Evaluating the Risk of *Toxoplasma gondii* Exposure for Hawaiian Monk Seals: a Conceptual Map and Research Directions

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Executive Summary

Hawaiian monk seals (*Neomonachus schauinslandi*) in the main Hawaiian Islands (Niihau to Hawaii Island; MHI) make up a crucial component of the species' recovery potential. Although seals in the MHI constitute just more than 20% of the total Hawaiian monk seal population (~300 seals in the MHI and ~1,100 in the Northwestern Hawaiian Islands estimated in 2019; Hawaiian Monk Seal Research Program 2019), positive trends in the MHI have been one greatest signs of population rebound (Baker and Johanos 2004, Baker et al. 2011). The Recovery Plan for the Hawaiian Monk Seal highlights the importance of the MHI with the priority statement to "Ensure the natural recovery of the Hawaiian monk seal in the MHI." An important component of safeguarding monk seal recovery in the MHI is pointed out in the Recovery Plan's following priority statement "Reduce the probability of the inadvertent introduction of infectious diseases into the Hawaiian monk seal population." Toxoplasmosis is a disease of primary concern for MHI monk seals, due to its apparently acute lethality and heavy impacts on breeding female seals. This parasite, *Toxoplasma gondii*, depends on cats to complete its life cycle; thus, in order to understand how this pathogen infects marine mammals, it is essential to understand aspects of the terrestrial ecosystem and land-to-sea transport. In this document, we propose a framework to characterize our understanding of the risk of environmental exposure to *T. gondii* for Hawaiian monk seals, evaluate data gaps and their influence on research and management strategies, and inform future recommendations for risk mitigation.

In this report we present a multi-step framework to use ecological data to model the risk of *T. gondii* exposure to Hawaiian monk seals.

- **Step 1: Establish the link between terrestrial pathogen source, runoff, and infection**

  As a first step we will conduct research to demonstrate the basic dynamics of a land-to-sea pathway linking the terrestrial source of toxoplasmosis (infectious oocysts shed in cat feces) to infection of Hawaiian monk seals in the marine environment.

- **Step 2: Build an environmental risk model evaluating monk seal exposure to *T. gondii***

  To construct a realistic model of the pathway of *T. gondii* exposure in Hawaiian monk seals, we must account for several complex processes (Oocyst Loading, Land-Sea Transport, Hawaiian Monk Seal Exposure) each requiring several data sources.

- **Step 3: Conduct sensitivity tests of the environmental risk model**

  We will systematically vary inputs and evaluate the degree of change in model outputs so that we can identify key variables influencing model sensitivity.

- **Step 4: Potentially iterative process to improve data inputs and model performance**

  We anticipate that the model may be sensitive to some inputs that require improved data. This will be an iterative process of improving data and re-evaluating performance.

Note: The process of iterating Steps 2–4 is likely to be a multi-year process, and findings from these steps will direct and possibly change the exact manifestations of Steps 5–6.

- **Step 5: Use environmental risk model to evaluate spatial variation in risk factors**

  The intention of constructing the environmental risk model is to produce a useful tool to evaluate whether "hot spots" of oocyst contamination exist or correlate with areas of toxoplasmosis strandings in Hawaiian monk seals.

- **Step 6: Use model to evaluate impact of varied management scenarios**

  We will work carefully to communicate both the modeling process and outputs in ways that are transparent and informative to the various partners and stakeholders involved.

Data required to inform this research effort can be thought of in terms of three main components of analysis: Oocyst loading (accumulation of infectious material on the landscape), Land-to-sea transport (hydrological processes transporting terrestrial pathogens to the marine environment), and Hawaiian monk seal exposure (routes by which seals encounter and become infected by the pathogen). In this document we discuss the strengths and limitations of available data related to each of these analytical components, as well as future research that could bolster each.

- *Oocyst loading*–data are sparse, assumptions will allow coarse estimation, sensitivity testing will be important and could help prioritize areas for data improvement, such as repeatable indices of cat density and distribution, estimates of differential shedding by cat class (companion, stray, colony, feral).
- *Land-to-sea transport*–the modeling frameworks are well developed, ecological data are available from reliable sources at fine-grained resolutions.
- *Hawaiian monk seal exposure*–data are of moderate quality, sightings data are available for all seals but can be sparse for some individuals; telemetry data offer potential for more precise estimates of space use, but fewer animals have been tracked.

Opportunities identified for future studies and partnership to serve research goals:

- ***Oocyst loading: Cat populations & T. gondii prevalence***–Improving these data sources through several means would lead to more accurate inference from model outputs.
  - Improve access to existing data regarding cat populations.
  - Develop a cat population index that can inform current models and provide metrics to evaluate management actions for cat overpopulation.
  - Use molecular methods to assess diversity of *T. gondii* strains in cats and other hosts (potentially including rodents, chickens, or other sentinel species).
- ***Land-to-Sea transport***–The current modeling framework focuses on spatial, rather than temporal, variability in risk of oocyst runoff into Hawaii's nearshore environment.
  - Conduct additional modeling to understand the influence of large rain and runoff events.
- ***Hawaiian monk seal exposure & susceptibility***–Additional research on key aspects of Hawaiian monk seal biology may help to understand individual variation in risk of *T. gondii*.
  - Conduct prospective health sampling for population-level serosurvey.

- Conduct female-focused space use and foraging studies.
- Sample potential prey items to test for oocysts.
- Conduct additional monk seal diet studies to evaluate prey specializations.

- ***Evaluate model outputs against field data***–It may be helpful to have proxies that would allow model validation, and future monitoring, based on empirical field data.
  - Evaluate diet items as proxies for monitoring the spatial and temporal distribution of oocyst contamination.
  - Evaluate eDNA methods for detecting signs of cat feces in seawater.
- ***Engagement strategy***–Making the environmental risk model transparent and easy to understand will be an important part of this effort.
  - Create graphical and interactive model products.

I. Background

Hawaiian monk seals (*Neomonachus schauinslandi*) in the main Hawaiian Islands (Niihau to Hawaii Island; MHI) make up a crucial component of the species' recovery potential. Although seals in the MHI constitute just more than 20% of the total Hawaiian monk seal population (~300 seals in the MHI and ~1,100 in the Northwestern Hawaiian Islands estimated in 2019; Hawaiian Monk Seal Research Program 2019), positive trends in the MHI have been one greatest signs of population rebound (Baker and Johanos 2004, Baker et al. 2011). After decades of precipitous decline (Kenyon 1973, DeLong et al. 1976, Johnson et al. 1982), the rate of decline slowed from the 1980s through early 2000s (Carretta et al. 2004) and, by 2013, the range-wide population was showing signs of a stabilizing-to-positive trend (Baker et al. 2016). The MHI plays a key role in the positive trend with the highest growth rate (Baker et al. 2011; Hawaiian Monk Seal Research Program 2019) and reproductive rate (Robinson et al. 2020) throughout the monk seals' range. The Recovery Plan for the Hawaiian Monk Seal highlights the importance of the MHI with the priority statement to "*Ensure the natural recovery of the Hawaiian monk seal in the MHI*" (National Marine Fisheries Service 2007). An important component of safeguarding monk seal recovery in the MHI is pointed out in the Recovery Plan's following priority statement "*Reduce the probability of the inadvertent introduction of infectious diseases into the Hawaiian monk seal population*" (National Marine Fisheries Service 2007). Toxoplasmosis is a disease of primary concern for MHI monk seals, due to its apparently acute lethality, and it is caused by infection with the protozoal parasite, *Toxoplasma gondii*. In this document, we propose a framework to characterize our understanding of the risk of environmental exposure to *T. gondii* for Hawaiian monk seals, evaluate data gaps and their influence on research and management strategies, and inform future recommendations for risk mitigation.

Toxoplasmosis is the leading disease impacting monk seals in the MHI and is among the top three causes of death in the MHI (along with anthropogenic trauma and net-related drownings; Harting et al. 2020). Toxoplasmosis is particularly impactful to population dynamics as adult females appear to be especially susceptible. Toxoplasmosis was responsible for 40% of confirmed mortalities in adult females in the MHI from 2004 to 2019 (Harting et al. 2020). This disease has led to lost pregnancies, has been vertically transmitted leading to neonatal mortality (Barbieri et al. 2016), and diminishes reproductive potential of the population with the loss of breeding females. Recent estimates suggest that toxoplasmosis could be dampening the potential growth rate of the MHI monk seal population growth by approximately 14% (current estimated growth rate, $\lambda = 1.043$, would be $\lambda=1.050$ if toxoplasmosis mortalities were removed; Harting et al. 2020).

Toxoplasmosis-related mortalities have become increasingly prevalent in the MHI since the early 2000s (Barbieri et al. 2016). Toxoplasmosis was first identified in a wild Hawaiian monk seal carcass examined in 2004 (Honnold et al. 2005). Fifteen additional toxoplasmosis-related mortalities have been reported after testing animals stranded from 2001 through early 2020 (Barbieri et al. 2016, Harting et al. 2020). This increase is likely coincident with increased opportunities for exposure as the MHI monk seal population has rebounded in this portion of its range over this time frame. Reports from other species demonstrate that the pathogen has long been established in the MHI; a spinner dolphin death on Oahu in 1990 was caused by toxoplasmosis (Migaki et al. 1990), several Hawaiian bird species have been impacted by the disease (Work et al. 2000, Work et al. 2002), and early studies of toxoplasmosis revealed its high prevalence in cats on Oahu (Wallace 1971).

