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# TERRESTRIAL PATHOGEN POLLUTANT, *TOXOPLASMA GONDII*, THREATENS HAWAIIAN MONK SEALS (*NEOMONACHUS SCHAUINSLANDI*) FOLLOWING HEAVY RUNOFF EVENTS

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**ABSTRACT:** Toxoplasmosis is a major threat to Hawaiian monk seals (*Neomonachus schauinslandi*) in the main Hawaiian Islands where seal habitat overlaps with substantial human and domestic cat populations. As the definitive hosts, members of the Felidae are the sole sources contaminating the environment with infectious oocysts; these oocysts can be transported into the marine environment, thereby threatening marine mammals. To understand environmental factors influencing Hawaiian monk seal exposure to *Toxoplasma gondii*, we examined monk seal strandings from toxoplasmosis in relationship to location and rainfall patterns throughout the main Hawaiian Islands. Using a case-control study design, we compared mortalities due to toxoplasmosis (cases) with those from other causes (controls). We found that cases were up to 25 times more likely than controls to occur after heavy runoff events. The greatest odds ratio was observed when rainfall occurred 3 wk before strandings, potentially indicating important timelines in the disease process. Our results suggest that heavy rainfall frequently delivers sufficient numbers of oocysts to infect Hawaiian monk seals. With infectious doses as low as a single oocyst, any contaminated runoff constitutes a risk to Hawaii's endangered monk seal.

**Key words:** Hawaiian monk seal, marine mammals, odds ratio, pathogen pollution, toxoplasmosis.

## INTRODUCTION

Hawaiian monk seals (*Neomonachus schauinslandi*) in the human-populated main Hawaiian Islands (MHI; Ni'ihau to Hawai'i Island; Fig. 1) make up a crucial component of the species' recovery potential. After decades of precipitous decline (Kenyon 1973; Fiscus et al. 1978; Johnson et al. 1982), the range-wide population has shown signs of a stabilizing-to-positive trend since the mid-2010s (Baker et al. 2016). Seals in the MHI constitute only approximately 20% of the total Hawaiian monk seal population (approximately 300 seals in the MHI and 1,100 in the Northwestern Hawaiian Islands; Carretta et al. 2022). Nevertheless, this population plays a key role in the recovery trend, with the highest growth rate (Baker et al. 2011) and reproductive rate (Robinson et al. 2021) of those seen throughout the monk seals' range.

Toxoplasmosis, a disease caused by the protozoan parasite *Toxoplasma gondii*, was

first identified as the cause of death in a wild Hawaiian monk seal in 2004 (Honnold et al. 2005). Such mortalities have become increasingly prevalent in the MHI since the early 2000s: eight cases were detected during 1982–2015 from 306 monk seal carcasses and a further seven from 38 carcasses examined from 2016 to August 2021 in the human- (and cat)-populated MHI (Barbieri et al. 2016; Harting et al. 2021). Only one case has been detected from the Northwestern Hawaiian Islands (remote, without human or cat populations) since regular monitoring and carcass examination began in 1982 (Barbieri et al. 2016). Reports from other species (cats, birds, and spinner dolphins [*Stenella longirostris*]) indicate that *T. gondii* has long been established in the MHI (Wallace 1971; Migaki et al. 1990; Work et al. 2000, 2002). The increase in monk seal cases is probably coincident with increased opportunities for exposure as the MHI seal population has rebounded over this time frame (Baker et al. 2011, 2016).

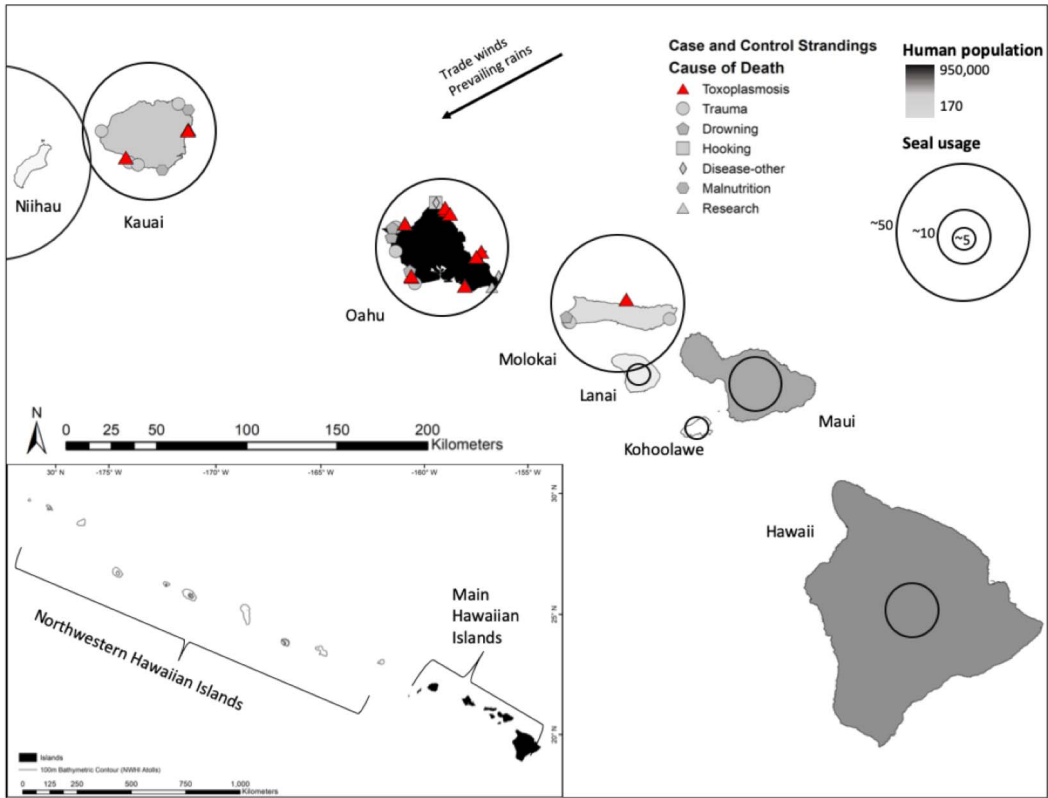


FIGURE 1. Map of the main Hawaiian Islands that comprises the study area where we evaluated associations between high runoff events and Hawaiian monk seal (*Neomonachus schauinslandi*) strandings of varied causes during 2005–21. The distributions of both the human and seal populations across the main Hawaiian Islands are shown. Locations of strandings identified as toxoplasmosis cases (bold triangles) and controls (other shapes) used in this study are indicated.

