Quantifying the presence of feral cat colonies and *Toxoplasma gondii* in relation to bird conservation areas on O'ahu, Hawai'i

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**Abstract**

Free-ranging feral cats (*Felis catus*) are increasingly found in colonies loosely managed by people. These colonies increase cat densities and, hence, pose threats to wildlife via disease and predation, particularly in insular ecosystems where native species have smaller populations and reduced pathogen exposure compared to continental systems. Given such concerns, our objectives were to: (a) identify feral cat colonies on the island of O'ahu in the vicinity of important native bird sites; and (b) test for *Toxoplasma gondii*, a parasite-causing disease (Toxoplasmosis) of concern to native birds, at cat colony sites. We identified 32 important native bird locations and surveyed public lands near these sites to determine presence of cat colonies. Where cat colonies were present, we collected feces and used molecular tools to identify the presence of *T. gondii*. We identified 25 cat colonies near an important bird area and collected feces at four of these colonies, with three testing positive for *T. gondii*. The presence of cats near a majority of native bird areas suggests that cats may impose a serious threat to bird species. Our finding of *T. gondii* at three of the four colonies where cat feces were collected raises serious health concerns for humans, birds, and many other terrestrial and aquatic organisms. Native birds in Hawai'i, including highly endangered species, are susceptible to both predation and *T. gondii*, and finding its presence in locations relatively near to important native bird areas provides further evidence that reducing free-ranging feral cat numbers is critical for reducing impacts on birds.

**Keywords**
cat colony, *Felis catus*, invasive species, island ecosystems, *Toxoplasma gondii*

1 | INTRODUCTION

Cats (*Felis catus*) have been associated with humans for 5,000–10,000 years (Driscoll et al., 2007; Hu et al., 2014) and were subsequently introduced by humans throughout the world (Medina et al., 2011). Humans have introduced cats to at least 5% of the world’s roughly 179,000 small to medium islands (Loss, Will, & Marra, 2013;
Medina et al., 2011), which has resulted in a variety of ecological problems (Medina, Bonnaud, Vidal, & Nagales, 2014, Shionosaki, Yamada, Ishikawa, & Shibata, 2015, Doherty, Glen, Nimmo, Ritchie, & Dickman, 2016). Native island faunas are especially susceptible to cat predation because they have little or no behavioral, morphological, or life history traits to aid in defense. Furthermore, cats carry a multitude of pathogens, such as Toxoplasma gondii, many of which may be detrimental to both native species and people (Aguirre et al., 2019; Lepczyk, Lohr, & Duffy, 2015). As a result, cats are listed among the world’s 100 worst invasive species and have been directly linked to animal species populations’ declines and extinctions in numerous studies on islands (Doherty et al., 2016; Lowe, Browne, Boudjelas, & De Poorter, 2000; Medina et al., 2011).

European ships introduced domestic cats to the Hawaiian Islands in the late 1700s (King, 1984), and within decades feral cats were noted in the wild (Brackenridge, 1841). Today, cats are the only felid in the Hawaiian Islands, and unfortunately are common and widespread throughout the main Hawaiian Islands. Since their introduction in Hawai‘i, cats have been documented to depredate a wide variety of bird species, including palila (Loxoides bailleui), Hawaiian petrel (Pterodroma sandwichensis), Hawai‘i ‘amakih (Chlorodrepanis virens), and Hawai‘i ‘elepaio (Chasiempis sandwichensis; Hess, Banko, Goltz, Danner, & Brinck, 2004, Hess, 2011), and likely impact many other species (Table A1). In addition to cats’ lethal predatory habits, they are the only known definitive host in Hawai‘i of the parasite T. gondii, which causes the disease toxoplasmosis (Dubey, 2010). T. gondii can infect any warm-blooded host, ranging from humans to both marine and terrestrial wildlife species (Aguirre et al., 2019). Within Hawai‘i, these species include ‘alalā (Corvus hawaiiensis), nēnē (Branta sandvicensis), Hawaiian monk seal (Monachus schauinslandi), spinner dolphin (Stenella longirostris), and Atlantic bottlenosed dolphin (Tursiops truncatus; Barbieri et al., 2016; Dubey, 2010; Inskeep, Gardiner, Harris, Dubey, & Goldston, 1990; Migaki, Sawa, & Dubey, 1990; Mikaelian, Boisclair, Dubey, Kennedy, & Martineau, 2000; Work et al., 2000, 2016; Work, Massey, Lindsay, & Dubey, 2002). Thus, cats potentially pose a very serious threat to native birds and mammals across the Hawaiian Islands.