T. gondii has a complex life cycle and is capable of infecting a wide range of warm-blooded species; however, it can only complete its life cycle (sexual reproduction resulting in oocysts) inside the gut of felids (either domestic or wild cats; Hutchison et al. 1969, Dubey et al. 1970). These oocysts are extremely hardy, persisting and maintaining infectivity for >1 year 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). Infected felids can shed millions of oocysts in their feces, leading to substantial environmental contamination (Dubey et al. 1970, Dubey 1995, Fritz et al. 2012). These oocysts can then be transported from the terrestrial to the marine environment where marine mammals such as Hawaiian monk seals may become infected (numerous demonstrations reviewed in Table 1).

While the general life cycle and infection pathways of *T. gondii* are well described, there is much we need to understand specific to environmental routes of exposure in Hawaiian monk seals in order to mitigate the threat to this species. In the Technical Workshop on Toxoplasmosis in Hawaiian Monk Seals, NOAA's Hawaiian Monk Seal Research Program (HMSRP) gathered experts in marine biology, epidemiology, and veterinary medicine to discuss and prioritize research needs related to toxoplasmosis in monk seals (for a full list of objectives, see Hawaiian Monk Seal Research Program 2020). One priority area, to *generate new data streams and analyses to address critical knowledge gaps, which could be realistically filled and have the greatest potential to inform management needs*, was broken down into three key research activities:

- Investigate correlations between large rainfall events and known cases of toxoplasmosis in Hawaiian monk seals;
- Estimate cat abundance across Hawaii's landscape (with existing data and partners);
- Use existing information on disease ecology and epidemiology to develop a risk model that will serve as a transparent, scientifically informed structure for conceptualizing and communicating about disease risk and potential mitigation strategies.

Filling these knowledge gaps has the potential to directly inform management activities to reduce risks of *T. gondii* exposure in Hawaiian monk seals (Strategic Plan; Pacific Islands Regional Office 2020[1]). Here we present a stepwise framework for addressing these research objectives while engaging with managers and other partners. In the following sections, we will

II. Review current research using spatial and ecological approaches to asses risk factors and pathogen spread associated with *T. gondii* infections in other ecosystems;
III. Present a framework for modeling Hawaiian monk seal risk of exposure to *T. gondii*, taking into account complex ecology and land-to-sea processes;
IV. Describe available data sets and highlight data gaps or other needs to bolster research quality;
V. Highlight opportunities for involvement of research collaborators and other stakeholders.

[1] Pacific Islands Regional Office. 2020. Strategic Plan for the Management of Toxoplasmosis in Hawaiian Monk Seals. NOAA Internal Report, in prep.

II. Review of current research related to *T. gondii* environmental risk modeling

T. gondii has been detected in numerous marine mammal species around the globe and in every ocean basin, thereby demonstrating the ability of this terrestrial pathogen to infiltrate marine environments (reviewed in Table 1). Many marine mammals likely become infected by eating prey that may have accumulated oocysts or through direct consumption of oocysts suspended in seawater (Conrad et al. 2005, Massie et al. 2010). While they do not become infected themselves, animals such as fish and invertebrates are capable of up-taking oocycsts and serving as transport hosts (physically vectoring the oocysts to capable hosts) (Lindsay et al. 2001, Massie et al. 2010, Krusor et al. 2015). Some predators may be infected by consuming *T. gondii* organisms encysted in tissues of infected prey (Jensen et al. 2010), but this is an unlikely route of infection for monk seals which do not consume warm-blooded prey (see Section IV). Additionally, animals including monk seals may acquire *T. gondii* through vertical transmission (Barbieri et al. 2016). Because oocyst contamination of marine environments poses the greatest threat to monk seals, we will focus on understanding factors influencing terrestrial oocyst loading (accumulation on the landscape) and transport from land to sea.

To evaluate levels of oocyst exposure in marine ecosystems, we must first understand the variation in oocyst loading by felid definitive hosts in the terrestrial watersheds feeding into marine environments. The abundance of felid hosts and their rates of *T. gondii* infection and oocyst shedding determine the level of oocyst contamination available for transport to the marine environment. Host abundance and shedding rates may vary with individual or environmental factors. A study of the ecology of *T. gondii* in cats in France found that infection occurred equally among domestic (*Felis catus*) and wild cats (*Felis silvestris*), but odds of infection increased in older cats, in dense farm areas, and in cool/moist years (Afonso et al. 2013). A study on the U.S. west coast found both domestic and wild cats infected with *T. gondii* strains that infected and killed California sea otters (Type X; VanWormer et al., 2014; Shapiro et al. 2019), although the greater abundance of domestic cats made them more likely to be the primary contributors of oocysts transported to the marine environment (VanWormer et al. 2014; 2016).

There is a strong body of research demonstrating that hydrological processes can deliver oocysts from wide catchment areas into the marine environment (reviewed in Table 2). In California, cases of protozoal disease in sea otters have been linked to heavy rainfall and streamflow events bringing greater flushes of oocysts to coastal waters (Shapiro et al. 2012a, Shapiro et al. 2019). This study found that more acute disease was more closely and consistently linked with water flow events, showing strong support for increased risk of infection following major runoff events (Shapiro et al. 2012a). Hydrological models simulating watersheds have shown that oocysts can be carried to coastal waters from far inland areas, with the timing of higher water flow (in this case snow melt) influencing the maximal oocyst load (Simon et al. 2013). Watershed features like wetlands and estuaries can increase water retention and sediment capture, and multiple studies have shown that vegetated wetland areas have the potential to capture and retain oocysts, decreasing the contamination of downstream water bodies (Shapiro et al. 2010, Hogan et al. 2013, Simon et al. 2013). But note that oocyst accumulation in estuarine environments could lead to oocyst contamination of prey species living in these productive habitats (Simon et al. 2013).

By incorporating landscape variables and individual host risk factors, some studies have added to the hydrological modeling framework to create increasingly realistic simulations of the *T. gondii* pathway for marine mammals. To model exposure risks to California sea otters on the west coast, VanWormer et al. (2016) incorporated cat ownership surveys, published wildlife density estimates (bobcat and puma), and field surveys of *T. gondii* infection and shedding rates in domestic and wild cats to create a thorough estimate of oocyst loading on the landscape. By incorporating land use variables in a hydrologic transport model, they were able to demonstrate that areas of greatest *T. gondii* prevalence in sea otters correlated with more developed areas which had higher domestic cat densities and greater runoff (less infiltration) contributing oocysts to sea otter habitat (VanWormer et al. 2016). Burgess and others (2018 and in prep) built on this approach by adding components accounting for diffusion of oocysts in nearshore waters and sea otter home ranges to account for individual exposure risk. They found that land use and land cover features including high density of housing and cropland (associated with higher domestic cat densities) increased risk of *T. gondii* infection in otters, while forest cover decreased it (Burgess et al. 2018). Individual factors including older age and male sex increased infection risk, and consumption of snails also increased risk (Burgess et al. 2018). When plotting animal territories relative to oocyst contamination, they showed that otters with territories closer to major freshwater outflows and including kelp beds (which can accumulate oocysts and host snails) had elevated *T. gondii* infection risk (Burgess et al. in prep). We believe that a similar approach, taking advantage of substantial seal space use data (see Section IV) and modeling oocyst transport into Hawaii's nearshore habitats, could help reveal ecological factors associated with *T. gondii* exposure for Hawaiian monk seals.

III. Proposed framework for modeling Hawaiian monk seal risk of *T. gondii* exposure

Step 1: Establish the link between terrestrial pathogen source, runoff, and infection

As a first step we will conduct research to demonstrate the basic dynamics of a land-to-sea pathway linking the terrestrial source of toxoplasmosis (infectious oocysts shed in cat feces) to the infection of Hawaiian monk seals in the marine environment. To accomplish this, we will address the first of the research objectives from the Technical Workshop on Toxoplasmosis in Hawaiian Monk Seals: **Investigate correlations between large rainfall events and known cases of toxoplasmosis in Hawaiian monk seals.** If cases of toxoplasmosis are associated with increased rainfall or surface water runoff, this would demonstrate that a) Hawaii's source of infectious material is local (flushed from our islands to nearshore waters rather than circulating basin-wide), b) the Hawaiian Islands experience a land-to-sea pathogen flow similar to that seen in other systems (Table 2), and c) major runoff events have the potential to precipitate acute infection leading to monk seal strandings.

