Research Article

Ordinary Boldness or Parasitic Mind Control? A Case of Crocodile Nest-Raiding Raccoons in the Florida Keys

Michael V. Cove[1], Kelly A. Crandall[2], Joseph Redinger[3], Isaac Lord[3], Arya Sanjar[2], Shauna Sayers[2], Jeremy Dixon[3], Brent S. Pease[2]

1. North Carolina Museum of Natural Sciences, Raleigh, United States; 2. Forestry Program, School of Forestry and Horticulture, Northern Illinois University, United States; 3. United States Fish and Wildlife Service, Falls Church, United States

The protozoan parasite *Toxoplasma gondii* can alter behavior by heightening the boldness of multiple taxa to increase predation by their definitive host—Felids. We screened northern raccoons (*Procyon lotor*) for *Toxoplasma*-infection and monitored American crocodile (*Crocodylus acutus*) nests with camera traps in the Florida Keys, USA. On four occasions, we documented raccoons investigating a monitored active nest until the female crocodile chased them off. We quantified the elapsed reaction time (ERT) as the number of seconds between raccoon retreat and crocodile surfacing into the photo frame, with longer times representing faster responses of retreat prior to the crocodile attack. Three of the incidents resulted in ERTs of 4-8 seconds (mean = 6 seconds) before the crocodile surfaced. Yet, the radio-collared raccoon from our study had a 0 second ERT and narrowly escaped, which may be the result of chronic seropositive (titer = 1:200) and mind-altering effects of *Toxoplasma* beyond ordinary raccoon boldness. That individual successfully depredated a crocodile nest the following season. We hypothesize that *Toxoplasma*-infection is a Simpson's paradox wherein short-term net benefits appear positive (e.g., successful nestpredation due to boldness), but overall they may result in lower fitness (e.g., lower survivorship due to boldness). Camera traps can elucidate behavioral changes in species interactions by explicitly quantifying reaction times.

Corresponding author: Michael V. Cove mvcove@ncsu.edu

Dealing with parasites is par for the course in free-ranging wildlife. Yet, the effects of many parasites either in isolated infestation or as comorbidities in intermediate or definitive hosts are often poorly documented or understood. There is growing evidence that parasites have evolved the ability to alter the

behavior of their intermediate hosts to increase the probability of reaching their definitive host to sexually reproduce[1]. Ultimately, host behavioral shifts will result in increased fitness among parasites. *Toxoplasma gondii* is one such parasitic protozoan that affects intermediate hosts and has been shown to increase the boldness of multiple taxa to ultimately increase potential predation and elevation to their definitive host—Felids[2][3].

Although it is fatal in many intermediate and non-target hosts[4][5], early research revealed that rodents (*Mus* and *Rattus*, e.g., primary prey of small cats) exposed to *Toxoplasma* were more likely to have delayed reaction speeds to stimuli, display an affinity for domestic cat (*Felis catus*) urine, and behave more erratically in the laboratory[6][7]. These observations suggest infected prey exhibit a range of behavioral changes which may increase the probability of potential predation. Further, *Toxoplasma* has been associated with other negative behavioral responses in wildlife such as dopey fox syndrome in Australia[8], boldness leading to lower survivorship in infected spotted hyena (*Crocuta crocuta*) cubs that were more likely to interact with lions (*Panthera leo*[9]), and was most recently related to boldness in gray wolves (*Canis lupus*) elevating them to park leaders in Yellowstone National Park, USA[2]. Although the latter case suggests some positive influence of the protozoan by increasing boldness, it is likely that behavioral changes that go beyond ordinary boldness could be maladaptive when individuals increase risk-taking behaviors while also experiencing reduced facultative capabilities that may ultimately result in lower survivorship. To our knowledge, this has not been studied in free-ranging wildlife because it would require long-term tracking and survival data along with periodic parasitic assays.