In the Hawaiian Islands, as elsewhere around the world, feral cats are often managed in open colonies (Lohr & Lepczyk, 2014; Longcore, Rich, & Sullivan, 2009). These colonies range in size from a few to hundreds of cats and are typically open to immigration and free-fed by caretakers (e.g., Peterson, Hartis, Rodriguez, Green, & Lepczyk, 2012). Because cat colonies artificially concentrate cats at a small location, the colonies pose an increased concern to native birds in the adjacent areas due to both pathogens, such as T. gondii, and predation issues (Courchamp, Chapuis, & Pascal, 2003; Lepczyk & Duffy, 2018). Of particular concern is that T. gondii and the associated disease, toxoplasmosis, has been found in people and wildlife in Hawai‘i for over half a century (Tilden, 1953; Work et al., 2002), and the parasite is present in cats living in colonies (Davis et al., 2018). Hence, cat colonies may be of particular concern to the long-term conservation of native birds living in close proximity due to both predation and disease exposure, both in continental and island ecosystems.

Though cat colonies are of conservation concern to native wildlife in Hawai‘i, little to no work has been targeted towards evaluating them in terms of disease and predation risk. Hence, given the ecological impacts that cats pose to native birds in island ecosystems, our goal was to assess the potential risk of cat colonies near important native bird areas in Hawai‘i. We focus on native birds because the Hawaiian Islands are home to a variety of rare and evolutionarily unique assemblage of birds, many of which are rapidly declining or going extinct (Banko & Banko, 2009; Pratt, 2009). Furthermore, of the 152 land bird species originally present in the archipelago, 110 have gone extinct since the arrival of humans, with 33 of the 42 remaining endemic birds listed as endangered or threatened, resulting in one of the most endangered avifaunas in the world (American Bird Conservancy, 2016; Walther 2016). Finally, except for sea turtles, two bat species, and the Hawaiian monk seal, the islands have no native herpetofauna or mammals, making birds the primary native terrestrial wildlife of conservation concern (Lepczyk, Hess, & Johnson, 2011). Furthermore, the birds that are present in islands have evolved in the absence of mammalian predators and thus lack behavioral responses to them. Considering these points in the context of our overarching goal, our objectives were to: (a) identify feral cat colonies on the island of O‘ahu that were in the vicinity of important native bird sites; and (b) test for T. gondii at cat colony sites using molecular methods.

2 METHODS

To address our research objectives, we compiled a list of important native bird sites that housed one or more native bird species on the island of O‘ahu from published agency reports, field guides, and first-hand accounts from bird conservation professionals. We considered important native bird sites to be those that were regularly used by native birds as noted from these three sources. We did not use an abundance threshold as a criterion for
designated as an important native bird site due to lack of abundance estimates at many locations and because low abundance does not necessarily mean low conservation value to many bird species given that many Hawaiian species are rare, threatened, or endangered. To ensure the accuracy and inclusiveness of our list of important native bird sites, the list was reviewed and edited by Dr. Eric Vanderwerf of Pacific Rim Conservation, an expert on Hawaiian avifauna. The process resulted in the identification of 61 locations that potentially supported native birds on the island (Table A2 and Figure A1). Using these 61 locations we then identified all public parks and lands, including schools, beaches, and arboretums that fell within a 1.61 km (1 mile) buffer from each important native bird site, resulting in 33 locations (Figure 1; Note that two locations within 250 m of one another [He’eia State Park and He’eia Boat Ramp] were considered as a single site and thus total sample was considered to be 32 locations). We chose public parks and lands because these locations are where cat colonies are commonly found on the island, likely due to their relative ease of access for feeding and abandoning cats throughout the day, and lack of enforcement for feeding feral animals. Sites that had restricted access or required permit or paid entry were excluded from consideration as these sites tend not to have colonies, unless being maintained by an on-site employee. We selected the 1.61 km buffer as it approximates the upper range of 15.2 ha for feral cat home ranges in urban areas, based on linear distance. The transects were run roughly in the middle of a site, where the cover consisted primarily of parking lots/impervious surface, grassy areas, and shrubs, with mixed solitary trees. Given that each site differed in area and the relative amount of cat activity observed, each transect was unique in length and the time required to survey it. Sites were visited at least once, and if no indication was found of any cat presence or cat feeding activity (i.e., presence of cat food, food containers, water, etc.) after a thorough search, they were not evaluated again. The remainder of the sites were visited between 1 and 3 times (Table 1). Because our objectives focused on identifying T. gondii, we did not seek to determine relative abundance or population estimates of colonies via distance sampling.