As a preliminary examination of this question, we used a case-control approach to examine the odds of toxoplasmosis cases vs. other types of strandings being associated with large surface-water runoff events (following large rainfall). We selected 7 cases (all confirmed toxoplasmosis cases in seals stranded on Oahu from 2014 to 2020, excluding fetal or neonatal cases) and 10 controls (seals found dead on Oahu over the same time period as a result of other causes including drowning, trauma, other diseases). We used logistic regression to calculate odds ratios (OR) and perform significance tests to determine whether exposure to heavy runoff events (indicated by stream gauges reading two standard deviations above average flow levels; USGS) was elevated prior to stranding. Because the duration of onset of toxoplasmosis in monk seals is unknown, we evaluated the OR of heavy runoff events during each week at 1–6 weeks prior to stranding. We found elevated odds of toxoplasmosis occurring 1 (OR: undefined due to 0 occurrence in controls), 2 (OR: 3.6), or 3 (OR: 12.0) weeks after heavy runoff events. The highest (and only statistically significant, $p < 0.05$) OR indicated that risk of toxoplasmosis cases was particularly elevated 3 weeks after large runoff events, potentially providing an indicator of time to acute outcomes.

Several follow-up steps will be taken to strengthen this line of research. We will expand the scope of this analysis to include all cases of toxoplasmosis from all islands across all years (although we will still exclude non-independent fetal or neonatal cases). In addition to analyzing OR between cases and controls, we will use a randomization routine to calculate the probability of the observed number of toxoplasmosis cases falling in proximity to large runoff events by chance. And, finally, we will examine whether toxoplasmosis cases are more likely to occur in windward watersheds where large terrestrial-flushing runoff events occur more frequently.

Step 2: Build an environmental risk model evaluating monk seal exposure to *T. gondii*

A central focus of our research will address the research objectives from the Technical Workshop on Toxoplasmosis in Hawaiian Monk Seals: **Use existing information on disease ecology and epidemiology to develop a risk model that will serve as a transparent, scientifically informed structure for conceptualizing and communicating about disease risk**

and potential management strategies to reduce it. As a necessary part of constructing such a risk model, we will also have to address the objective to: *Estimate cat abundance across Hawaii's landscape (with existing data and partners).* To construct a realistic model of the pathway of *T. gondii* exposure in Hawaiian monk seals, we must account for several complex processes (Figure 1). Each of these components requires several data sources to characterize variables and estimate model parameters. We will describe each of the available data sets (and, at times, their limitations) in Section IV.

- **Oocyst Loading**: First, oocysts must be deposited on the landscape by the felid hosts of *T. gondii*. This can be estimated as a product of the number and distribution of cats on the landscape, as well as the rates of *T. gondii* infection and oocyst shedding by domestic cats (the only felid species in the Hawaiian Islands).
- **Land-Sea Transport**: Next, the infectious oocysts must be transported from the terrestrial environment to the marine environment (typically via rain-driven runoff events) where monk seals can ingest them. This depends on island hydrology, which is, itself, described by a complex model based on rainfall and various geologic features that shape the infiltration or flow of water through Hawaii's watersheds. Oocyst loads will be an additional input into the hydrological model, providing an output of expected oocyst runoff into coastal areas.
- **Hawaiian Monk Seal Exposure**: Finally, a monk seal must consume an infectious oocyst; either directly from the water column or benthos, or indirectly through prey items that have accumulated oocysts. Thus, this risk should be influenced by the seal's space use (the level of oocyst runoff to which it is exposed) as well as diet and individual risk factors.

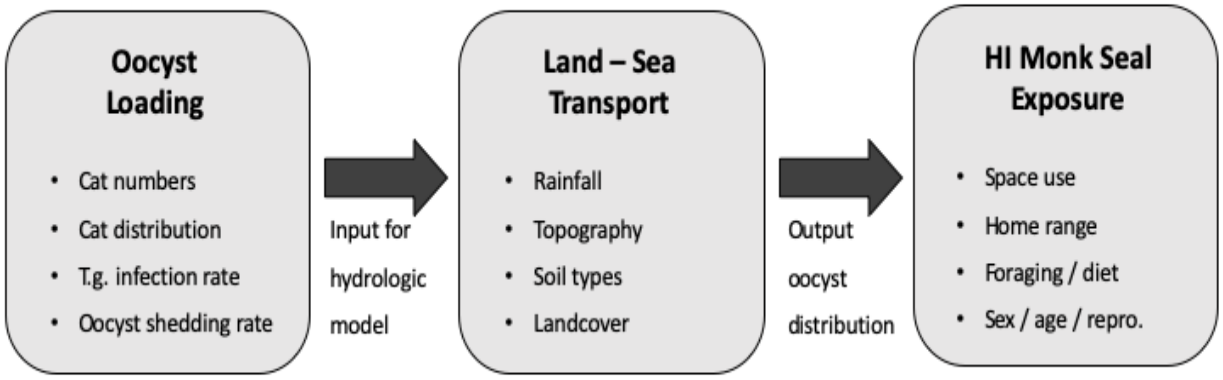

Figure 1. Schematic of components of the T. gondii risk model for Hawaiian monk seals. The flow chart shows the key variables involved in each component of our risk evaluation. Text under arrows indicates how each component feeds into the next.

Step 3: Conduct sensitivity tests of the environmental risk model

While many excellent data sources exist to inform the environmental risk model, most are subject to uncertainty, and some model inputs may be extrapolated from literature or small-scale studies (see Section IV). We will systematically vary inputs and evaluate the degree of change in

model outputs so that we can identify key variables influencing model sensitivity. Through this analysis, we will identify areas in which the current data produce robust inference, but we will also pinpoint data sources that will be most impactful to invest in improving.

Step 4: Potentially iterative process to improve data inputs and model performance

We anticipate that the model may be sensitive to some inputs that require improved data. For instance, if varying cat colony densities were to dramatically alter model results, we might determine that more precise information about colony distribution was needed before relying on a final model. This is likely to be an iterative process of improving data and re-evaluating model performance (i.e. repeating a cycle of Steps 2–4).

Step 5: Use 'final' environmental risk model to evaluate spatial variation in risk factors

The intention of constructing the environmental risk model is to produce a useful tool to evaluate whether 'hot spots' of oocyst contamination exist or correlate with areas of toxoplasmosis strandings in Hawaiian monk seals. However, because such conclusions have the potential to influence risk mitigations or management decisions, we believe it will only be responsible to undertake this step once Steps 2–4 demonstrate robust model performance. If areas of concentrated oocyst contamination exist (and correlate with monk seal strandings), these could provide a useful focus for management and mitigation activities. However, it is important to note that, given the apparent high susceptibility of monk seals to toxoplasmosis, even low levels of oocyst contamination might be sufficient to put seals at risk. Even if risk mitigation efforts cannot be limited to 'hot spots,' the environmental risk model can still provide a useful simulation tool to evaluate potential impacts of varied management options.

Step 6: Use model to evaluate impact of varied management scenarios

Our environmental risk model can serve as a bridge between science (characterizing risks of *T. gondii* exposure) and management (developing strategies to mitigate risks to monk seals). We will engage with partners in NOAA's Hawaiian monk seal management team, as well as other partners and stakeholders, to develop a set of management scenarios of interest. We will collaboratively determine the parameters to be altered under each scenario and then use iterative model runs to simulate the impact of each of these scenarios on oocyst contamination into Hawaiian monk seal marine habitat. We will work carefully to communicate both the modeling process and outputs in ways that are transparent and informative to the various partners and stakeholders involved.

IV. Overview of existing data sets and limitations (summarized in Table 3)

Oocyst Loading

Following the general approach of VanWormer et al. (2016), once we have estimated the numbers of cats and their distribution across the islands, we will apply a multiplier based on oocyst shedding prevalence and the estimated number of oocysts shed per cat. Thus, oocyst loading for a given area on the map will be:

Oocyst loading = cats per area × oocyst shedding prevalence × oocysts shed per event

Here we will describe the data required and data sets available to estimate each component of this equation.

Estimating cats per area

Classification of cats — An important starting point is designating distinct cat classes to be covered by our research, as there are different biological, epidemiological, management, and human value factors associated with cats according to their level of association with humans. The home range or territory size of cats tends to vary widely according their reliance on human care and habitat type utilized. For instance, un-owned cats typically exhibit wider movement, and more nocturnal activity than pet cats (Horn et al. 2011, Cove et al. 2018). Home-range size of free-roaming cats has been reliably correlated with levels human development (and corresponding potential for direct or indirect care from humans), with cats in less developed areas requiring larger territories to satisfy their needs (14× average difference across a 22-study meta-analysis in Hall et al. 2016, 7× difference in Hanmer et al. 2017, 4× difference in Cove et al. 2018). These difference in habitat use correspond to differences in diet, with more feral (less human-associated) animals relying more heavily on wild prey, which in turn may influence the prevalence of *T. gondii* in cats with different lifestyles (Levy and Crawford 2004; Afonso et al. 2006). In fact, a study in California found that feral cats (with no reliance on human care) were seven times more likely to shed *T. gondii*-like oocysts than managed stray cats (food provided by humans) (VanWormer et al. 2013).

