Detecting and quantifying parasite-induced host mortality from intensity data: method comparisons and limitations

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Abstract

Parasites can often have significant impacts on host populations through parasite-induced host mortality. Detecting and quantifying the magnitude of parasite- induced mortality, particularly for macroparasites in which pathology is linked to to the intensity of infection, can provide important insight into parasite dynamics and host population management. This is, however, notoriously difficult in practice as the only data often available are measures of parasite intensities. In this study we provide a novel method for detecting and quantifying parasite-induced mortality in wildlife populations from intensity data and, using simulations, we show that our method is more reliable for detecting and quantifying parasite-induced host mortality than the methods that are currently available. We also show that this method provides quantitative estimates of parasite-induced mortality in empirical data that are consist with previously published qualitative estimates. However, we stress that this method, along with all methods for estimating parasite-induced mortality in host populations for intensity data alone, has a number critical assumptions that limit their applicability in the real world. We conclude that methods for estimating and detecting parasite-induced mortality from intensity data should be used only as an exploratory tool for informing more rigorous studies of parasite-induced host mortality.

Introduction

Infectious agents can have major impacts on animal populations through changing population dynamics and stability (Dobson & Hudson 1992), altering predator-prey interactions (Joly & Messier 2004), and even causing species' decline and extinction (De Castro & Bolker 2005; McCallum 2012). Accurately estimating the impact of these infectious agents in wildlife is critical to understanding what regulates host and

parasite populations, making predictions about disease transmission, and managing disease outbreaks (Langwig *et al.* 2015). The impact of microparasite pathogens, such as rabies (Coyne *et al.* 1989), bovine TB (Cox *et al.* 2005), and rinderpest (Tillé *et al.* 1991), is typically quantified based on the presence or absence of disease, and does not account for the number of infectious agents present. This method is sufficient for many bacterial and viral agents that reproduce within a host, however for macroparasites, pathology is linked to the intensity of infection and hosts cannot be simply categorized as infected and uninfected (Anderson & May 1979; Lafferty & Kuris 2002). Helminths exhibiting this intensity dependent pathology have significant impacts on human health (Brooker *et al.* 2004), domestic livestock economics (Roeber *et al.* 2013), and wildlife survival (Kirk 2003; Logiudice 2003). While it is generally assumed that some fraction of wild host populations must succumb to parasitic infections, it is notoriously difficult to actually quantify parasite-induced host mortality (PIHM) in wild animal populations (McCallum 2000).

Ideally, parasite-induced host mortality is quantified by experimentally infecting and tracking individual hosts in the wild population; however, for logistical and ethical reasons this method is rarely feasible (McCallum 2000). Data on parasite intensity is much easier to collect and has often been used to identify the presence of PIHM (Crofton 1971; Lester 1977, 1984; Lanciani & Boyett 1989; Royce & Rossignol 1990; Ferguson *et al.* 2011) and to quantify the relationship between infection intensity and host mortality (Adjei *et al.* 1986).

Crofton (1971) first proposed that PIHM could be identified by comparing the observed parasite distribution in the host population to the distribution predicted in the absence of parasite-induced mortality. We briefly introduce the Crofton Method here and provide a more detailed explanation of its implementation in *Supplementary Material (SI)* 1. This method assumes that, prior to host mortality, infection intensity in the host population follows a negative binomial distribution and the tail of the distribution is truncated as intensity dependent pathology removes the most heavily infected hosts.

Assuming mortality occurs only in heavily infected hosts, evidence of this parasite-induced mortality should then be detectable by iteratively fitting a negative binomial distribution to hosts with lower and lower parasite loads, and comparing these truncated predicted distributions to the corresponding truncated observed parasite data. [FIGURE]

The Crofton Method may be able to detect the presence of PIHM, but it does not quantify the relationship between infection intensity and host survival probability. Adjei *et al.* (1986) suggested that this relationship could be calculated by first using the Crofton Method to estimate the pre-mortality parasite distribution and then using this distribution to calculate the probability of host survival with increasing parasite intensity. To do this, Adjei *et al.* (1986) modeled host survival as a logistic function and then used a generalized linear model (GLM) to estimate the logistic parameters (see *SI* 2 for a technical description of the Adjei Method). Adjei *et al.* suggested that this method could provide an estimate for the parasite intensity at which a host has a 50% chance of suffering parasite-induced mortalith (LD_{50}). However, to implement this method the observed data must be modified to fit the GLM framework and subjectively binned when mean infection intensity is high or sample sizes are small (see SI 2 for details).

After 30 years, and despite clear limitations (McCallum 2000), these methods (particularly the Crofton Method) are still discussed among parasitologists and are the primary techniques for examining population level impacts of parasitism using parasite intensity data. In these methods, PIHM can only be identified by visually examining plots and, with no clear decision rule, it can be difficult to determine the significance of PIHM across different host-parasite systems. The survival function given by the Adjei Method offers one solution; however, this method requires manipulating the original data and its accuracy has never been validated.