Toxoplasmosis is now the leading disease impacting monk seals and is one of the three major causes of death in the MHI, along with anthropogenic trauma and net-related drownings (Harting et al. 2021). Recent estimates suggest that toxoplasmosis could be dampening the potential growth rate of the MHI monk seal population, particularly through lost reproductive potential with the deaths of adult females, lost pregnancies, and neonatal mortality of pups following vertical transmission (Barbieri et al. 2016; Harting et al. 2021).

*Toxoplasma gondii* is capable of infecting a wide range of warm-blooded intermediate hosts, but can only complete sexual reproduction, producing oocysts, in the intestines of members of the Felidae, the definitive hosts (Hutchison et al. 1969). Infected felids may

shed millions of oocysts in their feces, leading to substantial environmental contamination (Dubey et al. 1970; Dubey 1995; Fritz et al. 2012). Torrey and Yolken (2013) reported that the environmental load of oocysts in the US is more than 1.2 million metric tons. Sporulated oocysts are extremely hardy, persisting and maintaining infectivity for >1 yr in soil, freshwater, or saltwater, making them the critical parasite stage that drives the environmental transmission of *T. gondii* (Miller et al. 1972; Dubey 1998; Lindsay and Dubey 2009). *Toxoplasma gondii* has caused infections and strandings in marine mammals worldwide and in every ocean basin, demonstrating the ability of this terrestrial pathogen to infiltrate marine environments (Dubey et al. 2003; Gibson et al. 2011). Extensive research has demonstrat-

ed that hydrologic processes may deliver oocysts from wide catchment areas into the marine environment (Conrad et al. 2005; VanWormer et al. 2016). For example, in California, cases of protozoal disease in sea otters (*Enhydra lutris nereis*) have been linked to heavy rainfall events leading to elevated freshwater runoff flushing oocysts to coastal waters (Shapiro et al. 2012, 2019).

In this study, we aimed to demonstrate the dynamics of a land-to-sea pathway linking the terrestrial source of toxoplasmosis to the infection of Hawaiian monk seals in the marine environment. We used a case-control study design to investigate correlations between high freshwater runoff events and known cases of toxoplasmosis in Hawaiian monk seals. If cases of toxoplasmosis are associated with increased surface water runoff, this would demonstrate that 1) Hawaii's source of infectious material is local (flushed from the islands to nearshore waters) and 2) high runoff events have the potential to precipitate acute disease, leading to monk seal strandings. In addition, we evaluated the timing of elevated stranding risk to gain insight into the disease process and examine the spatial distribution of toxoplasmosis cases.

## MATERIALS AND METHODS

### Study area

Our study was conducted within the main (populated) Hawaiian Islands. Although monk seals use all islands, toxoplasmosis cases have been confirmed only on three islands—Kaua'i, O'ahu, and Moloka'i—making these islands our focus. Each of these islands receives similar monk seal use (based on number of unique individuals sighted within a given year; Fig. 1). However, the human population varies by orders of magnitude among these islands (O'ahu, ~950,000; Kaua'i, ~67,000; Moloka'i, ~7,000; US Census 2015; Fig. 1) and may influence the detection of monk seals, which are typically reported by community members or volunteers.

### Sampling and diagnostics

Hawaiian monk seals in the MHI are monitored through a well-established network including survey efforts by the National Oceanographic and Atmospheric Administration (NOAA) and

partner institutions, along with citizen science reporting. Sighting reports from all sources are recorded in a NOAA database (Pacific Islands Fisheries Science Center 2020a). Trained teams respond as soon as possible any time a stranding is detected. Depending on the level of decomposition, varying levels of examination lead to variable certainty in diagnosis (for details, see Harting et al. 2021). We restricted our analysis to carcasses that were necropsied and assigned a probable cause of mortality. Numerous samples were collected at necropsy, including blood, tissue, and swabs. Fixed samples were examined histologically by board-certified veterinary pathologists according to standard methods.

### Case-control criteria

We included toxoplasmosis cases and suspect cases from the earliest detections in the MHI from 2004 to 2021. Cases ( $n=12$ ) and suspect cases ( $n=1$ ) included in this analysis were determined based on the case definitions described in Barbieri et al. (2016). In brief, for cases this required the presence of *T. gondii* organisms (cysts or tachyzoites) in association with histopathologic lesions that were severe enough to cause death and confirmed through immunohistochemistry (IHC). Seals with tissues that tested positive for *T. gondii* by PCR or were serum positive for protozoal immunoglobulin G antibodies alone were considered suspect cases (Barbieri et al. 2016). We excluded one case of a neonatal pup that was found dead 1 d separated from the dam. Although the case definition (presence of organisms and associated lesions) was met for both the neonate and the dam, these cases were considered to be a product of a single exposure event (to the female and then passed vertically to the pup). One suspect case (*T. gondii* detected by PCR, but no IHC conducted) was excluded, because although *T. gondii* infection was probable, the case did not meet the case definition criteria, and the carcass was detected offshore, making it impossible to link to a particular stranding location. One other suspect case (based on detection of protozoal cysts, histologic lesions consistent with protozoal disease, *T. gondii* detected by PCR rather than IHC) was included because toxoplasmosis was considered 90% probable as the cause of death (Harting et al. 2021) and location information was available to evaluate exposure. In total, we included nine cases on O'ahu, three on Kaua'i, and one on Moloka'i (see Supplementary Material Table S1). Within this dataset, we noted two clusters of cases: one cluster in which three animals stranded within 3 d in 2018 and one cluster in which two animals stranded within 8 d in 2020.