In addition to impacting the animals' biology and epidemiology, varying levels of human-cat-association have strong impacts on human values and the management options deemed appropriate for different types of cats. In their National Cat Management Strategy (NZ Gov 2017), the government of New Zealand legislatively categorized cats into four classes based on human-associations: companion cats, stray cats in managed colonies, unmanaged stray cats, and feral cats (defined as below). Public opinion surveys showed that concern for cat impacts to wildlife (through predation) fell on a gradient by cat class (highest for feral cats, lowest for companion cats; Walker et al. 2017). Support for cat management strategies fell along a similar gradient, with only mild management options supported for companion cats (cat exclusion zones, limits on ownership numbers, microchipping, registration, and spaying/neuteing), more intensive management favored for stray cats (Trap-Neuter-Return (TNR) for colony strays, TNR along with lethal control for unmanaged strays), while lethal control measures were favored for feral cats (Walker et al. 2017). Similarly, a recent US study including Hawaiian stakeholders found that people expressed different views of owned pet cats vs. unowned stray or feral cats (Leong et al. 2020). Even among those agreeing that cat overpopulation was a problem, acceptable

management solutions varied widely according to the values that different stakeholders associated with cats or specific classes of cats (Leong et al. 2020).

Operational definitions of designated cat classes — In this research effort, we will classify cats into four distinct categories. These designations mirror those defined by New Zealand's Cat Management Strategy, and coincide with the designations put forth in the NOAA PIRO Toxoplasmosis Strategic Plan[2]. The cat classes are ordered below from most to least association with human care or activities.

1) *Companion cats* (household-associated pets): live with humans and are dependent on humans for their care and welfare.
2) *Colony cats* (strays associated with colonies/feeding stations): do not live in human households, but have many of their needs, such as food, directly supplied by humans.
3) *Stray cats* (neighborhood/human-associated strays): live around centers of human habitation and have their needs supplied indirectly by humans by scavenging.
4) *Feral cats* (wildland /mauka/non-human-associated strays): generally do not live around centers of human habitation, have none of their needs provided for by humans, rely on hunting wild prey.

Cat estimates & distribution by cat class — The amount and quality of data available varies for each of the cat classes in Hawaii. In general, data are sparse, but provide a foundation for estimation given some assumptions (detailed below). In presenting the available data, we also offer suggestions for future efforts to address data gaps and improve quality. One key data limitation is that one of the primary sources of data regarding companion, stray, and colony cats in Hawaii is only indirectly available. Results from telephone surveys about cat care and ownership (conducted by Honolulu-based market research company Ward Research, Inc., on behalf of the Humane Society of the United States; HSUS), are only publicly available in the form of an Executive Summary of the 2012 study (presented by Inga Gibson, Hawaii State Director of the HSUS at The HSUS Cats Outdoors Conference December 2012). Should full reports or more recent surveys be made available, a full examination of survey questions, methods, and results would allow improved inference from these data.

Companion Cats & Stray Cats — Because cats can be solitary and/or secretive, they can be difficult to count directly, making telephone-based surveys a valuable tool in estimating cat populations, especially for companion cats that spend time associated with private residences. Telephone-based surveys have provided estimates forming the basis of previous research estimating *T. gondii* oocyst loading from companion and stray cats (Dabritz et al. 2006; VanWormer et al. 2016). Here we will rely on the Ward Research Survey (2012) as the primary source for companion cat and stray cat numbers. The survey found that 19% of Hawaii residents owned a pet cat or fed a stray/feral cat. Of cat owners 58% (Oahu)/46% (other islands) owned a single cat, while 42% (Oahu)/54% (other islands) owned multiple cats, and a majority of cat owners allowed their pets to roam freely outside (52% on Oahu/71% other islands).

[2] Pacific Islands Regional Office. 2020. Strategic Plan for the Management of Toxoplasmosis in Hawaiian Monk Seals. NOAA Internal Report, in prep.

In extrapolating these numbers to the population, we made a number of assumptions, typically erring on the side of making more conservative estimates. First, we assumed that residents interviewed represented the cat ownership/feeding of their entire household, so we conservatively applied the 19% of respondents owning/feeding cats to Hawaii households (average 2.8 residents per households, U.S. Census Bureau, 2016) rather than the full census of residents. We interpreted "multiple cats" to mean the lowest number possible: 2. Because the summarized survey results are presented in terms of the proportion of Hawaii residents that "own a pet cat or feed a stray/feral cat at least once a week," we cannot readily separate our estimates of these 2 classes of cats. Thus, as a conservative starting point, we may assume that stray cats are accounted for in the estimated number of outdoor cats per household. However, it is reasonable to assume that not all stray cats are accounted for in those directly fed by humans, so it would likely improve accuracy to add some number of stray cats (perhaps as a function of land cover and population density).

Given the above numbers and assumptions, we would estimate the number of outdoor companion and stray cats as follows for on example island (Oahu) as follows:

Cats in single-cat households (19% cat households × 58% single cat × 1 cat)

+ Cats in multi-cat households (19% cat households × 42% multi cat × 2 cat)

× Proportion of outdoor cats (52%)

= 0.14 outdoor cats per household on Oahu

This number of outdoor cats per household can then be used to estimate the number of outdoor companion and stray cats in each area of a map by multiplying this number by the number of households/km for each census block (based on the 2015 U.S. Census American Community Survey, U.S. Census Bureau, 2015).

Colony Cats — In Hawaii, cat colonies are not contained or spatially demarcated, and are typically loosely organized around feeding stations (typically informal, not involving a structure or shelter for feeding). Individuals can register as cat colony caretakers (Williams 2009), but such registries are not publicly available. Therefore, documenting cat colony numbers and/or locations is not straightforward. For this study, we have compiled cat colony information from multiple data sources, identifying 51 known cat colonies on Oahu (we continue to seek similar data for other islands). Cats were classified as colony cats (as opposed to stray cats) when multiple adult cats were seen together in one place, or at least one adult cat was observed along with evidence of large-scale feeding in the area. Lepczyk et al. (2020) identified 25 cat colony sites near important bird areas on Oahu. Additional colony locations were identified through social media posts describing observations of groups of free-roaming cats (posts regarding single stray cats or groups of kittens were treated as disregarded; NOAA PIRO unpublished, 2019). Whenever possible, we used reported counts to estimate the number of cats associated with a colony (knowing that numbers observed in a single instance would provide a conservative measure). Lepczyk et al. (2020) provided counts at each site where cats were detected (max observed during 1–3 visits, range:1–99), and 21 of 26 social media posts included text or photos providing a count of cats (range: 2–200). In the other five instances, we assumed the average colony size of 21 cats (as reported in the Ward Research, Inc., Cat Caregiver Survey).

Our current list of known cat colonies is certainly a vast underestimate. While colonies we've mapped on Oahu total just over 1,000 colony cats, a 2012 study estimated 16,7000 colony cats

on Oahu (Lohr et al. 2013), based on a registry of approximately 1,200 colony caretakers (Williams 2009) and surveys indicating that each caretaker on Oahu managed approximately 13.9 cats (Zasloff and Hart 1998). The more recent survey from Ward Research, Inc., indicates that current numbers are likely even higher (21 cats/colony). We may be able to improve our estimate of colony cat distribution by using known colony locations to determine relationships between colony locations and human density, land cover, or other mappable features, and then distribute a number of randomized colonies according to observed patterns.

Feral cats — Studies describing the number or distribution of feral cats in Hawaii are limited in scope and/or dated. However, studies in Hawaii and elsewhere suggest that land cover can serve as a useful proxy to estimate numbers of feral cats that could maintain territories in various landscape types. Studies on Hawaii Island have shown that while human care maintains high densities of (stray and colony) cats in coastal communities, feral cats exist at lower densities in montane and forest habitats (Goltz et al. 2008; Hess et al. 2009). Feral cats inhabiting the woodlands of Mauna Kea exhibited among the largest home ranges reported in the literature (772 ha for females, 1,418 ha for males; Hess et al. 2009). But these large ranges do not constitute exclusive territories, Goltz et al. (2008) captured up to 5 individual cats in a single trap. Together these numbers suggest that cat density could be less than 1 cat/km^2 in montane areas. A study in a different island system, the Florida Keys, found similarly low densities of feral cats (~1 cat/km^2) in remote areas far from human food sources, with densities increasing to ~4 cats/km^2 in more suburban areas (Cove et al. 2018). These studies can provide guidelines for realistic numbers that could be used to estimate feral cat distributions across the Hawaiian landscape in our modeling effort. We propose to use a multiple imputation approach, using a range of cat densities (e.g., 0.5–5 cats/km^2) assigned to undeveloped land cover classes (rangeland, forest). Our sensitivity analysis will provide an important check on the influence that these inexact estimates have on the final model.

Estimating oocyst shedding prevalence

While antibody tests routinely demonstrate high proportions of cats become infected with *T. gondii* (Vollaire et al. 2005), oocyst shedding can be short-term or intermittent and there is considerable variation in reported prevalence of cats actively shedding *T. gondii* oocysts. Studies in Hawaii have consistently shown high *T. gondii* infection prevalence based on serology (29% in Wallace 1971, 37% in Danner et al. 2007) but have shown a range of shedding prevalence from 0.6% to (oocysts detected in 6/1023 feces microscopically examined; Wallace 1971), to 7.2% (*T. gondii* DNA detected in 5/69 feces genetically examined; Davis et al. 2018). Keeping in mind that these values will function as something of a multiplier per cat, and the goal is not to estimate an exact number of oocysts reaching the ocean but to approximate levels of variation in oocyst contamination across the landscape. Thus, a value within the observed range should provide a reasonable estimate. However, it is worth noting that VanWormer et al. (2016) found that their oocyst loading model was sensitive to the shedding prevalence input for different classes of cats, illustrating the value of deriving locally realistic estimates of shedding prevalence. Accuracy of our model would likely benefit from information comparing *T. gondii* shedding prevalence in each of the four classes of cats under study. In the meantime, this is a parameter will be an important focus of our sensitivity analysis.

