

NEW METRICS FOR EVOLVING RISKS  
TO HUMAN AND WILDLIFE HEALTH

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# Abstract

Humans are altering the global environment at a rapid pace, which impacts ecosystems and creates opportunities for the spread of infectious diseases to new species. Healthy ecosystems are often dependent on healthy human populations and vice versa. However, resolving risks to human and wildlife populations requires insights provided by a scale of data that is not often available. New modeling approaches can be used to maximize the use of available data to achieve critical insights. In this dissertation, my collaborators and I develop new analytical and statistical approaches to understand the magnitude of risks to the health of human or wildlife populations and provide practical knowledge to mitigate those risks. We first examine the seasonal risk of spillover of a pathogenic acanthocephalan parasite to southern sea otters, which we find is driven by intermediate host population dynamics and sea otter foraging behavior. Then, we investigate the relationship between the genome sizes of viruses and their potential to spillover into new species by comparing the genomes of zoonotic and non-zoonotic viruses. We find that larger genomes increase spillover risk. The risk of death from viruses is difficult to discern due to incomplete



sampling of affected individuals. We develop a statistical method to calculate the true risk of death for COVID-19 across countries and find this risk in the early stages of the pandemic was approximately 1%. Lastly, we investigate through a diverse set of population genetic and epidemiological modeling how emerging gene drive technology can mitigate human risk to schistosomiasis. We find that schistosomiasis can be reduced under select conditions, such as low selfing rate in snail intermediate hosts. Together these chapters demonstrate how to leverage new quantitative approaches to guide conservation and disease management decisions, each important aspects of human and wildlife health.

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# Chapter 1

## Introduction

The Earth is increasingly managed to facilitate the flow of resources from nature to people. Rising human demands on the environment to secure food, water, and energy has altered an estimated 70% of Earth's land, and soon we will have the capacity to cultivate Earth's oceans (Shukla et al., 2019). Despite not inhabiting the ocean, we have contributed to its degradation through pollution, overfishing, and introduction of non-native species to ecosystems. Globalization exposes humans and wildlife to new pathogens at a growing pace (Kilpatrick, 2011). These trends are compounded by a changing climate, which shifts conditions for species and forces them to adapt or risk extirpation (Chakraborty, 2013). The first step in mitigating these risks is identifying them, and the second is quantifying their importance (Jardine et al., 2003). Yet, inadequately accomplishing the second step, and sometimes even the first, beleaguers our efforts to deal with emerging threats. The primary

reason these early steps are challenging is that ecosystem-level problems are inherently complex, and cryptic relationships are interwoven. This dissertation addresses these obstacles in two ways: (1) it develops new quantitative methods to simplify otherwise intractable biological problems, and (2) it uses these methods to advance our scientific understanding of evolving risks to human and wildlife health.

Some species face substantial population threats despite human intervention. Large, predatory animals in both terrestrial and marine environments have experienced widespread declines and extinctions due to anthropogenic activities. Once thought extinct after extensive fur trade, the southern sea otter population has rebounded in the last century after intensive conservation efforts. However, the population remains less than one-quarter of its historical size and faces multiple threats limiting further population growth, including a myriad of introduced pathogens (Tinker et al., 2021). Nearly all infectious disease-related mortality (contributing to 63% of deaths) is due to pathogen spillover, some of which is human-mediated (M. A. Miller et al., 2020a). In Chapter Two, we study how complex parasite transmission dynamics between mammalian, avian, and crustacean hosts causes seasonal swings in infection of a pathogenic acanthocephalan in southern sea otters. Despite contributing to 25% of sea otter deaths, little is known about this parasite; our work resolves drivers of infection and mortality in southern sea otters.

Pathogen spillover occurs in both wildlife and humans. Globalization has increased the

rate at which these pathogens spread and may increase the incidence of future human pandemics (J.-W. Lee & McKibbin, 2004). Viral pandemics have been associated new genetic variants that arise due to mutation or recombination, and although the scientific community has identified characteristics of genetic variants that contribute to cross-species transmission on a virus-specific basis, much is unknown about the general indicators of spillover potential for viruses (Olival et al., 2017a). We address this knowledge gap in Chapter Three by comparing the genome sizes of all known zoonotic viruses to those that are non-zoonotic in vertebrates and humans, hypothesizing that larger viral genomes improve the odds of genetic variability and plasticity enabling viruses to cross species boundaries.

Pandemic threats are nevermore relevant than in the past two years, in which humanity has experienced one of its worst contemporary health crises due to the spillover of the SARS-COV-2 virus. Difficulty in containing the virus is blamed in part on the lack of available testing, especially in the early phases of the outbreak. Data to interpret the extent of viral spread and the severity of disease caused by this novel virus was, therefore, lacking. Estimates for these metrics relied on seroprevalence studies, which were small in scope. Because COVID-19 severity depends on age, healthcare availability, and other region-specific features, generalizing these estimates is unreliable (Verity et al., 2020b). To remedy these limitations, in Chapter Four we develop a statistical method which simplifies to a linear regression describing the relationship between testing capacity and the case fatality ratio of observed in each country. The intercept of the regression is interpreted as the

true severity of COVID-19 across these countries by accounting for missing data.

Future treatments of human infectious diseases may rely on genetic technologies that mediate infection by altering the intermediate host or vector. Current progress has been made on several fronts to reduce mosquito-borne transmission of malaria and viruses like dengue and Zika by genetically modifying mosquitoes, primarily to reduce the fitness of vector populations (Hammond et al., 2016). However, little attention has been given to the development of similar technologies in snails that host schistosome parasites that cause 200 million cases of schistosomiasis in humans each year (Gryseels et al., 2006). Gene drive technology, which can propagate modified genes at a faster rate through a population, could be used to boost innate immunity in snails and thereby reduce pathogen transmission. Progress towards implementing this technology in mosquitoes relies on the synergy of laboratory, field, and modeling efforts to understand the potential benefits and risks associated with release of gene drive organisms (Golnar et al., 2021). Laboratory efforts to engineer drive constructs in schistosome host snails are underway, and no modeling efforts have yet informed the potential use of this technology to reduce schistosomiasis transmission (Tennessen et al., 2020a). Importantly, self-fertilization in snails presents challenges to drives that rely on cross-fertilization. In Chapter Five, we derive a new modeling approach to integrate evolutionary and epidemiological dynamics of schistosome transmission which describes the conditions under which a gene drive can succeed to mitigate the immense human health risk of schistosomiasis.

In the appendices we devise new approaches to evaluate one of the largest risks to ecosystem health in our oceans: overfishing. In recent years, wealthy nations have succeeded in more effectively managing their own waters, but many fisheries are expansive and cross international boundaries. Furthermore, exploitation of fisheries in less wealthy nations by other countries is a humanitarian issue that requires effective oversight (McCauley et al., 2018). Globally, most fished and bycatch species are considered data-limited, meaning no conventional stock assessment is possible because pertinent data is unavailable (Dowling, Smith, et al., 2019). Fisheries in the poorest nations are often the least studied, hindering potential management efforts for at-risk species. Semi-quantitative approaches have been developed in the past two decades to assess species' risk of population decline below sustainable levels due to fishing activities, which rely on expert input to score key attributes of biological productivity and species' susceptibility to overlapping fishing activities (Hobday et al., 2011a)). These methods have seen widespread adoption and are commonly used to prioritize management and conservation efforts in wealthy and poor countries alike. Appendix A finds that the most used method in this field is biased in a way that miscategorizes species in three risk categories. We develop a new approach based on statistical considerations that eliminates biases and provide software packages in R, Python, and Excel for use by scientists and managers.

Often species experience threats from multiple stressors, and it becomes critical to understand how these sources collectively contribute to risk of population loss. In fisheries,

these stressors can be multiple types of fishing gear that selectively target certain species but have unintended impact on others (Micheli et al., 2014a). For other species, including terrestrial organisms, stressors may be climate-induced or have direct human origin (e.g. pollution or habitat destruction). In recent years, indices of cumulative impacts have been devised to address the challenges of assessing the impacts of multiple stressors (Halpern & Fujita, 2013). However, data-limitations are an even greater hurdle for these analyses than for assessments of single stressors. In Appendix B, we extend our work from Appendix A to multiple stressors and generalize from fisheries to ecosystems to provide a statistically robust and comprehensive ecological risk assessment in data-limited contexts. We implement our software with a user-friendly website to facilitate use by a broad audience.

As humans continue to modify the earth and the species within it, we must update our approaches to understand and respond to the challenges that this can create. These novel approaches to illuminate and characterize poorly understood risks to wildlife and human health collectively advance the field of epidemiology and bridge the gaps between theory and practice.



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## **Chapter 2**

**Population dynamics of seabirds and  
crustacean prey result in seasonal  
spillover of *Profilicollis altmani* to  
California sea otters over decades**

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## 2.1 Abstract

Southern sea otters (*Enhydra lutris nereis*) face numerous risks to population recovery from infectious diseases, shark attacks, and toxins. The most significant contributor to fatal infectious disease in a previous study was acanthocephalan peritonitis (AP) caused by the parasite *Profilicollis altmani*, which was wholly or partially responsible for nearly one-quarter of deaths. Despite the importance of AP-associated sea otter mortality, the transmission dynamics of the parasite in its avian definitive hosts and *Emerita analoga* crustacean intermediate hosts have not been resolved. Using 30 years of AP necropsy findings encompassing 4460 otters, 20 years of beach monitoring of *E. analoga* harboring juvenile *P. altmani* encompassing 84212 core samples, and long-term population monitoring of wintering seabirds within the extant sea otter range, we found that spillover risk

to southern sea otters was primarily influenced by increasing abundance of *E. analoga* in the late spring and early summer. Peak detection of fatal AP in sea otters coincided with peak *E. analoga* consumption in the spring. Wintering scoters (*Melanitta spp.*), implicated as primary hosts of *P. altmani*, contributed marginally to parasite transmission, suggesting that other avian hosts with longer residence times along CA beaches sustain transmission year-round. Immature sea otters inhabiting sandy coastlines near or in Monterey Bay and Morro Bay were at highest risk of AP. Long-term trend analyses suggest that seasonal and geographic patterns of AP prevalence are shifting, possibly due to shifts in avian host abundance and distribution, varying otter diet composition, and/or environmental forces. Positive associations between *E. analoga* abundance and ocean temperatures could increase transmission of *P. altmani* to sea otters and birds in future ocean warming conditions. This study underscores the importance of long-term monitoring of sea otters and other host populations to assess the evolving risk that AP poses to sea otter population growth. This work can inform population recovery efforts in the historical range of the southern sea otter.

Keywords: *acanthocephalan* | *compartmental model* | *Emerita analoga* | *parasite* | *peritonitis*

## 2.2 Introduction

Southern sea otters (*Enhydra lutris nereis*) play a pivotal role in California's coastal ecosystem as a keystone predator (J. A. Estes & Palmisano, 1974). Their top-down regulation of near-shore communities leads to stable and diverse seagrass and kelp forest habitats (Hughes et al., 2013). Their proximity to land exposes them to a wide range of anthropogenic stressors, including land-based pathogens like *Toxoplasma gondii*, *Sarcocystis neurona*, *Coccidioides immitis*, and cardiac dysfunction secondary to domoic acid exposure, which can be associated with coastal nutrient loading (CORNELL et al., 1979; M. Miller et al., 2002; M. A. Miller, Conrad, et al., 2010; Moriarty et al., 2021). Despite decades of intensive population recovery efforts, southern sea otter population growth has virtually stagnated over the past decade, and they remain listed as Threatened by the federal Endangered Species Act. Failed expansion at the northern and southern extent of the population's current range has restricted population growth, as currently occupied areas are thought to be at or near carrying capacity (Tinker, 2021). Within preferred habitats such as bays, estuaries, and kelp forests colonized by sea otters, both resources and resource-associated health risks are unevenly distributed (C. K. Johnson et al., 2009; M. A. Miller, Kudela, et al., 2010). Density-dependent competition for prey and declines in prey populations like abalone can result in unequal resource access. As a result, less competitive otters may be forced to alter their foraging behavior by targeting new foraging grounds or by being less selective in their diet (J. Estes et al., 2003; Tinker et al., 2008). Prey choice



impacts disease risk in sea otters, as pathogens are strongly associated with specific prey species. For example, otters consuming small marine snails are more likely to be infected by *T. gondii*, while those feeding on abalone experience a lower risk of infection (C. K. Johnson et al., 2009). Potential health risks associated with consumption of particular prey items have been reported for *S. neurona* infection (clams; (Burgess et al., 2020)) and domoic acid intoxication (crustaceans; (M. A. Miller et al., 2020b; Moriarty et al., 2021)) in southern sea otters.

Many forces in addition to otter behavior govern disease susceptibility and exposure; additional factors include pathogen-specific attributes and environmental conditions. As the smallest marine mammals, sea otters have high metabolic demands to thermoregulate in cold oceanic environments, thus consuming one-quarter of their body weight daily (Thometz et al., 2014). High consumption exposes them to pathogens within infected prey. Infectious disease is a very important source of southern sea otter mortality; in a recent study, when all infectious cases of death were combined, infectious disease was the most prevalent primary or contributing cause of death (63%), not including additional cases where infectious disease was considered a sequela to another cause of death (M. A. Miller et al., 2020b). One especially common infectious condition is acanthocephalan peritonitis, which was shown to contribute to nearly one-quarter of southern sea otter deaths in a comprehensive retrospective study of otters necropsied 1998-2012 (M. A. Miller et al., 2020b). However, few studies have investigated the epidemiological factors driving transmission of

the parasite causing this important disease (Kreuder et al., 2003). Identifying transmission pathways and risk factors for pathogenic acanthocephalan infection is crucial to inform management decisions that aid population recovery.

Southern sea otters are known to harbor two genera of parasites in the phylum Acanthocephala: *Corynosoma* and *Profilicollis*. *Corynosoma enhydri* is found in nearly all necropsied sea otters and can contribute to sublethal morbidity but is not associated with mortality (Mayer et al., 2003; Rausch, 1953; Shanebeck et al., 2020). Sea otters are definitive hosts for *C. enhydri* and the intermediate crustacean host is unknown. Work by Hennessy and Morejohn (S. Hennessy & Morejohn, 1977; S. L. Hennessy, 1972), followed by Mayer et al. (Mayer et al., 2003), identified infection by three species of parasites in the *Profilicollis* genus based on examination of the gastrointestinal tracts of southern sea otters at gross necropsy. Two species, *P. altmani* and *P. kenti* were implicated in acanthocephalan peritonitis cases, because they were found perforating the intestinal wall or within the peritoneal cavity (Mayer et al., 2003). The type-species of these parasites were identified in different hosts, and morphological variation alongside observer differences accounted for differences in species classification (Perry, 1942; Van Cleave, 1947). Recent taxonomic changes have synonymized *P. altmani* and *P. kenti* (Amin et al., 2013; Amin et al., 2022). *Profilicollis altmani* (= *P. kenti*) is trophically transmitted between various seabird taxa and pacific mole crabs (*Emerita analoga*) (Karl Jr, 1967).

Similar to *T. gondii* and *S. neurona* infections, *P. altmani* transmission could be influenced by sea otter foraging behavior. Juvenile stages of *P. altmani* are harbored in *E. analoga* or transport hosts, spiny mole crabs (*Blepharipoda occidentalis*), and are transmitted trophically to otters consuming these crabs. Seabirds (including larids, anatids, and charadriiformes) serve as definitive hosts and harbor adult worms embedded in the intestines (Karl Jr, 1967). Recent population declines of many seabird species have been attributed to habitat degradation, particularly in summer breeding sites in the subarctic regions of northern Canada and Alaska (Warnock et al., 2021). Because *P. altmani* is not known to reproduce in sea otters, transmission relies on the presence of avian definitive hosts in coastal California. Long-term changes in bird abundance could coincide with risk of spillover infections in sea otters. Many definitive hosts of *P. altmani* are migratory and use California bays and estuaries as wintering or stopover feeding areas (Amin et al., 2022; Henkel, 2004; Karl Jr, 1967). Periodic use of California waters by seabirds, particularly those inhabited by *E. analoga*, may contribute to seasonal parasite transmission to both seabirds and sea otters (Fig. 2.1A). In fact, seasonal variation in acanthocephalan peritonitis was reported in a prior mortality survey in southern sea otters (M. A. Miller et al., 2020b). However, the observed spring peak of sea otter deaths in California due to acanthocephalan peritonitis does not temporally coincide with peak populations and habitat use by most avian definitive hosts. Not all potential avian hosts are migratory, and seasonal use of the California coast differs by species (Fig. 2.1B). The relevance of each seabird species

to transmission is unknown, but clarifying potential transmission patterns is essential to predict long- and short-term patterns of *P. altmani* spillover from seabirds to sea otters.

Importantly, seasonal breeding patterns also occur for *E. analoga* along sandy beaches in California (Fig. 2.1A, (Dugan et al., 1991)). Adult mole crabs breed in the summer and into fall, with mature eggs released a month later into the water column as zoeal larvae (Cox & Dudley, 1968). Free-floating larvae develop through five stages in four or more months and then settle on beaches as megalopae, which molt to a small adult form (Sorte et al., 2001). Adult mole crabs filter feed in the swash zone and can ingest suspended acanthors (hatched eggs) of *P. altmani*. Ingested acanthors enter the haemocoel of the crabs as acanthellae and mature to cystacanths (juvenile worms), which are infectious to sea otters and seabirds (Fig. 2.1C, (Karl Jr, 1967)). Recruitment of megalopae has been shown to occur throughout the year with some seasonal variation (Barnes & Wenner, 1968). Adult mole crabs can reproduce twice: their first summer after molting and the following summer (Barnes & Wenner, 1968; Dugan et al., 1991). However, most studies on seasonal variation of *E. analoga* abundance have been short-term and confined to beaches within a small geographic range. Estimates of abundance and seasonal trends differ by location (Barnes & Wenner, 1968; Cox & Dudley, 1968) and no study has examined variation in abundance and demographic structure over the entire southern sea otter range. In addition, no reports document variation in cystacanth abundance in *E. analoga* throughout the sea otter range. Each of these measurements is crucial to understand the epidemiology of the

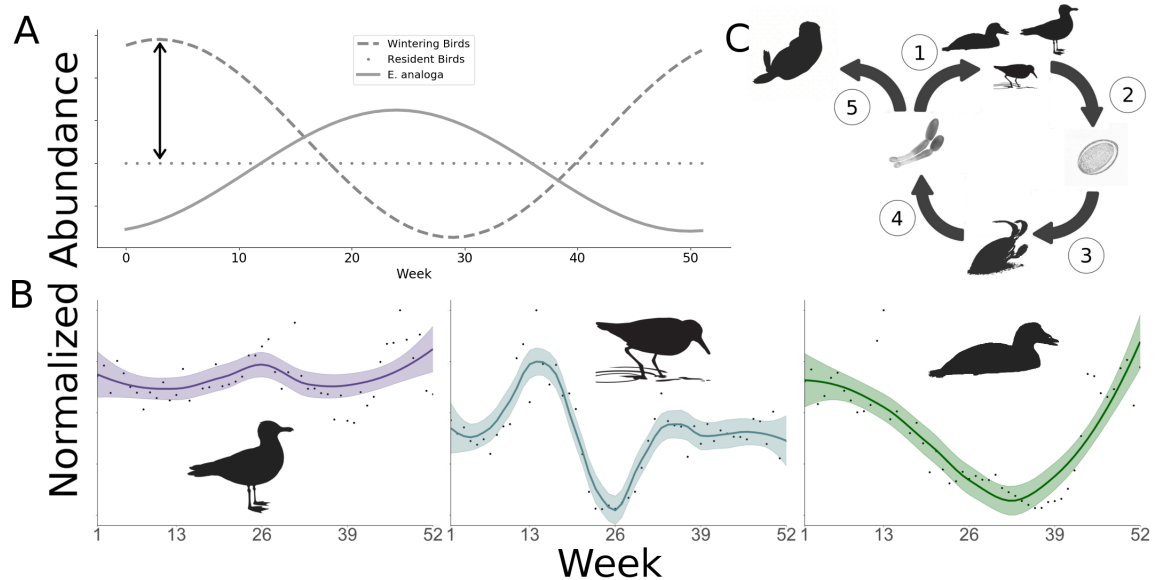


Figure 2.1: Host population dynamics can influence the transmission of *P. altmani*, which determines the availability of the parasite to southern sea otters. (A) Population sizes of hosts within the southern sea otter range are highly variable. *E. analoga* populations bloom in the early summer during seasonal reproduction on CA beaches. Definitive bird host species exhibit a wide range of migration patterns, contributing to transmission at different times of the year. The relative importance of migrant and resident bird populations in the transmission of *P. altmani* influences the seasonal risk of spillover to otters. (B) LOESS regressions of weekly population abundance estimates normalized to the maximum for each species. Left, *Larus occidentalis*; resident bird hosts like the western gull contribute to year-round transmission. Middle, *Calidris alba*; mixed migrants like the sanderling use CA beaches as stop-over and residential feeding grounds except during summer breeding. Right, *Melanitta perspicillata*; seasonal migrants like the surf scoter use CA for feeding grounds in the winter, contributing to parasite transmission during part of the year. (A) The life cycle of the parasite is indirect, requiring a definitive seabird or shorebird host and an intermediate *E. analoga* host. 1. Immature *P. altmani* mature in approximately seven weeks within the gastrointestinal tracts of birds. 2. Adult female worms produce eggs shed in bird feces, which hatch into acanthors suspended in the water column. 3. *E. analoga* crabs ingest acanthors while filter-feeding. Development of the parasite occurs within the crab, wherein larval acanthellae grow to infective cystacanths and migrate from the digestive tract to the haemocoel. 4. Cystacanths are ingested via predation or scavenging on *E. analoga*. Each proboscis everts to attach to the intestinal mucosa in the definitive host. 5. Southern sea otters are incidentally infested after consuming parasitized *E. analoga*.

parasite in its crustacean and seabird hosts, as well as to model the environmental dynamics and risk of pathogen spillover to threatened sea otters.

Since the late 1960s, nearly ten thousand stranded southern sea otters have been recovered and necropsied to determine sources of mortality. In the earliest work from the 1970s, Hennessy and Morejohn noted a 6% prevalence (5/81) of *P. altmani* infection (adjusted for updated taxonomy) (S. Hennessy & Morejohn, 1977). Although *P. altmani* was not noted as a significant source of mortality at that time, its prevalence and impact on mortality was not consistently noted until 1992, when the presence and severity of acanthocephalan peritonitis cases began to be systematically recorded as part of gross necropsy examinations. In addition, *E. analoga* populations and cystacanth infections have been systematically documented on sandy beaches throughout the sea otter range since 2001 by the Long-term Monitoring Program and Experiential Training for Students (LiMPETS) program, a partnership between scientists, teachers, students, and community scientist volunteers. Shorebird surveys have been conducted in central California by the United States Department of Fish and Wildlife and the CA Department of Fish and Wildlife for many decades (Richmond et al., 2014). These long-term datasets provide rich information to investigate potential patterns and factors contributing acanthocephalan peritonitis in southern sea otters. We supplemented these long-term datasets with seasonal measurements of crab and avian host abundance and fit a periodic epidemiological model to cystacanth abundance data using density-dependent parasite recruitment and fecundity relationships resolved from avian

necropsies, with a particular focus on surf scoters (*Melanitta perspicillata*) and gulls (*Larus spp.*). To test whether seasonal population dynamics of migratory bird and mole crab hosts predicted acanthocephalan peritonitis risk in otters, we estimated the contribution of each host to transmission and evaluated a time-series forecast of acanthocephalan peritonitis cases with and without our epidemiological model. We then incorporated interannual variability in population size of migratory bird and mole crab hosts to understand whether long-term population trends enhanced forecasting. We also characterized sea otter-specific variables features which increase the risk of acanthocephalan infection. This work is the first study that provides temporally-matched modeling of avian, crab, and otter hosts and does so in a single predictive framework that employs a much greater time span and sample set than any prior study. We interpret these risk analyses in the context of sea otter ecology to provide guidance in future southern sea otter management and recovery efforts.