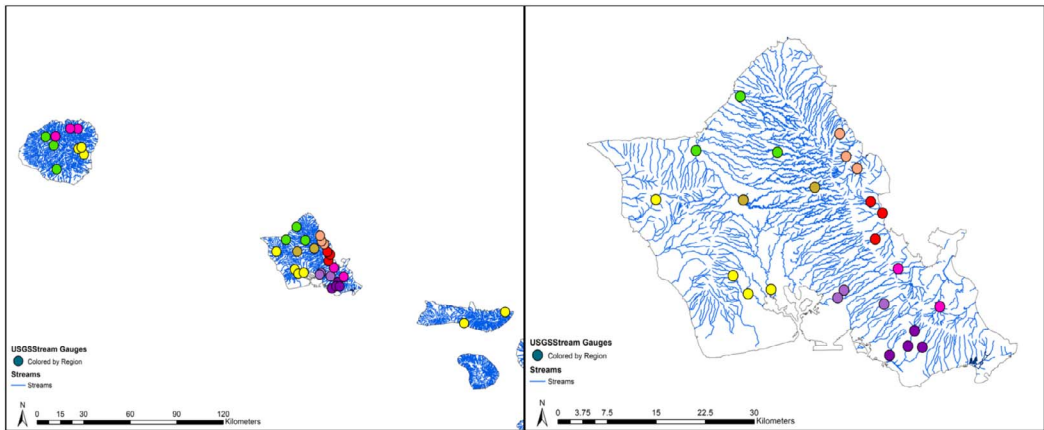


FIGURE 2. Hydrologic maps showing the stream gauge locations used to assess runoff intensity relative to potential risk of toxoplasmosis for Hawaiian monk seals (*Neomonachus schauinslandi*) on the Hawaiian Islands of Kaua'i, O'ahu, and Moloka'i. Different shades signify different regional groupings of stream gauge data.

**Controls:** To identify appropriate control animals, we evaluated stranding events with known or probable causes of death (Harting et al. 2021) and selected seals from the same island and as close as possible in time to each case seal (typically within the same year, always within 2 yr). Where multiple qualifying controls were available, the number of controls may exceed the number of cases. We excluded neonatal mortalities, nursing pups, and recently weaned pups (not yet expected to be foraging independently). In addition, we excluded one seal (an adult male on O'ahu) that was found dead in a fishing net but that had exhibited a high *T. gondii* titer (1:81,920 via immunofluorescence assay) during a research-related examination 2 mo before death. During research handlings, blood is typically collected and screened for several pathogens, including *T. gondii*, but this finding of positive titers in otherwise healthy animals is a rarity because most toxoplasmosis cases are associated with lethal strandings (Pacific Islands Fisheries Science Center 2020b). Although this animal's death was presumed to be unrelated to *T. gondii*, his exposure history might not have been consistent with control animals. We included a total of 11 controls on O'ahu: 7 on Kaua'i and 4 on Moloka'i (see Supplementary Material Table S1).

### Runoff data

In the Hawaiian ecosystem (without snow/ice melt), freshwater runoff derives from rainfall events. We found that stream gauges provided the most widely distributed data source for measuring water inputs or outflows across the islands. We evaluated land-to-sea runoff based on daily mean stream discharge (cubic feet per

second) data from stream gauge data spanning 2000–21 (USGS 2021). We computed flow exceedance probabilities (EPs), the percentage of days that stream flow at a particular site is likely to exceed a given level, at each gauge station to serve as a metric defining “high” runoff events. We first added 0.01 ft<sup>3</sup>/s to all values to avoid zeros and then log-transformed values to normalize the data. Finally, we assigned quantiles based on the 20-yr dataset of mean daily discharge (following USGS protocols; Risley et al. 2008). Because we were unsure what level of discharge might most affect monk seal risk of *T. gondii* exposure, we evaluated high runoff events defined at three EP levels: 5% corresponding to the upper end of a 90% confidence interval (EP<sub>5</sub>), 2.5% (upper end of a 95% confidence interval [EP<sub>2.5</sub>]), and 1% (upper end of a 98% confidence interval [EP<sub>1</sub>]). We grouped stream gauges in adjacent watersheds into regions so that each stranding was associated with three or four gauges representing the region of the stranding (except on Moloka'i where only a single gauge had adequate flow data; Fig. 2).

### Statistical analysis

**Odds ratio:** We used an odds ratio test (computed in the R package epitools; Aragon et al. 2017) to assess the differential odds of strandings due to toxoplasmosis versus other causes relative to exposure to high runoff events. We judged significance of the odds ratio based on a Fisher exact *P* value with correction for small sample size. Although diagnostic evaluation of toxoplasmosis in Hawaiian monk seals suggests that it is acutely lethal, little is known about the timeline between exposure and disease. There-

fore, we evaluated the association of high runoff events over 1-wk intervals in the 1–8 wk before each stranding.

**Randomizations:** In addition to comparing the exposure history of cases to controls, we used a randomization procedure to determine the probability that the exposure patterns observed in toxoplasmosis cases would have occurred by chance given local rainfall patterns (R Core Team 2013). We generated a set of nine random dates spanning 2004–21, mimicking the number and time span of toxoplasmosis cases on O‘ahu, the island with the most cases. Each random date was randomly assigned to an O‘ahu region and then stream flow data were compiled from the appropriate gauges. We generated datasets spanning 1 wk before each random date (noting that in a randomized dataset, a single week can serve as the comparison to any week 1–8). We calculated the number of high runoff events in each random dataset and determined the probability that the random dataset would meet or exceed the number of events in the observed dataset based on 1,000 replications. In addition, we used the randomizations to evaluate the probability that five of the nine O‘ahu cases would occur on the windward (rainier) side of the island by chance.