At locations where cats were found, we also inspected the site thoroughly for cat feces. Cat feces were collected following University of Hawai‘i biohazard protocols and placed in plastic bags for transport to the university. Fecal samples were then frozen (−20°C) until processed. Because feces were infrequently located through random searches, we also remained at locations that had colonies and observed cats for possible defecations, which increased the number of samples obtained in a given evening’s sampling period.

We used approximately 25 mg of macerated feces from each sample for molecular analyses to determine presence of coccidian parasites. Fecal floats were not performed prior to collecting the subsample for molecular analyses. In order to break open the hardy oocysts of coccidian parasites, we put samples through five rapid freeze/thaw cycles using liquid nitrogen (−80°C, 4 min) and a hot water bath set to 57°C (4 min) (Manore, Harper, Aguilar, Weeze, & Shapiro, 2019; Staggs, Keely, Ware, et al., 2015). We then froze (−20°C) the samples again until DNA extraction and PCR analyses. Briefly, we extracted DNA using Qiagen’s DNeasy Blood and Tissue Kit according to the manufacturer’s protocol; extracted DNA was eluted in 100 μL of elution buffer and stored at −20°C until PCRs were performed.

We used previously published pan-coccidian ApiITS1 primers (Gibson et al., 2011) anchored in the 18S and 5.8S small subunit (SSU) rDNA gene array to screen all samples for the presence of coccidian parasites. These nested primers amplify across the internal transcribed spaces 1 (ITS-1) region to distinguish between closely related and novel species of coccidian parasites. We conducted PCR using 3 μL of each DNA extraction with 5 μL of PCR buffer (10X containing MgCl2; Sigma, St. Louis, MO), 5 μL of 2 mM dNTP (Sigma-Aldrich, DNTP100-1KT), 1 μM of each primer, and 1.5 μL of Taq DNA Polymerase (Sigma-Aldrich, D1806), in a total reaction volume of 50 μL. We then carried out PCR amplification for 35 cycles (94°C for 40 s, 58°C for 40 s, 72°C for 40 s, followed by one 10-min extension at 72°C). Extracted DNA from T. gondii was used as a positive control, and molecular-grade water was used as negative controls (n = 4). All PCRs were nested; the second PCR reaction was done with 1 μL of the PCR mixture from the primary PCR and all other concentrations remained the same. We visualized amplicons in gel-red (Biotium Inc., Hayward, CA) stained 1% agarose gels and purified using SAP-Exo according to manufacturer’s instructions. We sequenced positive amplicons using Sanger sequencing.
Results were quality checked by visual examination using FinchTV (Version 1.5.0, Geospiza Inc.) and BLASTed for identification.

3 | RESULTS

Of the 32 public areas investigated, 25 (78.1%) had cats (Table 1). The number of cats observed at these 25 locations during a single survey ranged from 1–99, with a mean of 23.4 cats observed per survey per site with cats present. Furthermore, the number of cats observed at each site varied greatly (Table 1). Some colonies were spread over large areas, such as the colonies at Koko Head District Park (~24 ha) and Kea’īwa Heiau State Park (~155 ha). Other colonies were confined to smaller areas, such as the colonies observed at Sunset Beach Elementary (~3 ha) and Diamond Head Road (~2 ha). A total of 23 (71.9%) of the sites had colonies included a wide abundance of cats, but averaged 23.4 per colony. Both the large percentage of sites that had cat colonies, coupled with the colony size, is of notable concern in terms of the potential risk of cat predation on birds.