## 2.3 Results

### 2.3.1 Epidemiological Model

We hypothesized that known patterns of migration of seabird hosts wintering in California within the southern sea otter range and observed seasonal reproduction of *E. analoga* along California beaches impacts the rate of transmission of *P. altmani* to southern sea otters in the coastal environment. Several known definitive hosts of *P. altmani*, particularly diving

ducks, spend half of the year feeding in California estuaries and along the coast. Their presence during winter months precedes the reproduction period of *E. analoga* in summer and fall, and it is unclear whether significant sea otter transmission aligned with peak abundance of either definitive or intermediate hosts. To resolve these population-dependent transmission dynamics, we constructed a compartmental epidemiological model with prepatent and infectious classes for both seabird and crustacean hosts. Population dynamics were modeled explicitly with sinusoidal model fits to population survey data of *M. perspicillata* and *E. analoga* abundance in the California coastal region (Fig. 2.10). The winter *M. perspicillata* coastal population peak aligned well with those for other diving duck and wintering seabird hosts (Fig. 2.9).

After estimating model parameters, we optimized the model to fit simulated mean per capita cystacanth density to biweekly cystacanth density data collected via parasite surveys from 2001-2021 in dissected *E. analoga* (Fig. 2.2A). Two transmission rate parameters and background transmission not attributable to population dynamics in *E. analoga* and wintering seabirds were estimated through optimization. The background transmission parameters were null models assuming constant transmission through time. High values relative to the mean of the host population functions supported nearly homogeneous transmission rates and little importance of seasonal host population variation in the transmission of the parasite. The best fitting model supported a 6% contribution from population variability of wintering bird hosts to transmission (Fig. 2.2C). Although this contribution was not



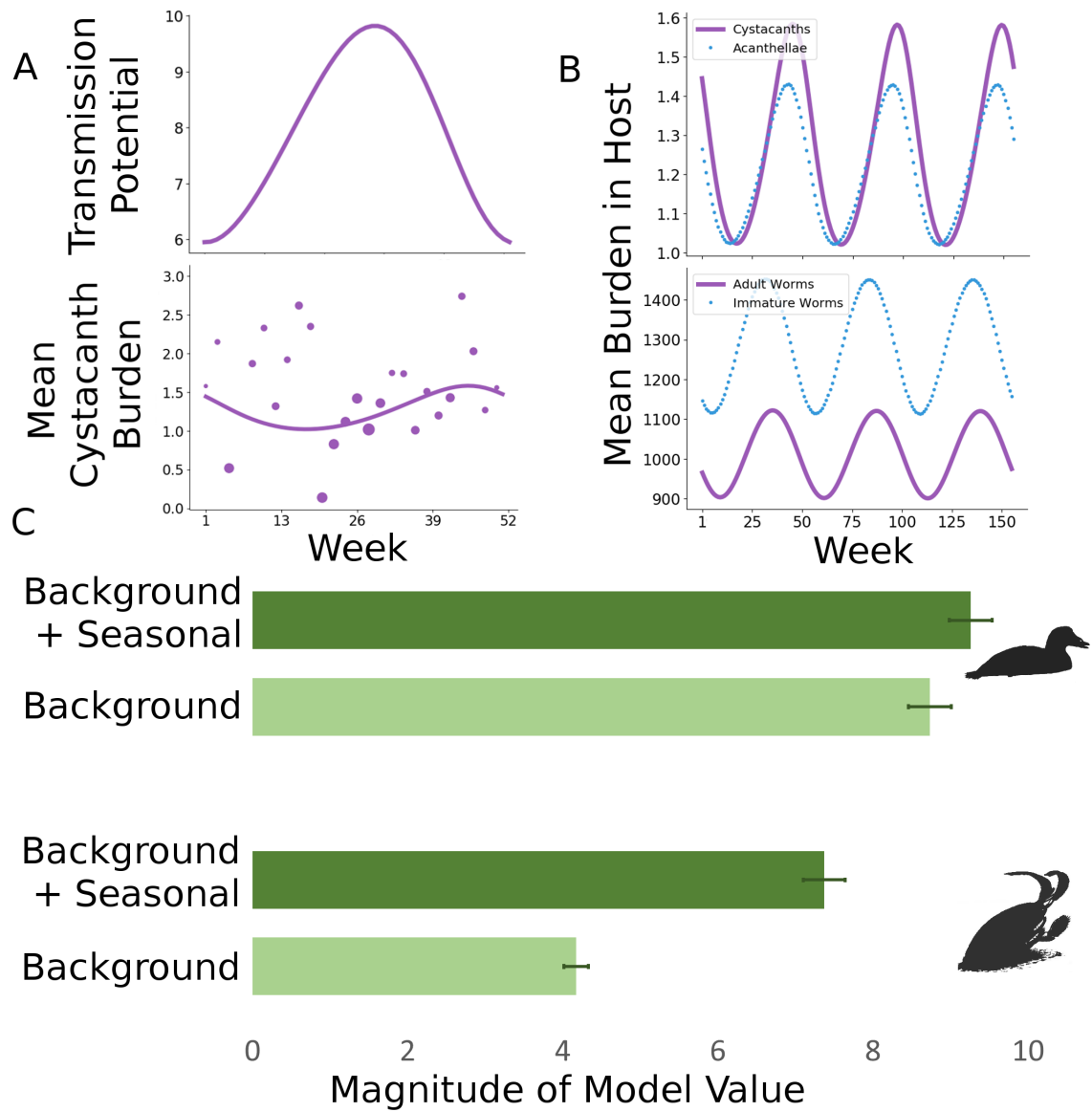


Figure 2.2: Figure caption continued on the following page.

Figure 2.2: Transmission of *P. altmani* occurred throughout the year with seasonal pulses near *E. analoga* reproductive peaks. Weaker seasonal effects were measured for transmission due to wintering birds. (A) Bottom: the epidemiological model was fitted to biweekly mean per capita cystacanth burden in *E. analoga* observed within the southern sea otter range between 2001-2021. Weights of each data point in model fitting are displayed by size. Top: cystacanth transmission potential in the environment represents the background and seasonal *E. analoga* population contribution to transmission multiplied by mean per capita cystacanth burden. Transmission risk peaked in the 28th week of the year (early July). (B) Three years of model output for mean burden of each parasite stage in crustacean and avian hosts. Top: The mean cystacanth burden in the *E. analoga* population by week. Development of acanthellae in *E. analoga* preceded accumulation of cystacanths each year. Bottom: Immature and adult *P. altmani* worm burdens fluctuated seasonally by  $\pm 10\%$ . (C) The relative contributions of seasonal fluctuations of wintering populations of seabirds and *E. analoga* to the transmission of *P. altmani* differed. Top: Wintering birds did not significantly alter the transmission dynamics of *P. altmani* in the southern sea otter range. Bottom: Spring recruitment and the summer reproductive peak of *E. analoga* were responsible for pronounced increases in transmission of *P. altmani*. Background + seasonal bars represent the sum of an optimized parameter,  $r$ , and the corresponding mean of the fitted host population function, whereas background bars represent only the optimized parameter,  $r$ .

significantly different ( $t = 1.35, df = 75, p = 0.096$ ) from constant background transmission, even marginal increases in transmission may lead to seasonal risk when compounded with increased *E. analoga* abundance. Our model revealed that 43% of transmission is explained by the population dynamics of *E. analoga*, which was a significant increase from background transmission ( $t = 10.18, df = 74, p < 0.0001$ ).

The resulting spillover risk for sea otters depends in part on the availability of cystacanths in the environment. We define transmission potential as the product of the mean per capita cystacanth burden (state variable  $C$ , see also Fig. 2.2A bottom row) in *E. analoga* crabs and the sum of the seasonal (state variable  $E$ ) and background (optimized parameter  $r_2$ ) contributions to transmission. Transmission potential peaks in early July each year (Fig.

2.2A). Without accounting for seasonal differences in otter consumption of *E. analoga* or paratenic hosts, immature worm densities in sea otter intestines should peak at this time. The model predicts a 60% increase in spillover risk during summer compared to winter.

### 2.3.2 Forecasting AP in Sea Otters

To test whether this potential seasonal spillover risk predicted by our epidemiological model was associated with an increased incidence of AP in sea otters, we incorporated the output of our epidemiological model into an auto-regressive integrated moving average (ARIMA) forecasting model. The ARIMA model was trained on the first 80% of the time series of AP prevalence (1992-2015). We measured the out of sample correlation between the forecast predictions from the endogenous fit of the ARIMA model to the latter 20% of the time series of AP prevalence (2016-2021), revealing a weakly positive correlation (Pearson's correlation coefficient = 0.085). We then sampled four evenly spaced values of transmission potential calculated from the epidemiological model output (see Fig. 2.2A top row).

AP prevalence in sea otters was evaluated in relation to predefined 13 week seasons between January 1 and December 31, and thus represents the average incidence across each season. However, the transmission potential calculated from the epidemiological model varies within each 13 week period. Therefore, we evaluated how the ARIMA + epidemiological model performance varied by sample week. By incorporating these 13 model

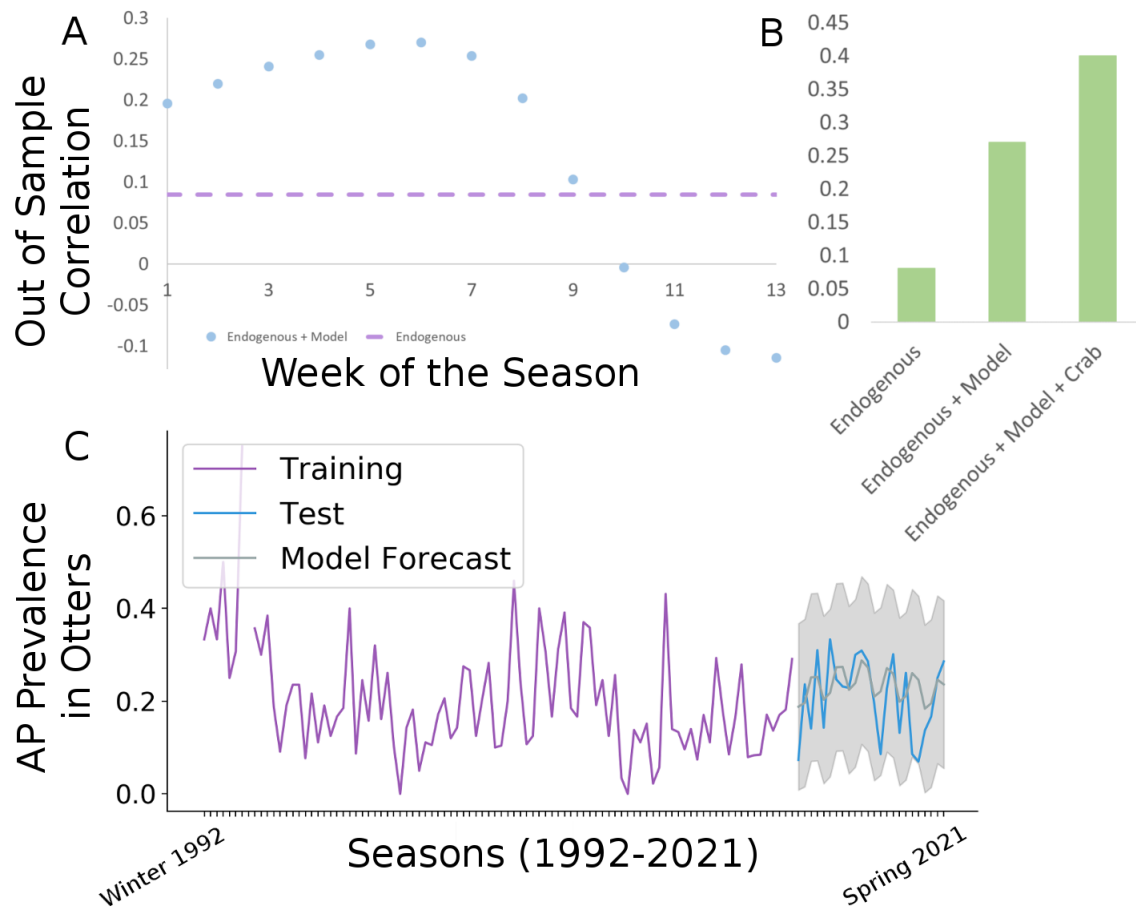


Figure 2.3: The seasonal epidemiological model and annual variation in *E. analoga* abundance improve forecasting performance of ARIMA time series model evaluating seasonal acanthocephalan peritonitis prevalence in otters. Each season represents 13 week increments. (A) ARIMA prediction is best when incorporating epidemiological model values prior to the middle of each season. These out of sample correlation values represent the endogenous + epidemiological model forecast compared to the endogenous forecast alone (dashed line). Further results use the epidemiological model output of the 6th week of each season for performance evaluation. (B) ARIMA + epidemiological model + annual variation in *E. analoga* abundance produces the best performance predicting out of sample AP prevalence compared to simpler models. (C) The 30 year time series of AP prevalence in otters is split into 80% training and 20% testing. The ARIMA model was trained on the time series from 1992-2015. The epidemiological model was added as an exogenous regressor for 1992-2001, while the crab abundance data was added as an exogenous regressor for 2001-2021 because data is unavailable prior to 2001. The endogenous ARIMA + epidemiological model + annual variation in *E. analoga* abundance model forecast for years 2016-2021 is displayed in gray with associated 95% confidence intervals.

variants as exogenous regressors in the ARIMA model, we found that the transmission potential from weeks preceding the median of each season better predicted sea otter AP prevalence (Fig. 2.3A). This coincides with our presumption that there is a delay between ingestion of cystacanth-laden *E. analoga* and subsequent sea otter death due to AP. Limited observations in rehabilitated sea otters suggest this delay may be less than two weeks in acute cases (Karl Mayer, personal comm.). Out of sample prediction declines precipitously, and the correlation can even become negative, when using transmission potential values from the latter portion of each season to predict within season sea otter AP. Using values from the sixth week of each season produced the best results (Pearson's correlation coefficient = 0.27). This corresponded to weeks 6, 19, 32, and 45 of each year, which are in months February, May, August, and November. Transmission potential values consisted of four values repeated for each of the 30 years of the analysis.

The endogenous ARIMA model incorporating epidemiological model output as an exogenous regressor produces much improved prediction compared to the endogenous model alone. However, epidemiological model represents only the seasonal variation in transmission. Interannual variation could also be present due to variability in host abundance. To understand whether interannual variation in host abundance improves forecasting, we incorporated yearly estimates of mean abundance of both *E. analoga* and *M. perspicillata* as exogenous regressors. These yearly estimates were multiplied by the state variables *E* and *M*, respectively, to retrieve values in each season. Addition of annual abundance data

for *E. analoga* improved forecasting (Pearson's correlation coefficient = 0.40) (Fig. 2.3B); however, annual abundance data for *M. perspicillata* did not improve forecasting when added as a third exogenous regressor (Pearson's correlation coefficient = 0.38). Model evaluation with all three added variables plus the endogenous ARIMA model revealed that both the seasonal epidemiological model ( $z = -2.91, p = 0.004$ ) and annual crab abundance ( $z = 3.212, p = 0.001$ ) as well as the moving average term in the ARIMA model ( $z = -20.9, p < 0.0001$ ) were significant contributors to forecasting performance, while the autoregressive term ( $z = 0.51, p = 0.611$ ) and *M. perspicillata* abundance ( $z = 1.90, p = 0.058$ ) were insignificant contributors (Fig. 2.3C).

### 2.3.3 Risk Factors for AP

Two components are necessary to influence the incidence of AP in the southern sea otter population: availability of *P. altmani* in the environment due to transmission among its hosts and sea otter predation on the intermediate hosts harboring cystacanths. We've shown that availability (transmission potential) peaks in the early summer and that this availability predicts incidence of AP in otters through time. Previous work using smaller datasets confined to shorter windows of time has identified elevated, but statistically non-significant, fatal AP in otters in late spring as well as clustering of cases near Moss Landing in Monterey Bay near sandy beach habitat (M. A. Miller et al., 2020b). We extended these previous findings by investigating risk factors, including otter behavior, contributing to AP

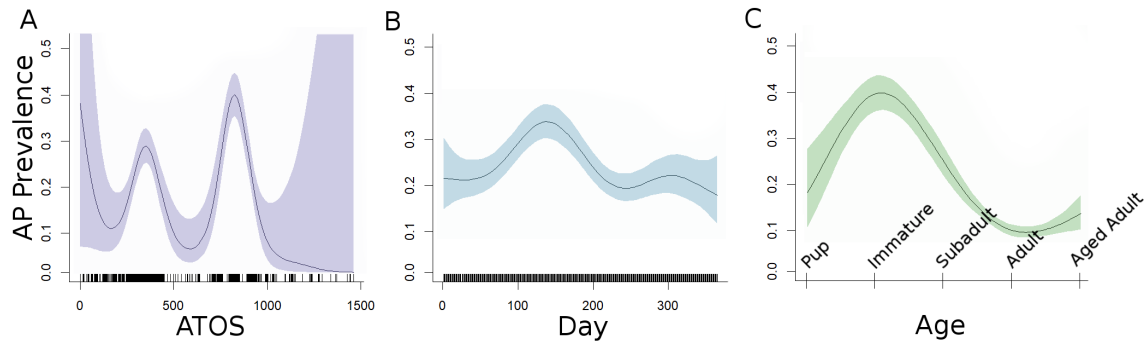


Figure 2.4: Geography, season, and sea otter age are significant predictors of acanthocephalan peritonitis. (A) GAM output of linear distance north to south along the CA coastline (As The Otter Swims) reveals hotspots of AP prevalence near Monterey Bay (ATOS 200-500) and Morro Bay / Pismo Beach (ATOS 700-1000). (B) A clear seasonal peak emerges between days 100-200 of the year with a smaller bump in AP prevalence in the fall. (C) Immature otters (3 mo – 1 year) are at highest risk of AP, and risk declines throughout the lifespan of otters with a slight bump in prevalence in aged adults.

prevalence across our 30 year study period, which includes an order of magnitude larger sample of otter carcasses ( $n=4460$ ) that met inclusion criteria than in previous studies.

A logistic generalized additive model (GAM) comparing presence of AP to the linear distance along the CA coastline (ATOS = As The Otter Swims, Fig. 2.4A), day of the year (Fig. 2.4B), and sea otter age (Fig. 2.4C) with sex included as a factor for ATOS and day of the year, demonstrated that ATOS ( $\chi^2 = 76.8$ ,  $\text{edf} = 7.46$ ,  $p < 0.0001$ ), day of the year ( $\chi^2 = 41.5$ ,  $\text{edf} = 5.73$ ,  $p < 0.0001$ ), and sea otter age ( $\chi^2 = 209$ ,  $\text{edf} = 3.85$ ,  $p < 0.0001$ ) were all highly significant predictors of AP incidence. Both interaction terms were insignificant: (ATOS:Sex,  $p = 0.155$ ; Day:Sex,  $p = 0.781$ ). This model was compared to all other subset model variants and yielded higher deviance explained (10.7%). The low overall deviance explained from the best fitting model is due to the complexity of death

due to AP and other confounding factors, including competing sources of mortality that obscure relationships between these variables and AP incidence.

We observed two primary clusters of AP in or near Monterey Bay and Morro Bay, which both possess sandy coastlines with *E. analoga* populations. In contrast, rocky coastlines like that of Big Sur posed little AP risk. Distinct seasonality of AP was observed; a greater than 50% increase occurs in late spring, peaking in the last week of May. This immediately precedes the calculated peak in transmission potential shown in Fig. 2.2A, which displays a broader peak but similar increase in risk between trough and peak. Young sea otters are more heavily affected by AP than old sea otters, with immature otters between approximately 3 months and 1 year of age most impacted. Pups in this analysis excluded the youngest individuals who possessed only milk teeth at the time of death. Milk-feeding pups are unlikely to be exposed to solid food, including *E. analoga*. No significant difference was observed between male and female AP prevalence as a factor in the GAM nor in a Fisher's exact test (odds ratio male:female = 0.96,  $p = 0.45$ ). These risk factors predispose southern sea otters to AP, though their feeding behavior directly influences their exposure to *P. altmani* only if consuming *E. analoga* or potentially *Blepharipoda occidentalis*. We examined whether sea otters who had recently consumed *E. analoga* were more likely to be found with AP. Otters found with *E. analoga* remnants in their GI upon necropsy were nearly twice as likely to also have AP (odds ratio = 1.95,  $p < 0.0001$ ). We investigated the seasonality of *E. analoga* consumption with a logistic GAM of presence of *E. analoga* in



the GI and day of the year (Fig. 2.5B).

Results showed a close alignment in the shape of the seasonal prevalence of *E. analoga* consumption and the seasonal prevalence of AP. This includes the timing of the peak in the late spring and the magnitude of the increase between the trough and the peak. However, because transit time in sea otter GIs is short and recording of prey is not detailed in the earlier years of the necropsy data collection, the number of otters with positive *E. analoga* identification in their GI was only 109 of nearly 4200 for which prey data was recorded. We were unable to find a statistically significant seasonal relationship in the GAM analysis ( $p = 0.25$ ), though more complete sampling in future years or including *B. occidentalis* prey in the analysis may improve resolution. As in previous studies, which have indicated emaciation associated with AP, we found that body fat was significantly reduced in AP cases. This was true across all subcutaneous body fat categories scored 0 = none to 4 = abundant ( $\chi^2 = 321.1$ ,  $df = 4$ ,  $p < 0.0001$ ) and between each pairwise comparison (odds ratio compared to 4: 1.48, 1.52, 2.95, 5.07 in descending order of body fat;  $p < 0.01$  for all pairwise comparisons). In addition to morbidity and mortality due to bacterial infection associated with AP, energetic demands from the immune system in AP cases contributes to emaciation, which is evident from our data.

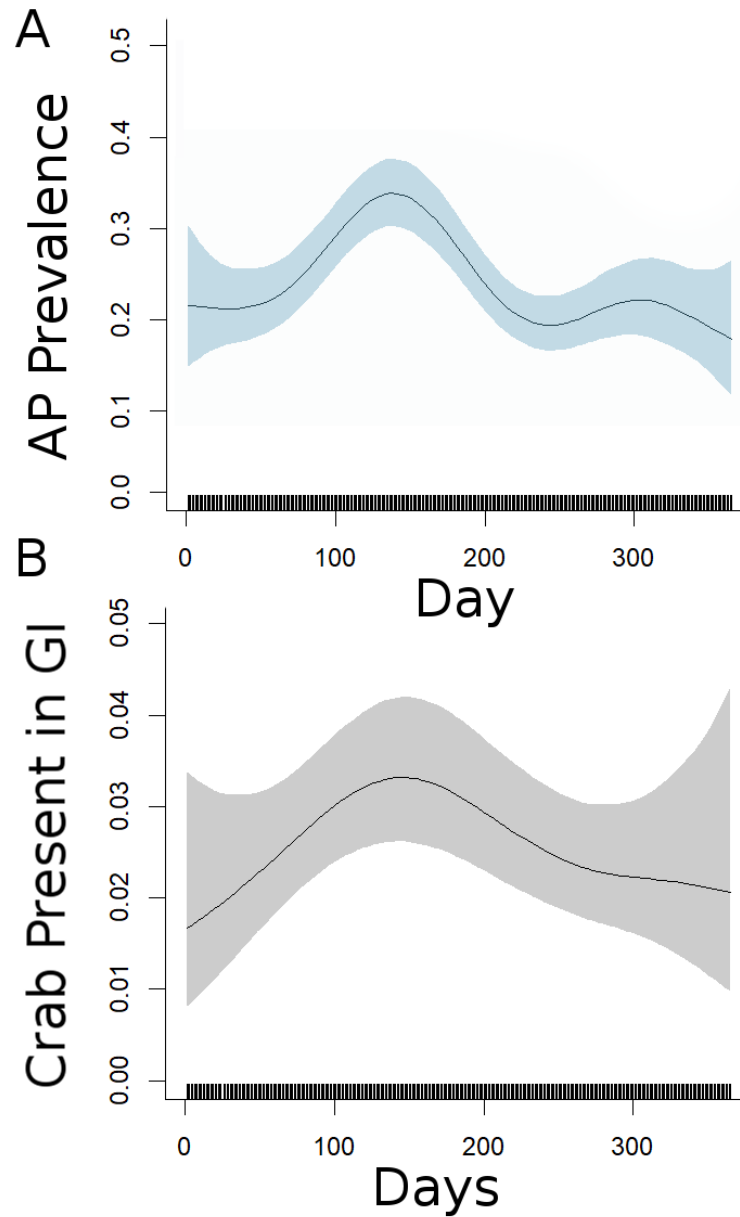


Figure 2.5: The presence of *E. analoga* crabs in the GI of southern sea otters on necropsy examination reveals the same seasonal pattern as the prevalence of AP. (A) AP prevalence by day of the year peaks between days 100 and 200. (B) *E. analoga* prevalence in the GI also peaks between days 100 and 200.