## RESULTS

### Runoff exposure of cases and controls

In exploring the runoff data before each stranding, there appeared to be a clear concentration of high runoff events in the month before toxoplasmosis cases on O‘ahu (Fig. 3A). Nearly all (eight of nine) cases were exposed to EP<sub>5</sub> events in the month (and specifically in the third week) before the stranding date, with half of the cases being exposed to more than 10 such events. Meanwhile, only 6 of 11 controls were exposed to (always fewer than 10) EP<sub>5</sub> events. However, other islands did not exhibit such a clear pattern. On Kaua‘i and Moloka‘i, high runoff events appeared to be equally distributed among both cases and controls (Fig. 3B).

### Statistical analysis

Odds ratios were elevated indicating that toxoplasmosis cases were more likely to be exposed to high runoff events than controls (Table 1). The highest odds ratio, 25.66 (using the EP<sub>5</sub> metric), was detected on O‘ahu 3 wk

before stranding date (Fig. 4). Both EP<sub>2</sub> and EP<sub>1</sub> metrics were also elevated at 3 wk before stranding date (Fig. 4). Some odds ratios were significant in the all-islands dataset, but patterns generally tracked those in the O‘ahu-only dataset (which had the majority of cases). Randomizations showed very low probability that the observed number of high runoff events would occur by chance in any given week before strandings on randomly generated dates (Table 1). However, the randomizations did not show a significant pattern in the spatial distribution of cases on O‘ahu, with a probability of 0.503 that five of the nine cases could randomly occur in the Windward regions.

## DISCUSSION

Both case-control and randomization analyses showed a significantly elevated risk of toxoplasmosis cases associated with exposure to high runoff events. This pattern was clear despite the small size of the Hawaiian monk seal population and thus the low sample numbers for cases and controls. Both of the case clusters, from 2018 and 2020, closely followed major runoff events, adding to the detected signal. Although this association is expected given the existing literature on pathogen pollution of *T. gondii* (Aguirre et al. 2019), demonstrating this route of exposure for Hawaiian monk seals clarifies disease dynamics and risk factors and provides a scientific foundation for risk management actions.

### Disease dynamics

Odds ratios were highest 2–4 wk before stranding, with a peak 3 wk before the stranding date. There were zero controls with exposure within week 3, which might have driven up the odds ratio by coincidence. However, even without considering control seals, week 3 was statistically significant: seven of the eight O‘ahu cases were exposed to EP<sub>5</sub> runoff events within the third week before stranding, whereas other weeks showed three to six case seals with EP<sub>5</sub> exposure. Random-

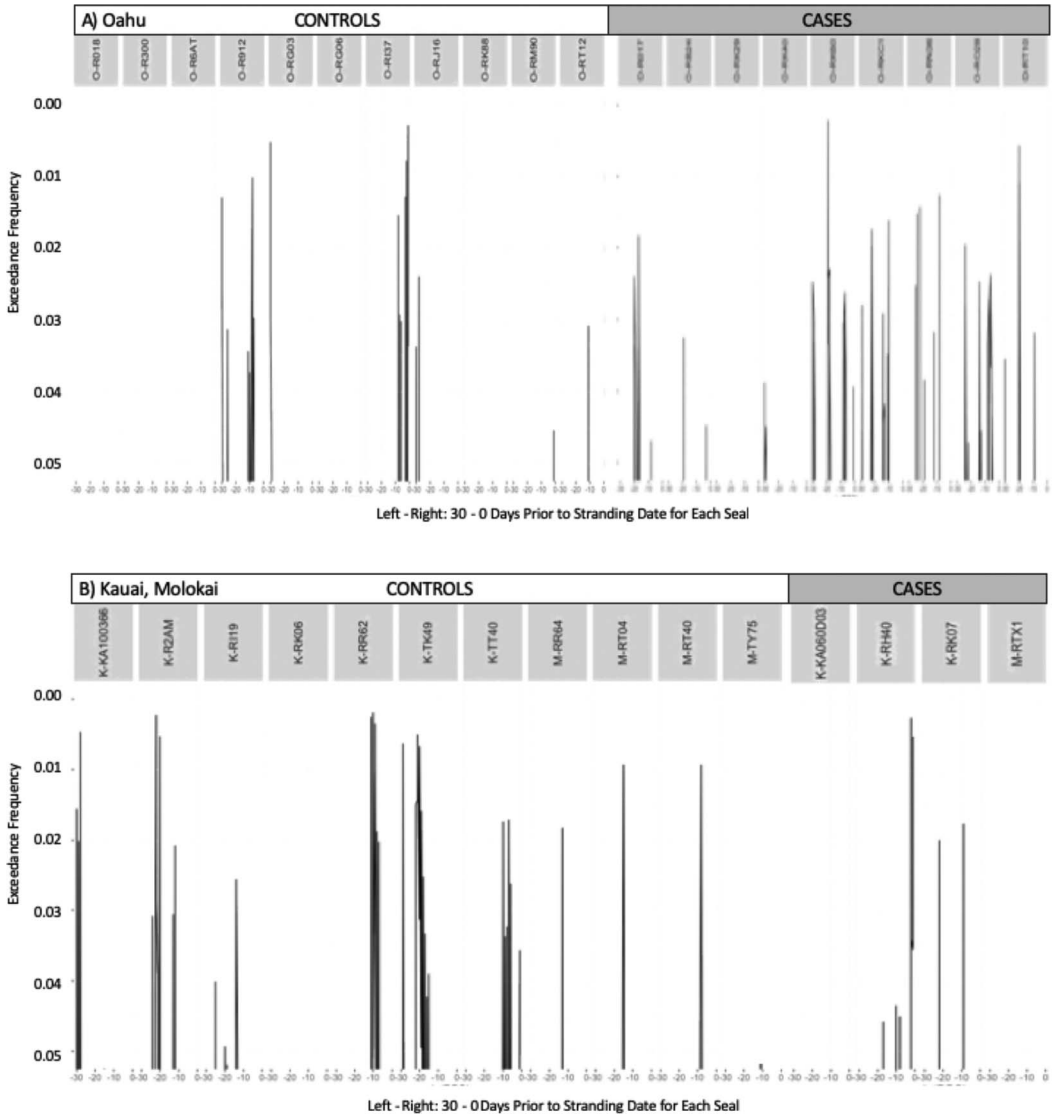


FIGURE 3. Time relationship graphs, with each line in the graphs showing an exposure to a high runoff event in the month preceding a Hawaiian monk seal (*Neomonachus schauinslandi*) stranding event in the main Hawaiian Islands: (A) O'ahu and (B) Kaua'i and Molokai. The frequency of these exposures in cases versus controls was used to assess toxoplasmosis risk.