As part of a natural history study of northern raccoons (*Procyon lotor*) in the Florida Keys and their efficacy as sentinel species for the prevalence of raccoon roundworm (*Baylisascaris procyonis*) and *Toxoplasma* in the endemic range of the Key Largo woodrat[10][11], we screened 23 captured raccoons for *Toxoplasma*-infection to assess the impacts of the protozoan on raccoon behavior and fitness by collecting blood samples from each raccoon and separating sera to test for anti-*Toxoplasma* immunoglobulin G antibodies via modified agglutination tests[12]. Samples with a detectable antibody titer level of 1:25 or greater were considered positive, with additional dilutions 1:50, 2:40, and $\geq$ 1:200 tested as well, which informed us which individuals have been infected more recently, as opposed to chronic infection[13][2]. We also fastened radio-collars to all 23 raccoons for tracking to assess their behavior and estimate survival as it relates to urbanization and *Toxoplasma* infection. We predicted that

chronic *Toxoplasma* seroprevalence would be associated with bolder behavior and higher mortality in tracked raccoons because of increased risk-taking.

Concurrently, as part of annual American crocodile (*Crocodylus acutus*) nest monitoring, we used camera traps to survey potential nest predators. Cameras (Reconyx PC800, RECONYX, Inc., Holmen WI, USA) were deployed on cinder blocks 2-3m from any active crocodile nests and set with high motion sensitivity and continuous photos when motion-activated (1sec between photos). On four occasions, we documented raccoons rooting around an active crocodile nest until the female crocodile ultimately rushed to defend against the potential nest predators. As an index of boldness, we quantified the elapsed reaction time (ERT) of the raccoons in the image sequences, by measuring the elapsed time from when the raccoon retreats before the crocodile surfaces into the photo frame—where shorter ERTs suggest increased boldness because the raccoons remain at the nest until the crocodile gets closer.

Three crocodile-raccoon interactions resulted in ERTs of 8 seconds (6 July 2022), 6 seconds (9 July 2022), and 4 seconds (22 July 2022) before the crocodile surfaced into the photo frame (Figure 1, Panels a-d). However, the other documented incident was of a radio-collared raccoon, which resulted in a 0 second ERT before the crocodile was in the frame and the raccoon retreated—just narrowly escaping the predator strike. The raccoon was identified as RA51 based on GPS tracks and was one of our study animals that tested positive with the highest titer level among all individuals (1:200), suggesting recent exposure and acute infection with *Toxoplasma gondii*. The individual was a small (5 kg) adult male raccoon and throughout his capture and subsequent work up and release, he presented as neurologically sound and had no detected physical abnormalities, suggesting he was healthy and not suffering from any other comorbidities.

