, G.F., Jarman, S., Beja, P., 2019. How much is enough? Effects of technical and biological replication on metabarcoding dietary analysis. *Mol. Ecol.* 28, 165–175. <https://doi.org/10.1111/mec.14779>
- McGregor, H., Legge, S., Jones, M.E., Johnson, C.N., 2015. Feral Cats Are Better Killers in Open Habitats, Revealed by Animal-Borne Video. *PLOS ONE* 10, e0133915.
<https://doi.org/10.1371/journal.pone.0133915>
- McInnes, J.C., Alderman, R., Deagle, B.E., Lea, M., Raymond, B., Jarman, S.N., 2017. Optimised scat collection protocols for dietary DNA metabarcoding in vertebrates. *Methods Ecol. Evol.* 8, 192–202. <https://doi.org/10.1111/2041-210X.12677>
- Medina, F.M., Bonnaud, E., Vidal, E., Tershy, B.R., Zavaleta, E.S., Josh Donlan, C., Keitt, B.S., Corre, M., Horwath, S.V., Nogales, M., 2011. A global review of the impacts of invasive cats on island endangered vertebrates. *Glob. Change Biol.* 17, 3503–3510.
<https://doi.org/10.1111/j.1365-2486.2011.02464.x>
- Moseby, K.E., Peacock, D.E., Read, J.L., 2015. Catastrophic cat predation: A call for predator profiling in wildlife protection programs. *Biol. Conserv.* 191, 331–340.
<https://doi.org/10.1016/j.biocon.2015.07.026>

- Nichols, R.V., Vollmers, C., Newsom, L.A., Wang, Y., Heintzman, P.D., Leighton, M., Green, R.E., Shapiro, B., 2018. Minimizing polymerase biases in metabarcoding. *Mol. Ecol. Resour.* 18, 927–939. <https://doi.org/10.1111/1755-0998.12895>
- Piontek, A.M., Wojtylak-Jurkiewicz, E., Schmidt, K., Gajda, A., Lesiak, M., Wierzbowska, I.A., 2021. Analysis of cat diet across an urbanisation gradient. *Urban Ecosyst.* 24, 59–69. <https://doi.org/10.1007/s11252-020-01017-y>
- Plimpton, L.D., Henger, C.S., Munshi-South, J., Tufts, D., Kross, S., Diuk-Wasser, M., 2021. Use of molecular scatology to assess the diet of feral cats living in urban colonies. *J. Urban Ecol.* 7, juab022. <https://doi.org/10.1093/jue/juab022>
- Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N., Taberlet, P., 2012. Who is eating what: diet assessment using next generation sequencing. *Mol. Ecol.* 21, 1931–1950. <https://doi.org/10.1111/j.1365-294X.2011.05403.x>
- Pongrácz, P., Turi, L., Dobos, P., Burányi, V., Schally, G., Biró, Z., 2024. Do as I say, not as I do – The paradoxical relationship between actual cat keeping practices and knowledge about wildcat conservation among Hungarian cat owners. *Biol. Conserv.* 293, 110586. <https://doi.org/10.1016/j.biocon.2024.110586>
- Pyšek, P., Jarošík, V., Hulme, P.E., Kühn, I., Wild, J., Arianoutsou, M., Bacher, S., Chiron, F., Didžiulis, V., Essl, F., Genovesi, P., Gherardi, F., Hejda, M., Kark, S., Lambdon, P.W., Desprez-Loustau, M.-L., Nentwig, W., Pergl, J., Poboljšaj, K., Rabitsch, W., Roques, A., Roy, D.B., Shirley, S., Solarz, W., Vilà, M., Winter, M., 2010. Disentangling the role of environmental and human pressures on biological invasions across Europe. *Proc. Natl. Acad. Sci.* 107, 12157–12162. <https://doi.org/10.1073/pnas.1002314107>
- Seymour, C.L., Simmons, R.E., Morling, F., George, S.T., Peters, K., O’Riain, M.J., 2020. Caught on camera: The impacts of urban domestic cats on wild prey in an African city and neighbouring protected areas. *Glob. Ecol. Conserv.* 23, e01198. <https://doi.org/10.1016/j.gecco.2020.e01198>
- Shehzad, W., Riaz, T., Nawaz, M.A., Miquel, C., Poillot, C., Shah, S.A., Pompanon, F., Coissac, E., Taberlet, P., 2012. Carnivore diet analysis based on next-generation sequencing: application to the leopard cat (*Prionailurus bengalensis*) in Pakistan. *Mol. Ecol.* 21, 1951–1965. <https://doi.org/10.1111/j.1365-294X.2011.05424.x>
- Sih, A., Bolnick, D.I., Luttbeg, B., Orrock, J.L., Peacor, S.D., Pintor, L.M., Preisser, E., Rehage, J.S., Vonesh, J.R., 2010. Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos* 119, 610–621. <https://doi.org/10.1111/j.1600-0706.2009.18039.x>
- Telles, N.J., Simon, B.T., Scallan, E.M., Gould, E.N., Papich, M.G., He, Y., Lee, M.-T., Lidbury, J.A., Steiner, J.M., Kathrani, A., Katherine Tolbert, M., 2022. Evaluation of gastrointestinal transit times and pH in healthy cats using a continuous pH monitoring system. *J. Feline Med. Surg.* 24, 954–961. <https://doi.org/10.1177/1098612X211062096>
- Thomas, R.L., Fellowes, M.D.E., Baker, P.J., 2012. Spatio-Temporal Variation in Predation by Urban Domestic Cats (*Felis catus*) and the Acceptability of Possible Management Actions in the UK. *PLoS ONE* 7, e49369. <https://doi.org/10.1371/journal.pone.0049369>
- Thuo, D., Furlan, E., Broekhuis, F., Kamau, J., Macdonald, K., Gleeson, D.M., 2019. Food from faeces: Evaluating the efficacy of scat DNA metabarcoding in dietary analyses. *PLOS ONE* 14, e0225805. <https://doi.org/10.1371/journal.pone.0225805>
- Van Der Heyde, M., Bateman, P.W., Bunce, M., Wardell-Johnson, G., White, N.E., Nevill, P., 2021. Scat DNA provides important data for effective monitoring of mammal and bird

- biodiversity. *Biodivers. Conserv.* 30, 3585–3602. <https://doi.org/10.1007/s10531-021-02264-x>
- van Heezik, Y., Smyth, A., Adams, A., Gordon, J., 2010. Do domestic cats impose an unsustainable harvest on urban bird populations? *Biol. Conserv.* 143, 121–130. <https://doi.org/10.1016/j.biocon.2009.09.013>
- Vestheim, H., Jarman, S.N., 2008. Blocking primers to enhance PCR amplification of rare sequences in mixed samples – a case study on prey DNA in Antarctic krill stomachs. *Front. Zool.* 5, 12. <https://doi.org/10.1186/1742-9994-5-12>
- Zhang, Z., Schwartz, S., Wagner, L., Miller, W., 2000. A Greedy Algorithm for Aligning DNA Sequences. *J. Comput. Biol.* 7, 203–214. <https://doi.org/10.1089/10665270050081478>