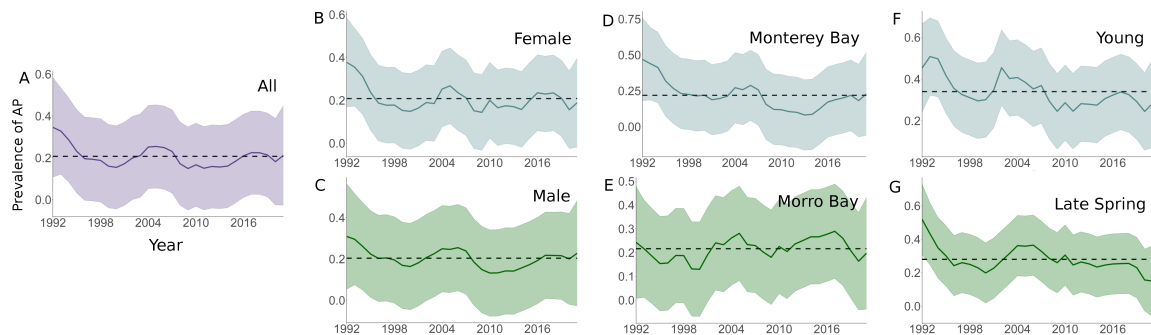


Figure 2.6: Long-term shifts in the prevalence of acanthocephalan peritonitis are evident for some southern sea otter (*Enhydra lutris nereis*) groups, while the prevalence for other groups is stationary. (A) The prevalence of AP varies around the mean with insignificant peaks and troughs from 1992-2021 for all otters. Long-term directional trends between sexes are not apparent. (B) Female otters exhibit short-term variability, while (C) patterns for male otters reveal small decadal oscillations. (D) Otters inhabiting Monterey Bay exhibited a declining risk of AP over time, while those recovered near (E) Morro Bay did not. AP risk also declined over the study period for (F) young otters (immature and subadult) and (G) otters dying during late spring. Trends were modeled as first-order random walk processes displayed by dark curves with shaded bands representing 95% Bayesian credible intervals. The horizontal dashed line is the mean AP prevalence across 30 years in each category. Significant deviations of the trend from the mean indicate significant directional shifts in AP prevalence, which were further evaluated with non-parametric statistical tests.

### 2.3.4 Long-term Changes in AP

To uncover the longitudinal impact of *P. altmani* on high risk categories of otters, we modeled long-term trends in the prevalence of acanthocephalan peritonitis among each group. The Bayesian model can help to discern short-term fluctuations from long-term shifts in AP prevalence.

We found that the prevalence of AP among all recovered otters varied around the mean with insignificant peaks and troughs from 1992-2021 (Fig. 2.6A,  $\tau = -0.12, p = 0.18$ ). After an initial decline from 1992, little variability is observed in AP prevalence among

female otters (Fig. 2.6B,  $\tau = -0.14, p = 0.14$ ). AP prevalence in male otters displayed small decadal oscillations which were more pronounced than for females, but no significant trend emerged (Fig. 2.6C,  $\tau = -0.16, p = 0.11$ ). Peaks and troughs aligned for both female and male otters, though these fluctuations were more pronounced in males and therefore contributed more heavily to short-term changes in AP prevalence observed for all otters. In high-risk geographic areas, Monterey Bay and the Morro Bay area, we saw a noticeable decline in AP prevalence for Monterey Bay otters (Fig. 2.6D,  $\tau = -0.36, p = 0.003$ ), whereas otters near Morro Bay experienced an insignificant but discernible increase (Fig. 2.6E,  $\tau = 0.16, p = 0.11$ ). Otters in high-risk age categories, immature and subadult, experienced a noticeable decline in AP, which reflects a mild trend towards uniform risk across age classes over time (Fig. 2.6F,  $\tau = -0.26, p = 0.021$ ). The seasonal peak in AP risk previously observed also appears to become less important through time as AP prevalence between the 100th and 200th days of the year significantly declined over 30 years (Fig. 2.6G,  $\tau = -0.29, p = 0.013$ ).

During a portion of the study period (2018-2020), we performed full longitudinal resections of the entire length of the GI for 80 otter carcasses immature or older to enumerate *P. altmani*. Methods were replicated from earlier work by Mayer (Mayer et al., 2003), which followed from an earlier study by Hennessy and Morejohn conducted in the 1970s (S. Hennessy & Morejohn, 1977). Each study enumerating *P. altmani* was conducted approximately two decades apart. Although sampling was not truly random due to geographical

biases of recovered carcasses, the prevalence of *P. altmani* in southern sea otters increased dramatically from 6% (5/81, after correcting for species identification) in the 1970s to 46% in the 1990s (109/238) and has stabilized at 44% (35/80) in recent years. Age was not recorded systematically in the first study, but there was no significant difference between the ages of the 1990s cohort and the recent cohort ( $\chi^2 = 4.54, p = 0.22$ ). The negligible difference in prevalence observed aligns with the observation that AP prevalence has changed little in the whole southern sea otter population in the past three decades. The increase from the 1970s cannot be explained by our data but could be due to increased consumption of *E. analoga*, particularly by inexperienced foragers, when competition for food is high. Sea otter densities in recovery areas increased steadily from the 1970s to the 1990s and have since plateaued (Lafferty & Tinker, 2014).

## 2.4 Discussion

We leveraged several long-term datasets to investigate the influence of host population dynamics on the spillover transmission of a pathogenic acanthocephalan, *P. altmani*, to southern sea otters. Uncovering the relative importance of migratory definitive hosts and ephemeral intermediate hosts of this parasite required an integrated approach which included a novel analytical epidemiological model parameterized by literature estimates, our own measurements, and statistical fits to long-term datasets. This epidemiological model was used to assess seasonal patterns of transmission which were incorporated as a derived

variable in a time series analysis of the prevalence of acanthocephalan peritonitis. We also assessed demographic and behavioral factors in otters that could contribute to increased risk of AP, including seasonal consumption of the intermediate host, *E. analoga*. Several important categories of otters were then assessed for long-term shifts in AP prevalence. Characterizing the nature of transmission of this parasite across multiple decades provides a more complete understanding of the health risks for the federally listed, threatened sea otter population in California and sheds light on the environmental forces and biological interactions contributing to the transmission of an understudied parasite phylum.

Acanthocephalan parasites are uncommon in humans and therefore, receive little scientific attention outside of aviculture, where high intestinal burdens in birds can lead to morbidity and mortality (Hynes & Nicholas, 1963). In wild migratory bird populations, such as in *M. perspicillata*, mortality events are documented and have been associated with peritonitis or enteritis (Garbus et al., 2018; Goodman, 2019). *P. altmani* is an apparent cosmopolitan parasite, capable of infecting diving ducks, shore birds, and gulls in North and South America (Amin et al., 2022). Little genetic differentiation between cystacanths recovered from *Emerita* populations suggests that migratory birds are responsible for transmitting the parasite across land masses to different ocean basins. Most bird species are able to survive high densities of adult *P. altmani*, with numbers exceeding one thousand in *Melanitta spp.* documented in our study and others (Karl Jr, 1967). Gulls and shorebirds are sometimes found with hundreds in much smaller GI length and surface area than

diving ducks. In our study we document one new host species, *Larus californicus*, which maintained hundreds of egg-shedding adults. *P. altmani* has been documented in nearly all locations *Emerita spp.* are known to inhabit. Southern sea otters overlap with the range of *Emerita analoga*, which extends up to Oregon. For this reason, southern sea otters are affected by *P. altmani*, while northern sea otters in Alaska are not. In contrast to definitive bird hosts, we find that sea otters are unable to sustain high *P. altmani* burden without substantial risk of intestinal perforation leading to peritonitis. With genetic and morphological evidence, we differentiated between *P. altmani* and a second species of *Profilicollis* parasite which was formerly identified as morphologically similar to *P. major*. However, we preliminarily conclude that it is instead a hybrid between *P. botulus* and *P. altmani*, pending further investigation to describe this finding in detail. This hybrid species is unable to perforate the intestinal wall of sea otters, presumably due to the short length of its neck and proboscis, rendering it incapable of causing acanthocephalan peritonitis. *P. altmani* alone was found in peritoneum or omentum in AP cases sampled in the 80 carcasses in which detailed sampling was performed.

Our work has confirmed that AP prevalence in southern sea otters is associated primarily with increased population abundance of the intermediate host, *E. analoga*. This is true for intra-annual variation in population size incorporated in our epidemiological model and for inter-annual variation in population size, which varied in a nearly decadal cycle between 2001 and 2021 (Fig. 2.16) with peaks associated with some El Niño years. There is a

moderately strong correlation between *E. analoga* abundance and the Oceanic Niño Index (ONI) in the months preceding recruitment to California beaches (Fig. 2.17), suggesting sensitivity of pelagic larval survival and recruitment to ocean temperatures. Larger *E. analoga* populations in California will lead to increased transmission of *P. altmani* in years of high ocean temperatures. Greater frequencies of El Niño events and ocean warming under climate change conditions may, therefore, contribute to greater AP risk in southern sea otters. All INLA trends reveal peaks in AP around 2004, which suggests a ubiquitous external force increasing transmission during that time. This time was associated with high Pacific Decadal Oscillation (PDO) index and ONI values. However, other periods of high PDO index and ONI values are not associated with high AP prevalence, suggesting a more complex role of the environment and other contributing factors, including otter diet preferences, in driving fluctuations in AP prevalence. El Niño events may also indirectly impact otter feeding through associated shifts in abundance of other prey. Additionally, there is a strong temperature-dependent relationship for cystacanth development time (Fig. 2.13). Warmer ocean temperatures are likely to increase the rate of development of *P. altmani* cystacanths in *E. analoga*, which could increase the rate of transmission of this parasite and alter the seasonal risk of infection for birds and sea otters.

We found a close association between otter feeding on *E. analoga* and AP, which appears to vary seasonally with AP prevalence. The reason for late spring feeding on *E. analoga* is not immediately apparent but may be associated with increased biomass of crabs



on CA beaches during this time, leading up to breeding (Fig. 2.18). Per capita biomass declines at this time as new recruits settle on the beaches. Our own observations during necropsy suggest otters prefer to feed on gravid females, which are larger and harbor more cystacanths. There is a moderately strong correlation between crab size and cystacanth burden (Pearson's correlation coefficient = 0.48, Fig. 2.19). However, gravid females are most common on beaches during the summer months proceeding breeding (Fig. 2.20). The explanation for spring feeding and associated AP may have to do with both the increased total abundance and biomass of crabs and increases in the otter age classes most likely to develop AP from feeding on these crabs. Previous tagging and observation efforts have supported a seasonal increase in winter pupping in southern sea otters, though other work suggests no seasonality in pupping (Jameson & Johnson, 1993; Riedman et al., 1994). Each study was confined to a small geographic region and sample otter population to make sweeping conclusions about population dynamics across the range and through time. We evaluated the seasonality of mortality across the entire necropsy dataset between 1992-2021 and found a strongly cyclic pattern of high mortality of pups relative to total mortality in February and March and low mortality in fall months. This peak was followed approximately two months later by a peak in immature otter mortality relative to total mortality (Fig. 2.23). These patterns may be explained by pup separation from mothers in rough winter conditions and seasonal disease risk from domoic acid and AP affecting immature otters in the spring (M. A. Miller et al., 2020b; Moriarty et al., 2021). However, these data may also

suggest seasonal reproduction reflected in high proportional mortality of young age classes proceeding winter pupping. If so, high numbers of older pups and immature otters learning to feed on easy prey like *E. analoga* could lead to a spike in spring consumption of *E. analoga* and subsequent spike of AP in the otter population.

The significance of *E. analoga* population size in transmission was likely due to increased population-level uptake of acanthors and corresponding higher consumption of *E. analoga* by bird hosts when *E. analoga* were abundant. 57% of transmission variation was unexplained by seasonal fluctuations in *E. analoga* population size, indicating either a high contribution of transmission through paratenic hosts like *B. occidentalis* and/or preferential predation on larger *E. analoga* which were harbored higher cystacanth burdens (Fig. 2.19). Higher cystacanth burden in large crabs may further enhance transmission when *E. analoga* are low in number. Meanwhile, transmission of *P. altmani* from avian hosts peaked in fall (Fig. 2.2B), though high levels of transmission occurred throughout the year. This was likely a result of higher parasite contribution by resident or mixed migrant avian hosts (Fig. 2.9). Many mixed migrants use California as a stopover site to and from arctic breeding grounds, wintering and feeding along the coasts of South America where *P. altmani* is also prevalent in *Emerita spp.* (Oliva et al., 2008). Unlike winter migrants who likely arrive with few to no surviving *P. altmani* adults from the previous winter (Rebecca Cole, personal comm.), these mixed migrants may harbor adults throughout the year and shed

eggs during stopover visits to the California coast. The time to ingest significant quantities of cystacanths in addition to the seven-week prepatent period means shedding of eggs into the environment by wintering birds occurs primarily during the latter half of the winter. Because residency periods within California among these species are not synchronous, continuous transmission of *P. altmani* from definitive hosts to the coastal environment is possible. Future research on feeding preferences, particularly size-biases, among the various bird hosts would improve our understanding of fine-scale transmission dynamics of the parasite. Most known bird hosts are capable of consuming *E. analoga* individuals whole, though scavengers and shore birds have been observed partially consuming alive or dead crabs. This behavior may reduce consumption of cystacanths if birds are able to avoid them in the haemocoel.

Evaluating the explicit role of each bird species in the transmission ecology of *P. altmani* requires greater understanding of their respective parasite burdens, feeding habits, and time spent on the coast vs inland or in estuaries. Rainfall is associated with temporary shorebird migration inland (Shuford et al., 1998; Warnock et al., 2021), which may reduce time spent consuming *E. analoga* and shedding eggs into the marine environment. Further research is necessary to understand whether the small but statistically significant declines in seasonality of AP in otters is due to host dynamics or otter diet preferences. Declines in wintering migratory bird populations, such as *M. perspicillata* (Fig. 2.16), could increase the importance of transmission at other times of the year, resulting in greater uniformity in

AP risk to sea otters between seasons.

High AP risk areas along the CA coast are those where sandy beaches are common, thereby sustaining populations of *E. analoga*. High otter densities in bays and inlets often coincide with these habitats, contributing to long-term exposure to *P. altmani*. Southern sea otter population recovery is limited by failed range expansion in part due to low kelp canopy cover and high white shark densities at the northern and southern ends of the sea otter range. Conservation approaches to expand the sea otter range are informed by mortality risks in introduced areas. Potential future habitats include estuaries like San Francisco Bay (Rudebusch et al., 2020), which pose risks due to human activity and future work to understand the potential impact of known terrestrial pathogens of otters, such as *Toxoplasma gondii*, *Sarcocystis neurona*, and *Coccidioides spp.*, as well as domoic acid and microcystins is necessary. However, estuary habitat poses little to no risk for the two highest contributors to southern sea otter mortality in their extant range: shark bites and acanthocephalan peritonitis. *E. analoga* require wave-swept sandy beach habitat and are not suited for estuaries with little wave action and small soil particle size typical of these habitats. Inspection of recovered *M. perspicillata* carcasses wintering in San Francisco Bay have revealed negligible *P. altmani* densities in their GIs (Jessie Beck, unpublished). With over half of current sea otter mortality attributed in whole or in part to shark bites and AP, estuary habitat may be a promising target for future recovery efforts. As management efforts towards range expansion proceed, we recommend thorough assessment of the range

of major health threats in proposed future habitats.

This study provides comprehensive insight into the transmission of *P. altmani* and resolves this species as the sole contributor to sea otter AP and associated mortality. The intensive efforts over the past several decades to document incidence of AP in southern sea otters and population densities of *E. analoga* with and without cystacanths allowed us to understand temporal patterns of disease in otters in a way that is unique among wildlife diseases. Similarly, long-term population monitoring of bird hosts enabled us to distinguish the roles of population dynamics of intermediate and definitive hosts in transmission of *P. altmani*. This work demonstrates the importance of long-term monitoring efforts in understanding wildlife disease, which has strong implications for conservation efforts, particularly for threatened or endangered species like southern sea otters. Insight provided by this work can contribute to ongoing research and conservation efforts to restore southern sea otter populations in their historical range. Additionally, the unique integration of epidemiological modeling and longitudinal analyses presented in this study can serve as a template for exploring long-term patterns of infectious diseases in humans and wildlife.

## 2.5 Methods

### 2.5.1 Southern sea otter necropsies

In California, all confirmed reports of stranded southern sea otters are investigated and, if verified, have been recorded in a sea otter stranding database since 1968. Carcass recovery and examination follows procedures described in (Pattison et al., 1997), including documentation of the stranding location, demographic information (e.g. age class, estimated age, sex, weight and total length), and any external and internal findings from gross necropsy. The cause(s) of death are also determined when possible. For most cases only a primary cause of death is determined, but a subset of cases receive more detailed examinations, with contributing causes of death also recorded. Beginning in 1992, the presence/absence, relative abundance, and location (intestinal or abdominal) of the acanthocephalan parasites *C. enhydri* and *Profilicollis spp.* were systematically recorded, in addition to whether acanthocephalan peritonitis was considered a primary or contributing cause of death. For most cases, the acanthocephalan burden was assessed via gross inspection of the omentum and peritoneal cavity, as well as 3-10 areas of the intestinal mucosa, noting independently the relative abundance of *C. enhydri* and *Profilicollis spp.* Quantities were classified as none (no acanthocephalans observed), mild (1-9 acanthi in one location or <20 total), moderate (10-19 acanthocephalans in one location or 20-59 total), or heavy ( $\geq 20$  in one location or  $\geq 60$  total). A subset of cases (n=80) were examined in greater

detail to assess acanthocephalan presence/absence and abundance with higher precision (S. Hennessy & Morejohn, 1977; Mayer et al., 2003).

The southern sea otter specimens examined specifically for this study were obtained through the Southern Sea Otter Stranding Network between 2018 and 2020 and were necropsied at the California Department of Fish and Wildlife's Marine Wildlife Veterinary Care and Research Center in Santa Cruz, CA. The selection criteria encompassed carcasses that were minimally decomposed sea otters of all age classes with no or minimal scavenging prior to examination. During necropsy the acanthocephalan burden was first assessed for the peritoneal cavity (including careful examination of the omentum), then the gastrointestinal (GI) tracts were removed for more detailed processing.

### **2.5.2 *Profilicollis* spp. sampling in southern sea otters**

To facilitate mucosal examination, the mesentery between each bowel loop was incised so the intestinal loops could be laid out in longitudinal rows. Next transverse cuts were made to segment the intestines into approximately half meter long sections. Each section was then cut longitudinally to reveal the mucosa. After assessing the intestinal contents for prey items and melena (digested blood), the mucosa was rinsed lightly with tap water to remove sediment and digesta that could hinder detection of acanthocephalans. All acanthocephalan parasites were counted, including any parasites that had become detached from the mucosa

during rinsing. Separate counts were performed for enumeration of *C.enhydri* and *Profilicollis spp.*; morphological differences between these genera, such as angled orientation of the proboscis and the small size of the proboscis relative to the body in *C. enhydri*, facilitate gross identification during necropsy. The resulting counts were recorded to two significant digits (e.g. 158 rounded to 160 or 1236 rounded to 1200) to ensure counts were not more precise than could be accurately measured. For each examined otter with confirmed infection, five or more *C. enhydri* individuals were collected with forceps, washed with tap water and placed collectively in a screw cap collection tube filled with 95% ethanol for potential future examination. When available, ten or more individual *Profilicollis spp.* were sampled from each otter, with parasite sampling completed both relation to the relative parasite density across the GI tract (i.e. higher sampling effort in areas of intestine with higher mucosal parasite density), and relative to the size distribution of the *Profilicollis spp.* parasites within each otter. These parasites were also washed with tap water and stored collectively in a screw cap collection tube filled with 95% ethanol. The omentum was also examined for intact or partially phagocytized acanthocephalans. All peritoneal and omental acanthocephalans were morphologically consistent with *Profilicollis spp.*, and those with an intact proboscis were excised, washed, and stored in 95% ethanol. After transportation to a laboratory, all *Profilicollis spp.* specimens were separated, washed in distilled water, and placed in separate screw cap collection tubes filled with 95% ethanol.



### 2.5.3 *Profilicollis spp.* sampling in seabirds and shorebirds

Seabird and shorebird carcasses were collected for this project between 2018-2019. Carcasses were either found beachcast and collected during standardized beach surveys by BeachCOMBERS (Beach Coastal Ocean Mammal and Bird Education and Research Surveys) Program volunteers, or carcasses were collected postmortem in rehabilitative care by the Society for the Prevention of Cruelty to Animals for Monterey County (MSPCA) Wildlife Rescue and Rehabilitation Center. All carcasses were saved frozen (-20°C) until time of necropsy. Birds were examined via systematic gross necropsy at the California Department of Fish and Wildlife, Office of Spill Prevention and Response, Marine Wildlife Veterinary Care and Research Center (CDFW-OSPR MWVCRC) and gastrointestinal tracts (esophagus to cloaca) were removed and stored frozen (-20°C) in plastic bags until further analysis or were examined the same day. During subsequent gastrointestinal examination, intestinal loops were laid out longitudinally after severing the mesentery between adjacent bowel loops. In heavily parasitized birds, some acanthocephalans had perforated through one intestinal wall and into an adjacent bowel loop, resulting in areas of parasite-mediated intestinal adhesion. Effort was made to separate these loops without severing the proboscis of adherent parasites. Transverse cuts were made at intervals throughout the intestines, dividing them into 20cm long segments. As described above for sea otters, each intestinal segment was opened longitudinally, rinsed, and the *Profilicollis spp.* parasite burden was assessed. When intestinal acanthocephalan burdens were greater than one-thousand, the

total counts were extrapolated based on counts of subsamples within each GI segment. In these latter cases, the intestinal segment that was most densely colonized by *Profilicollis spp.* was noted for each case, and the center of this segment served as the basis for selection of five 2 x 2cm sampling areas for parasite enumeration. The five sampling areas encompassed the central, highest density site plus two areas located proximal, and two areas oriented distal to the highest density area, with each 2 x 2 cm sample site separated by approximately 8cm. At each sampling site, adult and juvenile acanthocephalans were counted separately. Grossly, adults are distinctly longer ( $> 1\text{cm}$ ), possess a larger body relative to the size of the neck and proboscis, and are opaque or stained by exposure to intestinal contents. When distinguishing adults and juveniles was difficult grossly based on size and body shape, indeterminate parasites were collected, washed with tap water, and stored in 95% ethanol-filled tubes for later microscopic examination and identification. When available, ten or more individual acanthocephalans were collected to proportionally represent the density and size distribution of individuals observed in each bird, as described above for sea otters. These specimens were washed and stored in ethanol as described above, and were washed in distilled water and stored in separate 95% ethanol filled tubes after transport to a laboratory.