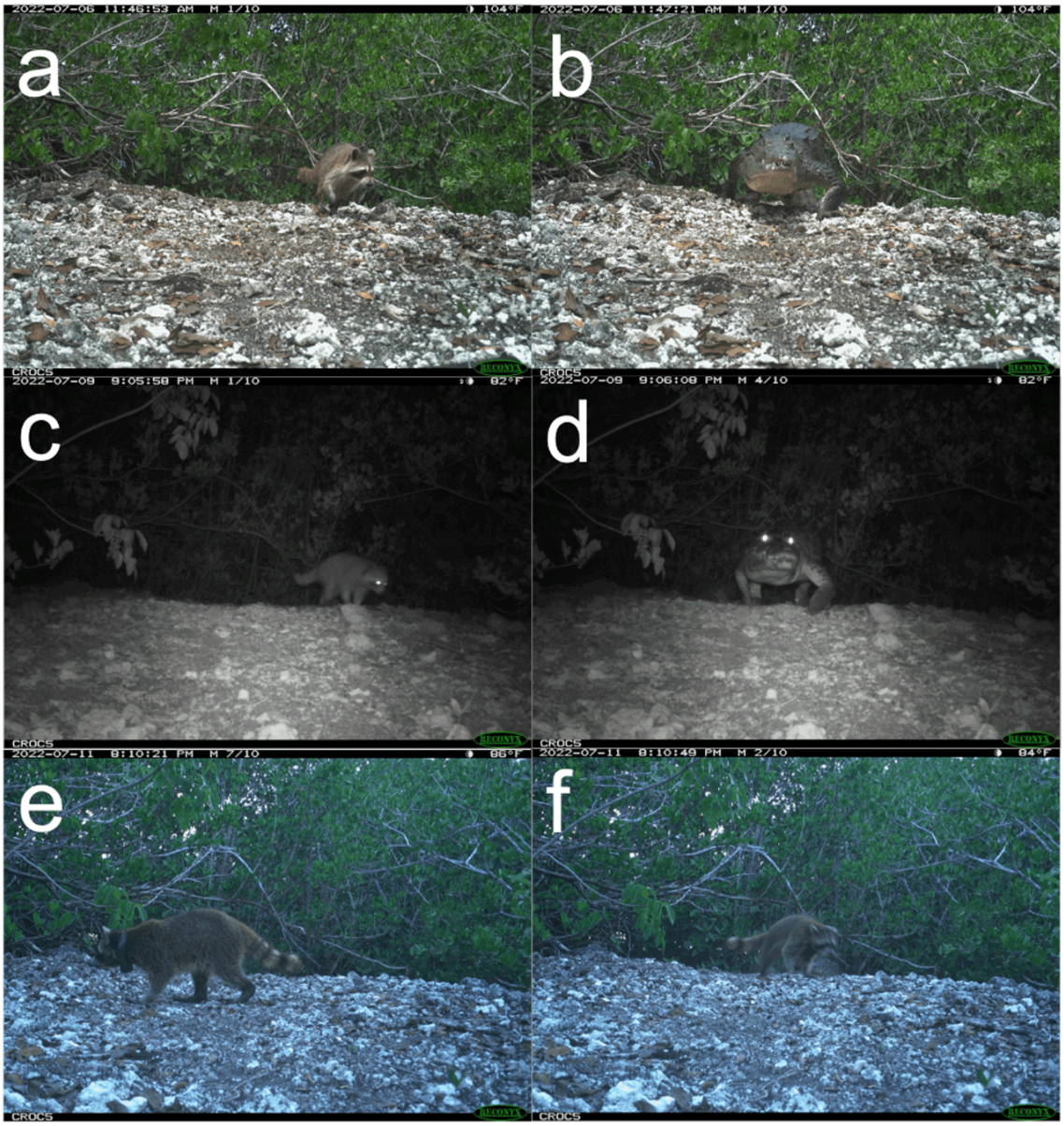

Figure 1. Selected photo sequences of northern raccoons (*Procyon lotor*) interacting with an American crocodile (*Crocodylus acutus*) nest in Key Largo, Florida, USA, 6-11 July 2022. Panels a-d) show two cases where the raccoons retreated before the crocodile entered the photo frames (8 and 6 seconds, respectively). Panels e-f) show the apparent increased boldness observed in the radio-collared raccoon with an acute *Toxoplasma*-infection that narrowly escaped the crocodile seen surfacing just behind it. Photo Credit: Isaac Lord, Joseph Redinger, and Kelly Crandall.

Furthermore, on 29 April 2023, the radio-collared raccoon RA51 was again captured on a camera trap successfully depredating a crocodile's nest (Figure 2), which resulted in a complete loss of the clutch for

the federally threatened reptile. This is the first recorded instance of a raccoon depredating a crocodile nest on Key Largo, but nest predation has been documented in other areas of crocodiles' range in southern Florida[14]. This individual was not the only raccoon to encounter the crocodile nest and further suggests that its successful nest predation was due to increased boldness. While raccoons are bold and adaptable broadly, the bold behavior of RA51 is most parsimoniously associated with long-term parasitic infection. This leads us to hypothesize that *Toxoplasma*-infection in nonfelids may represent a Simpson's paradox–a statistical phenomenon where a trend appears in several groups but disappears or reverses when the groups are aggregated[15]. In this sense, the "groups" are short-term tracking periods of individual animals and the aggregated data would be the long-term life outcomes of many individuals in the population. Therefore, the net benefits of *Toxoplasma*-infection are positive in the short-term (e.g., successful nest predation due to increased boldness), but as we extend the sampling window to include the entire life of the individual and others in the population, the overall result likely leads to lower fitness in the long-term (e.g., lower survivorship due to boldness). Further examination of broader survivorship analyses of the raccoons of the Florida Keys and other populations such as the wolves of Yellowstone will help differentiate and elucidate the net effects of chronic *Toxoplasma*-infection in nonfelids[2].