Intensity data should be used to estimate parasite impacts on host populations only if unbiased and accurate methods exist. In this study, we first propose a novel method for detecting and quantifying PIHM. We next use simulations to compare our method with the previous Adjei Method to test the ability of both to (1) detect occurrence of PIHM

and (2) estimate the lethal parasite load (LD_{50}) and the associated survival function. We then apply both methods to real datasets previously used in PIHM analyses and compare the results. Finally, we discuss the limitations of inferring PIHM from intensity data and whether any method for inferring PIHM has a place in quantitative parasitology.

Methods

A novel, likelihood-based method for estimating PIHM

Here we propose a novel, likelihood-based method (henceforth Likelihood Method) that does not require binning or data alteration, reduces the number of parameters to be estimated, is highly generalizable, and uses standard statistical techniques to determine PIHM significance. The Likelihood Method begins with the same assumptions as the Adjei Method: namely that infection of a host, parasite-induced mortality of a host, an the sampling of a host population occur at distinct time intervals during a hosts life. As discussed by Adjei *et al.*, this is not necessarily unrealistic as studies have shown that infection and host mortality are often age and/or body size dependent [citations].

The Likelihood Method then assumes that prior to mortality the parasite distribution can be described by the distribution $g(x; \boldsymbol{\phi})$, which specifies the probability of a host having x parasites when it is observed. $\boldsymbol{\phi}$ is a vector of parameters that describes the shape this distribution.

The method then assumes that the probability of a host surviving with x parasites from infection until sampling is given by $h(\text{survival}; x, \boldsymbol{\theta})$ where $\boldsymbol{\theta}$ specifies any additional parameters needed to define the host survival function.

With these two assumptions, we can define a probability distribution that gives the probability of having a parasite load of x parasites conditional on host survival. Using standard rules of conditional probability this distribution can be written as

$$P(x|\text{survival}) = \frac{P(\text{survival}|x) * P(x)}{P(\text{survival})} \quad (1)$$

$P(\text{survival}|x)$ is the survival function $h(\text{survival}; x, \boldsymbol{\theta})$, $P(x)$ is the pre-mortality parasite distribution $g(x; \boldsymbol{\phi})$ and $P(\text{survival}) = \sum_{x=0}^{\infty} P(\text{survival}|x) * P(x) = \sum_{x=0}^{\infty} h(\text{survival}; x, \boldsymbol{\theta}) * g(x; \phi)$. Therefore equation 1 can be written as

$$P(x|\text{survival}) = \frac{h(\text{survival}; x, \boldsymbol{\theta}) * g(x; \boldsymbol{\phi})}{\sum_{x=0}^{\infty} h(\text{survival}; x, \boldsymbol{\theta}) * g(x; \boldsymbol{\phi})} \quad (2)$$

Using this probability distribution, one can then find the parameters $\boldsymbol{\theta}$ and $\boldsymbol{\phi}$ that maximize the likelihood of an observed host-parasite dataset. To estimate the significance of PIHM in a host-parasite system, a likelihood ratio test can be used in which the full model is given by equation 2 and the reduced model is given by the pre-mortality distribution $g(x; \boldsymbol{\phi})$. If PIHM is not significant in the system, the resulting likelihood ratio statistic should approximately follow a χ^2 distribution with degree of freedom equal to the number of parameters in the full model with parasite-induced mortality minus the number of parameters in the reduced model without parasite-induced mortality [citation].

Equation 2 could be parameterized in many different ways depending on the parasite system of interest. In this study, we adopt the typical assumption that the pre- mortality parasite distribution $g(x; \boldsymbol{\phi})$ follows a negative binomial distribution with the parameters mean parasite intensity (μ_p) and aggregation (k_p) before mortality, respectively (smaller k_p indicates more aggregation) (Crofton 1971; Anderson & May 1978; Adjei *et al.* 1986). The negative binomial distribution can arise as the equilibrium parasite distribution under a variety of different biological and statistical assumptions (Kendall 1948; Boswell & Patil 1970; Calabrese *et al.* 2011). However, it is also an incredibly flexible distribution that fits many host-parasite systems regardless of whether the underlying mechanisms lead to an exact negative binomial distribution (Shaw *et al.* 1998).

Choosing an appropriate function for $h(\text{survival}; x, \boldsymbol{\theta})$ will depend on the system

under consideration. Many theoretical models of parasite-induced host mortality assume that the parasite-induced death rate of hosts is a linear function of parasite intensity (Anderson & May 1978; Dobson & Hudson 1992; Barbour & Pugliese 2000). It has been previously noted that parasite-induced mortality can be nearly impossible to detect from intensity data when the host survival function is a linear function of parasite intensity as the post-mortality distribution will be of a similar form as the pre-mortality distribution (Lanciani & Boyett 1989). For example, Barbour & Pugliese (2000) showed that both simple host parasite models without parasite-induced host mortality and those with linear parasite-induced host mortality produced Poisson distributions at equilibrium. More complex, non-linear survival functions were needed to produce over-dispersed of under-dispersed post-mortality distributions. This is not unrealistic as empirical evidence has shown that non-linear host-survival functions are not uncommon (Benesh 2011) [phrasing].