#### 2.5.4 Identification of *Profilicollis* spp.

The taxonomy of the *Profilicollis* genus has changed dramatically over decades of investigation due to discoveries of presumed different acanthocephalan species in different avian hosts across a broad geographical area (Amin et al., 2013; Amin et al., 2022; Karl Jr, 1967). Disparate classification between observers has plagued the taxonomy, with *P. altmani* now considered synonymous with *P. kenti* and *P. texensis*. *P. bullocki* has been identified as a synonym, but genetic evidence from our work suggests otherwise. More research is needed to clarify the taxonomy of the *Profilicollis* genus. *Profilicollis altmani* and *P. kenti* were considered divergent due to differences in body size and shape, and variation in hook counts on the proboscis. However, later observations noted clear morphological transitions in avian hosts as the parasites matured from cystacanths to mature adults and took on characteristics originally prescribed to *P. kenti* (Karl Jr, 1967). This includes a spherical proboscis, parasite lengthening and clearer body segmentation, and an increased number and greater variability of the numbers of rows of hooks. Individuals can possess between 23 and 30 distinguishable longitudinal rows of hooks and 12 or more hooks per row. We also noted this transition across the range of ages of specimens we collected. *Profilicollis texensis* was identified in a sanderling along the Gulf coast of the United (J. D. Webster, 1948). Its geographic separation led to distinct categorization at the time, although later genetic characterization has confirmed the cosmopolitan nature of *P. altmani* infection of *E. analoga* throughout this crustacean's geographic range (Goulding & Sarah Cohen, 2014).

Previous work has identified up to four acanthocephalan species infecting sea otters, but parasite egg production and fecal shedding has only been confirmed for animals infected with *C. enhydri*. In contrast sea otters do not appear to serve as definitive hosts for *Profilicollis* spp., although excysted cystacanths and maturing parasites have been recovered from sea otter intestines. Mayer previously implicated *P. altmani* and *P. kenti* in cases of acanthocephalan-induced peritonitis based on detection of morphologically compatible parasites found in the omentum or fully penetrating the intestinal wall (Mayer et al., 2003). These identifications plus a third presumed species in the *Profilicollis* genus were inferred based on earlier work from Hennessy and Morejohn (S. Hennessy & Morejohn, 1977). A third species possessing 16 longitudinal rows of hooks and 7-9 hooks per row, referred to as *P. major*, was not implicated in peritonitis cases in sea otters (Mayer et al., 2003). One key objective of the current study is to revisit classification of *Profilicollis* species in general, as well as the range of species infecting sea otters through application of an updated understanding of morphology-based taxonomy, in combination with molecular identification. Individual specimens with intact proboscises were examined under a compound light microscope to count the longitudinal rows of hooks and hooks per row on each proboscis. For each specimen the body shape, trunk spines, sex, and length were also noted.

In total 188 acanthocephalans specimens from avian hosts and 215 from sea otters were examined, revealing two distinct morphological types. The first type fit prior morphological descriptions of *P. altmani*, with variation as described above between juvenile and adult

forms. In contrast, a second morphological type matched the description of *P. major* as identified by Hennessy and Morejohn (S. Hennessy & Morejohn, 1977). This second morphospecies was distinct from *P. altmani* in possessing a more cylindrical (less bulbous) proboscis and shorter neck relative to body size. The characteristics presented could not be distinguished from *P. botulus*, a closely related species to *P. altmani* that has been found in European and Canadian (British Columbia) diving ducks (Ching, 1989; Thompson, 1985).

To facilitate definitive parasite identification using molecular methods, we designed five sets of primers, four targeting rRNA loci and one targeting CO1, using *P. altmani* GenBank accession ID KF835281.1 (Goulding & Sarah Cohen, 2014). Forward and reverse primer descriptions are available in Table 2.1. We extracted genomic DNA by emulsifying each whole parasite in an Eppendorf tube using a sterile pestle. Each sample was digested overnight with proteinase K at 50 C. Phenol-chloroform extraction was facilitated by use of sterilized vacuum grease for phase separation. After ethanol precipitation, DNA pellets were suspended in 10mM Tris-HCl (pH 8.5) and stored at -20 C. The DNA concentration was measured using a NanoDrop spectrophotometer. Five separate PCR reactions were performed for each individual parasite; one for each locus. The volume for all reactions was 50 microliters: 5 microliters Taq buffer, 1 microliter dNTP, 1 microliter template DNA at 10 ng/microliter, 0.25 microliter Taq polymerase, 40.75 microliters RNase free water, 1 microliter forward primer at 50 pM, and 1 microliter reverse primer at 50 pM. A gradient reaction was performed at a range around the expected mean annealing temperature for

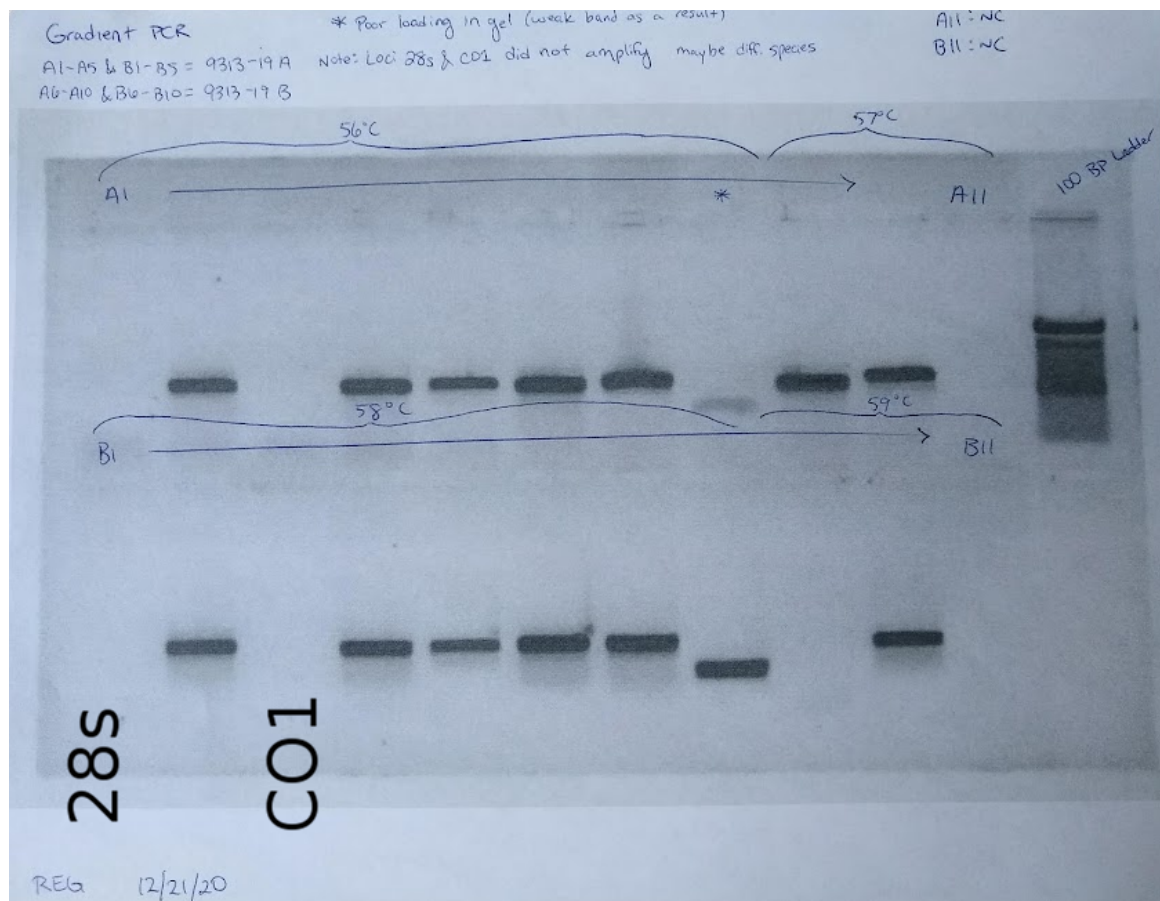


Figure 2.7: Example gel electrophoresis with differential amplification of primers between two *Profilicollis* spp.. Partial gradient PCR is shown for two individuals. Lanes 1 and 3 showed no amplification for individual A, which was morphologically identified as the *P. botulus*/*P. major* morphospecies.

each primer (given by IDT). 30 PCR cycles at 56 C annealing temperature were performed for each reaction mixture. Five microliters of each sample were run on EtBr agarose gel (1%) and PCR reactions were repeated until either a distinct band was visible within the gel, or no band was visualized after three sequential PCR runs.

The success rate was high, and few repeat runs were necessary except for loci A and C for the *P. botulus*/*P. major* morphospecies, and loci A-D for *C. enhydri*. Amplification

did not occur in any attempts, even with lower annealing temperatures, for 2/5 loci in the *P. botulus*/*P. major* morphospecies samples, and 4/5 loci in the *C. enhydri* samples (Fig. 2.7). Therefore, primers for locus E (CO1) appear to be universal across sea otter acanthocephalan species, while primers for locus A and C are specific to *P. altmani*. These results alone strongly support the morphological distinction between *P. altmani* and *P. botulus*/*P. major*, but to verify the phylogenetic relationship we performed Sanger sequencing (GeneWiz) on all amplified loci for 23 *P. altmani*, 6 *P. botulus*/*P. major*, and 3 *C. enhydri* samples. Indigo (Gear Genomics, supported by EMBL) was used to call alleles in SNV discovery compared to the *P. altmani* GenBank accession ID KF835281.1 reference sequence for each locus. We constructed an unrooted phylogeny with the ClustalW2 neighbor-joining algorithm (Larkin et al., 2007), concatenating the recovered alleles for loci B,D, and E and comparing to concatenated representative GenBank sequences for *P. altmani* and *P. botulus* with a *P. bullocki* outgroup (Fig. 2.8).

Allele calling revealed two distinct haplotypes at approximately 50% frequency for the six *P. botulus*/*P. major* individuals. Sequence concordance was 100% between the six individuals for each haplotype. Haplotypes were divergent at all three loci with multiple SNVs and indels. In contrast, *P. altmani* sequences revealed a single allele for each locus, therefore one haplotype that was 100% concordant between all twenty-three individuals. Phylogenetic comparison with these sequences and GenBank sequences from the three closest relatives revealed 100% concordance between our *P. altmani* sequences and GenBank

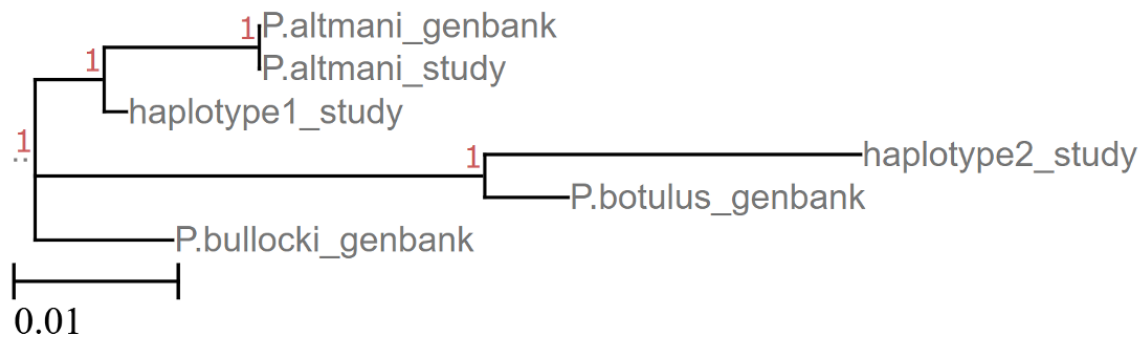


Figure 2.8: Phylogenetic-distance calibrated unrooted tree with branch support containing aligned *Profilicollis* spp. sequences retrieved from GenBank and sequences from our study. The two rRNA gene haplotypes evident from our study for the *P. botulus*/*P. major* morphotype species group separately: one two *P. altmani* and the other to *P. botulus*. However, each is distinct from GenBank sequences for the respective species.

sequences, therefore no phylogenetic distance. However, each haplotype of the *P. botulus*/*P. major* individuals was genetically distinct from all GenBank sequences, and each haplotype grouped separately with *P. botulus* and *P. altmani*. This result is highly unusual due to the strict conservation of rRNA genes sampled by these loci. rRNA genes are the hallmark of concerted evolution to maintain homogeneous copies throughout the genome, yet we find two distinct haplotypes maintained identically across all six individuals samples from different sea otters. We tentatively suggest this represents a newly identified species that could have arisen from a hybridization event between *P. altmani* and *P. botulus*, though we are investigating this possibility in greater detail for future work.

Five of the twenty-three *P. altmani* individuals were recovered from the omentum and were also genetically identical at all three loci to each other and to the other eighteen recovered from the intestinal mucosa.



|          |                       | Table 2.1: Primer set metadata                                      |             |
|----------|-----------------------|---------------------------------------------------------------------|-------------|
| Locus    | Gene                  | Sequence                                                            | Length (nt) |
| <i>A</i> | 28S RNA               | F: CGA CGC TTG TTG TTT GGT ATG<br>R: CCA CTT GGC GCT CTC TAT TT     | 582         |
| <i>B</i> | 28S RNA               | F: TAC TCG GAG GCA GGG AAT AA<br>R: GCT ACC TCG AGA GAG TCA TAG T   | 582         |
| <i>C</i> | ITS1-5.8S-ITS2<br>RNA | F: GCC ACT GCT TGT TGG TAA TTC<br>R: CGC TAA CTC ACC TTC GTC AA     | 328         |
| <i>D</i> | 18S RNA               | F: GCA TTC GCC AAC GCT TTA TC<br>R: GTA CAA AGG GCA GGG ACT TAA T   | 515         |
| <i>E</i> | CO1                   | F: TGG GTT GAG AAT GGT GGT TC<br>R: CCC TAA AGT TAA AGG AGG GTA CAT | 580         |

### 2.5.5 Data curation

Due to sparse recording of data on *Profilicollis spp.* prior to 1992 our final sample set encompassed southern sea otters examined by CDFW from 1992 through 2021 of both sexes and all age classes except for fetuses and pups with only milk teeth present at necropsy. These young otters do not have the same opportunity for *P. altmani* exposure and were excluded. Scavenged animals and animals for which acanthocephalan peritonitis was unassessed or undetermined were also excluded from analyses. Otters recovered outside of 34-38 degrees latitude were excluded due to sparse carcass recovery outside this range. In total 4460 otters were included in the final sample set. We designed software to verify the accuracy of all data columns used in association with this study. This was accomplished by first identifying potential data entry errors such as duplications and mismatches between column data containing overlapping information. All potential errors were manually checked and corrected. Two hundred sea otters from the sample set were randomly selected for cross referencing with archival case data to assess data coding accuracy; the total error rate (any error across all data columns for each sea otter counts as an errant row) was less than 5%.

Two datasets collected by the Long-term Monitoring Program and Experiential Training for Students (LiMPETS) between 2001 and 2021 were used in this study. The first dataset includes population abundance estimates of *E. analoga* in sandy beach habitat sites in central and southern California. Data from sample sites between 34 and 38 degrees

latitude were included in this study. Data collection as part of the long-term sandy beach monitoring program conducted by LiMPETS occurs at sixty-five sites, fifty-four of which are within the 34-38 degrees latitude boundaries of our study. Sampling occur regularly (4 or more times per year) at some sites, while other sites are not surveyed consistently. At each site *E. analoga* crabs are surveyed along a 50m stretch of beach. The section of beach is divided into two areas: one for investigating the current boundaries of crab habitat based on beach conditions, while the other is reserved for systematic sampling. In the sampled area, three to eight transects are randomly placed perpendicular to the waterline, extending from the high tide strand line to the lower end of the swash zone (water depth approx. 10-20cm). Along each transect ten core samples are collected. The 10cm core is pressed into the sand approximately 10cm deep. The sand removed by the core is strained with a mesh bag by pouring the sand into the bag and dunking the bag in a bucket of water until sand is removed and any crabs remain. The length of the carapace of each crab is determined with calipers to the nearest millimeter, and the sex is determined by the presence of pleopods in females. Ovigerous (gravid) females are separately recorded. Animals are returned to the swash zone between each sample measurement. In total 84212 core samples were considered in our analyses.

The second dataset collected by LiMPETS includes *P. altmani* cystacanth abundance in a subset of the crabs counted. When cystacanth burden is assessed, approximately thirty randomly selected crabs are placed in zip lock bags and frozen for more than four hours to

euthanize them. The crabs are thawed and dissected, with attention given to the haemocoel where cystacanths are present. The number of cystacanths is enumerated for each crab. We used the same latitudinal inclusion criteria for the cystacanth dataset, in total considering 5956 dissected crabs in our analyses.

Long-term data on *M. perspicillata* abundance in the San Francisco estuary in the Lower Pacific Flyway were total counts observed from aerial and ground surveys conducted by US and CA Fish and Wildlife (aerial) as well as the San Francisco Bay Bird Observatory and US Geological Survey (ground) each year in early January. Detailed survey methods are available in Midwinter Waterfowl Survey reports (Richmond et al., 2014). Seasonal data on *M. perspicillata* and other avian hosts of *P. altmani* was obtained from a range of scientific and citizen science surveys conducted between 1968 and 2021 included in the eBird database (Sullivan et al., 2009). Data used adhered to the eBird checklist and included species, location, time, survey duration, number of observers, and total individuals counted in each survey. Counts per unit effort (effort = survey duration \* number of observers) were averaged across the dataset, binned by week, for California surveys within the 34-38 latitude range (Fig. 2.9).

### 2.5.6 Epidemiological model

We describe the transmission dynamics of *Profilicollis altmani* with a system of ordinary differential equations representing two infectious states: mean burden of adult worms

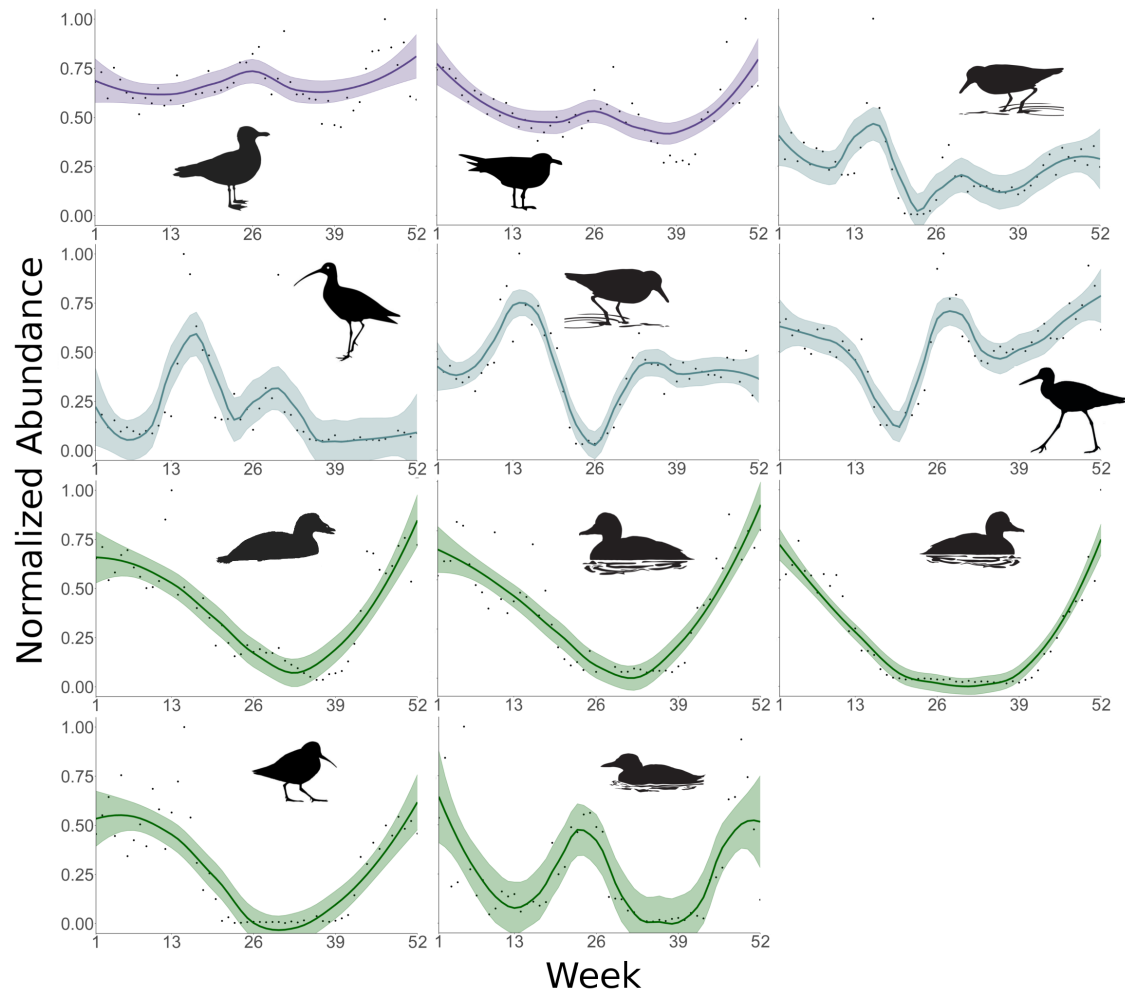


Figure 2.9: Known definitive hosts of *P. altmani* are diverse in their time spent along CA coastlines. Some species are residential, migrating short distances but spending a significant fraction of their time within the southern sea otter range. Others are migratory but use CA as both stop-over and residential feeding grounds. These mixed migrants can contribute to seasonal variation in *P. altmani* transmission, but their abundance in CA depends on the migration routes and distance. Some species are only present during winter migrations (or for *Cerorhinca monocerata*, also summer breeding). LOESS regressions of weekly population abundance estimates normalized to the maximum for each species show the population dynamics of these three distinctive migration types. Each migration type is color-coded: resident = purple, mixed = blue, winter = green. First row: Left, *Larus occidentalis* (resident); Middle, *Larus californicus* (resident); Right, *Calidris mauri* (mixed). Second row: Left, *Numenius hudsonicus* (mixed); Middle, *Calidris alba* (mixed); Right, *Tringa semipalmata* (mixed). Third row: Left, *Melanitta perspicillata* (winter); Middle, *Aythya marila* (winter); Right, *Aythya affinis* (winter). Fourth row: Left, *Calidris alpina* (winter); Middle, *Cerorhinca monocerata* (winter).

which are reproductively mature in definitive bird hosts ( $W$ ) and mean cystacanth burden in intermediate crab hosts ( $C$ ). A prepatency period for both infectious states requires two latent states: mean burden of juvenile recruits which are reproductively immature in definitive bird hosts ( $R$ ) and mean acanthellae burden in intermediate crab hosts ( $A$ ). Host population dynamics are described by seasonal and resident populations, which are time varying and constant, respectively. Seasonally, migratory waterfowl and shorebirds serve as transient hosts to *P. altmani*, and their population size in the sea otter range is summarized by  $M$ . The contribution of the residential bird population to the transmission of *P. altmani* is summarized by  $r_2$ , which is a parameter optimized in model fitting. We describe the population size of *E. analoga* within the sea otter range with a similar periodic function ( $E$ ). Recruitment of megalopae to sandy beaches occurs year-round, but is strongest in the late winter and spring (Cox & Dudley, 1968). However, positive size-selectivity by bird and otter predators in addition to the presence of transport hosts like *Blepharipoda occidentalis*, which scavenges *E. analoga* and lives subtidally, could reduce the effect of seasonal recruitment of *E. analoga* on transmission. We summarize the contribution of this non-seasonal cystacanth reservoir with  $r_1$ , which is also a parameter for background transmission optimized in model fitting.  $\lambda_1$  and  $\lambda_2$  are the two other parameters optimized in model fitting and are the transmission rates of cystacanths to bird hosts ( $\lambda_1$ ) and acanthors to crab hosts ( $\lambda_2$ ).

$$\frac{dR}{dt} = \lambda_1 e^{-zW} C(E + r_1) - \left(\rho_1 + \frac{1}{M + r_2} \frac{dM^+}{dt}\right) R \quad (2.1)$$

$$\frac{dW}{dt} = \rho_1 R - \left(\mu_1 + \frac{1}{M + r_2} \frac{dM^+}{dt}\right) W \quad (2.2)$$

$$\frac{dA}{dt} = \lambda_2 (\gamma - \eta W) W (M + r_2) - \left(\rho_2 + \frac{1}{E + r_1} \frac{dE^+}{dt}\right) A \quad (2.3)$$

$$\frac{dC}{dt} = \rho_2 A - \left(\mu_2 + \frac{1}{E + r_1} \frac{dE^+}{dt}\right) C \quad (2.4)$$

$$\frac{dM}{dt} = \omega \alpha \cos(\omega t + \phi_1) \quad (2.5)$$

$$\frac{dE}{dt} = \omega \beta \cos(\omega t + \phi_2) \quad (2.6)$$

The population size of migratory birds in the southern sea otter range at time  $t$  is approximated as

$$M(t) = \alpha \sin(\omega t + \phi_1) \quad (2.7)$$

See Fig. 2.10 for model fit to abundance data.  $\alpha$  is the seasonal amplitude,  $\omega$  is the scaling constant to set the period to fifty-two weeks.  $\phi_1$  is the phase of the sinusoidal curve. The population size of *E. analoga* in the southern sea otter range at time  $t$  is approximated as

$$E(t) = \beta \sin(\omega t + \phi_2) \quad (2.8)$$

See Fig. 2.10 for model fit to abundance data.  $\beta$  is the seasonal amplitude of the *E. analoga* population. The derivatives of  $M(t)$  and  $E(t)$  are included in the system of ODEs above alongside their positive components,  $\frac{dM^+}{dt}$  and  $\frac{dE^+}{dt}$ . The mean burden of infectious and prepatent stages of *P. altmani* in bird and crab hosts is diluted by the influx of naive hosts. The settling of megalopae which mature to adulthood and filter feed in the swash zone of sandy beaches provides new, parasite-free recruits. Likewise, the migration of wintering birds to California shores after months away from *P. altmani* transmission sites provides new opportunity for transmission and reduced mean worm burden in the collective bird host population. With mean prepatency period of seven weeks and life expectancy of the reproductive adult twice as long, we assume the vast majority of infections have cleared by the following year. In addition to juvenile bird recruits and adult deaths since the previous year, the birds immigrating to California shores are assumed negligible reservoirs of *P. altmani* adults. Opportunistic inspection of the GI tracts of migratory hosts in summer breeding grounds, especially in the weeks immediately prior to the return migration to California, will illuminate the reliability of this assumption. Immigration and recruitment dilute the mean parasitic burden among host populations by introducing naive hosts, while death decreases the mean parasitic burden proportionally, and emigration has no effect. This last assumption requires further investigation, as the mean worm burden of migrants can differ from residents due to disparate feeding habits and time spent in California. We find higher counts and densities ( $cm^{-2}$ ) of *P. altmani* in surf scoter GI tracts than in western



gulls, which migrate little but are likely feeding on fewer *E. analoga* due to their generalist diet (Shaffer et al., 2017). The trigonometric functions  $\frac{dM}{dt}$  and  $\frac{dE}{dt}$  representing rates of population size change can be decomposed as the sum of immigration and emigration rates. We leverage the property of trigonometric functions to produce a periodic Fourier synthesis upon addition to solve for immigration and emigration rates. We take the migration rate of wintering birds as an example which applies equally well to the population growth rate of *E. analoga*. Generally, the migration rate should be decomposed as

$$\frac{dM}{dt} = \frac{dM^+}{dt} - \frac{dM^-}{dt} \quad (2.9)$$

the two terms representing immigration and emigration rates, respectively. When the terms are equal, migration is neutral, therefore population growth in California is zero. We assume that these separate rates take the same functional form as the net rate (i.e. sinusoidal functions with the same period). However, peak emigration will not be aligned with peak immigration, and each will have different phases. Additionally, although the amplitudes of each separate rate will be the same, these amplitudes may differ from the amplitude of the net rate function. We express this relationship as

$$\frac{dM}{dt} = k\omega\alpha\cos(\omega t + \phi_1) = \omega\alpha\cos(\omega t + x) - \omega\alpha\cos(\omega t + y) \quad (2.10)$$

$x$ ,  $y$ , and  $k$  are values to be solved.