izations offered additional evidence that the observed temporal clustering of exposures would be highly unlikely by chance. Detection of extensive cellular inflammation and necrosis paired with a high abundance of *T. gondii* organisms in toxoplasmosis cases has suggested that the progression of disease after infection can rapidly disseminate, leading to acute death (Barbieri et al. 2016), but there

has been little information to determine the time of exposure in wild Hawaiian monk seals. Our findings, indicating an elevated risk of stranding approximately 3 wk after exposure to heavy runoff, suggest that morbidity and mortality in infected Hawaiian monk seals develop relatively soon after exposure. Although some species (e.g., humans) may experience latent or subclinical infections

TABLE 1. Number of case and control Hawaiian monk seals (*Neomonachus schauinslandi*) exposed to high runoff events at varying time points before stranding in the main Hawaiian Islands (2005–21). Superscript numbers with case and control numbers indicate the probability level of observing the given number of exposures in 1,000 replicates with  $n$  randomized dates. ORs (displayed as odds of exposure for cases:controls) show that cases were generally more likely than controls to be exposed to high runoff events in the weeks before the stranding date. Superscripts on OR values indicate a significant  $P$  value based on a Fisher exact test.<sup>a</sup>

Weeks PSD	Metrics by exceedance probability								
	EP <sub>5</sub>			EP <sub>2</sub>			EP <sub>1</sub>		
	Controls	Cases	OR	Controls	Cases	OR	Controls	Cases	OR
O'ahu only, $n$	11	9		11	9		11	9	
8	6 <sup>0.05</sup>	4	0.48	3	3	0.86	0	3 <sup>0.05</sup>	4.71 <sup>0.01</sup>
7	5	4	0.67	3	3	0.86	1	2	1.25
6	3	5 <sup>0.01</sup>	2.00	3	5 <sup>0.05</sup>	2.00	2	2	0.75
5	5	6 <sup>0.05</sup>	1.50	4 <sup>0.01</sup>	6 <sup>0.005</sup>	2.10	2	1	0.33
4	1	3	2.14	0	3	4.71 <sup>0.01</sup>	0	0	0.00
3	0 <sup>0.05</sup>	7 <sup>0.005</sup>	25.67 <sup>0.005</sup>	0	3	4.71 <sup>0.01</sup>	0	2	2.75
2	3	6 <sup>0.05</sup>	3.00	2	3	1.29	1	1	0.56
1	2	3	1.29	1	1	0.56	1	1	0.56
All islands, $n$	22	13		22	13		22	13	
8	10 <sup>0.05</sup>	7 <sup>0.05</sup>	1.09	6	5 <sup>0.05</sup>	1.27	2	5 <sup>0.005</sup>	3.70 <sup>0.01</sup>
7	8	6 <sup>0.01</sup>	1.17	3	5 <sup>0.05</sup>	2.64	1	2	1.75
6	7	5	1.04	5	5 <sup>0.05</sup>	1.57	3	2	0.79
5	7	6 <sup>0.01</sup>	1.41	6	6 <sup>0.05</sup>	1.71	4	1	0.28
4	4	4	1.44	2	4	2.67	2	0	0.00
3	4	8 <sup>0.005</sup>	4.8 <sup>0.05</sup>	3	3	1.30	3	2	0.79
2	10 <sup>0.05</sup>	8 <sup>0.005</sup>	1.45	7 <sup>0.01</sup>	4	0.75	3	1	0.37
1	4	4	1.44	3	2	0.79	1	2	1.75

<sup>a</sup> PSD = prior to stranding date; EP<sub>5</sub> = exceedance probability 5%; EP<sub>2</sub> = exceedance probability 2.5%; EP<sub>1</sub> = exceedance probability 1%; OR = odds ratio.

leading to severe disease primarily with immunosuppression (Dubey and Jones 2008), we did not detect this pattern in Hawaiian monk seals.

### Risk factors

All of the high runoff metrics showed association with elevated exposure risk in toxoplasmosis cases. Because the EP<sub>5</sub> threshold was more frequently crossed, the accumulation of these events led to the highest odds ratios. Fewer events exceeded the EP<sub>2</sub> and EP<sub>1</sub> thresholds, but these events were also associated with cases (Fig. 3), although their rarity in the overall data may have led to lower odds ratios. With a small dataset, it is difficult to measure the added risk that might come with additional runoff, but our findings indicate that exposure to runoff events in the

upper 5% is sufficient to pose significant toxoplasmosis stranding risk to monk seals. It is probable that risk is at least equally high with increased runoff, making any extremely high runoff high risk.