Of colonies identified, at least 75% (n = 3) of the sites where cat feces were obtained (n = 4 total) tested positive for T. gondii. Given that we did not conduct fecal floats, we cannot be certain that the T. gondii DNA detected in cat feces was associated with oocysts; it may be associated with infected prey, and the DNA from the prey’s T. gondii tissue cysts was detected in the cat feces. Further, fecal floats are typically conducted to concentrate the oocysts, if present. Since we did not do this, it is likely we obtained false negatives as a cat would have to be shedding high numbers of oocysts in order for them to be detected using our methods and lack of concentration techniques. Therefore, our results may actually underestimate the number of cats infected with and/or shedding T. gondii oocysts into the terrestrial and near-shore environments of Hawai‘i.

T. gondii is a zoonotic pathogen that poses a risk to humans (Aguirre et al., 2019; Ikeda, 2000) and the native wildlife of Hawai‘i. Specifically, T. gondii has been documented to infect and kill the ‘alalā, the most endangered corvid in the world (Work et al., 2000). Acute, fatal toxoplasmosis has also been documented in two other species of birds found in the Hawaiian Islands—nēnē and the red-footed booby (Sula sula; Work et al., 2002). Indeed, T. gondii is the most common infectious disease in nēnē (Work, Dagenais, Rameyer, & Breeden, 2015). The impact of T. gondii on native wildlife of Hawai‘i extends beyond the terrestrial and into the coastal marine waters, where it has been documented as an important cause of mortality in the endangered Hawaiian monk seal (Barbieri et al., 2016).

Though one sample amplified using the ITS1 primers and had an amplicon consistent with the size expected for T. gondii (~550 bp), we were unable to get clean a sequence for this sample. We therefore did not include this sample in our results as being positive for a protozoal infection. In general, sequence quality for most samples was poor, which is to be expected when dealing with scat samples due to the degraded nature of the DNA in general and also inhibitors in feces prior to and after the DNA extraction. This may have been minimized had we conducted fecal floats and only extracted the top 100 μL of the float. Further, we could likely have improved the quality of DNA by using a DNA extraction kit specifically for feces. Regardless, clean sequence traces for six samples provided sequences of approximately ~190–450 base pairs that were able to be identified as T. gondii, and in a...
seventh sample both *T. gondii* and *C. felis*, via BLAST searches (Table 2).

Although *T. gondii* was identified, based on the methods employed to sample feces, we cannot make a statement regarding the prevalence of *T. gondii* in feral cats on O‘ahu. We did not conduct fecal floats to concentrate any *T. gondii* oocysts that may have been present. Further, we did not homogenize the individual scat samples. Therefore, a false negative cannot be ruled out. Furthermore, because only a small number of infected cats may actively be shedding oocysts in their feces at any given point in time (Dabritz et al., 2007), fecal analysis provides only a partial picture of pathogen prevalence in comparison to approaches such as serology, or testing for antibodies to *T. gondii* in the blood. In fact, as Tenter, Heckeroth, and Weiss (2000) noted, up to 74% of cat populations may show antibodies to *T. gondii*. Given that feces were collected at four locations and three tested positive for *T. gondii* and the ability of oocysts to persist for an extended time in the environment (Tenter et al., 2000), our results suggest that the parasite may be quite prevalent on the island. In particular, recent work on O‘ahu has also found *T. gondii* at cat colonies at the University of Hawai‘i at Mānoa campus and from feral cats removed from a seabird nesting site Ka‘ena Point Natural Area Reserve when a predator proof fence was installed (Davis et al., 2018). Furthermore, *T. gondii* has been identified in nēnē carcasses from the islands of Hawai‘i, Moloka‘i, Kaua‘i, and Maui (Work et al., 2015, 2016). Taken together, our results highlight the potential threat of feral cats and the non-native parasites they shed to the native wildlife of Hawai‘i.