This relationship simplifies to

$$k\cos(\omega t + \phi_1) = \cos(\omega t + x) - \cos(\omega t + y) \quad (2.11)$$

and using trigonometric identities we substitute the right-hand side in several steps:

$$= -2\sin(0.5(\frac{2\pi t}{365} + x + \frac{2\pi t}{365} + y))\sin(0.5(\frac{2\pi t}{365} + x - \frac{2\pi t}{365} - y)) \quad (2.12)$$

$$= -2\sin(\frac{2\pi t}{365} + \frac{x+y}{2})\sin(\frac{x-y}{2}) \quad (2.13)$$

$$= -2[\sin(\frac{2\pi t}{365})\cos(\frac{x+y}{2}) + \cos(\frac{2\pi t}{365})\sin(\frac{x+y}{2})]\sin(\frac{x-y}{2}) \quad (2.14)$$

$$= -2[\sin(\frac{2\pi t}{365})\frac{\sin x - \sin y}{2} + \cos(\frac{2\pi t}{365})\frac{\cos x - \cos y}{2}] \quad (2.15)$$

Given the above and the following relations,

$$\cos(\omega t + \phi_1) = \cos(\omega t)\cos(-\phi_1) + \sin(\omega t)\sin(-\phi_1) \quad (2.16)$$

it follows that

$$k[\cos(\frac{2\pi t}{365})\cos(-\phi_1) + \sin(\frac{2\pi t}{365})\sin(-\phi_1)] \quad (2.17)$$

$$= -\cos(\frac{2\pi t}{365})(\cos y - \cos x) - \sin(\frac{2\pi t}{365})(\sin x - \sin y) \quad (2.18)$$

A solution to this equation includes this system of equations

$$\frac{\cos y - \cos x}{-k} = \cos(-\phi_1) \quad (2.19)$$

$$\frac{\sin x - \sin y}{-k} = \sin(-\phi_1) \quad (2.20)$$

when solved yields

$$\frac{\cos y - \cos x}{\cos(\phi_1)} = \frac{\sin y - \sin x}{\sin(\phi_1)} \quad (2.21)$$

Implicit solutions for  $x$  and  $y$  are not unique, so we choose the simplest and most parsimonious solution for  $x$ , which assumes that the peak immigration rate aligns with the peak in population growth rate (i.e  $x = \phi_1$ ). Solving for  $y$  reveals that the peak emigration rate aligns with the trough in population growth rate (i.e.  $y = \phi_1 + \pi$ ). This result is biologically realistic, as little to no overlap is known between immigrating and emigrating birds.

$$\frac{dM}{dt} = \frac{\alpha\omega}{k} \left[ \cos\left(\frac{2\pi t}{365} + \phi_1\right) - \cos\left(\frac{2\pi t}{365} + \pi + \phi_1\right) \right] \quad (2.22)$$

$$= \frac{\alpha\omega}{k} \left[ 1 + \cos\left(\frac{2\pi t}{365} + \phi_1\right) - 1 + \cos\left(\frac{2\pi t}{365} + \pi + \phi_1\right) \right] \quad (2.23)$$

The trivial addition of ones to each term above allows us to separate immigration and emigration rates by ensuring neither intersects the x-axis. Each term therefore represents the positive and negative components of migration, respectively.

$$\frac{dM^+}{dt} = \frac{\alpha\omega}{k} [1 + \cos(\frac{2\pi t}{365} + \phi_1)] \quad (2.24)$$

$$\frac{dM^-}{dt} = \frac{\alpha\omega}{k} [1 + \cos(\frac{2\pi t}{365} + \pi + \phi_1)] \quad (2.25)$$

The same exercise holds true for the sinusoidal function representing *E. analoga* population dynamics, and we represent recruitment of newly molted, cystacanth-free crabs with the equation

$$\frac{dE^+}{dt} = \frac{\beta\omega}{k} [1 + \cos(\frac{2\pi t}{365} + \phi_2)] \quad (2.26)$$

### 2.5.7 Model parameters estimation

Table 2.2: Parameter descriptions

| Parameter  | Description                                                                                                                          | Value                                                       | Reference                                                                                           |
|------------|--------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| $R(t = 0)$ | Initial mean burden of <i>P. altmani</i> recruits (immature) in the definitive bird hosts within the sea otter range                 | 1300 $\frac{\text{worms}}{\text{bird}}$                     | Calculated analytically by solving for the equilibrium at initial conditions                        |
| $W(t = 0)$ | Initial mean burden of <i>P. altmani</i> adult worms (reproductively mature) in the definitive bird hosts within the sea otter range | 1000 $\frac{\text{worms}}{\text{bird}}$                     | Average count of adult worms per bird host in our study and others (see text for discussion)        |
| $A(t = 0)$ | Initial mean burden of <i>P. altmani</i> acanthellae in the crab intermediate hosts, <i>E. analoga</i> , within the sea otter range  | 1.1 $\frac{\text{acanthellae}}{\text{crab}}$                | Calculated analytically by solving for the equilibrium at initial conditions                        |
| $C(t = 0)$ | Initial mean burden of <i>P. altmani</i> cystacanths in the crab intermediate hosts, <i>E. analoga</i> , within the sea otter range  | 1.5 $\frac{\text{cystacanths}}{\text{crab}}$                | Approximation from data at week 52 (Fig. 2.2)                                                       |
| $M(t = 0)$ | Initial spatial density of migratory, definitive bird hosts within the sea otter range                                               | 0.87 $\frac{\text{bird}}{\text{observer min}}$              | Estimated intercept of sinusoidal regression (Fig. 2.10)                                            |
| $E(t = 0)$ | Initial spatial density of crab intermediate hosts, <i>E. analoga</i> , within the sea otter range                                   | 0.92 $\frac{\text{crab}}{\text{core sample}}$               | Estimated intercept of sinusoidal regression (Fig. 2.10)                                            |
| $k$        | Scaling variable to adjust the decomposed periodic functions describing seasonal population dynamics of hosts                        | 2                                                           | Calculated analytically by solving for emigration and immigration rates in sinusoidal decomposition |
| $\alpha$   | Amplitude of M(t)                                                                                                                    | $0.416 \pm 0.023$ $\frac{\text{bird}}{\text{observer min}}$ | Average value estimated as the amplitude in sinusoidal regression (Fig. 2.10)                       |
| $\beta$    | Amplitude of E(t)                                                                                                                    | $2.44 \pm 0.16$ $\frac{\text{crab}}{\text{core sample}}$    | Average value estimated as the amplitude in sinusoidal regression (Fig. 2.10)                       |

Table 2.2: Parameter descriptions (continued)

| Parameter | Description                                                                                           | Value                                        | Reference                                                                                                                                         |
|-----------|-------------------------------------------------------------------------------------------------------|----------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| $\omega$  | Seasonal frequency constant                                                                           | $\frac{2\pi}{52} wk^{-1}$                    | Direct calculation: 52 weeks per year                                                                                                             |
| $\phi_1$  | Seasonal phase shift of $M(t)$                                                                        | $0.97 \pm 0.06 wk^{-1}$                      | Estimated parameter of sinusoidal regression (Fig. 2.10)                                                                                          |
| $\phi_2$  | Seasonal phase shift of $E(t)$                                                                        | $-1.22 \pm 0.13 wk^{-1}$                     | Estimated parameter of sinusoidal regression (Fig. 2.10)                                                                                          |
| $z$       | Density-dependent saturation constant for $R(t)$ recruitment to the GI tract in definitive bird hosts | 0.0006                                       | Estimated slope of semilog regression (Fig. 2.11)                                                                                                 |
| $\mu_1$   | Death rate of adult <i>P. altmani</i>                                                                 | $0.175 wk^{-1}$                              | Death rate of adult <i>P. botulus</i> , closest relative and with similar life history and definitive hosts to <i>P. altmani</i> (Thompson, 1985) |
| $\mu_2$   | Death rate of cystacanth <i>P. altmani</i>                                                            | $0.07 wk^{-1}$                               | Death rate of settled <i>E. analoga</i> (cystacanth mortality is assumed negligible outside of host mortality) (Diehl, 2001)                      |
| $\eta$    | Density-dependent constant for $W(t)$ fecundity                                                       | $1.07 \frac{\text{eggs}}{\text{adult worm}}$ | Estimated slope from linear regression (Fig. 2.12)                                                                                                |
| $\gamma$  | Maximum (low adult worm density) fecundity of adult worms                                             | $9900 wk^{-1}$                               | Estimated intercept from linear regression (Fig. 2.12)                                                                                            |
| $\rho_1$  | Maturation rate of immature (recruits) to adult worms                                                 | $0.14 wk^{-1}$                               | Reciprocal of average time to reproductive maturity in <i>P. altmani</i> (Karl Jr, 1967)                                                          |
| $\rho_2$  | Maturation rate of acanthellae to cystacanths                                                         | $0.1 wk^{-1}$                                | Reciprocal of estimated intersection of regression at 14 C (Fig. 2.13)                                                                            |

Table 2.2: Parameter descriptions (continued)

| Parameter   | Description                                                                                          | Value                                                                        | Reference              |
|-------------|------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|------------------------|
| $\lambda_1$ | Maximum (low adult worm density) transmission rate of cystacanths to immature recruits in bird hosts | $37.08 \pm 0.225$                                                            | Optimized in ODE model |
| $\lambda_2$ | Transmission rate of shed acanthors to acanthellae in <i>E. analoga</i>                              | $1.69 * 10^{-9} \pm 5.13 * 10^{-13}$                                         | Optimized in ODE model |
| $r_1$       | Spatial density equivalent of intermediate host reservoir within the sea otter range                 | $4.17 \pm 0.16 \frac{\text{crabs}}{\text{core sample}}$                      | Optimized in ODE model |
| $r_2$       | Spatial density of residential, definitive bird hosts within the sea otter range                     | $8.73 \pm 0.28 \frac{\text{bird host equivalents}}{\text{observer minutes}}$ | Optimized in ODE model |
| $t$         | Time in weeks                                                                                        |                                                                              |                        |

Previous accounts of adult worm burdens in definitive hosts of *P. altmani* vary greatly due in part to the size of the bird and thus the mucosal surface area in the intestines, but also the bird feeding habits and timing of carcass collection with respect to migration or bird age can affect density estimates. We found densities exceeding 20 adult worms per square centimeter of mid and anterior intestine of *M. perspicillata* and total counts of adults greater than 3000 in some individuals. However, this contrasts other estimates of mean abundance of 209 and 1482 in *M. perspicillata* and *M. deglandi*, respectively (Karl Jr, 1967). All samples have been opportunistic rather than systematic, our measurements included. Many scoters examined in our study were recovered from a supposed acanthocephaliasis die-off event, and we suspect such high densities observed are not characteristic of mean burden in the scoter population, of which surf scoters comprise more than 98% (Richmond et al., 2014). White-winged and black scoters are not modeled in our study due to their low abundance in CA waters. We find densities exceeding 15 adult worms per square centimeter of mid and anterior intestine of *Larus occidentalis* and *L. californicus* and overall adult worm abundance greater than 700 in rare cases. To our knowledge, these reports are the first for *P. altmani* in the California gull. Other gull species, *Larus dominicanus*, *Larus Belcheri*, *Larus argentatus*, *Leucophaeus pipixcan*, *Leucophaeus modestus* are reported hosts (Amin et al., 2022), though little evidence has been recorded for fecundity of adult worms in these hosts. However, based on our observations of egg release in two gull species as well as surf scoters, it is clear that *P. altmani* reproduction is not specialized to



bird taxa, even at the level of order. Based on accounts of gravid adults and/or egg shedding in shorebirds, it is reasonable to assume that most bird species consuming *E. analoga* are potential definitive hosts and that this parasite is cosmopolitan. Other known host species common to California are listed in Fig. 2.9. We use *M. perspicillata* as our type species for winter migrants to analyze seasonal contribution to transmission of *P. altmani* for several reasons: *M. perspicillata* is the type definitive host for this species of parasite identified in (Perry, 1942); measurements of parasite density and fecundity were taken from necropsied birds, primarily in *M. perspicillata*; and *M. perspicillata* exhibits defined winter migration to CA that is representative of several known wintering host species.

We set initial conditions for mean adult parasite burden in wintering birds to 1000. This number is within the observed range of our study and previous studies, and variation of this value matters little for the outcome of the analysis, which used optimized transmission rates calibrated to fit this initial condition. At this mean burden, which is lower than the burden observed in our study, the average fraction of immature (recruits) to adult parasites we measured is not valid. Therefore, we estimate the initial mean immature parasite burden at equilibrium with the adult parasite population.

$$\frac{dR}{dt} = \frac{dW}{dt} = 0 \quad (2.27)$$

$$\lambda_1 e^{-zW} C(E + r_1) - (\rho_1 + \frac{1}{M + r_2} \frac{dM^+}{dt}) R = 0 \quad (2.28)$$

$$\rho_1 R - (\mu_1 + \frac{1}{M + r_2} \frac{dM^+}{dt}) W = 0 \quad (2.29)$$

$$\implies \lambda_1 e^{-zW} C(E + r_1) - (\rho_1 + \frac{1}{M + r_2} \frac{dM^+}{dt}) R = \rho_1 R - (\mu_1 + \frac{1}{M + r_2} \frac{dM^+}{dt}) W \quad (2.30)$$

$$\lambda_1 e^{-zW} C(E + r_1) + (\mu_1 + \frac{1}{M + r_2} \frac{dM^+}{dt}) W = \rho_1 R + (\rho_1 + \frac{1}{M + r_2} \frac{dM^+}{dt}) R \quad (2.31)$$

$$= (2\rho_1 + \frac{1}{M + r_2} \frac{dM^+}{dt}) R \quad (2.32)$$

$$R(t = 0) = \frac{\lambda_1 e^{-zW(t=0)} C(E(t = 0) + r_1) + (\mu_1 + \frac{1}{M(t=0)+r_2} \frac{dM(t=0)^+}{dt}) W(t = 0)}{2\rho_1 + \frac{1}{M(t=0)+r_2} \frac{dM(t=0)^+}{dt}} \quad (2.33)$$

An analogous calculation was performed to yield starting conditions for mean per capita acanthellae burden in *E. analoga*. Weeks 51-52 and weeks 1-2 cystacanth burden is approximately 1.5 per crab sampled. Therefore, we initiated  $C(t = 0) = 1.5$ . We solve for mean acanthallae burden at equilibrium conditions.

$$\frac{dA}{dt} = \frac{dC}{dt} = 0 \quad (2.34)$$

$$\lambda_2(\gamma - \eta W)W(M + r_2) - (\rho_2 + \frac{1}{E + r_1} \frac{dE^+}{dt})A = 0 \quad (2.35)$$

$$\rho_2 A - (\mu_2 + \frac{1}{E + r_1} \frac{dE^+}{dt})C = 0 \quad (2.36)$$

$$\lambda_2(\gamma - \eta W)W(M + r_2) - (\rho_2 + \frac{1}{E + r_1} \frac{dE^+}{dt})A = \rho_2 A - (\mu_2 + \frac{1}{E + r_1} \frac{dE^+}{dt})C \quad (2.37)$$

$$\lambda_2(\gamma - \eta W)W(M + r_2) + (\mu_2 + \frac{1}{E + r_1} \frac{dE^+}{dt})C = \rho_2 A + (\rho_2 + \frac{1}{E + r_1} \frac{dE^+}{dt})A \quad (2.38)$$

$$= (2\rho_2 + \frac{1}{E + r_1} \frac{dE^+}{dt})A \quad (2.39)$$

$$A = \frac{\lambda_2(\gamma - \eta W)W(M + r_2) + (\mu_2 + \frac{1}{E + r_1} \frac{dE^+}{dt})C}{2\rho_2 + \frac{1}{E + r_1} \frac{dE^+}{dt}} \quad (2.40)$$

Initial conditions for sinusoidal population functions for definitive and intermediate hosts were determined via least squares model fitting of sine functions  $M(t)$  and  $E(t)$  given above.  $\alpha, \beta, \phi_1, \phi_2$  were solved using the Minimize function in the `scipy.optimize` package. The intersection of each model with  $t = 0$  gives the initial condition. Model fits are given in Fig. 2.10.

For the values  $x$  and  $y$  determined in the decomposition of the migration rate functions,

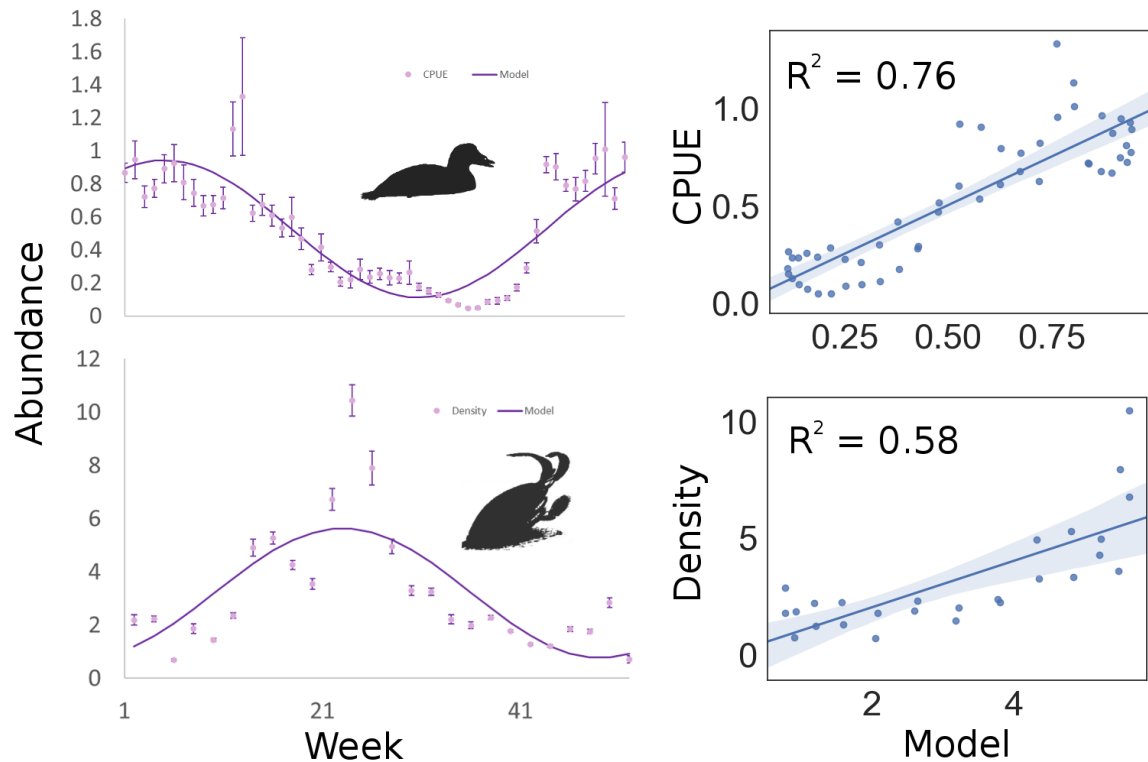


Figure 2.10: Sinusoidal models fit to population abundance data for scoters (top) and pacific mole crabs (bottom). Scoter data is the weekly average of counts per unit effort (observer minutes) within the sea otter range in the eBird database. Mole crab data is the biweekly average of counts per core sample within the sea otter range in the LiMPETS database. Strong correlations exist for both model fits to the data, though a better fitting model for mole crab abundance may include a higher order periodic function (4th or 6th order), which allows a steeper peak in summer and broader trough in winter. Decomposing this periodic function into immigration and emigration rates is not readily feasible, however. Thus, we opt for a first order sine function for model tractability.

alignment of the immigration rate peak with the overall population growth rate produces a value  $k = 2$ . This is easily found by inspection. For a seasonal period of 52 weeks,  $\omega$  is found directly. The density-dependent saturation constant for the rate of recruitment of immature worms to definitive host GI tracts is found as the slope of a semilog plot between total adult worm burden in each surf scoter and the fraction of immature to total worms found as the average across five sample counts done in each individual. Fig. 2.11 shows the regression line and the corresponding slope which is  $-z$ .