As one of the goals of this study is to compare this new Likelihood Method to the previously proposed Adjei Method, we adopt the host- survival function used in their study and assume host-survival is non-linear and follows a logistic function given by

$$h(\text{survival}; x, a, b) = \frac{e^{a - b\log(x)}}{1 + e^{a - b\log(x)}} \tag{3}$$

where $b/4$ determines the maximum rate of decline of host survival probability with increasing parasite load, analogous to the pathogenicity parameter α in traditional macroparasite models (Anderson & May 1978). When b is held constant, for every one unit increase in a the parasite intensity at which 99% of hosts survive increases by $1/b$. The equation $\exp(a/b)$ can also be used to calculate the parasite LD_{50}, here defined as the infection intensity at a host has a 50% dying. This function is commonly used in toxicology and survival analysis and has the useful properties of being bounded between 0 and 1 and being differentiable for all x. That being said, it is phenomenological and there is little justification to use it rather than it tends to survival data. However, given that a goal of

these analyses is to compare this method's results the those given by the Adjei Method it is natural to adopt the same host-survival function to facilitate comparison. When applying the likelihood method to other systems, other more mechanistic host-survival functions can be used in place of equation 3.

Evaluating the Adjei and Likelihood Methods

Question 1: Can we detect PIHM?

We tested the ability of the Adjei and the Likelihood Methods to identify the presence of PIHM on simulated data with known pre-mortality parameters. Consistent with the assumptions of the model that parasite infection, mortality, and sampling occur at distinct life stages of the host, we first created a pre-mortality host population by drawing N_p randomly infected hosts from a negative binomial distribution with parameters μ_p and k_p. This is equivalent to the period of hosts becoming infected with parasites given in Adjei *et al.* (1986). In the Adjei Method and Crofton Method, N_p is a necessary parameter that is defined as the number of hosts in the population before parasite-induced mortality. A more appropriate way to define this parameter is the number of hosts that would have been sampled had parasite-induced host mortality not occurred. This parameter is not necessary using the Likelihood Method because unlike the Adjei Method and Crofton Method which estimate parasite-induced mortality using absolute numbers of hosts, the Likelihood Method estimates parasite-induced mortality using probabilities. However, to compare the results of the Likelihood Method with the Adjei Method, we specified a value for N_p for all simulations.

Second, we chose values of a and b for the host survival function and calculated the probability of survival for all N_p hosts using equation 3. Then, for each host, we drew a random number from a uniform distribution between 0 and 1 and if the calculated host survival probability was less than this random number, the host experienced parasite-induced mortality. This was the period in which hosts died due to infection. The parasite

distribution in these simulated surviving hosts represented the parasite distribution in a wild host population that has undergone parasite-induced host mortality.

We used these simulated pre-mortality and post-mortality datasets to test the ability of both methods to correctly determine whether or not PIHM was occurring when the parameters N_p, μ_p and k_p were known. Although the parameters N_p, μ_p, and k_p are always unknown in real systems, a method that fails under these ideal simulation conditions will certainly also fail using less ideal, empirical data. In practice, for the Adjei Method, N_p, μ_p, and k_p are estimated using the Crofton Method (Adjei *et al.* 1986), while μ_p and k_p in the Likelihood Method can be estimated jointly with a and b or via the Crofton Method.

We used three different values of μ_p (10, 50, 100) and for each μ_p we examined three different survival functions that had gradual, moderate, and steep decreases in the host survival with increasing parasite intensity (Figure 1A). For a given μ_p, each survival function had the same LD_{50} ([$\mu_p = 10$, $LD_{50} = 7.39$], [$\mu_p = 50$, $LD_{50} = 35.57$], [$\mu_p = 100$, $LD_{50} = 77.3$]), but different values of a and b. We examined each μ_p-survival function pair at three levels of parasite aggregation, $k_p = 0.1$, 0.5, and 1 — realistic values of parasite aggregation in natural populations (Shaw *et al.* 1998). For each of these parameter combinations we simulated 150 datasets and tested the probability of each method correctly identifying PIHM in the post-mortality dataset (power) and incorrectly identifying PIHM in the pre-mortality dataset (Type I error). For each method, we used a likelihood ratio test to determine whether the full model with PIHM provided a significantly better fit than the reduced model without PIHM at significance level of 0.05. We tested each parameter combinations for pre-mortality population sizes of $N_p =$ [50, 100, 200, 300, 400, 500]. N_p is not technically the sample size on which the methods are being tested for the post-mortality data because PIHM reduces N_p for each simulated dataset. We therefore used the average number of surviving hosts over all 150 simulations for a given parameter combination as our measure of sample size in the power simulations.

In the next simulation, we tested the ability of only the Likelihood Method to correctly identify PIHM and estimate LD_{50} when the pre-mortality parameters are unknown. As a best-case scenario, we simulated host- parasite systems with $\mu_p = 10$ and $k = 1, 0.5, \text{and} 0.1$ [re-run for 0.5 and 0.1] as it is easier to detect PIHM from small samples sizes when mean parasite intensity is low. We then used the Likelihood Method to identify PIHM for gradual, moderate and steep survival functions when the pre-mortality parameters μ_p and k_p also needed to be estimated. We perform 500 simulations over a range of different samples sizes following the simulation procedure described above.