Hawaiian monk seals have substantial home ranges to accommodate their foraging activities; however, their core use areas are typically much smaller (149.2 km<sup>2</sup> mean home range, 23.2 km<sup>2</sup> mean core area; Wilson et al. 2017). Monk seals in the MHI make nearshore or offshore foraging trips typically lasting from 0.5 to 1 d, during which seals travel a total of 10–50 km and dive to depths of 20–50 m (Wilson et al. 2017). This makes their time concentrated in the nearshore areas where exposure to runoff and island-based pollution is most likely (Littnan et al. 2006; Lopez et al. 2012). Based on haul-out sightings, seals have



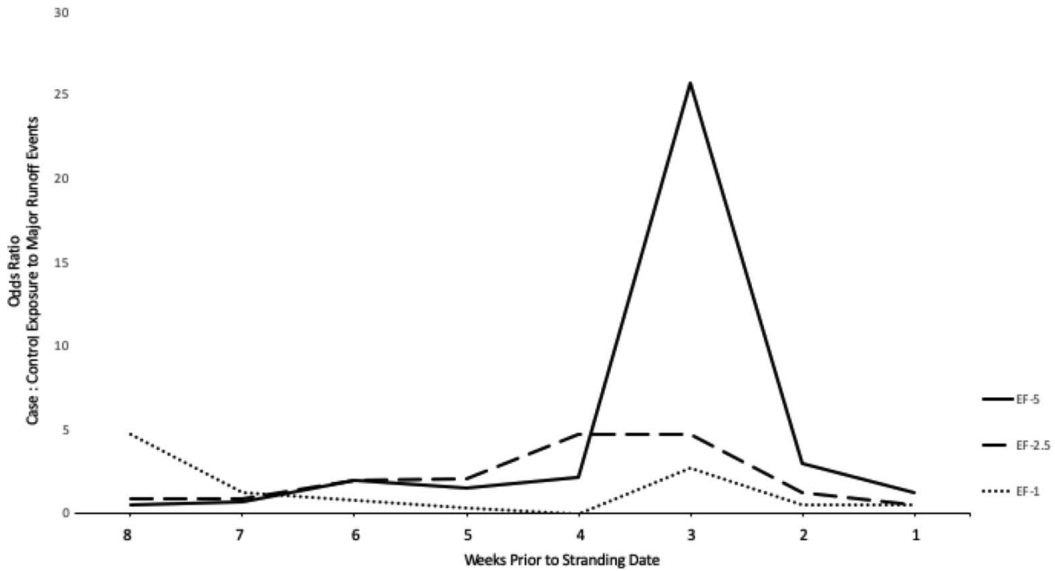


FIGURE 4. Line graph showing a peak in the odds ratio indicating an association of risk of toxoplasmosis in Hawaiian monk seals (*Neomonachus schauinslandi*) with exposure to major runoff events 3–4 wk before the stranding event, based on analysis of cases and controls on the island of O‘ahu, Hawai‘i, 2005–21.

a tendency to favor certain beaches or a cluster of nearby beaches, spending an average of 74.69% of their haul-out time in a single region (range, 21.71–100.00%; SD, 23.08%; Pacific Islands Fisheries Science Center 2020a). Thus, although monk seals can be far ranging, their typical regional fidelity suggests that their stranding location would probably be in or near the area recently used and that their exposure to *T. gondii* oocysts would be associated with oocyst contamination of that area. Most of the major rain events in the dataset led to high runoff in several regions (perhaps unsurprising given the severity of EP<sub>5</sub>, EP<sub>2</sub>, or EP<sub>1</sub> and the small size of the islands); therefore, this timeline of exposure after major rain is likely to be important whether a monk seal was exposed in its region of stranding or other regions.

It was clear that the O‘ahu data were highly influential on the all-islands analysis. Despite similar levels of monk seal use on O‘ahu, Kaua‘i, and Moloka‘i, toxoplasmosis-related strandings were far more prevalent on O‘ahu, making this island a more data-rich area for analysis. Although separating O‘ahu provided a clearer picture of disease dynamics on that

island, Kaua‘i (three cases) and Moloka‘i (one case) were too data limited to examine separately. However, generally the controls exposed to high runoff were mostly from Kaua‘i or Moloka‘i, and the cases on those islands were not strongly associated with high runoff events (Fig. 3).

There are several possible reasons for the lack of association with toxoplasmosis and runoff exposure on islands beyond O‘ahu. First, stream gauge data were not consistently available across all three islands. Despite having similar rugged topography and numerous small streams, Kaua‘i and Moloka‘i had many fewer USGS stream gauges than the more populous O‘ahu. On Kaua‘i stream regions were larger and on Moloka‘i only a single stream gauge had adequate data. Thus, the limited runoff data on these islands may have been less representative of the exposure experienced by seals.

The low number of cases detected on Kaua‘i or Moloka‘i might lead to inability to detect patterns. Most areas of Kaua‘i are accessible to agency staff or volunteers who monitor monk seals, and sighting data are of similar quality to O‘ahu. The ratio of seal

carcasses detected (as opposed to seals disappearing) and examined is also similar, so there is little reason to expect missed cases or biased cause-of-death determination on Kaua'i. Moloka'i is a more rural island, with only a few areas where monk seals are closely monitored, and a higher proportion of seals disappears from sighting data without carcass detection, although after carcass detection there is no reason to suspect an island-specific bias in cause-of-death determination.

Finally, a potential reason underlying the elevation in cases on O'ahu is the markedly higher human population and thus higher population of outdoor owned and feral cats. Hawai'i has no native felids, leaving domestic cats as the only contributors of oocyst pathogen pollution (Hess et al. 2007). Telephone surveys contracted by the Hawai'i Humane Society support the association of pet cats with the number of human households on the islands; numbers of stray cats (solo or in colonies) are less well tracked, but they are also expected to vary according to human feeding efforts (Ward Research Inc. 2018). Given the larger cat population, the documentation of more cases on O'ahu may be a function of increased pathogen pollution. On other islands, with fewer cats, it is possible that the association with runoff was not detected because the environmental oocyst loading would be lower and even high runoff events might not always bring as substantial a flush of infectious oocysts to coastal waters. Nevertheless, with infectious doses potentially as low as a single oocyst (Dubey et al. 2012), the risk may still be sufficient to negatively affect this endangered population—and indeed, although documented less frequently, seals on these islands still die from toxoplasmosis. Furthermore, given the hardiness of *T. gondii* oocysts and the circulation of water between islands, oocysts may be transported between islands, presenting a threat that is not temporally linked to the runoff process. Using particle movement models, Wren et al. (2016) found that self-recruitment (particles remaining near the island of origin) was high, but particle movement between islands could

be up to 30%. There was a general directionality of flow from southeast to northwest, with islands of O'ahu, Kaua'i, Ni'ihau, and Nihoa most connected. The Maui complex of islands (Maui, Moloka'i, Lana'i, and Kaho'olawe) and Hawai'i Island are separated by substantial channels and received lower interarea connectivity.