Our current findings have positively demonstrated that feral cats supported by people are near important native bird areas and house a disease of notable concern to birds, other wildlife, and humans. Furthermore, the locations of these cat colonies pose a risk to birds due to predation by cats. Importantly, however, our findings are likely conservative for several reasons. First, finding cat colonies and identifying the number of cats in each colony is a time-consuming process whereby only one to several locations can be accurately assessed on any given day. Thus, if we happened to survey a site at a time when no visible signs of supporting cats (e.g., cat food, water, dishes/bowls) or cats themselves, we would have excluded it from our analysis, when in fact it was a false negative. Second, our analysis only evaluated public lands because these locations are where cat colonies are commonly found on the island, likely due to their relative

FIGURE 1 Location of public lands, with the addition of site names for each location. The figure notes all locations that were investigated for feral cat colonies. Locations noted in red font were found to house feral cat colonies whereas those in white font denote locations where no feral cat colonies were found.
ease of access for feeding and abandoning cats throughout the day, and lack of enforcement for feeding wildlife. However, cat colonies can exist on business, residential, or other private lands. Third, the number of cats observed within a colony varied widely depending on environmental conditions and time of day, making accurate counts

### TABLE 1  Locations and summary statistics of cat colony surveys across 32 parks and public lands on O’ahu, Hawai’i

| Location                                    | # of visits | Cats present | Max. # of cats observed | Supported by people | Feces collected | Toxoplasma. gondii detected |
|---------------------------------------------|-------------|--------------|-------------------------|---------------------|-----------------|-----------------------------|
| Ali'i Beach Park                            | 1           | No           | 0                       | NA                  | NA              | NA                          |
| Black Point                                 | 1           | No           | 0                       | NA                  | NA              | NA                          |
| Diamond Head Road                           | 1           | Yes          | 6                       | Yes                 | 0               | NA                          |
| Hanauma Bay                                 | 1           | Yes          | 22                      | Yes                 | 0               | NA                          |
| Hawaii Prince Golf Club                     | 2           | Yes          | 9                       | Yes                 | 0               | NA                          |
| He'eia Boat Ramp/State Park                 | 3           | Yes          | 99                      | Yes                 | 12              | 1                           |
| Ho'omaluhia Botanical Gardens               | 1           | Yes          | 1                       | Yes                 | 0               | NA                          |
| Ka'ena Point end of Road North Shore Side   | 1           | No           | 0                       | NA                  | NA              | NA                          |
| Kailua Beach Park                           | 1           | Yes          | 8                       | Yes                 | 0               | NA                          |
| Kawaiinui Park                              | 1           | No           | 0                       | NA                  | 0               | NA                          |
| Kea'iwa Heiau State Park                    | 3           | Yes          | 35                      | Yes                 | 0               | NA                          |
| Ke'ehi Lagoon Park                          | 3           | Yes          | 50                      | Yes                 | 12              | 0                           |
| Koko Head District Park                     | 3           | Yes          | 97                      | Yes                 | 20              | 2                           |
| Kualoa Regional Park                        | 2           | Yes          | 21                      | Yes                 | 0               | NA                          |
| Lagoon Drive                                | 2           | No           | 0                       | NA                  | 12              | 1                           |
| Lyon Arboretum                              | 3           | Yes          | 5                       | Yes                 | 0               | NA                          |
| Malaekahana                                 | 1           | Yes          | 12                      | Yes                 | 0               | NA                          |
| Mokule'ia Beach Park                        | 1           | No           | 0                       | NA                  | NA              | NA                          |
| Oneula Beach Park                           | 1           | Yes          | 4                       | Yes                 | 0               | NA                          |
| Pua'ena Point Beach Park                    | 2           | Yes          | 11                      | Yes                 | 0               | NA                          |
| Pu'u O Mahuka Heiau                         | 1           | Yes          | 5                       | No                  | 0               | NA                          |
| Sand Island State Recreation Area           | 1           | Yes          | 88                      | Yes                 | 0               | NA                          |
| Sand Island Boat Ramp                       | 1           | Yes          | 53                      | Yes                 | 0               | NA                          |
| Sandy Beach Park                            | 2           | Yes          | 1                       | Yes                 | 0               | NA                          |
| Sunset Beach Elementary                     | 1           | Yes          | 8                       | Yes                 | 0               | NA                          |
| Ted Makalena Golf Course                    | 3           | Yes          | 17                      | Yes                 | 0               | NA                          |
| Turtle Bay Resort                           | 1           | Yes          | 4                       | No                  | 0               | NA                          |
| Waimea Bay Beach Park                       | 1           | Yes          | 11                      | Yes                 | 0               | NA                          |
| Waipio Soccer Park                          | 3           | Yes          | 8                       | Yes                 | 0               | NA                          |
| West Loch Community Shoreline Park          | 3           | Yes          | 1                       | Yes                 | 0               | NA                          |
| West Loch Homes next to West Loch Shoreline Park | 1   | Yes          | 9                       | Yes                 | 0               | NA                          |