Figure 2. Camera trap evidence of the same radio-collared raccoon (*Procyon lotor*) with an acute *Toxoplasma*-infection walking away from an American crocodile (*Crocodylus acutus*) nest in Key Largo, Florida, USA on 29 April 2023, with an egg in its jaws. Photo Credit: Arya Sanjar and Shauna Sayers.

In general, raccoons are curious and bold animals, which is associated with their capacity to adapt and thrive in human environments by consuming external resources[16][17], but we believe our current observations and the auxiliary information about *Toxoplasma* seroprevalence taken together support the hypothesis that the protozoan makes intermediate hosts behave more boldly and erratically. Although we cannot individually identify the raccoons in the three other sequences to know the *Toxoplasma* exposure of those individual(s), the high level of titer (1:200) observed in RA51 was less common in the population. Only 26.1% of the 23 screened raccoons tested positive at the 1:200 dilution, as compared to the overall positivity rate of 82.6% (19/23), which roughly corresponds with the frequency in the photo sequences herein. Irrespective of the current titer levels, the observed *Toxoplasma*-seroprevalence in raccoons of Key Largo was very high compared to previous surveys in Florida (18-29%[18]), which should raise concerns about the abundance of feral and free-ranging cats and their potential negative consequences for the endangered rodents of the island[10].

We present the first case of using camera traps to quantify elapsed reaction time (ERT) between prey and a potential predator to serve as an index of boldness in the prey. This simple metric may be useful in other applications to better understand species interactions and food webs, in line with recent spatiotemporal analyses (e.g., Gilbert et al.[19]). Ultimately, we recommend further research on the role and implications of *Toxoplasma gondii* infections affecting the behavior and fitness of free-living wildlife to better understand the protozoan's role in community interactions and food webs more broadly.

Statements and Declarations

Acknowledgements

This work was supported by the US Fish and Wildlife Service Inventory and Monitoring Program (Grant F21A0C406-00) and conducted under the Southern Illinois University at Carbondale IACUC Protocol 22-034. Thanks to Dr. M. Claudio for collecting samples and to the staff at SCWDS at the University of Georgia for their diagnostic services. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the United States Government.

Ethics Statement

The data from this manuscript will be made available upon reasonable request. The Funding Agency and grants are acknowledged in the Acknowledgements. We declare no conflicts of interest and all animal-related research was in accordance with the guidelines of the American Society of Mammalogists and approved by the Southern Illinois Institutional Animal Care and Use Committee Protocol 22-034.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability

Only observational and photographic data were collected for this study, but seroprevalence data will be published in the Dryad data repository upon acceptance of this manuscript.