Improving our understanding of environmental routes of *T. gondii* exposure is a first step in mitigating the conservation risk posed by this pathogen. Management of outdoor cat populations is a critical component of mitigating risk of *T. gondii* exposure, not only for monk seals but also to limit human exposure (Torrey and Yolken 2013) and protect ecosystems more broadly (Aguirre et al. 2019). Although controlling oocyst input is the ultimate key to risk mitigation, discharge into marine systems may also be mitigated by managing terrestrial runoff so that oocysts might be settled or filtered out before reaching the coastal environment (Shapiro et al. 2010; Hogan et al. 2013).

#### ACKNOWLEDGMENTS

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#### DATA AVAILABILITY

All data used in this study are publicly available through the National Marine Fisheries Service institutional data repository at <https://www.fisheries.noaa.gov/inport/item/5673>.

## SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at <http://dx.doi.org/10.7589/JWD-D-21-000179>.

## LITERATURE CITED

- Aguirre AA, Longcore T, Barbieri M, Dabritz D, Hill D, Klein PN, Lepezyk C, Lilly EL, McLeod R, et al. 2019. The one health approach to toxoplasmosis: Epidemiology, control, and prevention strategies. *EcoHealth* 16:378–390.
- Aragon TJ, Fay MP, Wollschlaeger D, Omidpanah A, Omidpanah MA. 2017. *Package 'epitools'*. <https://cran.r-project.org/web/packages/epitools/index.html>. Accessed March 2021.
- Baker JD, Harting AL, Johanos TC, Littnan CL. 2016. Estimating Hawaiian monk seal range-wide abundance and associated uncertainty. *Endanger Species Res* 31:317–324.
- Baker JD, Harting AL, Wurth TA, Johanos TC. 2011. Dramatic shifts in Hawaiian monk seal distribution predicted from divergent regional trends. *Mar Mamm Sci* 27:78–93.
- Barbieri MM, Kashinsky L, Rotstein DS, Colegrove KM, Haman KH, Magargal SL, Sweeny AR, Kaufman AC, Grigg ME, Littnan CL. 2016. Protozoal-related mortalities in endangered Hawaiian monk seals *Neomonachus schauinslandi*. *Dis Aquat Org* 121: 85–95.
- Carretta JV, Oleson EM, Forney KA, Muto MM, Weller DW, Lang AR, Baker JD, Hanson B, Orr A, et al. 2022. *U.S. Pacific marine mammal stock assessments: 2021*. NOAA Technical Memo NOAA-TM-NMFS-SWFSC-358291. <https://repository.library.noaa.gov/view/noaa/44406>. Accessed March 2021.
- Conrad PA, Miller MA, Kreuder C, James ER, Mazet J, Dabritz H, Jessup D, Gulland F, Grigg M. 2005. Transmission of *Toxoplasma*: Clues from the study of sea otters as sentinels of *Toxoplasma gondii* flow into the marine environment. *Int J Parasitol* 35:1155–1168.
- Dubey J. 1995. Duration of immunity to shedding of *Toxoplasma gondii* oocysts by cats. *J Parasitol* 81: 410–415.
- Dubey J. 1998. *Toxoplasma gondii* oocyst survival under defined temperatures. *J Parasitol* 84:862–865.
- Dubey J, Miller NL, Frenkel J. 1970. The *Toxoplasma gondii* oocyst from cat feces. *J Exp Med* 132:636–662.
- Dubey JP, Ferreira LR, Martins J, McLeod R. 2012. Oral oocyst-induced mouse model of toxoplasmosis: Effect of infection with *Toxoplasma gondii* strains of different genotypes, dose, and mouse strains (transgenic, out-bred, in-bred) on pathogenesis and mortality. *Parasitology* 139:1–13.
- Dubey JP, Jones JL. 2008. *Toxoplasma gondii* infection in humans and animals in the United States. *Int J Parasitol* 38:1257–1278.
- Dubey JP, Zarnke R, Thomas NJ, Wong SK, Van Bonn W, Briggs M, Davis JK, Ewing R, Mense M, et al. 2003. *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*, and *Sarcocystis canis*-like infections in marine mammals. *Vet Parasitol* 116:275–296.
- Fiscus CH, Johnson AM, Kenyon KW. 1978. *Hawaiian monk seal (Monachus schauinslandi) survey of the northwestern (Leeward) Hawaiian Islands, July 1978*. National Marine Fisheries Service report, Seattle, Washington. <https://repository.library.noaa.gov/view/noaa/30211>. Accessed March 2021.
- Fritz H, Barr B, Packham A, Melli A, Conrad PA. 2012. Methods to produce and safely work with large numbers of *Toxoplasma gondii* oocysts and bradyzoite cysts. *J Microbiol Methods* 88:47–52.
- Gibson AK, Raverty S, Lambourn DM, Huggins J, Magargal SL, Grigg ME. 2011. Polyparasitism is associated with increased disease severity in *Toxoplasma gondii*-infected marine sentinel species. *PLoS Negl Trop Dis* 5:e1142.
- Harting AL, Barbieri MM, Baker JD, Mercer TA, Johanos TC, Robinson SJ, Littnan CL, Colegrove KM, Rotstein DS. 2021. Population-level impacts of natural and anthropogenic causes-of-death for Hawaiian monk seals in the main Hawaiian Islands. *Mar Mamm Sci* 37:235–250.
- Hess SC, Hansen H, Banko PC. 2007. *Ecology of an invasive predator in Hawaii*. USDA National Wildlife Research Center Symposia Proceedings, University of Nebraska Press, Lincoln, Nebraska. <https://digitalcommons.unl.edu/nwrcinvasive>. Accessed March 2021.
- Hogan JN, Daniels ME, Watson FG, Oates SC, Miller MA, Conrad PA, Shapiro K, Hardin D, Dominik C, et al. 2013. Hydrologic and vegetative removal of *Cryptosporidium parvum*, *Giardia lamblia*, and *Toxoplasma gondii* surrogate microspheres in coastal wetlands. *Appl Environ Microbiol* 79:1859–1865.
- Honnold SP, Braun R, Scott DP, Sreekumar C, Dubey J. 2005. Toxoplasmosis in a Hawaiian monk seal (*Monachus schauinslandi*). *J Parasitol* 91:695–697.
- Hutchison W, Dunachie J, Siim JC, Work K. 1969. Life cycle of *Toxoplasma gondii*. *Br Med J* 4:806.
- Johnson A, DeLong RL, Fiscus CH, Kenyon KW. 1982. Population status of the Hawaiian monk seal (*Monachus schauinslandi*), 1978. *J Mammal* 63:415–421.
- Kenyon KW. 1973. The Hawaiian monk seal. *IUCN Nat Resour, Publ, New Ser, Suppl Pap* 39:88–97.
- Lindsay DS, Dubey J. 2009. Long-term survival of *Toxoplasma gondii* sporulated oocysts in seawater. *J Parasitol* 95:1019–1020.
- Littnan CL, Stewart BS, Yochem PK, Braun R. 2006. Survey for selected pathogens and evaluation of disease risk factors for endangered Hawaiian monk seals in the main Hawaiian Islands. *EcoHealth* 3:232–244.
- Lopez J, Boyd D, Ylitalo GM, Littnan CL, Pearce R. 2012. Persistent organic pollutants in the endangered Hawaiian monk seal (*Monachus schauinslandi*) from the main Hawaiian Islands. *Mar Pollut Bull* 64:2588–2598.