Estimating oocysts shed per event

It is acknowledged that the number of oocysts shed by a *T. gondii*-infected cat can have great individual variability (Dubey 1995; Fritz et al. 2012). However, a number of studies in diverse environments have relied on a central estimate of 50,000,000 oocysts per shedding event (Dabritz et al. 2007, Afonso et al. 2010, VanWormer et al. 2016). Some proportion of these oocysts will become bound to soil or vegetation making them unlikely to be mobilized in surface water runoff. We will use a conservative estimate of 1% mobilization after VanWormer et al. (2016), based on studies of the related parasite, *Cryptosporidium parvum* (Ferguson et al. 2007).

Land-to-Sea Transport

We plan to model the surface hydrology of Hawaiian Islands using the InVEST NDR model (Integrated Valuation of Ecosystem Services and Tradeoffs, Nutrient Delivery Ratio v3.6.0) (Hoyer and Chang 2014, Sharp et al. 2015, Falinski 2016, Redhead et al. 2018). The InVEST model is widely favored for its straightforward construction, rapid calculation, and strong spatial expression, and the NDR component in particular, has performed well in characterizing the relative magnitude nutrient export in diverse systems (Canqiang et al. 2012, Redhead et al. 2016). A nutrient transport model such as InVEST NDR will be the most appropriate to capture the hydrological dynamics of *T. gondii* oocysts given their microscopic size and long settling time (Shapiro et al. 2012b). In addition to oocyst loading, the model requires inputs including rainfall, topography (Digital Elevation Model, DEM), and land use/land cover (Figure 2).

- Topography/Elevation – USGS digital elevation model (DEM) at 10-m resolution[3].
    - Watershed delineation: The ArcHydro add-in uses the DEM to create subwatersheds and identify stream channels
- Land use/Land cover – NOAA's Coastal Change Analysis Program (C-CAP) land cover layer for the Hawaiian Islands provides resolution at 2.4m for land classification images from 2017 .
- Soil Type – Soils data will be accessed from the USDA-NRCS SSURGO database. Soil erodibility (also known as the k-factor) is a factor derived by soil type determined by grain size and other soil physical properties. The SSURGO database was last updated in 1972 for Hawai'i, but previous work has found coverage for most areas of the MHI.

In addition, we will use a more complex model that predicts daily export of sediment and nutrients using local daily rainfall. Currently the two options for models include SWMM and SWAT, both regularly used to model urban drainage and watershed hydrology, respectively.

- Rainfall – annual average precipitation data from (Giambelluca et al. 2013).
    - While annual rainfall data may not capture individual large rainfall events, this method will correctly characterize watersheds (or other spatial areas) that are more prone to frequent rain events.
- Streamflow – based on daily stream gauge data from the USGS .
    - It should be noted that stream gauges are more abundant on Oahu as compared to other islands, and even then, only approximately 20 gauges are available. So, watershed-level stream flow data are often unavailable; however, major rainfall events tend to be reflected in regional increases in

[3] https://www.pacioos.hawaii.edu/metadata/usgs_dem_10m_oahu.html.

[4] https://coast.noaa.gov/digitalcoast/tools/lca.html

[5] https://waterdata.usgs.gov/hi/nwis/current/?type=flow

streamflow, so even sparse stream gauges are likely to capture major flow events.

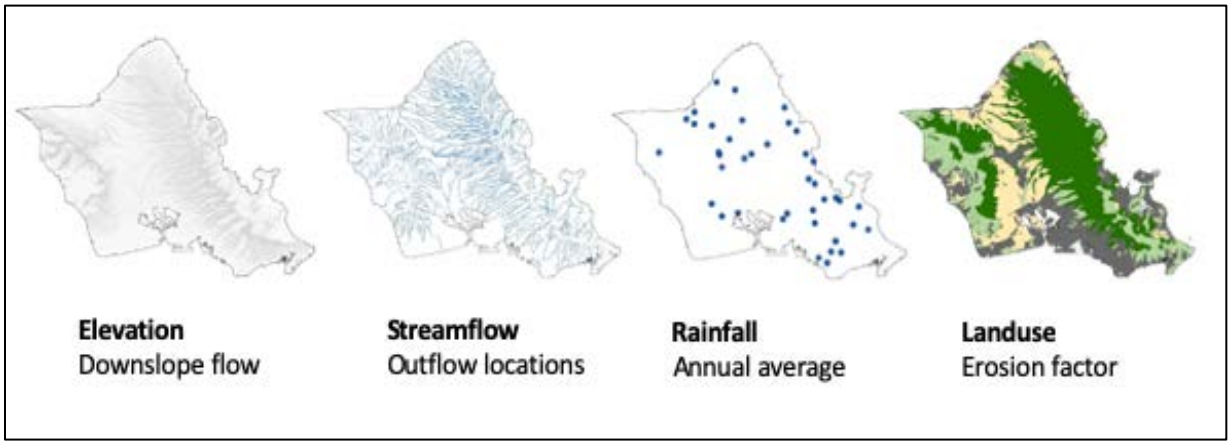

Figure 2. These maps show key landscape data layers that will inform the hydrological model describing land-to-sea transport of oocysts. Oahu is shown as an example island, but most data sets are of similar quality for all islands throughout the main Hawaiian Islands, with the exception that other islands have fewer rain/stream gauge stations.

Hawaiian Monk Seal Exposure

Toxoplasmosis case information

Even though toxoplasmosis is one of the leading causes of death for Hawaiian monk seals in the MHI, the low number of seals in the population means that the overall case numbers are still low. As of January 2020, 15 monk seal deaths associated with *T. gondii* had been recorded. Case designations and details are summarized in Table 4 (Barbieri et al. 2016; Harting et al. 2020). While toxoplasmosis is frequently fatal in Hawaiian monk seals, there is some evidence that not all exposures lead to acutely fatal infection. Positive serological titers for antibodies to *T. gondii* were detected in 2 of 18 apparently healthy seals sampled as part of an instrumentation study from 2004 to 2005 (Littnan et al. 2006). High antibody titers have also been noted in one captive seal which was receiving antibiotic therapy (B. Doesher, pers. comm.), and one apparently healthy seal sampled during instrumentation in 2017 (HMSRP, unpublished). These reported cases represent a minimum, considering that female seals predominate the number of confirmed deaths yet are underrepresented in the serology data because reproductive age females are deliberately avoided during elective research handling events. Further, carcass detection is imperfect, and not all carcasses can be fully examined due to accessibility, state of decomposition, or other factors. There is a likely spatial component to carcass detection bias; an evaluation of seals that have been confirmed dead (carcass detected) vs. presumed dead (not sighted in >3 years, no carcass detected) revealed that dead seals were twice as likely to be detected around Oahu or Kauai (47–49%) vs. Molokai (26%), and detection was sporadic on other islands.

Individual seal characteristics

The NOAA HMSRP Hawaiian monk seal sightings database has recorded all monk seal sightings since the 1980s and provides a rich source of individual seal information including age, sex, and history of sightings locations. We will incorporate this data to assess individual risk factors. Individual characteristics such as age and sex have been associated with risk of toxoplasmosis in other species (Burgess et al. 2018), and in monk seals adult females appear to be at elevated risk (Harting et al. 2020). Additionally, the HMSRP has archived ten thousands of blood and tissue samples from carcass examinations as well as sampling healthy seals, providing resources to evaluate other individual characteristics including genetic and health factors.

Potential Exposure — Location

Animal space use can provide a useful indication of exposure to environmental sources of pathogens (Littnan et al. 2006; Pepin et al. 2017; Burgess et al. 2018). We will use Hawaiian monk seal location data (both from sightings histories, and satellite telemetry) to calculate utilization distributions to assess the potential for overlap between areas of high seal use with coastal areas receiving runoff with high oocyst contamination. Existing data suggests that monk seals, particularly in the MHI, spend a substantial portion of their time in nearshore areas where (unfortunately) exposure to oocyst contamination may be greatest. Monk seals come to shore regularly to rest and spend weeks ashore for molting, pupping and nursing, and seals in the MHI spend an average of about 40% of their time ashore (Cahoon 2011; Wilson et al. 2017). MHI monk seals favor foraging locations close to the island used for hauling out, for instance seals from Kauai typically travel between Kauai and neighboring Niihau, while seals from Molokai often forage at Penguin Bank, a submerged bank extending offshore (Cahoon 2011; Wilson et al. 2017). Foraging trips in the MHI are typically much shorter than those in the NWHI, extending 10–50 km and lasting just 0.5–3.8 days (Cahoon 2011; Wilson et al. 2017). However, seals can also readily move between the main islands, which are separated by as little as 15–100 km (~35–37% of individuals; Littnan et al. 2006; Wilson et al. 2017). While there does not appear to be a sex-based bias in either dispersal rate (Johanos et al. 2014) or home range size (Stewart et al. 2006), space use does tend to increase with age, with weaned pups gradually increase their range in the months after weaning (Henderson and Johanos 1988; Norris et al. 2017).