Question 2: Can we estimate properties of the host survival function?

In the previous section we compared the ability of the Adjei Method and the Likelihood Method to correctly identify whether or not PIHM was a occurring in a system (i.e. a yes or no answer). In this section we compare the ability of the Adjei Method and the Likelihood Method to estimate properties of the survival function such as the parameters a, b and LD_{50}. To do this, we used the same simulation procedure and parameter combinations described above. For each parameter combination we simulated 150 datasets, estimated a, b, and LD_{50} and calculated the standardized bias and precision (Walther & Moore 2005) for these estimates. Because estimating properties of the host survival function requires more information than simply detecting PIHM, we used larger values of N_p for this simulation (N_p = [300, 500, 1000, 2000, 5000, 7500, 10000]). We used the average number of surviving hosts over all 150 simulations for a given parameter combination as our measure of sample size. Because parameters a and b showed similar patterns of bias and precision, we only show the results for a.

Application to real data

We tested the ability of the Adjei Method and the Likelihood Method to identify PIHM in 6 host-parasite datasets given in Crofton (1971) and 4 datasets given in Adjei *et al.* (1986) (Table 2). Crofton analyzed infection patterns in the snail *Gammarus pulex* infected with

the acanthocephalan *Polmorphus minutus*. Adjei *et al.* analyzed males and females of two species of lizard fish *Saurida tumbil* and *S. undosquamis* that were infected by the cestode *Callitetrarhynchus gracilis*.

In both earlier studies, the authors reported PIHM in some of the datasets and we tested whether the Adjei Method and/or the Likelihood Method also predicted PIHM. For the 6 datasets from Crofton (1971), we truncated the data at 4 parasites, applied the Crofton Method to estimate the pre-mortality distribution, and then ran the Likelihood Method and Adjei Method using these pre-mortality parameters. For the Adjei *et al.* (1986) datasets, we followed the same procedure as the authors and first truncated the data at 2 parasites and then fit the Crofton Method for the female fish of both species. Then, following the original authors' methods, we parameterized the male pre-mortality distributions for each species with the results from the females. Finally, we applied the Adjei Method and the Likelihood Method to determine whether or not PIHM was significant for these species and compared our results to those given by the authors. All fitting to data was done with the code provided in *SI* 4.

Results

Question 1: Detecting presence of PIHM

The power of the Adjei Method to detect PIHM in a system was close to unity for larger sample sizes and tended to decrease as sample size decreased (Figure 1C; *SI* 3 Figs 1-3). The Likelihood Method had a power close to unity for all parameter combinations and sample sizes considered. With gradual survival functions, the power of the Likelihood Method decreased slightly for small samples sizes (Fig. 1C, *SI* 3 Figs 1-3).

The Adjei Method showed highly inflated Type I error rates (i.e. falsely detected PIHM) for all parameter combinations that we considered (Fig. 1B; *SI* 3 Figs 1-3). This method also showed the unintuitive pattern of Type I error rate decreasing as

sample size decreased. This pattern was due to the issue of binning discussed in the *Introduction* and *SI* 2. For small samples sizes, the applicability of the Adjei Method is compromised without binning the observed data in some way. In contrast, the Likelihood Method showed a Type I error rate at or near the pre-set level of 0.05 for all parameter combinations and sample sizes considered (Fig. 1B; *SI* 3 Figs 1-3).

Question 2: Estimating the LD_{50} and survival function

The Likelihood Method gave asymptotically unbiased estimates of the LD_{50} for all combinations of parameters examined in this study (Fig. 2, *SI* 3 Figs 4-6). Even for the smallest sample sizes we considered, the Likelihood Method's estimate of LD_{50} was largely unbiased, with small biases occurring for host survival functions that were gradual. The precision of the Likelihood Method's LD_{50} estimates decreased (increasing coefficient of variation) as sample size decreased for all parameter combinations we examined (Fig 2, *SI* 3 Figs 4-6).

The Adjei Method produced biased estimates of the LD_{50} across nearly all parameter combinations (Fig 2, *SI* 3 Figs 4-6). For $\mu_p = 10$, the LD_{50} estimates from the Adjei Method were largely unbiased for large samples sizes, but as μ_p increased, the Adjei Method produced biased estimates of LD_{50} across all sample sizes, with bias increasing as sample size decreased (Figure 2, *SI* 3 Fig 4-6). The LD_{50} estimates from the Adjei Method showed large decreases in precision with the steepest survival function across all values of μ_p (Figure 2, *SI* 3 Fig 4-6).

In terms of the host survival function, the Likelihood Method gave unbiased estimates of survival function parameters when sample sizes were large, however as sample size decreased these estimates became severely biased (Fig. 2, *SI* Fig 7 - 9) The Adjei Method produced biased estimates of the host survival function across all sample sizes, with the bias consistently being larger when the survival function was steeper and μ_p was larger (Fig 2, *SI* 3 Figs 7-9).