*aLocations identified as “Yes” (n = 25) are locations used to determine average cat number across sites.

*bMax. # of cats observed during a single site visit.

*cYes indicates that people were observed feeding cats or that evidence of cat feeding, such as cat food or cat food containers, was apparent.

*dNA refers to locations where no feces collections were attempted, or there were no cats available to be supported by people; whereas zeros are sites that were attempted but in which no feces could be located or sites with cats but no evidence of human support.

*eFor locations where feces collections did not occur, the cells have been left blank, whereas locations that were sampled but yielded no positive results are indicated with a zero. In locations where T. gondii was identified the number refers to the number of fecal samples that tested positive.
extremely challenging. Although we did not specifically test for the effects of these factors, they are often found to influence free-ranging animal numbers. Furthermore, our goal was not focused on explicit enumeration of cats in colonies, but rather determining locations of cat colonies and occurrence of *T. gondii*. As a result, the counts of cats are the minimum number present. Fourth, we did not observe people feeding cats at many locations directly, but anecdotally we did note that where feeding was occurring the number of cats was much greater. Thus, feeding rates and times likely influence the number of locations where the parasite is present. Fifth, only a small number of infected cats (~1–6%) may actively be shedding oocysts in their feces at any given point in time (Dabritz et al., 2007; Lilly & Wortham, 2013). Hence, at a minimum, power analysis suggests that at least 20 fecal samples need to be collected per site in order to be confident of the presence or absence of the disease at study locations (Davis, 2013). Only one site met this minimum criterion, therefore the number of locations where the parasite is present is likely higher. In fact, as 75% (3/4) of sites where we did collect feces yielded positive results for *T. gondii* even without meeting the requirements of the power analysis, the parasite may be widely present in feral cats on O‘ahu and possibly other islands. Thus, our results should be considered *an absolute minimum*, and the occurrence of *T. gondii* in O‘ahu’s environment is almost certainly higher.

In order to improve upon the research presented here, we suggest the following elements be considered for future studies. First, as with all field studies, there is a tradeoff between the number of sites or plots that can be visited and the amount of information that can be collected at each site given the number of individuals collecting data. Thus, if the goal of the study is to increase detection of *T. gondii* from feces or count cats in a robust manner, we recommend selecting fewer sites and conducting a higher repetition of sampling at a given site or considering additional personnel or even the use of a citizen science monitoring approach. Second, if assessing *T. gondii* is of interest, a mechanism to improve the estimated prevalence of the parasite in feral cat populations would be to collect blood samples from cats for serological analyses. To monitor shedding rate of *T. gondii* oocysts in the environment, and thus better understand risk of exposure to native wildlife and humans, additional fecal samples should also be collected. To overcome the challenge of feces collections, it would be valuable to direct more attention to fewer sites in order to observe cats defecating. Finally, because many feral cat colonies are supplemented by people, it is imperative to understand the frequency and amounts of cat food being provided. Without such knowledge it is difficult to discern how cat numbers may be related to supplemental feeding.