The extensive time spent hauled out and the prevalence of near-island foraging trips suggests that sightings data may provide a reasonable initial assessment of monk seal space utilization and pathogen exposure. Sightings data offers the benefit that all seals are tracked year-round across many years by the biologists and citizen scientists that report detailed seal sightings (Robinson et al. 2020). Thus, sightings data offers a unique opportunity to assess an animal's long-term space-use patterns and tendencies to use areas near potential oocyst runoff sources. However, we must also acknowledge some shortcomings of sightings data; 1) records are generally limited to sightings of seals hauled out, so they do not reflect the full area utilized for foraging, 2) sightings are typically biased by human effort, so islands with greater volunteer effort have more thorough sightings data, and more populated or more accessible beaches tend to receive better coverage, 3) while they cover longer periods of time, sightings records for a given time period will be sparse compared to satellite telemetry data, thus resultant utilization distributions will be less precise (Figure 3).

In addition to sightings data, previous and ongoing studies have developed a resource of satellite telemetry data describing monk seal space use in the MHI (Littnan et al. 2006; Cahoon 2011;

Wilson et al. 2017). Satellite location data is available for 98 seals (33 females, 65 males) in the MHI. Only two of the seals among the toxoplasmosis cases have been tracked with satellite telemetry, and these were years prior to the time of stranding. While telemetry data will not directly represent infected seals, this data will be helpful in examining the range of variation to help interpret utilization distributions computed with sightings data. Satellite tracking has the benefit of recording seal locations through all hours of the day and regardless of proximity to land or accessible beaches; thus, it can provide a more comprehensive impression of seal space use (see Figure 4). Of course there are limitations; the short duration of satellite transmitter deployments, makes telemetry-based estimates of space use more precise but less representative of long-term patterns.

Potential Exposure — Diet

Hawaiian monk seals consume a wide variety of prey species, and do not show the level of prey specialization that has been associated with *T. gondii* risk in other marine mammals (Burgess et al. 2018). Rather than showing a taxonomic prey preference, monk seals target prey with a cryptic, benthic lifestyle, including species from more than 40 families of fish, cephalopods, and crustaceans (Goodman-Lowe 1998; Longenecker 2010; Iverson et al. 2011). Estimates of diet composition are imprecise and vary considerably depending on the method of diet analysis (Goodman-Lowe 1998; Iverson et al. 2011; Thompson 2011; Cahoon et al. 2013). Foraging behavior and dietary preferences have also been shown to vary with age and geographic region (Goodman-Lowe 1998; Iverson et al. 2011; Cahoon et al. 2013). Given the variability within the data, and lack of a clear dietary risk signal, we are not including monk seal diet in models of *T. gondii* risk at this time. However, should future research identify potential prey items that serve as effective oocyst accumulators, further targeted diet studies would be well-justified.

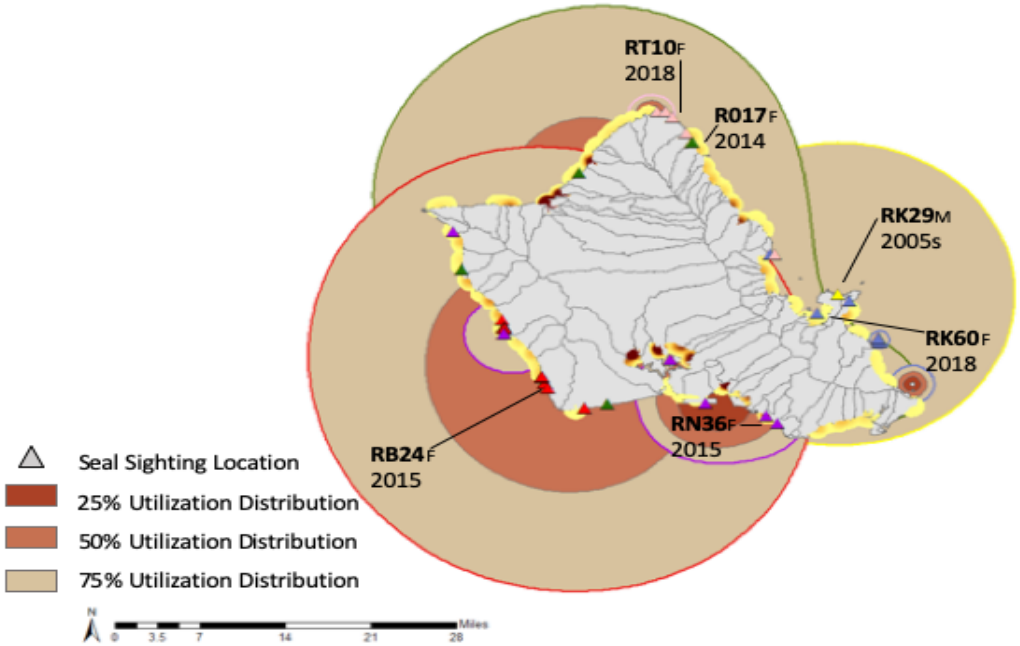

Figure 3. The map of Oahu shows the space utilization distributions of seals stranded with toxoplasmosis based on their sighting histories. The variation in size of utilization distribution is based on differences in locations visited as well as the number of data points available.

Figure 4. Three maps of Oahu show the variability in what would be determined high seal use areas (darker color) based on variable data sources: land-based, human-reported sightings; GPS satellite telemetry points including many animals instrumented on southern Oahu, Argos satellite telemetry points including many animals instrumented on Oahu's North Shore.

V. Opportunities for future work & partnership in bolstering research

The complexity of toxoplasmosis, and the associated research effort, lends itself to collaborative efforts, thereby creating many opportunities for partnership and future research to improve on the foundation set forth by our proposed research framework. Such collaborative effort also fits into priorities defined by the Technical Workshop on Toxoplasmosis in Hawaiian Monk Seals: *Leverage partnerships to improve scientific knowledge of Toxoplasma, including research that may require long-term effort or have indirect benefit to Hawaiian monk seal recovery*. Below we give a brief description of future research that could strengthen the various components of the presented research framework (with additional links to priorities noted in the Technical Workshop). We acknowledge that this list is not exhaustive, does not indicate an order of priority, and these opportunities will need to be balanced with additional priorities beyond the scope of this framework.

Oocyst loading: Cat populations & T. gondii prevalence – As noted in the previous section, data regarding cat population levels, distribution, and *T. gondii* shedding prevalence and molecular characterization are particularly sparse. As these data sources form the basis of the oocyst loading portion of the environmental risk model (VanWormer et al. 2016), we can expect that improving these data sources through several means would lead to more accurate inference from the model.

- Improve access to existing data regarding cat populations. Non-public data sources exist documenting cat ownership and colony caretaking, collaborative efforts with partners able to share these data could improve our modeling effort and help address questions of broader interest to other stakeholders as well.
- Develop a cat population index that can inform current models and provide metrics to evaluate management actions for cat overpopulation. While the widespread and cryptic nature of cats can make populations difficult to accurately census, repeatable surveys in a representative set of study areas could provide an index to assess baseline levels and detect population trends in response to management actions. Developing such an index would be most useful as a long-term endeavor and offers excellent opportunity for partnership to engage key stakeholders and ensure perpetuated effort. Better estimating cats on Hawaii's landscape was one of the priorities set forth from the Technical Workshop on Toxoplasmosis in Hawaiian Monk Seals.
- Use molecular methods to assess diversity of *T. gondii* strains in cats and other hosts (potentially including rodents, chickens, or other sentinel species). Little is known about *T. gondii* genotypes circulating in Hawaii; however, revealing the strain diversity of the pathogen can offer valuable insights to dynamics of spread through the local ecosystem and the potential for transport to the marine environment. Evaluating *T. gondii* strain genotypes circulating in Hawaii was one of the priorities set forth from the Technical Workshop on Toxoplasmosis in Hawaiian Monk Seals.

Land-to-Sea transport – The current spatial-hydrological modeling framework focuses on spatial, rather than temporal, variability in risk of oocyst runoff into the Hawaii nearshore environment. Additional modeling could be used to better understand the influence of large rain

and runoff events. Evaluating such routes of environmental exposure was one of the priorities set forth from the Technical Workshop on Toxoplasmosis in Hawaiian Monk Seals.

- Evaluate variation in oocyst contamination with varying precipitation levels. If oocyst contamination and seal deaths from toxoplasmosis are linked to large runoff events, we may wish to better quantify the increased infection risk expected with varying levels of storm events (e.g. 5-year, 50-year, 100-year storm events). Such inference requires additional complex hydrological modeling, which would likely require specialized partners and be limited to a few representative example watersheds.

Hawaiian monk seal exposure & susceptibility – Additional research on key aspects of Hawaiian monk seal biology may help to understand individual variation in risk of toxoplasmosis.