Detecting PIHM with unknown pre-mortality parameters

When all parameters were jointly estimated, the Likelihood Method showed highly context-dependent results when detecting PIHM in the best-case scenario of $\mu_p = 10$ and $k_p = 1$. The Likelihood Method's power of detecting PIHM was greater than 0.8 when host sample sizes exceeded 424 and 83 for survival functions that were moderate and steep, respectively (Fig 3). When the host survival function was gradual, the Likelihood Method never had a power greater than 0.8 for any post-mortality samples sizes we considered (Fig 3).

Application to real data

The previous authors qualitatively detected PIHM in 7 of the 10 datasets considered(Table 2). The Likelihood Method parameterized from the pre-mortality parameters of the Crofton Method detected significant PIHM in 6 of these 7 datasets at a significance level of 0.05. The only dataset in which the Likelihood Method did not detect a significant effect of PIHM was the Adjei dataset for female *S. tumbil*. For this dataset there was a marginally significant effect of PIHM ($\chi^2_{df=2} = 5.34; p = 0.069$). The Adjei Method detected PIHM in 9 of the 10 datasets (Table 2), consistent with our simulation results that the Adjei Method has a high Type I error rate.

Discussion

Quantifying the impact of parasitism on wild host populations is critical for managing wildlife populations and understanding parasite-host dynamics. Ideally the relationship between infection intensity and host survival would be measured experimentally, but for logistical and ethical reasons, this is often impossible (McCallum 2000). Looking for evidence of mortality in parasite distribution data requires the least amount of information, but is notoriously difficult to implement. The methodological flaws in the

Adjei Method limit its utility, so here we propose an alternative, likelihood-based, method to estimate host survival and the LD_{50} from observed parasite intensity data. This method is a significant improvement over the previous methods because it requires fewer parameters, provides a statistical decision rule for identifying PIHM and does not require any data manipulation.

Using simulated data, we found that the Likelihood Method always out performed the Adjei Method. For simply detecting the presence of PIHM, the Likelihood Method was both more powerful and had fewer false detection events (Type I errors). When both methods were applied to published datasets previously used in PIHM analyses, the Adjei Method tended to detect PIHM where it had not previously been reported, consistent with the high Type I error rate observed in our simulations. The Likelihood Method was also more precise and less biased in calculations of both the parasite LD_{50} and host survival curve over the parameter values we considered. However, while only the Likelihood Method produced precise and unbiased LD_{50} estimates, neither method could provide unbiased estimates of the host survival function at realistic sample sizes. These simulations demonstrate that the Likelihood Method is more powerful and precise than the previously proposed Adjei Method.

Although superior to the Adjei Method, the Likelihood Method is not universally applicable to real data. Our simulations showed when the when pre- mortality parameters were estimated directly, the Likelihood Method needed at least 83-424 samples to have 80% power for steep to moderate survival functions, an even larger sample size as the survival function became more gradual. While some of these sample sizes are reasonable for hosts such as invertebrates or small fish, even the smallest sample sizes are completely unfeasible for many vertebrates, particularly the species of conservation concern where addressing the impact of parasitism would be most important. An even larger sample size would be required to identify PIHM when parasites are highly aggregated, mean infection intensity is high, or parasite prevalence is low, all of which are common in many parasitic helminths. Moreover, our results are in agreement with previous work that has shown

that as host-survival functions become progressively more linear, PIHM becomes all but impossible to detect (Lanciani & Boyett 1989). This result, however, does not preclude the use of this method as non-linear survival functions are not uncommon in empirical host-parasite systems (Benesh 2011). Finally, while linear functions make PIHM undetectable, at the other extreme, steep, non-linear survival curves produce severely biased estimates of the survival function. Give the interaction between all of these different factors, the Likelihood Method is probably limited to detecting PIHM in systems where greater than 100 hosts can be collected, parasites are common and only moderately aggregated, and substantial host mortality occurs at relatively low parasite intensity.

While we have improved on the existing methods for quantifying PIHM from parasite intensity data, all such methods require several fundamental, and potentially problematic assumptions. Nearly all current methods derive from Crofton (1971) (but see Ferguson *et al.* 2011) and assume that, prior to any PIHM, parasites are distributed in the host population following a negative binomial distribution. But, it is fundamentally impossible to know what the pre-mortality parasite distribution was in a wild host population and it is widely recognized that different processes can lead to a variety of parasite distributions in hosts (Anderson & Gordon 1982; Duerr *et al.* 2003). However, the negative binomial is extremely flexible and there is substantial empirical and theoretical evidence to support the assumption that, prior to any PIHM, parasite distributions can be fit by a negative binomial distribution (Shaw & Dobson 1995; Shaw *et al.* 1998; Wilson *et al.* 2002).

Unfortunately, this flexibility in the distribution may also reduce our ability to detect PIHM. If a negative binomial can be fit to the observed post-mortality parasite distribution then, regardless of how lethal the parasite was, it will be impossible to detect PIHM because there is no need for a more complex model. Most observed parasite distributions are well fit by the negative binomial distribution (Shaw *et al.* 1998), suggesting that systems where these methods are applicable may be more the exception than the rule. Furthermore, even when truncation of the negative binomial distribution is

detected, it may be caused by other processes such as within host density dependence, age dependent variation in host resistance and/or heterogeneous infection rates (McCallum 2000; Anderson & Gordon 1982; Rousset *et al.* 1996). This means that in the event that PIHM is detected, it may actually not be the result of PIHM.