References

1. ^Dubey JP. Toxoplasmosis of Animals and Humans. 2nd ed. CRC Press Taylor and Francis Group, 2010.
2. a, b, c, dMeyer CJ, Cassidy KA, Stahler EE, Brandell EE, Anton CB, Stahler DR, Smith DW. "Parasitic Infection Increases Risk-Taking in a Social, Intermediate Host Carnivore." Communications Biology. 5 (1): 1180. doi:10.1038/s42003-022-04122-0.
3. ^Poirotte C, Kappeler PM, Ngoubangoye B, Bourgeois S, Moussodji M, Charpentier MJE. "Morbid Attraction to Leopard Urine in Toxoplasma-Infected Chimpanzees." Current Biology. 26 (3): R98–R99. doi:10.1016/j.cub.2015.12.020.
4. ^Hollings T, Jones M, Mooney N, Mccallum H. "Trophic Cascades Following the Disease-Induced Decline of an Apex Predator, the Tasmanian Devil." Conservation Biology. 28 (1): 63–75. doi:10.1111/cobi.12152.
5. ^Conrad PA, Miller MA, Kreuder C, James ER, Mazet J, Dabritz H, Jessup DA, Gulland F, Grigg ME. "Transmission of Toxoplasma: Clues from the Study of Sea Otters as Sentinels of Toxoplasma Gondii Flow into the Marine Environment." International Journal for Parasitology. 35 (11): 1155–1168. doi:10.1016/j.ijpara.2005.07.002.
6. ^Webster JP. "Prevalence and Transmission of Toxoplasma Gondii in Wild Brown Rats, Rattus Norvegicus." Parasitology. 108 (4): 407–411. doi:10.1017/S0031182000075958.
7. ^Vyas A, Kim SK, Giacomini N, Boothroyd JC, Sapolsky RM. "Behavioral Changes Induced by Toxoplasma Infection of Rodents Are Highly Specific to Aversion of Cat Odors." Proceedings of the National Academy of Sciences. 104 (15): 6442–6447. doi:10.1073/pnas.0608310104.
8. ^Milne G, Fujimoto C, Bean T, Peters HJ, Hemmington M, Taylor C, Fowkes RC, et al. "Infectious Causation of Abnormal Host Behavior: Toxoplasma Gondii and Its Potential Association With Dopey Fox Syndrome." Frontiers in Psychiatry. 11 (2020). doi:10.3389/fpsyt.2020.513536.
9. ^Gering E, Laubach ZM, Weber PSD, Soboll Hussey G, Lehmann KD, Montgomery TM, Turner JW, Perng W, Pioon MO, Holekamp KE, Getty T. "Toxoplasma gondii infections are associated with costly boldness toward felids in a wild host." Nature Communications. 12 (1): 3842.
10. a, bCrandall KA, Pease BS, Simmons AL, Adamovicz A, Cove MV. "Raccoon roundworm undetected in endangered Key Largo woodrat endemic range." Journal of Wildlife Diseases. 59 (3): 536-538. doi:10.7589/JWD-D-22-00133.
11. ^Crandall KA, Pease BS, Dixon J, Cove MV. "Human-derived food shrinks home ranges and alters resource selection of mammals at the urban-wild interface." Food Webs. 41: e00363.

12. ^ *Dubey JP, Hamir AN, Shen SK, Thulliez P, Rupprecht CE. "Experimental Toxoplasma gondii infection in raccoons (Procyon lotor)." The Journal of Parasitology. (1993): 548-552.*
13. ^ *Desmonts G, Naot Y, Remington JS. "Immunoglobulin M-Immunosorbent Agglutination Assay for Diagnosis of Infectious Diseases: Diagnosis of Acute Congenital and Acquired Toxoplasma Infections." Journal of Clinical Microbiology. 14 (5): 486–491. doi:10.1128/jcm.14.5.486-491.1981.*
14. ^ *Mazzotti FJ. "The American Crocodile in Florida Bay." Estuaries. 22 (2): 552–561. doi:10.2307/1353217.*
15. ^ *Pearl J. "Comment: understanding Simpson's paradox." In Probabilistic and causal inference: The works of Judea Pearl, pp. 399-412. 2022.*
16. ^ *Cove MV, Jones BM, Bossert AJ, Clever DR, Dunwoody RK, White BC, Jackson VL. "Use of camera traps to examine the mesopredator release hypothesis in a fragmented Midwestern landscape." The American Midland Naturalist. 168 (2): 456-465.*
17. ^ *Nicholson M, Cove MV. "Stable Isotopes Point to Anthropogenic Subsidies in Northern Raccoons at the Urban-Wild Interface." Food Webs. 31: e00233. doi:10.1016/j.fooweb.2022.e00233.*
18. ^ *Hancock K, Thiele LA, Zajac AM, Elvinger F, Lindsay DS. "Prevalence of antibodies to Toxoplasma gondii in raccoons (Procyon lotor) from an urban area of Northern Virginia." Journal of Parasitology. 91 (3): 694-695.*
19. ^ *Gilbert NA, Stenglein JL, Pauli JN, Zuckerberg B. "Human Disturbance Compresses the Spatiotemporal Niche." Proceedings of the National Academy of Sciences. 119 (52): e2206339119. doi:10.1073/pnas.2206339119.*

Supplementary data: available at https://doi.org/10.32388/TEHA80

Declarations

Funding: This work was supported by the US Fish and Wildlife Service Inventory and Monitoring Program (Grant F21A0C406-00).

Potential competing interests: We declare no conflicts of interest and all animal-related research was in accordance with the guidelines of the American Society of Mammalogists and approved by the Southern Illinois Institutional Animal Care and Use Committee Protocol 22-034.