- Conduct prospective health sampling for population-level serosurvey. Most of what we know about toxoplasmosis comes from stranded cases, but this reveals little about the time of infection or the progression from exposure to acute outcomes. By routinely sampling individuals across time, we may be able to better understand the progression of toxoplasmosis as well as sublethal impacts of the disease.
- Conduct female-focused space use and foraging studies. Female Hawaiian monk seals have been disproportionately impacted by *T. gondii*, as described in Harting et al. (2020). Yet, is unknown whether this may relate to differences in exposure to oocyst contamination or differences in physiological response once infected. The majority of instrument-based research in Hawaiian monk seals has focused on males or juvenile animals, avoiding adult females to prevent stress on potentially pregnant seals. It would be valuable to capitalize on narrow windows of safe opportunity (shortly after an adult female has weaned a pup and molted) to expand the use of bio-logging instruments to understand patterns of female space use and potential shifts throughout the breeding season. Because toxoplasmosis has impacted pregnant females and caused pup losses, it may also be valuable to investigate interactions between *T. gondii* infection and female reproductive biology.
- Sample potential prey items to test for oocysts. Evaluating routes of exposure through ingestion (monk seal diet) was one of the priorities set forth from the Technical Workshop on Toxoplasmosis in Hawaiian Monk Seals. Both invertebrates and fish have been shown to be effective at filtering oocysts out of seawater, making them potential sources of infection (Massie et al. 2010; Krusor et al. 2015; Coupe et al. 2018; Marino et al. 2019). Several marine invertebrates and fishes inhabiting nearshore waters around Hawaii could serve as transport hosts of oocysts to monk seals, monk seal prey, as well as humans. Thus, there could be joint benefit to our research, as well as public health, to further investigate the oocyst accumulation ability of a variety of Hawaiian species.
- Conduct additional monk seal diet studies. A number of biological, ecological, as well as management-related questions could be served by better understanding the composition of the diets of MHI monk seals. Given the diversity found in monk seal diets, high individual variability, and high variability between methods, this would not be a simple or short-term investigation and should involve strong partnerships with experts.

Evaluate model outputs against field data – The seemingly low numbers of toxoplasmosis cases in Hawaiian monk seals will make it difficult to statistically evaluate the outputs of our environmental risk model relative to seal health outcomes. It may be helpful to have proxies that would allow model validation, and future monitoring, based on empirical field data.

- Evaluate diet items as proxies for monitoring the spatial and temporal distribution of oocyst contamination. In conjunction with the research described above, near-shore filter feeders could provide a measure of oocyst contamination in the environment.
- Evaluate eDNA methods for detecting signs of cat feces in seawater. As eDNA methods advance, this may have potential to reveal levels of contamination with cat feces, potentially allowing validation of the land-to-sea model.

Engagement strategy – While the devastating impacts of toxoplasmosis to Hawaiian monk seals makes our research directions highly focused, management of this disease threat necessarily extends far beyond a single species or habitat. Recognizing the importance of building productive partnerships, we will engage management partners and stakeholders to obtain input on risk-reduction scenarios to evaluate in final modeling stages (as an extension of Step 6 in the above framework). Making the environmental risk model transparent and easy to understand will be an important part of this effort, and fit into the communication strategy called for in the Technical Workshop on Toxoplasmosis in Hawaiian Monk Seals.

- Create graphical and interactive model products. Graphical user interfaces allowing stakeholders to adjust model inputs and view outputs can be a powerful tool in helping them to understand a complex system. To this end, video game interfaces have provided a helpful and engaging platform for making complex models interesting to diverse stakeholders (for example, the NOAA Atlantis model). Partnering with web or game developers could be a creative solution to in future steps of communication and planning management scenarios.

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VII. Appendix of Tables

Table 1. Summary of published T. gondii screening in marine mammals / marine environments.

Author	Year	Location	Detection	Species	Finding
Central Pacific—Hawaii					
G. Migakit et al.	1990	**Hawaii, USA**	Histopath., PCR	HI Spinner Dolphin (Stenella longirostris)	1
Littnan et al.	2006	**Hawaii, USA**	Serology	HI Monk Seal (Neomonachus schauinslandi)	2/18
Barbieri et al.	2016	**Hawaii, USA**	Histopath., PCR	HI Monk Seal (Neomonachus schauinslandi)	7
Work et al.	2000, 2002	**Hawaii, USA**	Histopath.	Varied avian species	4 5
Western Pacific—Australia / New Zealand					
Donahoe et al.	2014	Australia	Histopath., PCR	New Zealand Fur Seal (Arctocephalus forsteri)	1
Roe et al.	2013	New Zealand	Histopath., PCR	Hector's / Maui Dolphin (Cephalorhynchus hectori)	7/28
Roe et al.	2017	New Zealand	Pathology, PCR	New Zealand Sea Lioon (Phocarctos hookeri)	1
Michael et al.	2016	New Zealand	Serology: ELISA, LAT, Wblot	New Zealand Sea Lioon (Phocarctos hookeri)	5/55
Eastern Pacific—USA West Coast					
Lambourn et al.	2001	Washington, USA	Serology: MAT	Harbor Seals (Phoca vitulina)	29/380
Gaydos et al.	2007	Washington, Alaska, USA	Serology: IFAT	River Otters (Lontra canadensis)	7/40
Miller et al.	2002	California, USA	Serology	California Sea Otter (Enhydra lutris nereis)	115/223

Author	Year	Location	Detection	Species	Finding
Conrad et al.	2005	California, USA	uncertain	California Sea Otter (Enhydra lutris nereis)	159/305 dead 98/257 live
Shapiro et al.	2012	California, USA	Histopath.	California Sea Otter (Enhydra lutris nereis)	11–19% / 128

Author	Year	Location	Detection	Species	Finding
Southern Pacific—South America					
Calvo-Mac et al.	2020	Chile		Marine otter (Lontra felina)	1/19
				Domestic cats (Felis catus)	4/50
Western Atlantic /Canadian Arctic					
Measures et al.	2004	E. Canada	Serology: MAT	Grey seal (Halichoerus grypus)	11/22
				Hooded seal (Cystophora cristata)	1/60
				Harp seals (Pagophilus groenlandicus)	0/112
Bachand et al.	2019	Nunavut, CAN	PCR, Serology: MAT	Ringed seals (Pusa hispida)	12/61
				Walruses (Odobenus rosmarus)	0/27
				Caribou (Rangifer tarandus)	8/31
				Ptarmigan (Lagopus lago- pus)	0/66
				Geese (Branta spp. Chen spp.)	14/156
Northern Atlantic/Europe					
Forman et al.	2009	England	Serology: Sabin Feldman Dye Test	Varied - Cetaceans	8/101
Jensen et al.	2010	Svalbard, Norway	Serology: Direct Agglutination	Polar bears (Ursus maritimus)	46%

Author	Year	Location	Detection	Species	Finding
				Ringed seals (Pusa hispida)	19%
				Bearded seals (Erignathus barbatus)	67%
				Harbour seals (Phoca vitulina)	0
				White whales (Delphinapterus leucas)	0
				Narwhals (Monodon monoceros)	0
van de Velde et al.	2016	N. Sea and E. Atlantic Ocean	PCR, Serology: MAT, ELISA, IFA	Harbour porpoise (Phocoena phocoena)	2/193 PCRs
				Varied - Marine Mammal	7–41%
Mediterranean					
Bigal et al.	2018	Israel	PCR	Bottlenose Dolphin (Tursiops truncatus)	3
Antarctica					
Rengifo-Herrera et al.	2012	Antarctic	Serology	Southern elephant seals (Mirounga leonina)	10/13
				Weddell seals (Leptonychotes weddellii)	13/31
				Antarctic fur seals (Arctocephalus gazella)	4/165
				Crabeater seals (Lobodon carcinophaga)	1/2
Multi-basin Surveys					
Gibson et al.	2011	Widespread	PCR	Varied - Marine Mammal	94/161

Author	Year	Location	Detection	Species	Finding
Dubey et al.	2003	Widespread	Serology: MAT	Sea otters (Enhydra lutris)	9/115
				Pacific harbor seals (Phoca vitulina)	51/311
				Sea lions (Zalophus californianus)	19/45
				Ringed seals (Phoca hispida)	5/32
				Bearded seals (Erignathus barbatus)	4/8
				Spotted seals (Phoca largha),	1/9
				Atl. bottlenose dolphins (Tursiops truncatus)	141/138
				Walruses (Odobenus rosmarus)	3/53

Table 2. Summary of published research applying spatial/ecological msodels to assess risks associated with T. gondii.