Given these numerous caveats, is there a place in parasitology for methods that estimate PIHM from intensity data? We are in agreement with Lester (1984) that, at the very least, methods for estimating PIHM can provide preliminary insight into whether or not PIHM is worth further exploration. However, we stress that these methods should only be used as an exploratory tool when assessing the role of PIHM in a system, and potential users should critically evaluate whether they think they have a large enough sample size and an appropriate host survival function/post-mortality distribution for the methods developed in this paper to be applicable. Even if they are applicable, inferring PIHM from distributional data is no substitute for field experiments and an in depth understanding of the natural history of the host-parasite system under consideration.

Acknowledgments

TODO

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Table 1: Definition of parameters and functions used in the main text

Parameter	Definition
μ_p	Pre-mortality mean parasite intensity
k_p	Pre-mortality parasite aggregation
N_p	Pre-mortality host population size
x	Number of parasites in a given host
$g(x; \mu_p, k_p)$	Pre-mortality negative binomial parasite distribution
$h(\text{survival}; x, a, b)$	The probability of host survival given a parasite load x and logistic parameters a and b
$b/4$	The maximum rate of decline in host survival probability with increasing parasite load
a	When b is held constant a one unit increase in a leads to a $1/b$ increase in the parasite intensity at which 99% of hosts survive
LD_{50}	$\exp(a/b)$, parasite intensity at which a host has a 50% chance of dying

Table 2: Comparison of the PIHM predictions of previously used host-parasite datasets to those given by the Adjei Method and the Likelihood Method. The first column specifies the identity of the dataset, the second column specifies whether or not the authors indicated that PIHM was occurring in the system based on a qualitative assessment, the third column indicates whether or not the Likelihood Method with pre-mortality parameters estimated from the Crofton Method detects significant PIHM, and the final column indicates whether the Adjei Method with pre-mortality parameters estimated from the Crofton Method detects PIHM. If a method detected significant PIHM the predicted LD_{50} is given in parentheses.

Data Set (sample size)	Author detected PIHM?	Likelihood Method?	Adjei Method?
Crofton, Station 1 ($n = 538$)	Yes	Yes (7.27)	Yes (9.33)
Crofton, Station 2 ($n = 507$)	Yes	Yes (6.92)	Yes (14.95)
Crofton, Station 3 ($n = 633$)	Yes	Yes (5.93)	Yes (5.98)
Crofton, Station 4 ($n = 486$)	No	No	Yes (7.99)
Crofton, Station 5 ($n = 276$)	No	No	Yes (10.58)
Crofton, Station 6 ($n = 191$)	No	No	No
Adjei, *S. tumbil* female ($n = 446$)	Yes (5.7)	No	Yes (6.37)
Adjei, *S. tumbil* male ($n = 452$)	Yes (3.4)	Yes (3.42)	Yes (3.66)
Adjei, *S. undosquamis* female ($n = 2573$)	Yes (3.2)	Yes (3.04)	Yes (3.11)
Adjei, *S. undosquamis* male ($n = 2440$)	Yes (1.8)	Yes (1.83)	Yes (1.78)