Death rates for established immature worms and acanthellae were assumed negligible, and loss of parasites from the transmission cycle was incorporated in death rates of adult worms and cystacanths. The death rate of *P. altmani* worms is unknown, but the death rate of adult *P. botulus*, the closest relative, is measured in experimental eider infections (Table 2.2). Similarly, *P. botulus* exhibits transmission through crab and diving duck hosts. We use this death rate in our model assuming similarities in life history make it a close approximation. The absence of *P. altmani* in *M. perspicillata* dissected in Alaska during the summer breeding season (Rebecca Cole, personal comm.) supports a short-lived adult worm with a life expectancy on the order of several weeks rather than several months. The life expectancy of *P. botulus* is less than 6 weeks, which aligns with this expectation. Migratory hosts favor short-lived parasites due to the confined window of opportunity to shed eggs in the environment of *E. analoga*. Fitness costs via peritonitis and energy loss in bird host during migration would be unfavorable to maintain long-term transmission, however

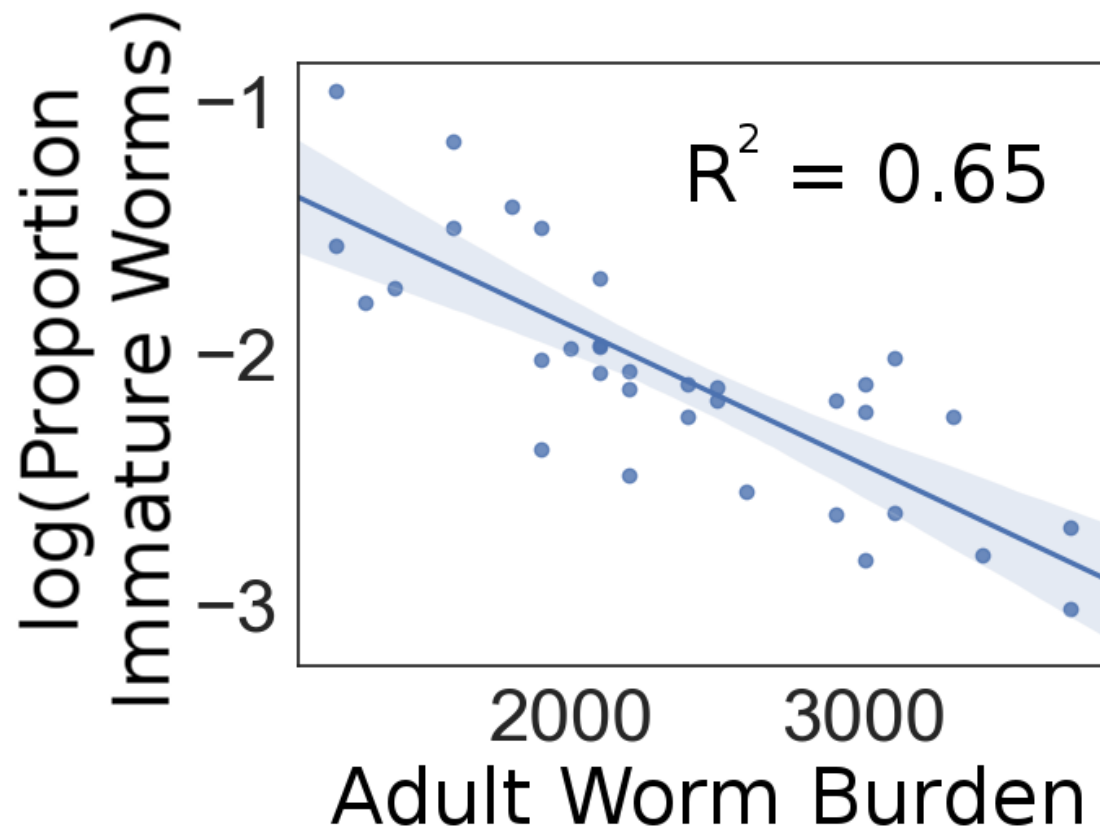


Figure 2.11: Regression of total worm burden in each scoter vs the log of the proportion of immature to total worms averaged across five samples (see methods above). We use this proportion to approximate the density-dependent recruitment rate relative to the maximum when adult worms are present at low density. Only *M. perspicillata* individuals were included in this measurement to minimize differences due to intestinal surface area that exists between species.

this is not necessarily true for residential hosts. Accurate measurements of the lifespan of *P. altmani* through experimental infections can clarify these assumptions. We also assume that cystacanths experience negligible mortality outside of host mortality, therefore, rate of loss of cystacanths is estimated by the death rate of *E. analoga*. Long-term experimental infections of *E. analoga* have not been conducted, and evaluation of cystacanth survival can verify whether mortality is dominated by crab death or not.

Density-dependent fecundity was observed for adult worms in bird hosts. During each bird dissection, prior to cutting the intestines longitudinally to examine the mucosa, a sample of digesta from the colon was removed and placed in a screw cap tube. 1g of digesta from each tube was thoroughly mixed with 20 mL 0.1M NaOH and strained through cheesecloth to remove debris. 5 samples each of 25 $\mu$ L were examined by light microscope under cover slips to count eggs. Sample counts were averaged and multiplied by 800 to get an egg per gram estimate. Weekly estimates assume scoters defecate 20 grams daily (reports from domestic ducks). Fig. 2.12 displays the log-log plot testing the significance of a density dependent relationship between worm burden and fecundity.

Whereas density-independence would produce a slope of 1 on a log-log plot, we find a slope of  $0.898 \pm 0.006$ . A Wald test gives a p-value less than 0.0001 when compared to the null slope of 1, indicating a significantly negative density-dependent relationship between per capita adult worm fecundity and worm burden. Plotting untransformed per capita fecundity against worm burden yields a slope of -1.07 and intercept at 9900, both

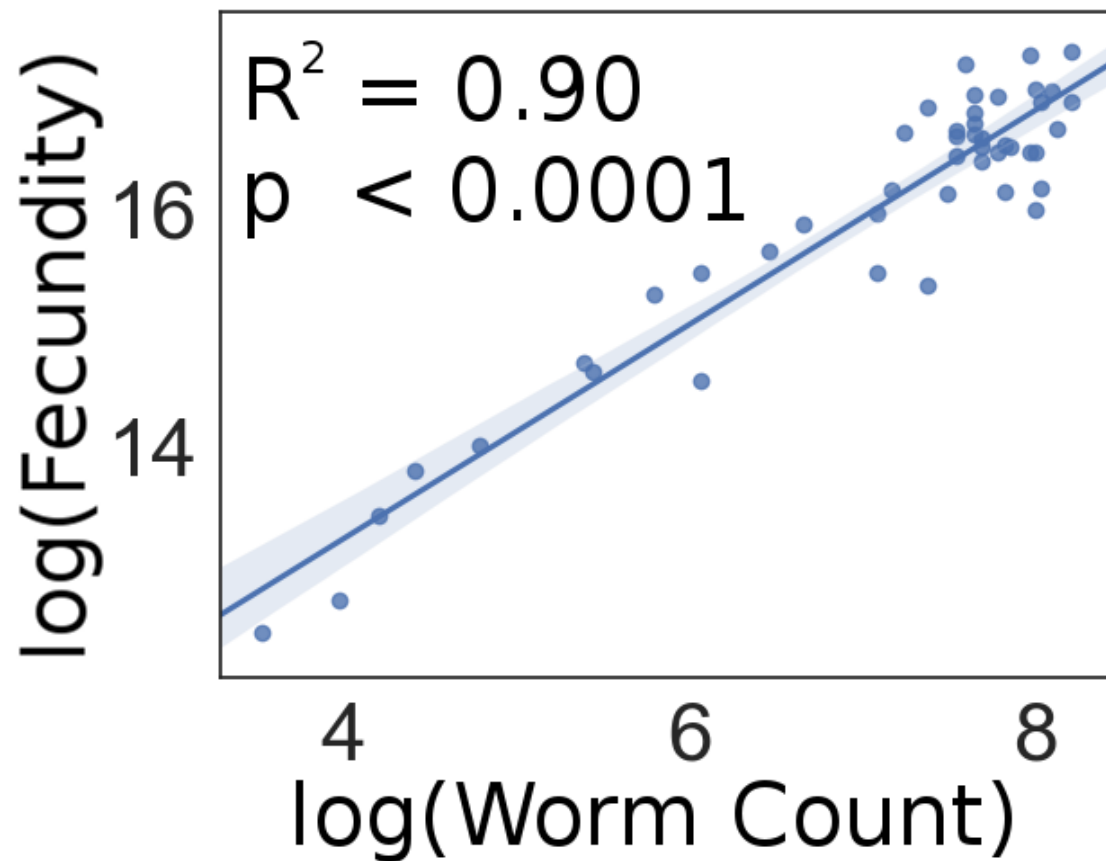


Figure 2.12: Regression of log total worm burden in each bird vs the log of the weekly estimated fecundity (total eggs). The log-log plot produces a strong fit, though plotting an untransformed plot of total worm burden vs estimated fecundity per worm produces an  $R^2$  value of 0.17, thus the log transformation condenses data points to produce the strong relationship.



parameter estimates used in the ODE model. Note that these estimates are not for fecundity per female worm. Of the 188 *P. altmani* specimens examined from birds (see above for details), we found a nearly even sex ratio of 0.92:1 male to female adult worms. This suggests the conclusions made regarding density dependence are unlikely to change should female worms have been separately counted in each bird, though the intercept would approximately double.

The prepatent period measured for immature *P. altmani* in domestic ducks is approximately 7 weeks. The maturation rate,  $\rho_1$ , of immature worms is the reciprocal of this period. The prepatent period measured for acanthellae maturing to infectious cystacanths is unknown; however, it is known to be strongly temperature dependent within and between acanthocephalan species. We produce a semilog plot of temperature vs the duration of prepatency across experimental estimates from acanthocephalans recorded in the literature. The average sea surface temperature in the sea otter range is approximately 14 degrees Celsius. Therefore, we use the value of the regression line at 14 degrees as an estimate of the prepatent period for *P. altmani*. The reciprocal of this estimate is the maturation rate,  $\rho_2$ , of acanthellae used in our model. Fig. 2.13 displays this regression of literature values and the intercepting value at 14 degrees.

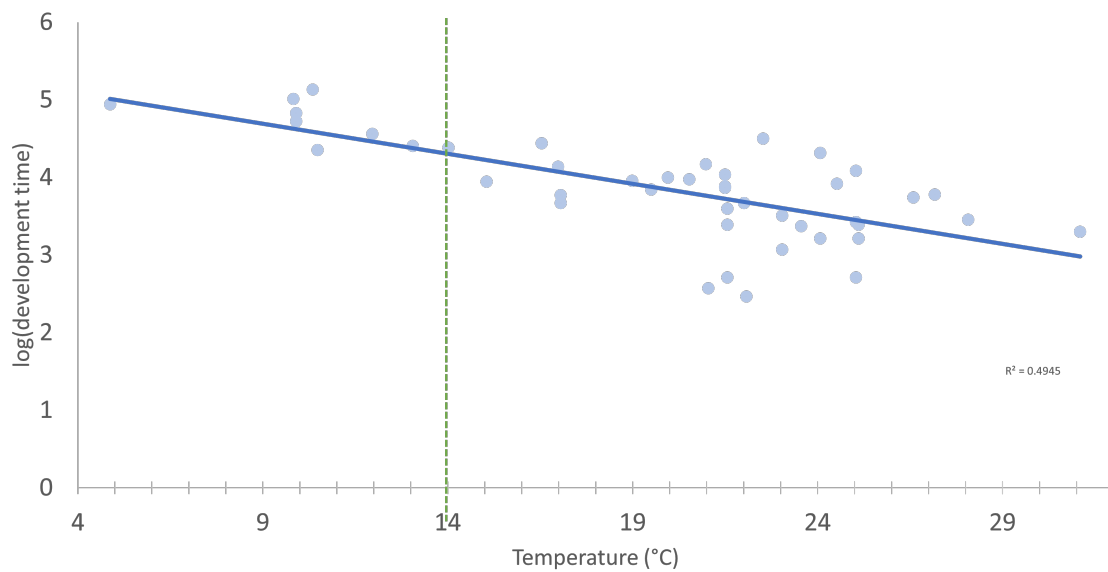


Figure 2.13: Regression of log-transformed development time (in days) of acanthocephalan cystacanths of many species by temperature (°C) reveals a significant temperature-dependent relationship for the rate of cystacanth development in intermediate crustacean hosts. The regression line value at 14 °C was used to approximate the development time of *P. altmani* cystacanths.

### 2.5.8 Epidemiological model optimization and statistics

Estimated parameters were substituted in the compartmental epidemiological model, and four variables were left to optimize in model fitting. These were the two transmission rates,  $\lambda_1$  and  $\lambda_2$ , and the estimates for background transmission,  $r_1$  and  $r_2$ , which describe the relative contribution of non-seasonal (constant) transmission to the observed patterns of parasite abundance measured in *E. analoga*. State variables were solved with a Python ODE solver ODEint while performing a global optimization routine with a genetic algorithm implemented in the Python package Differential Evolution. The model was optimized to biweekly mean per capita cystacanth burden observed between 2001-2021. Means were calculated across all samples within the sea otter range binned in two week increments (n=84212 core samples). Two week increments were used due to low sampling in winter months, particularly around holidays. Because each two week measurement represents a mean across twenty years of data collection, errors of each estimate approximate a normal distribution. Therefore, we optimized using a weighted least-squares objective function. Weights are inverse variance of the mean for each biweekly measurement.

$$f(x) = \min\left\{\sum_{i=1}^N W_i(\hat{y}_i - y_i)^2\right\} \quad (2.41)$$

$$W_i = \frac{n_i}{\sigma_i^2} \quad (2.42)$$

The standard errors of the estimates of each of the four optimized parameters was calculated from the Jacobian ( $J$ ) of the model result. The covariance matrix takes the form

$$\Sigma = \frac{(J^T J)^{-1}(RSS)}{22} \quad (2.43)$$

$RSS$  is the residual sum of squares, and 22 is the degrees of freedom in the model (26 data points minus 4 optimized parameters). Standard errors of the estimates of each mean from the least-squares sinusoidal regressions on *M. perspicillata* and *E. analoga* were obtained in similar fashion but using the inverted Hessian matrix obtained from the Minimize function in the Scipy.optimize package. Standard errors are the square roots of the diagonal of the covariance matrix,  $\Sigma$ .

$$\Sigma = \frac{H^{-1}(RSS)}{49} \quad (2.44)$$

The variance (of the mean) for background transmission estimates and the variance of the mean population estimates are used to calculate the joint standard error of seasonal and background transmission estimates.

$$SE_{joint} = \sqrt{var_{background} + var_{seasonal}} \quad (2.45)$$

One-tailed Welch's T-tests were performed to assess whether joint (seasonal + background) transmission is significantly higher than background transmission alone for both wintering birds represented by models fitted to *M. perspicillata* data and *E. analoga*.

### 2.5.9 Time series forecasting

Autoregressive integrated moving average (ARIMA) was used as a forecasting approach for the seasonal time series of southern sea otter AP prevalence. Data was aggregated by season: winter = weeks 1-13, spring = weeks 14-26, summer = weeks 27-39, fall = weeks 40-52. Estimates were available for all seasons for the 30 year time series except for the fall of 1993 because all deaths evaluated for AP during that season were pups with milk teeth, which excluded them from our data set. Some data points in early years consisted of few measurements, contributing to stochasticity. The time series was split into 80% training (years 1992-2015) and 20% testing (years 2016-2021). Using the function ARIMA in the Python package Statsmodels, we plotted the autocorrelation of the time series, first order differencing, and partial autocorrelation for the first order to determine the order of best fitting ARIMA model (Fig. 2.14).

ARIMA(1,1,1) and ARIMA(2,1,1) fit comparably well, with AIC values of -95 and

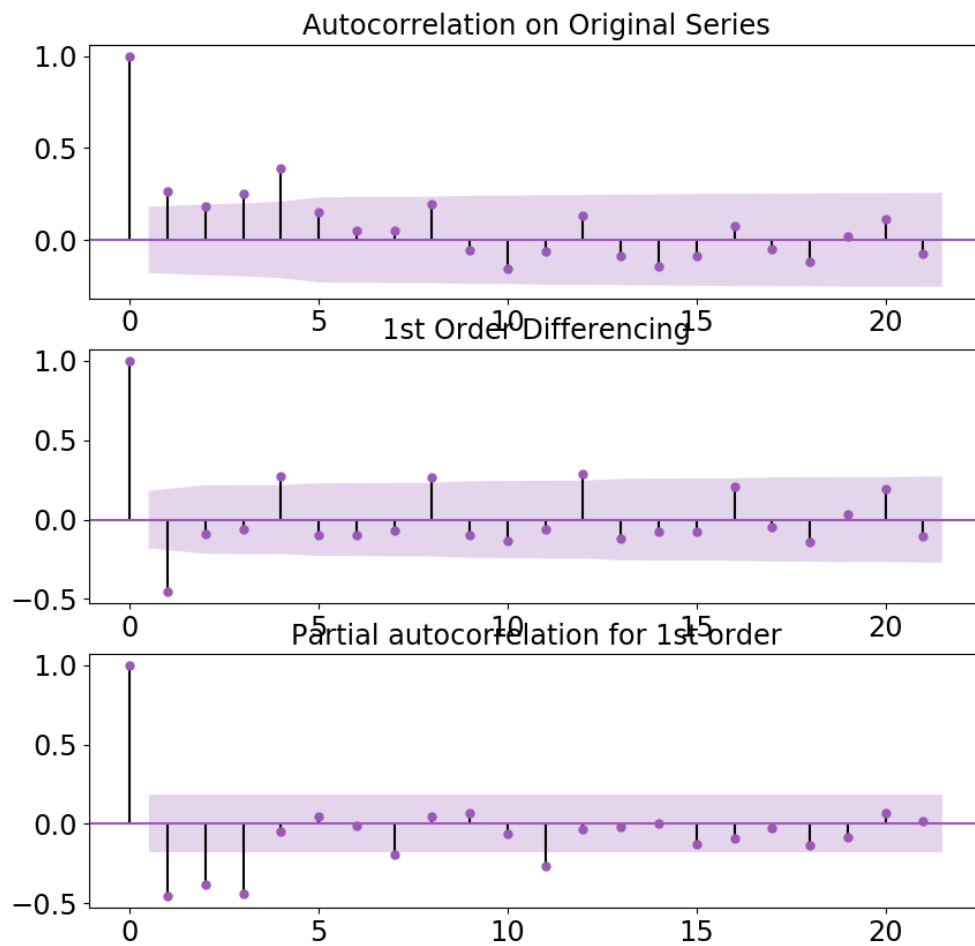


Figure 2.14: Diagnostic autocorrelation, 1st order differencing, and partial autocorrelation plots support the use of  $ARIMA(p,D,q) = ARIMA(1,1,1)$  model.

-96, respectively. The ARIMA(1,1,1) model outperformed the ARIMA(2,1,1) model during subsequent additions of exogenous variables and was used throughout the analysis for results and interpretation. Transmission potential calculated as the product of the intermediate host effective population size (*E. analoga* population function,  $E(t)$ , plus  $r_1$ ) and the mean cystacanth burden  $C(t)$  from the epidemiological model output was used as an exogenous regressor in the ARIMA forecast. We produced 13 model alternatives, which represented each of the 13 weeks sampled per season. Each model alternative consisted of four transmission potential values solved at a single week per season, spaced 13 weeks apart. For example, if we denote  $TP(t)$  as transmission potential solved at week  $t$ , one model choice would include the set  $\{TP(2), TP(15), TP(28), TP(41)\}$ . The four seasonal values were repeated for each year in the 30 year time series. The 13 model alternatives were evaluated via out of sample correlation of the ARIMA prediction and the test data set to understand whether choice of sampling week had an impact on the ARIMA performance. After finding the sixth week performed best, we incorporated two additional exogenous variables representing annual population abundances of *E. analoga* and *M. perspicillata*. Because the LiMPETS data set of *E. analoga* abundance was collected unevenly throughout each year, with a bias favoring summer and fall months, we standardized the yearly mean abundance estimate across all samples by calculating the residuals of each core sample from the fitted model  $E(t)$  estimate. The mean of residuals from each year was then

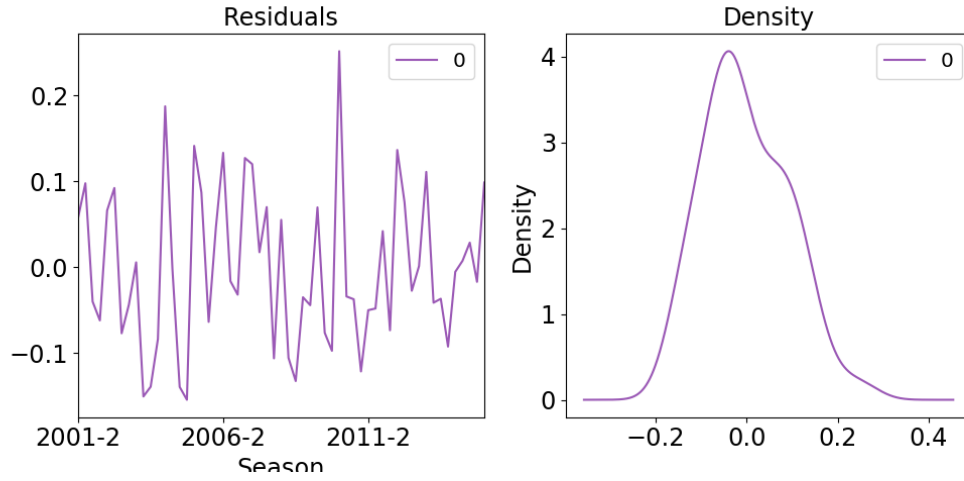


Figure 2.15: Residuals between in-sample (within the training set) data and the ARIMA predictions with significant exogenous regressors are approximately normally distributed and stationary around zero, indicating no systematic bias in the ARIMA fit.

calculated and multiplied by  $E(t)$  at  $t = \{6, 19, 32, 45\}$  to yield seasonal abundance estimates for each year. In this way, interannual variability in *E. analoga* abundance could be incorporated in the ARIMA forecast. Four seasonal values were calculated in the same manner for long-term midwinter survey data (Richmond et al., 2014) on *M. perspicillata* by multiplying each yearly abundance estimate by  $M(t)$  at  $t = \{6, 19, 32, 45\}$ .

### 2.5.10 Long-term trends

In our ARIMA approach, we discovered significant first-order autocorrelation affecting the time series of AP prevalence in southern sea otters. To deal with the temporal dependency of the AP prevalence and to explicitly model long-term trends, which minimized the effects of short-term variation on time series interpretation, we applied a classical first-order



random walk trend to our yearly AP time series for each sea otter group. The random walk trend ( $\mu_t$ ) was modeled as the state in the previous year plus a stochastic, normally distributed random variable ( $\epsilon$ ), or  $\mu_t = \mu_{t-1} + \epsilon_t$  in which  $\epsilon_t \propto N(0, \sigma^2)$ . All analyses assessing temporal trends were conducted in a Bayesian framework using INLA in the R-INLA package for R. Weakly informative penalized complexity priors for the parameters and hyperparameters were used for all analyses in INLA. Long-term trends for AP in each sea otter group were assessed as significant when the 95% credible intervals of the mean trend line did not overlap with the overall mean of the time series and were further supported by non-parametric Mann-Kendall statistical tests for monotonic trends. Statistical significance was evaluated at a 0.05  $\alpha$  level.

### 2.5.11 Statistical analyses

Sea otter-specific attributes representing linear distance along the California coastline (ATOS = As The Otter Swims), day of the year, age, and sex were evaluated in a generalized additive model (GAM) framework using the R package mgcv. Because AP is a binary value for each otter, taking values 0 or 1, we modeled each attribute with a logistic GAM using a binomial distribution and logit link function. Each variable was treated as a smoother to allow varied degrees of freedom in the model fit. Sex was treated as a categorical variable with two levels, male and female, and served as a factor in interaction terms within the model structure. The full model was specified as

$$y_i \sim B(n, p_i), \text{ where} \quad (2.46)$$

$$\text{logit}(p_i) = \beta_0 + f(ATOS_i) + f(day_i) + f(age_i) \quad (2.47)$$

$$+ f(ATOS_i, sex_i) + f(day_i, sex_i) + \epsilon_i, \text{ where}$$

$$\epsilon_i \sim N(0, \sigma^2) \quad (2.48)$$

The limited degrees of freedom in the age variable prevented an interaction term between age and sex. We compared this model with all subsets of the model comparing AIC and percent deviance explained. The full model as well as the models excluding interaction terms performed comparably well using these metrics. We additionally performed Fisher's exact tests (when two categories were compared) or Chi-squared tests (when more than two categories were compared) on AP prevalence between sexes, for otters with and without identified *E. analoga* remains in their GI, and for subcutaneous fat content.