Citation	Location	Disease Data	Analysis Methods	Risk Factor Variables	Findings
Miller et al., 2002	California, USA	223 California Sea Otter (Seroprevalence 52%)	Logistic regression	Sex	Tg > male
				Age	Tg > older
				Sample location	Tg > Morro Bay
				Proximity to freshwater runoff	Tg > more runoff
				Human population density	NS
				Proximity to sewage outflow	NS
Shapiro et al., 2012	California, USA	128 California sea otters (Histopathology; 11-19%)	Odds ratio tests via regression	Age	Tg > Adults
				Sex	NS
				Location	Some risk factors showed Bay-specific associations
				River flow	NS
				Rainfall	Tg > lower 60-day rainfall (at Monterey Bay; Note —stronger association between rain/river flow in more acute S. neurona)
Afonso et al., 2013	France	210 felids (Seroprevalence 62%) European wildcat, Domestic cat	Spatial and temporal cluster detection	Species (or hybrid based on genotype)	NS
				Sex	NS

Citation	Location	Disease Data	Analysis Methods	Risk Factor Variables	Findings
				Age	Tg > in adults
				Farm density	Tg > high farm density
				North Atlantic Oscilation index (weather pattern)	Tg > years with cool and moist winters
Simon et al., 2013	Canadian Arctic	Oocyst load - estimated	Watershed-based hydrological model	NA	NA
				Daily snowmelt / stream flow	Tg oocyst runoff > at beginning of the snowmelt
				River discharge location	Tg oocysts at low concentration at river outlets
				Habitat / landcover type	Tg oocyst accumulation in the estuarine areas likely sufficient to contaminate prey species
VanWormer et al., 2014	California, USA	373 carnivores (PCR screening) Feral domestic cats (30%), Mountain lions (14%), Bobcats (41%), Foxes (17%), Coyotes(4%)	Geographical cluster detection, Odds ratio tests via regression	carnivore group (feral domestic cat, wild felid, wild canid),	Tg Type X > in wild carnivores
				Age class (juvenile, adult)	NS
				Sex	NS
				Year	NS
				Season (wet, dry)	NS

Citation	Location	Disease Data	Analysis Methods	Risk Factor Variables	Findings
				Location	1 cluster of Tg Type II, 2 clusters of Tg Type X
				Landuse / Development	Type II cluster > developent, Type X clusters < development
VanWormer et al., 2016	California, USA	Oocyst load - estimated	GIS Hydrological model	NA	NA
				Transport time (slope, roughness)	Transport times < in developed areas
				Rainfall	Greater precipitation -> greater oocyst transport
				Cat distribution – based on phone surveys, landcover types	Domestic cats > developed areas, Developed areas with > domestic cats and < Transport times correlated w/ Tg
				Oocyst loading - based on published shedding rates	Oocysts > domestic cats
Burgess et al., 2018	California, USA	710 California sea otters (131 tracked) (Seroprevalence 26%)	Logistic model of weighted individual exposure risk	Age class	Tg > with age
			Logistic model of weighted individual exposure risk	Sex	Tg > Male
				Diet	Tg > snail specialists
				Landcover	Tg > crop cover, Tg < forest cover
				Road density	Tg > road density
				Census data for population density, housing density,	Tg > housing and population density

Table 3. Descriptions of variables and associated data sets to be used to model spatial and ecological processes relevant to Hawaiian monk seal risk of *T. gondii* exposure.

Variable	Description	Rationale	Data Source	Potential for Data Improvements
Oocyst Loading				
Companion cats	Estimates of outdoor cats/household × households	Important component of oocyst-shedding source, tied to pet treatment/management	Ward Research 2012 Survey Report summarized by Hawaii Humane Society	* More recent * Repeatable index * Spatially/environmentally explicit
Stray cats	Estimates of outdoor cats/household × households	Important component of oocyst-shedding source, tied to cat abandonment, food source management	Ward Research 2012 Survey Report summarized by Hawaii Humane Society	* More recent * Repeatable index * Spatially/environmentally explicit
Colony cats	Known colony locations, estimated average cats/colony	Important component of oocyst-shedding source, tied to cat abandonment, food source management, colony care-taking	Ward Research 2012 Survey Report summarized by Hawaii Humane Society; Lepczek et al., 2020; PIRO unpublished data	* Specific to HI environments * Repeatable index
Feral cats	Approximated by Landcover type and expected feral cat territory size	Important component of oocyst-shedding source, tied to predator control, natural lands management	Goltz et al., 2008; Hess et al., 2009	* More comprehensive * Repeatable index
Household density	Households per census block	Link to distribution of cats associated with human households/development	2015 US census update data	Data quality sufficient
Residential zoning	Property zone and type (area, # units)	May refine outdoor cat distribution based on residence type (yards)	Tax Map Key data	Data quality sufficient
Tg infection rates	Annual average infection rate for cats	Provides a measure of disease expected per cat population	Wallace 1971; Danner et al., 2007; Davis et al., 2018	* Specific to HI environments/cat populations

Variable	Description	Rationale	Data Source	Potential for Data Improvements
Oocyst shedding rates	Average shedding rate × average oocysts shed per event	Provides a measure of oocyst contamination per cat population	Dabritz et al. 2007; Afonso et al. 2010; VanWormer et al. 2013	* Specific to HI environments/cat populations
Land-Sea Transport				
Precipitation	Annual average rainfall level	Rainfall is a key factor in mobilizing oocysts deposited on land, and runoff to coastal waters	HI rain gauge data	Data quality sufficient
Streamflow	Daily gauge level from USGS	Streamflow is a key factor in transporting oocysts from land to coastal waters	https://waterdata.usgs.gov/hi/nwis/current/?type=flow	Data quality sufficient, though not even across islands
Topography	USGS DEM at 10-m res.	Elevation and slope are influential in water flow dynamics and erosion	https://www.pacioos.hawaii.edu/metadata/usgs_dem_10m_oahu.html	Data quality sufficient
Landuse / Landcover	MRLC classification, 1-5 m res.	Vegetative cover and land hardening/development are influential in water flow dynamics and erosion	https://www.mrlc.gov/data/noaa-2011-high-resolution-land-cover-hawaii-0	Data quality sufficient
Hawaiian Monk Seal Exposure				
T. gondii cases	Confirmed or suspected cases	Case information and location can be used to assess relationships with risk factors (note: few cases may often lead to qualitative rather than statistically significant conclusions)	HMSRP strandings/health monitoring data	* Higher/more consistent detection rates * Serial serological sampling to determine non-lethal exposure
Sex	Sex of known seals	Female monk seals are observed to have higher incidence of Tg strandings	HMSRP sightings database	Data quality sufficient

Variable	Description	Rationale	Data Source	Potential for Data Improvements
Age	Age of known seals	Breeding aged (adult) monk seals are observed to have higher incidence of Tg strandings	HMSRP sightings database	Data quality sufficient
Utilization distribution	Area used, and intensity of use, by known seals	Use of coastal areas will provide an estimate of potential oocyst exposure	HMSRP sightings database	* Higher resolution tracking, especially for female seals
Diet items	Prey items consumed by monk seals	Consumption of potential oocyst accumulators will provide an estimate of potential oocyst exposure	Not specified in model	* Improved analysis of individual diet variation in MHI

Table 4. Summary of T. gondii-related mortalities in Hawaiian monk seals (2004–2020).

Seal ID	Stranding date	Island	Year	Size	Sex	Case Designation* (Prob. T. g. COD**)	Diagnostic Notes***
R011	10/2/07	Maui	2007	A	F	Suspect (0.90)	Carcass in advanced decomposition, tissues autolyzed, T. gondii detected on PCR
RK29	9/14/05	Oahu	2005	A	M	Suspect (0.75)	Cessation of cardiac function with intense myocarditis, suspected association with Toxoplasma-like cysts observed in lung tissue
RK07	1/23/04	Kauai	2004	A	M	Confirmed (1.0)	Disseminated Toxoplasmosis, confirmed with IHC
KA060D03	5/22/06	Kauai	2006	J2	M	Confirmed (1.0)	Disseminated Toxoplasmosis, confirmed with IHC
RH40	3/17/10	Kauai	2010	A	M	Confirmed (1.0)	Disseminated Toxoplasmosis, confirmed with IHC

RTX1	1/25/10	Molokai	2010	P1	F	Confirmed (1.0)	Disseminated Toxoplasmosis, confirmed with IHC, presumed transmission from the dam which was not sighted again/ presumed dead but never detected/evaluated
R017	4/15/14	Oahu	2014	A	F	Confirmed (1.0)	Disseminated Toxoplasmosis, confirmed with IHC
RB24	3/17/15	Oahu	2015	A	F	Confirmed (1.0)	Disseminated Toxoplasmosis, confirmed with IHC, died after attempted rehabilitation
RGX2	3/11/15	Oahu	2015	P0	M	Non-case (1.0)	Death secondary to dam's (RB24) severe Tg infection, pup aborted ~one week prior to death of dam, though not detected in pup tissues, death was considered ultimately related to Tg.
RN36	11/13/15	Oahu	2015	A	F	Confirmed (1.0)	Disseminated Toxoplasmosis, confirmed with IHC
RK60	5/15/18	Oahu	2018	A	F	Confirmed (1.0)	Disseminated Toxoplasmosis, confirmed with IHC
RKD2	5/16/18	Oahu	2018	P1	F	Confirmed (1.0)	Disseminated Toxoplasmosis, confirmed with IHC, genetic tests confirmed pup to be offspring of RT10, also died from Tg.
RT10	5/17/18	Oahu	2018	A	F	Confirmed (1.0)	Disseminated Toxoplasmosis, confirmed with IHC
RKC1	1/26/20	Oahu	2020	J1	M	Confirmed (1.0)	Disseminated Toxoplasmosis, confirmed with IHC
RO28	4/1/20	Oahu	2020	A	F	Confirmed (1.0)	Disseminated Toxoplasmosis, confirmed with IHC, died after >2 months of rehabilitative care