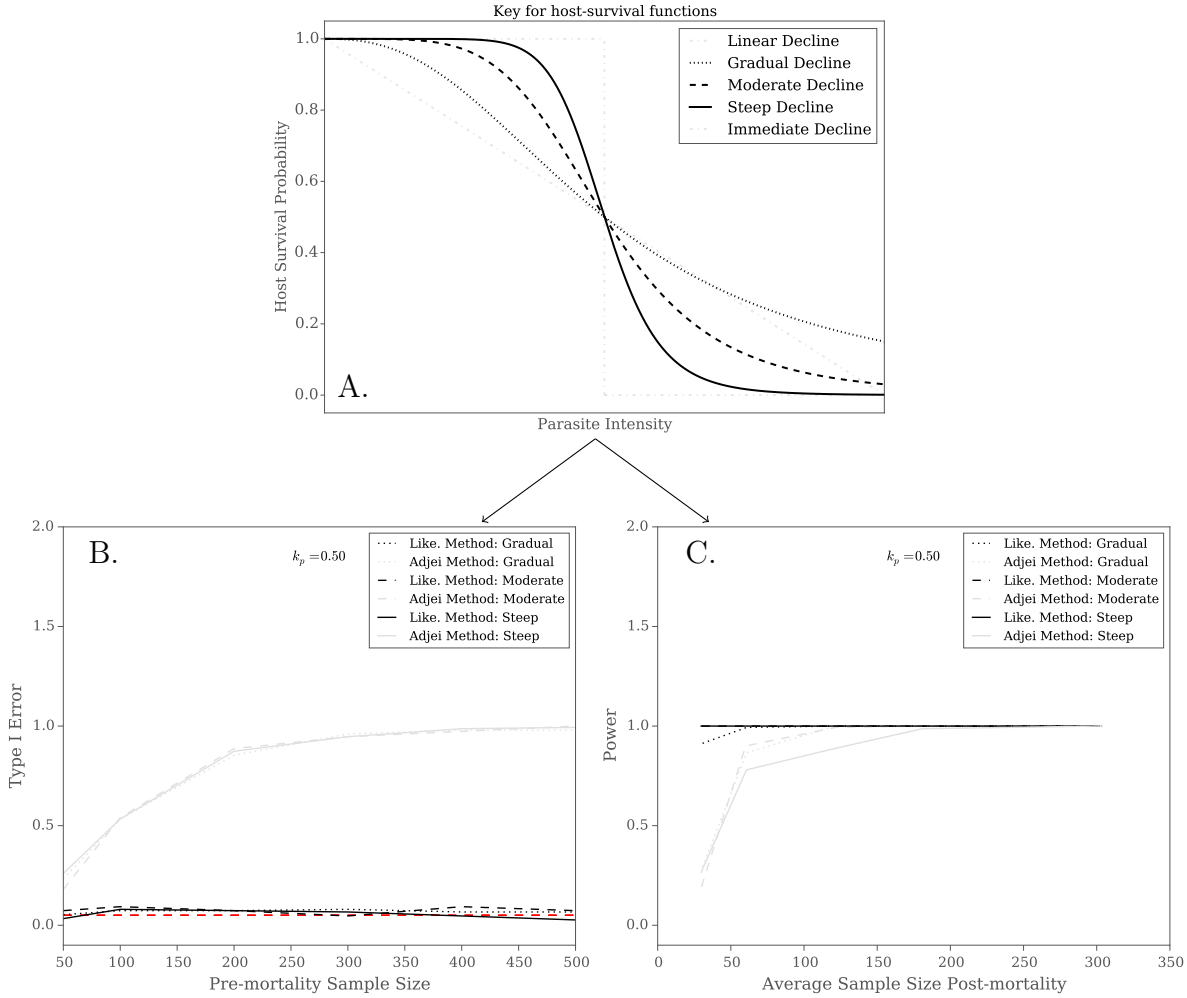

Figure 1: A) Five potential shapes for a host-survival functions. In the simulations we used a gradual survival function (dotted line), and moderate survival function (dashed line), and a steep survival function (solid line). The linear and immediate survival functions represent two potential extremes that we do not include in the simulations. For each of these survival functions and the parameter combinations described in the main text, we tested the Type I error and power of the Likelihood Method and Adjei Method. B) Gives the Type I error of each method over a range of pre-mortality sample sizes with a pre-mortality mean parasite intensity (μ_p) of 50 and pre-mortality parasite aggregation (k_p) at 0.5. The red line shows the pre-set significance level of 0.05. C) Gives the Power of each method for detecting PIHM over a range of post-mortality sample sizes for $\mu_p = 50$ and $k_p = 0.5$. In general, the Likelihood Method has higher power and lower Type I error than the Adjei Method. See the *SI* 3 Fig 1 - 3 for Type I Error and power results for all parameter combinations.