## 2.6 Acknowledgments

We thank members of the De Leo lab for continued support in this work. We also thank the LiMPETS program and those involved in sandy beach monitoring since its inception, with special dedication to the late John Pearse who helped initiate the program. We also thank the sea otter stranding network, including the Monterey Bay Aquarium, for recovery of sea

otter carcasses. We thank contributors to the sea otter necropsy program through the years, including Jack Ames, Mike Harris, Angie Reed, Francesca Batac, Karl Mayer, and Brian Hatfield. REG is funded by the Stanford Graduate Fellowship and the ARCS Fellowship. Laboratory work was funded by the Sea Otter Fund Trust and the Myers Trust.

## **2.7 Contributions**

Study design: all authors, data collection and curation: RG, KG, CY, CG, LH; analysis: RG; writing: RG with contributions from KG, CY, CG, MM; editing: all authors

## 2.8 Supplementary Information

Population abundance data was collected for *Melanitta perspicillata* and *Emerita analoga* to examine changes in population sizes through the time period of our study. Fig. 2.16 plots both time series for available data. Data for *M. perspicillata* was collected via annual aerial and ground midwinter waterfowl surveys by the US Fish and Wildlife Service and the CA Department of Fish and Wildlife. Scoter species are not readily differentiated with these surveys, though over 98% of individuals in California are known to be *M. perspicillata*. Count data was analyzed for the Lower Pacific Flyway from 1992-2015. Data from 2016-2021 was interpolated from deviations from the mean annual counts per unit effort of *M. perspicillata* in the eBird dataset previously described. No standard errors are available for these measurements. Because sampling in the LiMPETS data for *E. analoga* abundance is irregular across the coastline and occurs at different months each year, we standardized each measurement by calculating its residual from the sinusoidal population model fit to the entire dataset. The annual mean of these residuals and standard error are reported. Kendall's tau nonparametric test was used to determine whether time-series trends in count data were statistically significant at an alpha level of 0.05. Declines were evident for *M. perspicillata* ( $\tau = -0.578, p < 0.0001$ ) and were not detected for *E. analoga* ( $\tau = 0.067, p = 0.678$ ). The lack of support for the importance of *M. perspicillata* in variations in transmission of *P. altmani* in the main text is further bolstered by the notable decline in abundance of this host in the Lower Pacific Flyway yet no observed decline in

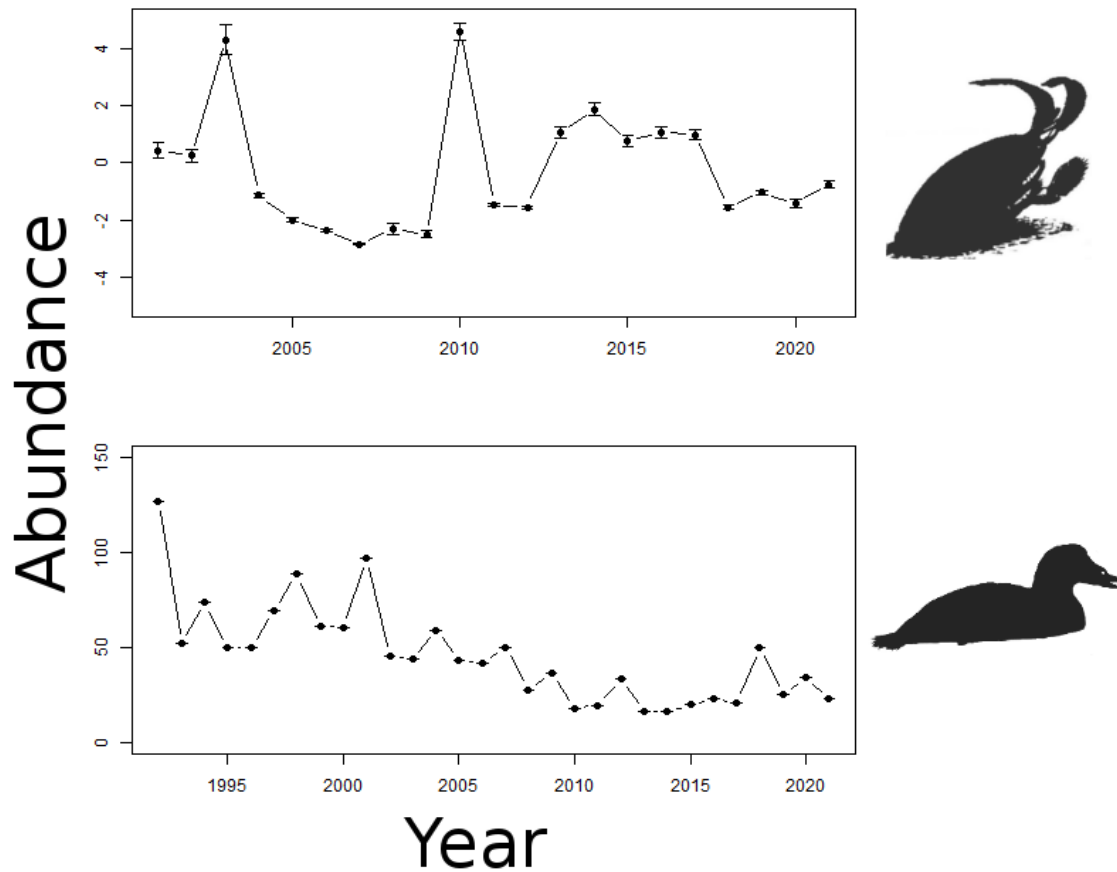


Figure 2.16: Long-term population data for *E. analoga* and *M. perspicillata*. Top: intermediate host populations remained stable through the sampled 21 year period. Spikes in abundance occurred in 2003 and 2010, both years associated with moderate ENSO conditions. Abundance was calculated as residuals from the modeled mean. Bottom: definitive host surf scoters declined dramatically through the sampled 30 year period. Abundance reflects raw counts from the Lower Pacific Flyway each year.

southern sea otter acanthocephalan peritonitis prevalence. Population trends for other diving ducks, shore birds, and gulls that are known or potential hosts of *P. altmani* are varied. Significant declines in sea otter acanthocephalan peritonitis in Monterey Bay could result from local population declines in definitive host abundance and is an important topic for future research.

Previous studies have identified environmental effects on local population abundance of *E. analoga* (Sorte et al., 2001). Although no significant trend emerges between 2001-2021 for the population size of this species, there is marked annual variation that may be due to environmental influences. Peaks in abundance are clear for two El Niño years: 2003 and 2010. We tested the correlation between the Oceanic Niño Index (ONI) reported as the monthly 3-month rolling average between 2000-2021 and the annual residual abundance of *E. analoga*. We found a moderately strong relationship between crab abundance and ONI that peaked in March of the year in which the adult crabs were counted (Fig. 2.17).

A strong pattern emerged across the months preceding summer breeding which corresponded to high relevance of ONI to periods of planktonic development. Correlation grew from the previous year's breeding period to the period of winter and spring recruitment of megalopae to California beaches. These results demonstrate the importance of sea surface temperature in zoeal survival, which directly impacts annual adult recruitment. The lack of correlation proceeding recruitment indicates a reduced importance of ENSO (El Niño Southern Oscillation) in adult crab survival.

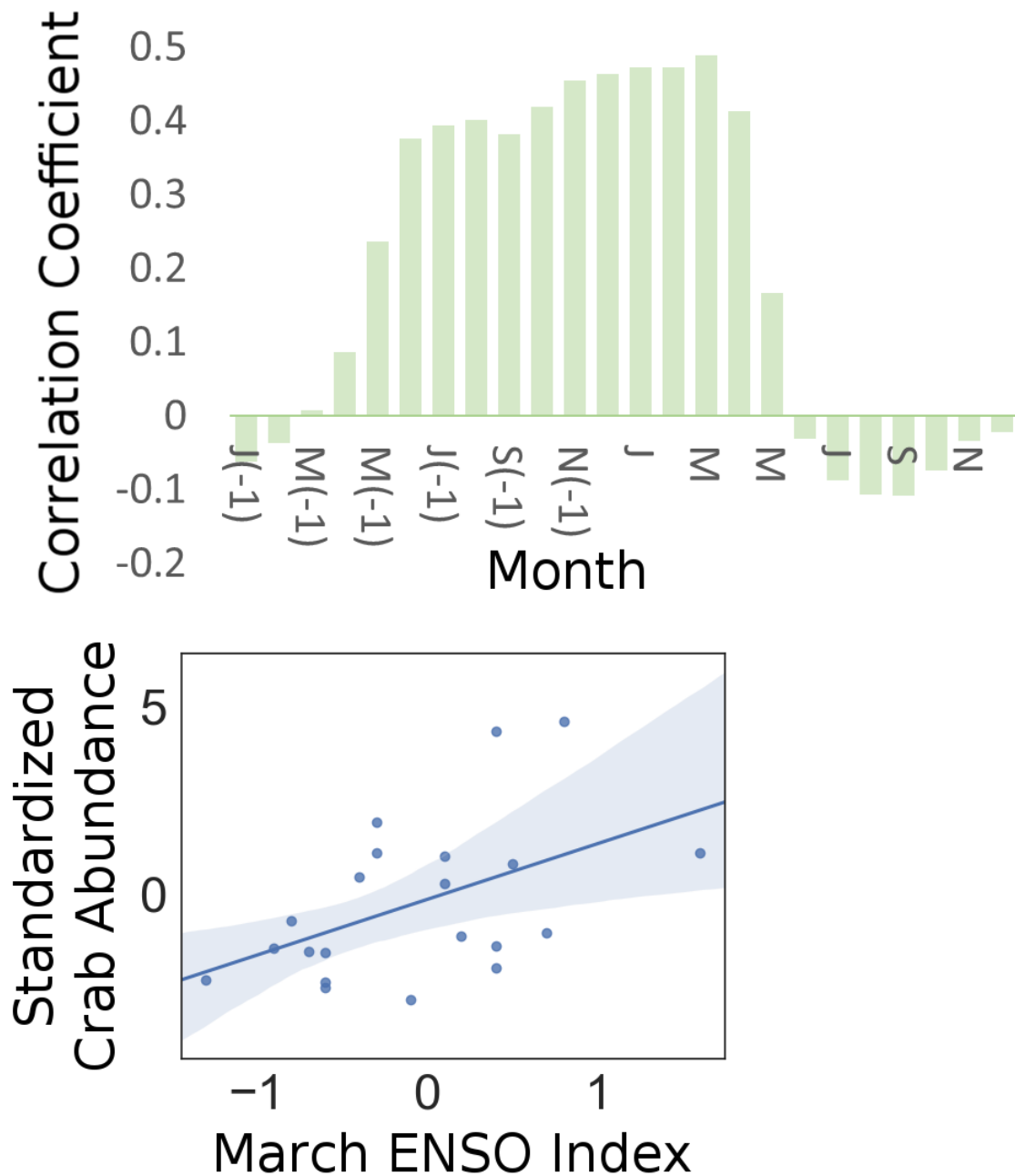


Figure 2.17: Mean *E. analoga* abundance each year is correlated with ENSO conditions preceding the breeding season. Warmer ocean temperatures evidently enhance zoeal survival and increase recruitment. March is the most sensitive month for survival, and aligns with late-stage zoeal arrival to California beaches.

Although the population size of *E. analoga* varies seasonally, peaking during breeding season in early summer, many of these individuals are newly molted recruits which are small in size and are likely to carry less cystacanth burden due to a shorter feeding period compared to older adults. We show in the main text that a seasonal decline in cystacanth burden occurs in spring when recruitment is presumably high (Fig. 2.2). To show that recruitment of young crabs is indeed occurring in these spring months, we estimate the relative mass of each crab as proportional to the cube of its body length; three is the approximate allometric scaling constant found in similar crab species. Using this approximation for all measured crabs ( $n=84212$ ) we plot the weekly total crab biomass averaged across years. By dividing this metric by the total crab count in each measured mean, we derive the mean per capita biomass. This plot is also shown. A LOESS regression was performed for each set of measurements for visualization (Fig. 2.18).

We find that total biomass peaks gently in the summer but remains remarkably constant throughout the year. Additionally, there is a dramatic decline in per capita biomass in the spring, indicative of recruitment of young adults preceding the breeding season. This accounts for the moderate reduction in mean cystacanth burden observed. Previous work has supported the observation that cystacanths do not impose large fitness costs on *E. analoga* hosts. Therefore, each host can accrue multiple cystacanths as it ages. Because *E. analoga* grow as they mature, particularly females, which can reach much greater sizes than males, larger crabs may harbor more parasites. We perform a linear regression comparing body



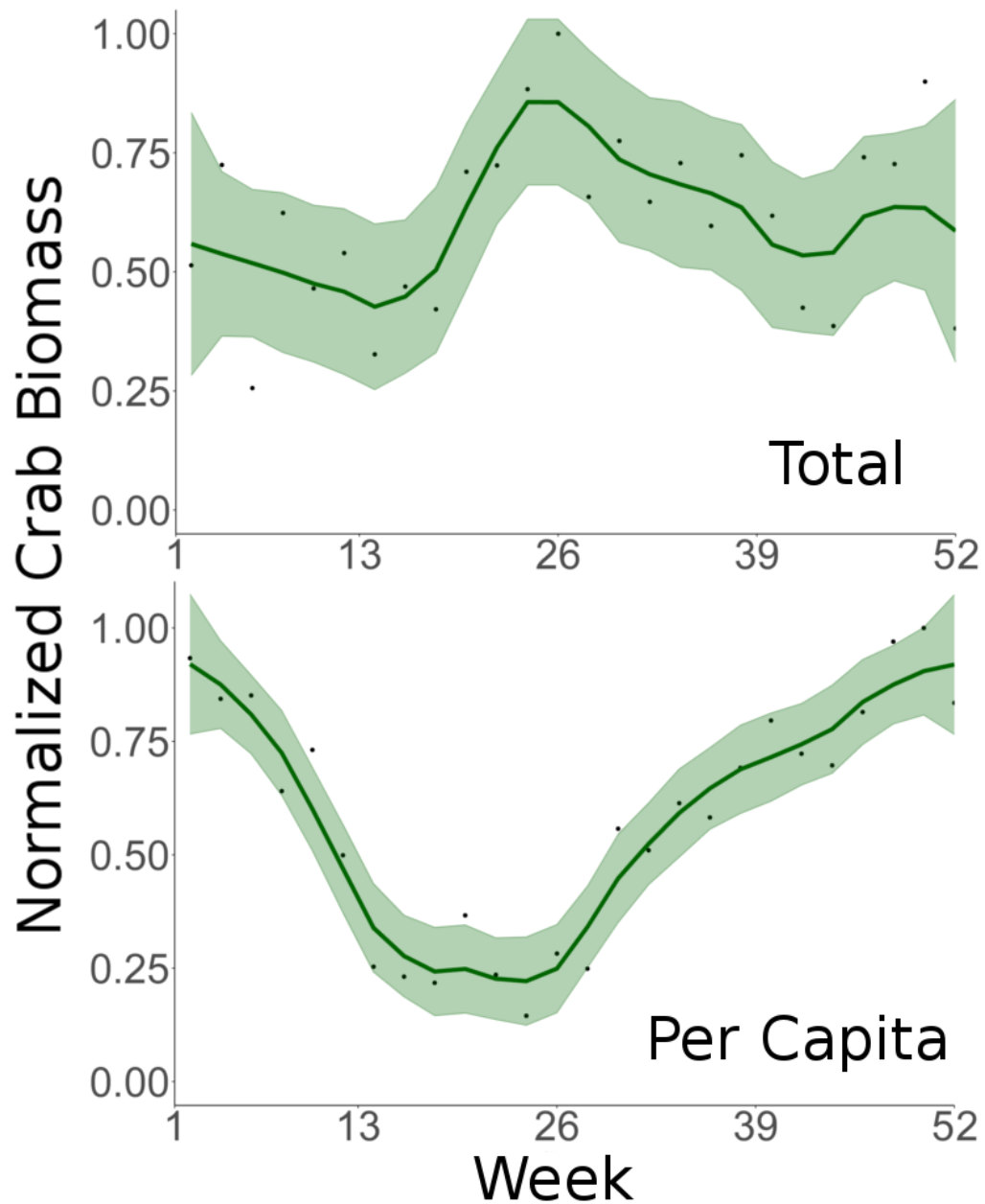


Figure 2.18: *E. analoga* biomass peaks in summer breeding season immediately following spring recruitment. Low per capita biomass in spring reflects this recruitment of small crabs to beaches. Data is normalized by the maximum weekly values in each plot. LOESS regressions reveal smoothed fits to the data for visualization.

length and parasite abundance found upon haemocoel dissection (Fig. 2.19).

The regression reveals a moderately strong relationship between body length of each crab and cystacanth burden (correlation = 0.40,  $p < 0.0014$ ,  $n=5957$ ) and the same relationship is supported with log transformation of the cystacanth burden (correlation = 0.48,  $p < 0.001$ ,  $n=5957$ ). However, this obscures variability between cystacanth burden and sex. Females ( $19.77 \pm 0.11mm$ ,  $n = 2344$ ) are far larger than males ( $14.26 \pm 0.08mm$ ,  $n = 2067$ ), and gravid females ( $23.29 \pm 0.11mm$ ,  $n = 1516$ ) are larger still. We performed a single factor ANOVA between cystacanth counts among these three groups and found a highly significant difference between the three groups ( $F=83.18$ ,  $p < 0.0001$ ). We performed post-hoc tests between the three pairs to find highly significant differences between each pair: female-gravid female (Tukey HSD:  $Q = 6.62$ ,  $p = 0.001$ ; Scheffe/Bonferroni/Holm:  $T=4.68$ ,  $p < 0.0001$ ), female-male (Tukey HSD:  $Q = 16.43$ ,  $p = 0.001$ ; Scheffe/Bonferroni/Holm:  $T=11.62$ ,  $p < 0.0001$ ), gravid female-male (Tukey HSD:  $Q = 21.11$ ,  $p = 0.001$ ; Scheffe/Bonferroni/Holm:  $T=14.92$ ,  $p < 0.0001$ ). Although not consistently noted in the long-term sea otter necropsy dataset, we have observed associations between consumption of gravid female *E. analoga* and presence of high densities of *P. altmani* between 1997-2022. Due to their larger size and egg mass, gravid females are likely preferred by foraging sea otters. The orange egg mass may also make gravid females more identifiable in the surf. Each of these factors could increase the likelihood sea otters foraging on *E. analoga* would consume high quantities of *P. altmani*, leading to acanthocephalan

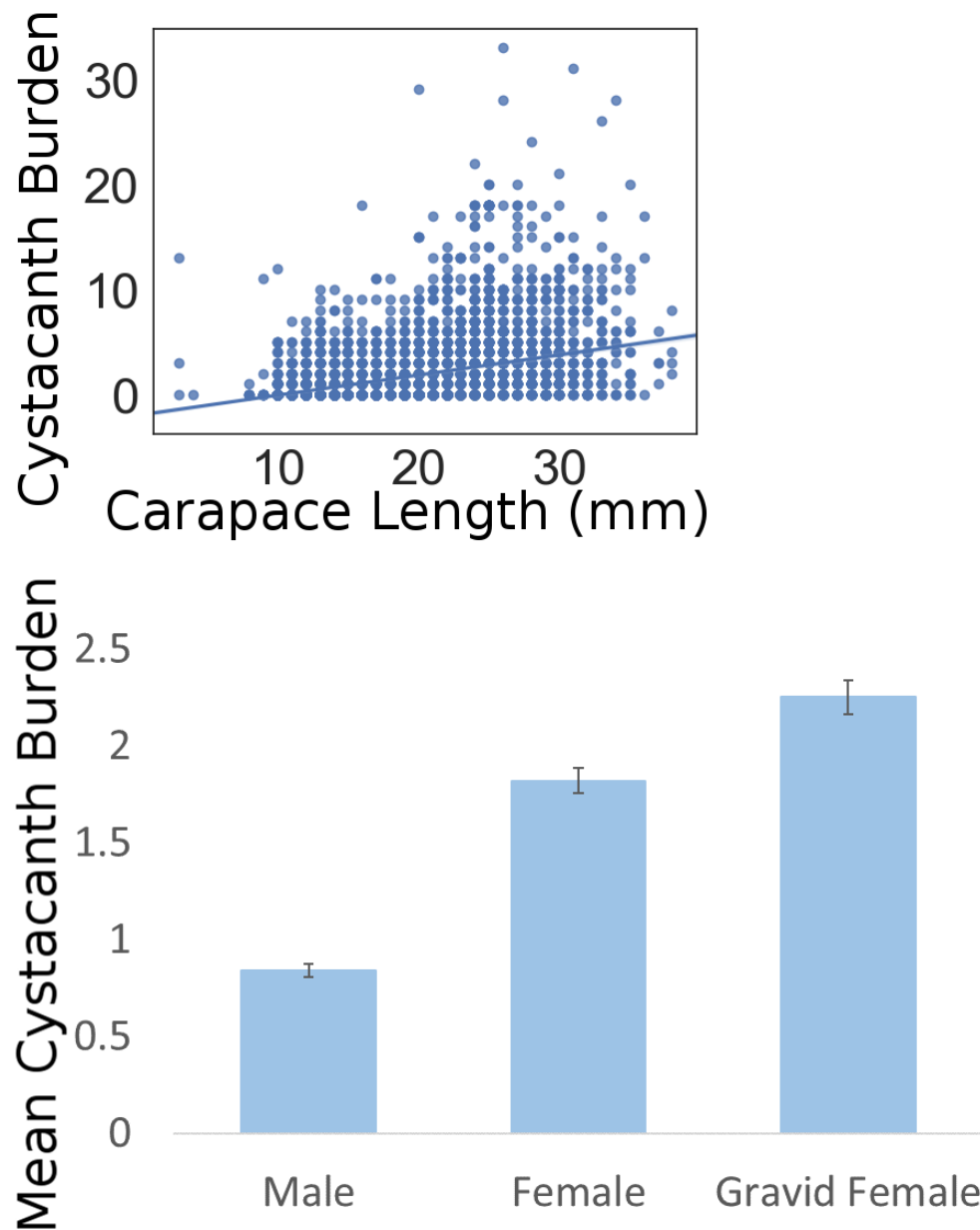


Figure 2.19: There is a positive relationship between the size of *E. analoga* and the cystacanth burden per crab. Regression on untransformed and log-transformed cystacanth burden data support this relationship. Greater burdens of cystacanths are found in gravid females than in non-gravid females, which both have greater burdens than males. The order of this ranking matches the size distribution of the crabs: gravid females are more massive than females, which are both more massive than males.