### 5 | CONSERVATION IMPLICATIONS

Our findings have a number of important repercussions for management of feral cats on the island of O‘ahu specifically, as well as more broadly across Hawai‘i and other island systems. The presence of feral cats at ~78% of public spaces near important native bird habitats on O‘ahu indicates cats are likely having a substantial impact on the populations of the island’s endangered birds due to their generalist predatory behavior and high mobility (Blancher, 2013; Bonnington, Gaston, & Evans, 2013; Loss et al., 2013). Though we did not attempt to quantify predation rates here, the reality of cat colonies and free-ranging feral cats on the landscape near important native bird areas have caused real world population effects for native birds (Doherty et al., 2016; Medina et al., 2011). In addition to
predation, cats also pose a significant threat in terms of disease transmission. In our results, the detection of *Toxoplasma gondii* at three of the four sites where cat feces were collected raises serious health concerns for humans, birds, and other native terrestrial and aquatic organisms. Native birds in Hawai`i, including highly endangered species such as `alalā, are susceptible to *Toxoplasma gondii*, and finding its presence in locations relatively near to important native bird areas provides further evidence that reducing feral cat numbers is critical for reducing impacts on birds. In the case of people, it should be noted that sites where *Toxoplasma gondii* was detected are high-use areas for a variety of activities by people. Specifically, these locations include parks and beaches which could provide opportunities for people or pets to come into contact with oocysts during aquatic activities (e.g., snorkeling or swimming) or on land, or while eating uncooked seafood that may be contaminated. Overall, given the numbers of cats at some colony sites and the detection of *Toxoplasma gondii*, disease transmission is a serious threat for birds and public health on O`ahu, and likely the other main Hawaiian Islands. As a result, our findings provide further evidence of the need to effectively manage feral cats on public lands in the state.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**AUTHOR CONTRIBUTIONS**

C.A.L., G.C.S., and C.F. conceptualized the project, K.E.H. analyzed the samples, and C.A.L., G.C.S., C.F., and K.E.H. wrote the manuscript.

**ETHICS STATEMENT**

All research was carried out in accordance with standard research practices.

**DATA AVAILABILITY STATEMENT**

All data are presented in the manuscript.

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FIGURE A1  Map of initial important bird sites O‘ahu, Hawai‘i listed in Table A2
| **Species** | **USFWS status** | **IUCN status** | **Location** | **Source** |
|-------------|------------------|-----------------|-------------|------------|
| Hawai‘i ‘Amakihi (*Chlorodrepanis virens*) | Unlisted | Least concern | Mauna Kea FR, Hawai‘i; Hanawi, Maui | Snetsinger, Fancy, Simon, & Jacobi, 1994; Hess, Banko, Goltz, Danner, & Brinck, 2004; Kowalsky, Pratt, & Simon, 2002 |
| Hawai‘i ‘Elepaio (*Chasiempis sandwichensis*) | Unlisted | Vulnerable | Mauna Kea FR, Hawai‘i | Snetsinger et al., 1994; Hess, Banko, Goltz, Danner, & Brinck, 2004 |
| Hawaiian Coot, ‘Alae ke‘oke‘o (*Fulica alai*) | Endangered | Vulnerable | Hanalei NWR, Kaua‘i | KNWRC, 2015 |
| Hawaiian Duck, Koloa (*Anas wyvilliana*) | Endangered | Endangered | Hanalei NWR, Kaua‘i | KNWRC, 2015 |
| Hawaiian Gallinule, ‘Alae ‘ula (*Gallinula galeata sandvicensis*) | Endangered | n/a | Hanalei NWR, Kaua‘i | KNWRC, 2015; KNWRC, 2018; Byrd & Zeilmaker, 1981 |
| Hawaiian Goose, Nēnē (*Branta sandvicensis*) | Endangered | Vulnerable | Volcanoes NP, Hawai‘i | Hoshide, Price, & Katahira, 1990; Hess & Banko, 2006 |
| Hawaiian Petrel, ‘Ua‘u (*Pterodroma sandwichensis*) | Endangered | Vulnerable | Mauna Loa, Hawai‘i, Haleakalā, Maui | Hess, Hansen, Nelson, Swift, & Banko, 2007; Judge, Lippert, Misajon, Hu, & Hess, 2012; Simons, 1983 |
| Hawaiian Stilt, Ae‘o (*Himantopus mexicanus knudseni*) | Endangered | n/a | Hanalei NWR, Kaua‘i, unspecified, Maui/ O‘ahu/Kaua‘i | KNWRC, 2015; Robinson, Reed, Skorupa, & Oring, 1999 |
| Tiwi (*Drepanis coccinea*) | Threatened | Vulnerable | Hakalau NWR, Hawai‘i, Mauna Kea FR, Hawai‘i | Smucker, Lindsay, & Mosher, 2000; Snetsinger et al., 1994 |
| Maui ‘Alauahio (*Paroreomyza montana*) | Unlisted | Endangered | Hanawi, Maui | Kowalsky et al., 2002 |
| Newell’s Shearwater, ‘A‘o (*Puffinus novelli*) | Threatened | Endangered | undisclosed, Kaua‘i | Ainley, Podolsky, DeForest, Spencer, & Nur, 2001; KESRP, 2015 |
| ‘Ōma‘o (*Myadestes obscurus*) | Unlisted | Vulnerable | Hakalau NWR, Hawai‘i | Smucker et al., 2000 |
| Palila (*Loxioides bailleui*) | Endangered | Crit. endangered | Mauna Kea FR, Hawai‘i | Laut, Banko, & Gray, 2003 |
| Wedge-tailed Shearwaters, ‘Ua‘u kani (*Ardenna pacifica*) | Unlisted | Least concern | Mālaekahana and Moku‘auia, O‘ahu | Smith, Polhemus, & VanderWerf, 2002 |