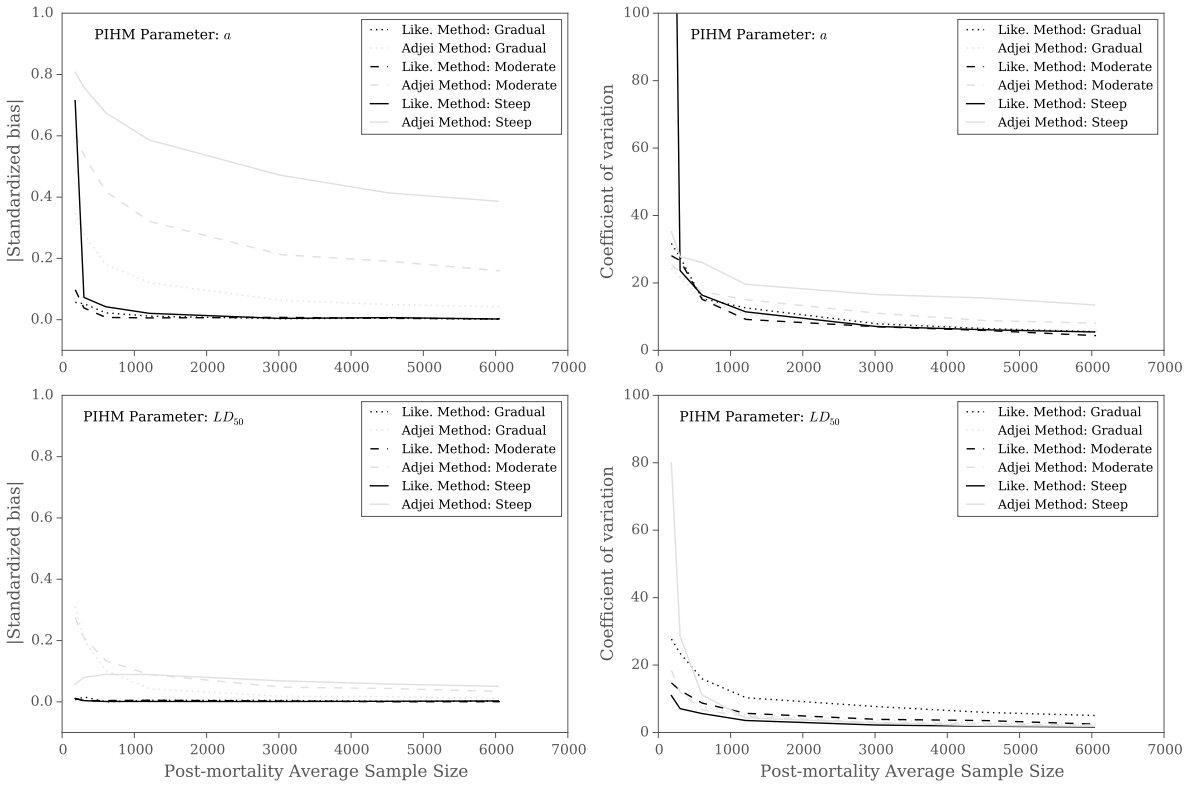

Figure 2: Bias and precision (coefficient of variation) for the Likelihood Method and Adjei Method estimates of the a parameter and the LD_{50} of the host survival function based on simulated PIHM data over a range of post-mortality sample sizes. As the coefficient of variation increases, precision decreases. The pre-mortality parameters for this simulation were $\mu_p = 50$ and $k_p = 0.5$. The figure shows the simulations for three different host survival functions (gradual, moderate, and steep), each with the same LD_{50}. Bias and precision results of LD_{50} and a for all other parameter combinations can be found in *SI* 3 Fig 4 - 9.

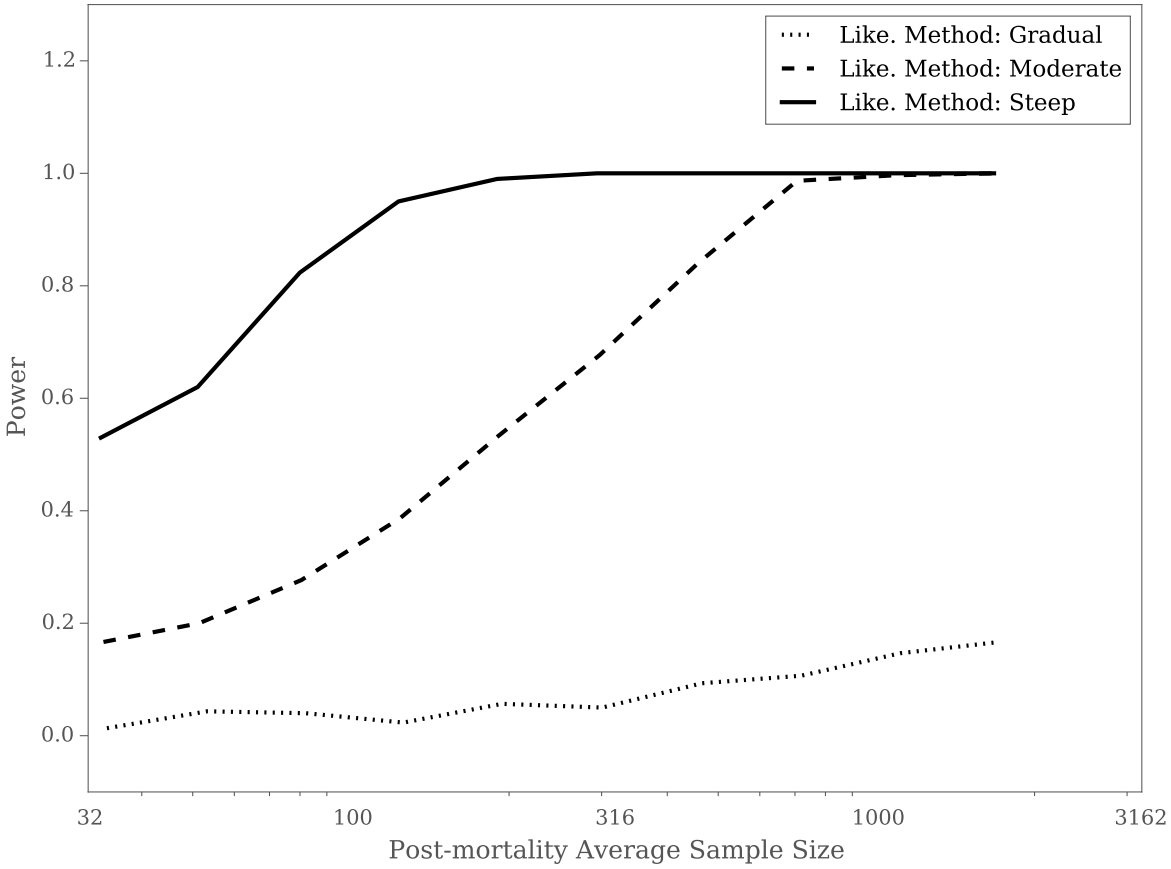

Figure 3: The power of the Likelihood Method to detect PIHM for gradual, moderate, and steep survival functions when all four parameters μ_p, k_p, a, and b were jointly estimated. The curves were generated from 500 simulations for 10 pre-mortality population sizes, N_p.