- Migaki G, Sawa T, Dubey J. 1990. Fatal disseminated toxoplasmosis in a spinner dolphin (*Stenella longirostris*). *Vet Pathol* 27:463–464.
- Miller NL, Frenkel J, Dubey J. 1972. Oral infections with *Toxoplasma* cysts and oocysts in felines, other mammals, and in birds. *J Parasitol* 58:928–937.
- Pacific Islands Fisheries Science Center. 2020a. *Hawaiian Monk Seal Research Program Hawaiian monk seal survey data collected in the Hawaiian Archipelago, 1982–2019*. US National Oceanographic Data Center. <https://inport.nmfs.noaa.gov/inport/item/5676>. Accessed March 2021.
- Pacific Islands Fisheries Science Center. 2020b. *HMSRP Hawaiian monk seal necropsy data*. US National Oceanographic Data Center. <https://www.fisheries.noaa.gov/inport/item/5673>. Accessed March 2021.
- R Core Team. 2013. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>. Accessed March 2021.
- Risley J, Stonewall A, Haluska T. 2008. *Estimating flow-duration and low-flow frequency statistics for unregulated streams in Oregon*. US Geological Survey, Reston, Virginia. <https://pubs.usgs.gov/sir/2008/5126>. Accessed March 2021.
- Robinson SJ, Harting AL, Mercer T, Johanos TC, Baker JD, Littnan CL. 2021. Sighting patterns reveal unobserved pupping events to revise reproductive rate estimates for Hawaiian monk seals in the main Hawaiian Islands. *Mar Mamm Sci* 37:420–432.
- Shapiro K, Bahia-Oliveira L, Dixon B, Dumètre A, de Wit LA, VanWormer E, Villena I. 2019. Environmental transmission of *Toxoplasma gondii*: Oocysts in water, soil and food. *Food Waterborne Parasitol* 15:e00049.
- Shapiro K, Conrad PA, Mazet JA, Wallender WW, Miller WA, Largier JL. 2010. Effect of estuarine wetland degradation on transport of *Toxoplasma gondii* surrogates from land to sea. *Appl Environ Microbiol* 76:6821–6828.
- Shapiro K, Miller M, Mazet J. 2012. Temporal association between land-based runoff events and California sea otter (*Enhydra lutris nereis*) protozoal mortalities. *J Wildl Dis* 48:394–404.
- Torrey EF, Yolken RH. 2013. *Toxoplasma* oocysts as a public health problem. *Trends Parasitol* 29:380–384.
- USGS (US Geological Survey). 2021. *USGS water data for the nation*. USGS, Reston, Virginia. <https://help.waterdata.usgs.gov>. Accessed March 2021.
- VanWormer E, Carpenter TE, Singh P, Shapiro K, Wallender WW, Conrad PA, Largier JL, Maneta MP, Mazet JA. 2016. Coastal development and precipitation drive pathogen flow from land to sea: Evidence from a *Toxoplasma gondii* and felid host system. *Sci Rep* 6:1–9.
- Wallace GD. 1971. Isolation of *Toxoplasma gondii* from the feces of naturally infected cats. *J Infect Dis* 124:227–228.
- Ward Research Inc. 2018. Public attitudes toward the Hawaiian Humane Society and animal related issues. A contracted report prepared for Hawaiian Humane Society, Honolulu, Hawaii.
- Wilson KC, Littnan CL, Read A. 2017. Movements and home ranges of monk seals in the main Hawaiian Islands. *Mar Mamm Sci* 33:1080–1096.
- Work TM, Massey JG, Lindsay DS, Dubey J. 2002. Toxoplasmosis in three species of native and introduced Hawaiian birds. *J Parasitol* 88:1040–1042.
- Work TM, Massey JG, Rideout BA, Gardiner CH, Ledig DB, Kwok O, Dubey J. 2000. Fatal toxoplasmosis in free-ranging endangered ‘Alala from Hawaii. *J Wildl Dis* 36:205–212.
- Wren JL, Kobayashi DR, Jia Y, Toonen RJ. 2016. Modeled population connectivity across the Hawaiian archipelago. *PLoS One* 11:e0167626.

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