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**a**The gallinule and stilt subspecies are not listed independent by IUCN so have a “n/a” entered.

**b**Mauna Kea FR is the State of Hawai‘is Mauna Kea Forest Reserve and is a “donut” of subalpine mixed dry forest around Mauna Kea; Hakalau NWR is the Hakalau Forest National Wildlife Refuge, a wet ‘ōhi‘a-koa forest on the lower eastern slope of Mauna Kea.
### Table A2: Locations of important bird sites on the island of O'ahu, Hawai'i

| Location name                                          |
|--------------------------------------------------------|
| 'Aiea Loop Trail                                       |
| Black Point                                            |
| Chevron Refinery                                       |
| Enchanted Lakes Shopping Center                        |
| End of Road Ka'ena (West Side)                         |
| Ewa Forest Reserve                                     |
| Hale'iwa/Pua'ena Point Beach Park                     |
| Hamakua Marsh                                          |
| Hanauma Bay                                           |
| Hawaii Prince Golf Club                                |
| He'eua Pond/Kaneohe Bay Boat Ramp                      |
| Hoakalei Country Club                                  |
| Ho'omaluhia Botanical Garden                           |
| James Campbell National Wildlife Refuge                |
| Ka'ena                                                 |
| Kahana Bay Beach Park                                 |
| Kahuku Golf Course                                    |
| Kaiaka Bay Beach Park                                 |
| Kailua District Park                                   |
| Kalaniana'ole Highway Cliffs                           |
| Kalihi Valley                                          |
| Kawainui Marsh                                         |
| Kawainui Park                                          |
| Kawela Bay Beach Park                                 |
| Ke'awa Heiau State Park                               |
| Keolu Hills Neighborhood Park                         |
| Koko Head Crater                                       |
| Kualoa Beach Park                                     |
| Lagoon Drive                                           |
| La'ie Beach Park                                       |
| Lyon Arboretum/Manoa Falls Trail                       |
| Makaha Valley                                          |
| Makapu'u                                               |
| Malaekahana                                            |
| Maunawili Park                                         |
| Mokule'ia Rock Quarry                                 |
| Nanakuli                                               |
| Nu'uanu Reservoir Area                                 |
| O'ahu Forest National Wildlife Refuge                  |
| Paiko Lagoon                                           |
| Pearl Harbor National Wildlife Refuge                  |
| Pearl Harbor National Wildlife Refuge Kalaeloa         |

(Continues)