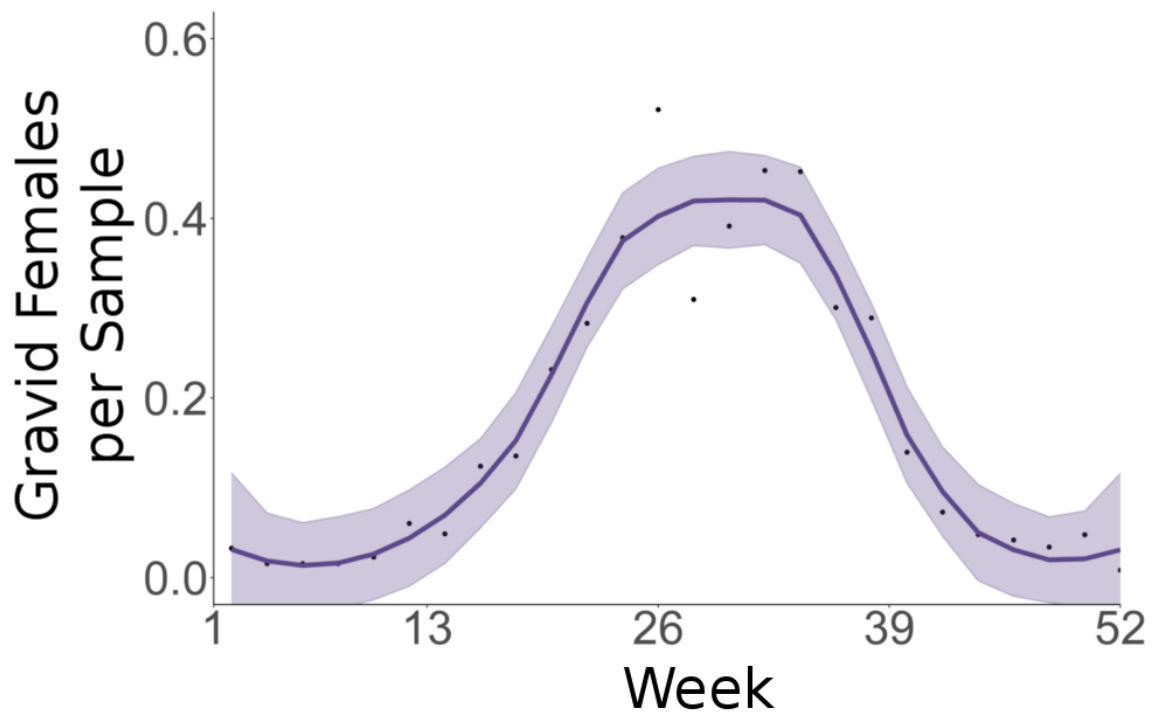


Figure 2.20: The abundance of ovigerous (gravid) female crabs peaks immediately following early summer reproduction. The early portion of this peak aligns with peak AP in sea otters. Observations of gravid females in otter carcasses with AP will need further corroboration via seasonal diet preferences of otters to make conclusions about the relationship between the availability of this category of *E. analoga* and AP prevalence.

peritonitis during seasonal breeding months (Fig. 2.20).

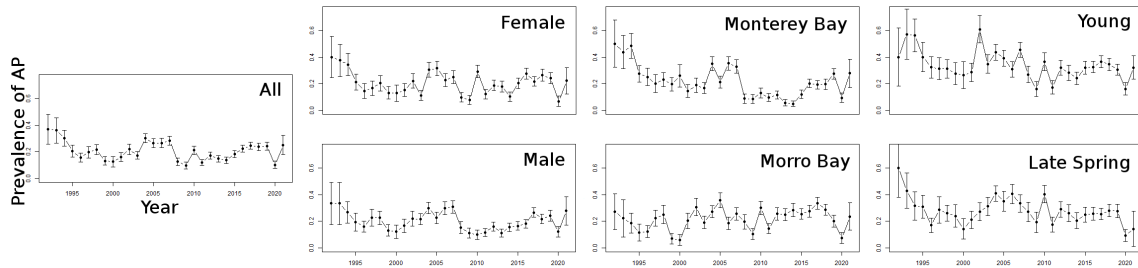


Figure 2.21: Longitudinal prevalence of acanthocephalan peritonitis for several important southern sea otter groups. Data reflect means and standard errors of the mean prevalence across years 1992-2021. These data are incorporated into the INLA models reported in Figure 2.6. Please refer to Figure 2.6 for more information of each panel.

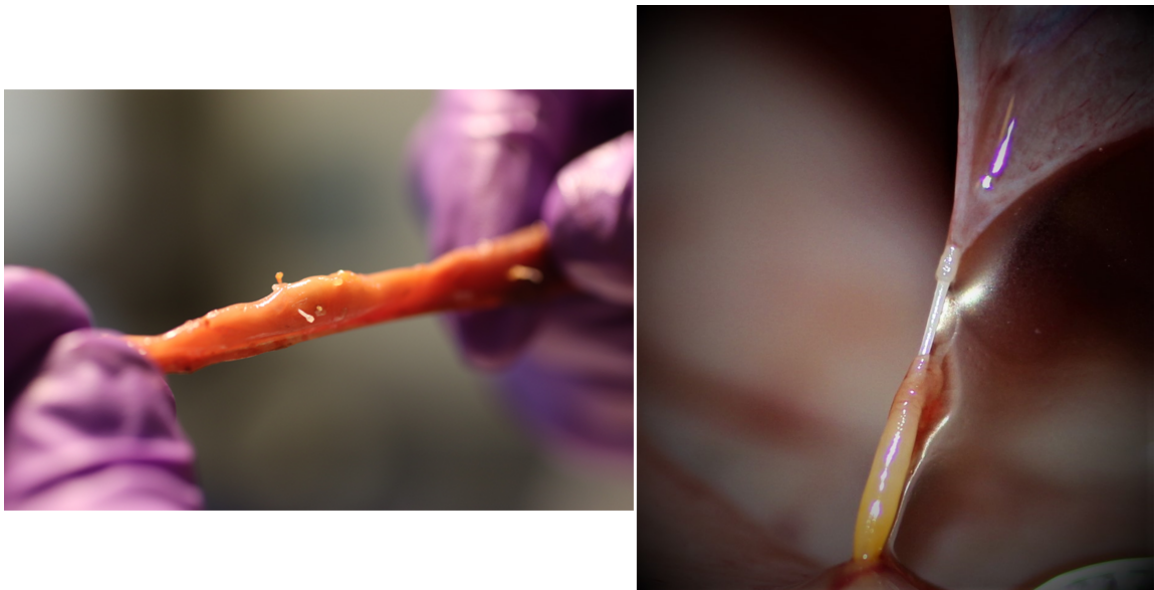


Figure 2.22: *P. altmani* induces peritonitis by perforating otter intestines, often causing bacterial infection. The omentum adheres to the parasite emerging from the serosa and recruits macrophages and neutrophils to phagocytize the parasite. However, many simultaneous perforations can increase the severity of peritonitis, leading to death. Photos depicting probosces of *P. altmani* perforating avian intestines (left) and sea otter intestines (right). The proboscis on the right is attached to the omentum of the otter.

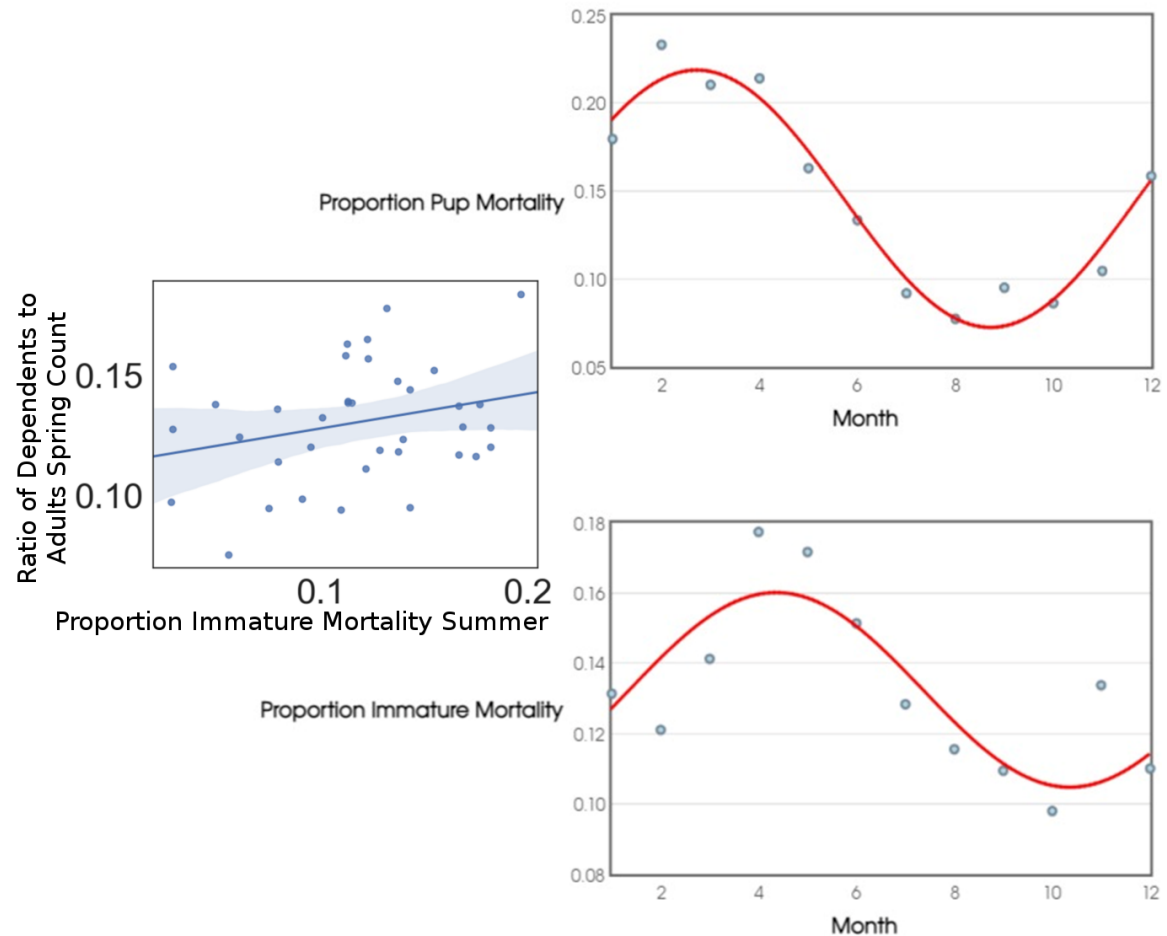


Figure 2.23: The proportion of pup mortality relative to all southern sea otter mortality in the entire mortality dataset (1968-2021) exhibited a distinct seasonal trend, which peaked in February-March. The proportion of immature mortality relative to all mortality also exhibited a seasonal trend, peaking approximately two months after the peak in pup mortality. A regression between the proportion of dependents observed in the Monterey Bay spring survey by USGS to the proportion of immature mortality in the following summer, which would correspond closest to dependent pups observed in spring, showed a mild correlation of 0.27. This evidence suggests a potential increase in pupping in winter months that may lead to high immature abundance in the spring and higher rates of *E. analoga* consumption across the sea otter population, contributing to the observed late spring peak in AP observed. However, it is challenging to distinguish these results from known increases in pup separation in winter months and spring increases in disease, like AP, among young otters, both leading to increased mortality in respective age classes.

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## **Chapter 3**

# **Larger Viral Genome Size Facilitates Emergence of Zoonotic Diseases**

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### 3.1 Abstract

Emergence of new viral diseases is linked to mutation or recombination events. The likelihood of cross-species transmission is related to phenotypic plasticity of a virus and its capacity to produce genetically variable progeny. Herein a model is described connecting the production of genetic variability with increasing genome size. Comparing all known zoonotic viral genome sizes to known non-zoonotic viral genome sizes in humans demonstrates that zoonotic viruses have significantly larger genomes. These results support the notion that large viral genomes are important in producing new zoonotic disease, and suggest that genome size may be a useful surrogate in screening for potential zoonotic viruses.

**Keywords:** *Coronavirus, Mutation, Recombination, Phenotypic Plasticity*



## 3.2 Introduction

Human population expansion continually redefines the interactions between humans and wildlife. Zoonotic diseases that emerge from these interactions account for over 60% of human infectious diseases (Taylor et al., 2001). Although this figure may represent over 2 billion new cases annually, zoonoses are characteristically underreported and misdiagnosed (Gebreyes et al., 2014). Because zoonoses affect humans and other vertebrates, they also generate substantial economic losses, as livestock death and human disability lead to loss of productivity (Hampson et al., 2015). For example, economic losses due to Ebola in 2014 were \$2.2 billion (World Bank, 2014). Zoonotic outbreaks also present an increasingly pressing risk to human health due to higher densities of people with little to no immunity to novel pathogens. They are often unpredictable in occurrence and are caused by new species against which there are no developed vaccines, treatment, or public health protocol. This is particularly true for viruses that have great capacity to mutate, even during the course of a pandemic (Jones et al., 2008; Taylor et al., 2001). This century has seen numerous epidemics and pandemics caused by zoonotic viruses like Ebola, Nipah, Avian Influenza, and Coronavirus (Jones et al., 2008).

In late 2019, a novel Coronavirus (SARS-CoV-2) emerged and has since killed over 75000 people, making it the deadliest Coronavirus pandemic in recent history (Li et al., 2020a; Worldometer, 2020a). The Wuhan province in China, where the virus emerged,

effectively quarantined its populace, and many countries restricted travel of their own citizens to China. Outbreaks in other countries have spurred similar action. Although human to human transmission is driving current transmission, its emergence was facilitated by close human-animal contact, much like its predecessors SARS and MERS (de Wit et al., 2016). Members of the Coronaviridae family are widely known to jump across host taxa to become infectious in new populations (Chu et al., 2011; Rest & Mindell, 2003). Non-pathogenic varieties in a reservoir population can become pathogenic in new species. This phenomenon is well documented in many viral zoonoses, yet Coronaviruses pose exceptional risk to human populations due to the variety of potential reservoirs and the virulence of the emergent pandemics they cause (Rest & Mindell, 2003). The ability of Coronaviruses to shift to new species is due in part to the mutation rate they exhibit, but they are not exceptional among viruses in this way. They are exceptional, however, in their high frequency of recombination and in possessing the largest of the ssRNA viral genomes (Makino et al., 1986; NCBI, 2020). These two characteristics provide ample opportunity for Coronaviruses to evolve new traits and establish themselves in new host species.

The capacity to produce genetic variability is a defining feature among viruses that are able to jump to new host species. Mutations and recombination events have been explored as a source of this variability. Both processes have been implicated in producing new viral strains or species responsible for observed pandemics (Parrish et al., 2008). The rates of mutation and recombination are known to vary across viral taxa and within viral

genomes (Drake & Holland, 1999). For example, RNA viruses characteristically mutate faster than DNA viruses due to polymerase infidelity, and hypermutable regions within some viral genomes have been demonstrated. Likewise, recombination processes vary considerably by viral type: reassortment is common in viruses with multi-segmented genomes, while homologous or non-homologous recombination is predominant in viruses with non-segmented genomes (Drake & Holland, 1999; Pérez-Losada et al., 2015).

A primary challenge for virologists and public health professionals is identifying characteristics of viruses that lead to higher likelihoods of new outbreaks (Taylor et al., 2001). The variety of mechanisms by which genetic variation can be produced makes finding clear patterns in zoonotic risk difficult (Gebreyes et al., 2014; Parrish et al., 2008). One yet unrecognized viral characteristic likely to facilitate cross-species infections is the size of the viral genome. Both the total number of mutations accumulated during replication and the probability of a recombination event increase with the size of a genome when replication mechanisms are conserved, though nucleotide substitutions from either process often lead to net reduction in fitness of progeny. The number of substitutions, thus potential for reduction in host-specific fitness, increases with genome size (Bull et al., 2007; Drummond et al., 2005; Jensen-Seaman et al., 2004). If genetic variability is instrumental in producing cross-species infections, genome size should be positively related to the likelihood of viral spillover events provided lethal substitutions do not substantially reduce the mean fitness of the viral population.

### 3.3 Results

Cross-species transmission for each viral particle is assumed to be a function of the phenotypic plasticity of the viral genome and the genetic variability produced during replication (C. K. Johnson et al., 2015; Parrish et al., 2008; Pérez-Losada et al., 2015). The probability of a transmission event occurring can be represented as:

$$Pr(Transmission) \propto \lambda(Plasticity(L)) + Variability(L) \quad (3.1)$$

where  $\lambda \in [0, \infty)$  is the weighted contribution of plasticity over variability in promoting cross-species transmission and  $L$  is the length of the viral genome. Although a relationship between genome size and phenotypic plasticity is probable, little work has enlightened the nature of this relationship. What is clear is that transcription and gene regulation networks can become more complex with increasing number of nucleotides (S. Ecker et al., 2018; Lauber et al., 2013). Variability produced with each replication event can be more accurately approximated. Variability is produced via mutation and recombination (reassortment, non-homologous and homologous). Per nucleotide rates of mutation ( $\mu$ ) and recombination ( $r$ ) can be used to derive nucleotide substitution rates by adjusting the recombination rate by the sequence divergence between co-infecting viruses ( $D \in [0, 1]$ ). The substitution rate via recombination is  $R = Dr$ . The accumulation of variability per

genome should increase linearly as  $(\mu + R(1 - \mu))L$ . When  $\mu$  and  $R$  are small, this variability can be expressed as  $(\mu + R)L$ . Lethal mutagenesis and incompatible recombinants reduce viability of viral products. Viability converges to  $e^{-\mu\gamma L}$  across mutational models (Bull et al., 2007) and is calculated as  $\rho^{\frac{9rL^2(D-r)}{4(DL-4)}}$  for recombination (Drummond et al., 2005), where  $\gamma$  is the proportion of lethal mutations and  $\rho$  is the genome-specific tolerance to recombination events. Per nucleotide variability ( $dV/dN$ ) produced with each replication event can be given as:

$$\frac{dV}{dN} = (\mu + R)(e^{-\mu\gamma L} + \rho^{\frac{9rL^2(D-r)}{4(DL-4)}} - 1) \quad (3.2)$$

Per genome variability ( $dV/dG$ ) can be given as:

$$\frac{dV}{dG} = L(\mu + R)(e^{-\mu\gamma L} + \rho^{\frac{9rL^2(D-r)}{4(DL-4)}} - 1) \quad (3.3)$$

Assuming the number of polymerase enzymes scales with genome size (Patton & Spencer, 2000; Rivera-Mulia & Gilbert, 2016), a rate of production of variability can be derived.

$$\frac{dV}{dt} = (kL/(I + L))(\mu + R)(e^{-\mu\gamma L} + \rho^{\frac{9rL^2(D-r)}{4(DL-4)}} - 1) \quad (3.4)$$

The efficiency of replication of each genome can be represented as  $L/(I + L)$ , where  $I$  is the product of the time required for replication initiation and termination, and  $k$ , the rate of polymerase-mediated replication. Figure 3.1 displays equations 3.2-3.4 parameterized to

represent positive sense ssRNA viruses.

Per nucleotide variability production decreases with genome size due to reduced fitness with accumulated substitutions (Fig. 3.1A). However, genome-level variability production increases almost linearly with genome size, revealing that the rate of fitness reduction from the accumulation of deleterious substitutions does not outpace the increased variability associated with increase in genome size (Fig. 3.1B). Because more genomes can be replicated in a fixed amount of time for smaller genomes, the total variability produced in unit time gives indication of whether population-level variability increases with genome size. In fact, Fig. 3.1C shows that population-level variability is maximized within the known size range of ssRNA(+) viral genomes. Variability production per unit time continues to decline for genome sizes larger than 50,000 nt, though no known ssRNA viruses have genomes larger than 35,000 nt (NCBI, 2020). The maximum variability production per unit time is dependent on the efficiency of replication. Because Coronaviruses have much larger genomes than other ssRNA(+) viruses, they have evolved 3'  $\rightarrow$  5' exoribonuclease repair and have lower mutation rates than other ssRNA(+) viruses (Minskaia et al., 2006). Substituting this mutation rate in equation 3.4 produces a curve (Fig. 3.4) with a maximum shifted to the right, reflecting that production of genetic variability is maximized at a larger genome size for Coronaviruses than for other ssRNA(+) viruses. Considering these theoretical results, variability is greater per virion produced for viruses with larger genomes.

Because population-level production of variability has been shown to improve viral survival via immune evasion (Drake & Holland, 1999), there is likely strong selection toward viral genome sizes close to the peak described by equation 3.4 (Fig. 3.1C). Even in the absence of phenotypic plasticity conferred by large genomes, viruses with large genomes have high potential to generate variability that promotes cross-species transmission events. Zoonotic viruses should, therefore, possess larger genomes than other viruses. To examine the relationship between viral genome size and likelihood of zoonosis, this study compiles a comprehensive list of all known viral zoonoses and leverages the entire NCBI database of viral genomes (NCBI, 2020).

Peer-reviewed literature from 1920 to 2019 was searched via the Web of Science electronic library for viral zoonoses. Search key words included “zoonotic”, “zoonoses”, “emerging disease”, “viral spillover”, “animal to human transmission”, and “animal reservoir virus”. After cross examination of all viruses yielded through this search, all confirmed zoonotic viruses transmitted from animals to humans in the past 100 years that also had a complete genome sequence were included in the analysis. This set encompasses 201 viral species from over 40 genera in groups 1, 3, 4, 5, and 6 of the Baltimore classification system, grouped by genome structure. Genome sizes for all viruses were retrieved from the NCBI genome database or from peer-reviewed reports of fully sequenced genomes. The entire NCBI viral genome database of 9238 sequenced genomes was downloaded and

filtered to remove incomplete, prokaryote, fungus, protist, and plant associated viral sequences. 2088 animal associated virus species in the five evaluated Baltimore groups remained after removal of zoonotic virus sequences. These viruses were further subset to human associated virus, of which there were 440. All species were categorized by genome structure according to the Baltimore classification system. Published phylogenetic distance matrices for each viral family were used to calibrate a phylogenetic logistic regression of genome size vs zoonotic status (binary variable) using Phylolm-R. Polytomies were inserted at the root of each viral family as necessary to standardize distance between families at 1. One-tailed Welch's T-tests for sample means were performed for Baltimore group 6 for which data was too sparse for regression analysis. Table 3.1 gives the results of these analyses, showing that zoonotic virus genomes are significantly larger than non-zoonotic virus genomes across viral groups.

Resolution of this relationship is strong except for viral group 4, where the mean genome size for zoonotic viruses approaches being larger than the mean genome size for other animal viruses (at  $\alpha = 0.05$ ). The distributions of genome sizes are visualized in Fig. 3.2.

### 3.4 Discussion

This finding strongly suggests that large genome size facilitates emergence of viral zoonoses. The signal is clear despite the large diversity of viruses within each Baltimore group and



despite not accounting for viruses that share or jump between animal host species. Viruses with a wide host range likely share characteristics with zoonotic viruses, and based on these results, should have large genomes as well. For example, numerous species of Coronavirus are included in the non-zoonotic list ( $n=42$ ) because they are not directly implicated in zoonoses, yet they are known to exhibit wide host ranges (Chu et al., 2011; Rest & Minde, 2003). Specifically, several bat Coronaviruses are in the non-zoonotic list, though researchers largely regard these viruses as the close relatives to SARS and MERS CoV (Lu et al., 2020). This fact likely impaired the resolution of the relationship between genome size and cross-species transmission, resulting in the single non-significant p-value across evaluated groups. Large genomes are likely to facilitate zoonotic transmission via high mutational load per genome replication or high probability of recombination; both processes produce variability by which cross-species infection may occur. Within several generations, the per genome variability harbored in larger genomes has potential to vastly exceed that of smaller genomes. Genome sizes for some viral groups, especially dsDNA viruses, are tightly correlated with increasing number of genes ( $R^2 = 0.91$ , Fig. 3.5). More coding regions within the genome could lead to greater plasticity and expanded host range, as reliance on host replication machinery is reduced (Lauber et al., 2013; Nagy & Pogany, 2012).

Among the viral groups there exists much variation in replication strategy, thus there is potential for differences in the predominant strategy for producing genetic variability

leading to zoonotic transmission events (Pérez-Losada et al., 2015). Though mutation rate is 100 fold lower per nucleotide for dsDNA viruses than for RNA viruses, their much larger genomes enable a comparable likelihood of accumulating at least one mutation during replication. A negative correlation between mutation rate and genome size has been noted for a small set of viruses for which mutation rate has been directly measured (Drake & Holland, 1999). Much of the correlation for RNA viruses results from measurement of bacteriophage mutation rate, and this interesting relationship should be further explored as it pertains to zoonotic potential. Coronaviruses have evolved 3' → 5' proofreading capabilities to accommodate mutagenesis associated with a large genome (Minskaia et al., 2006). As recombination is generally associated with lower reduction in fitness compared to mutation, recombination may be the preferred mechanism to generate genetic variability for the viruses with the largest genomes. dsDNA viruses are known to have high per nucleotide rates of non-homologous recombination. Because of the large genome sizes of dsDNA viruses, the likelihood of at least one recombination event occurring during replication of the genome is high (Pérez-Losada et al., 2015; Simon-Loriere & Holmes, 2011). The many coded genes of dsDNA viruses allow more easily incorporation of recombinant DNA without deleterious effects and may reduce host specificity through phenotypic plasticity (Drummond et al., 2005; Pérez-Losada et al., 2015). In contrast, Papillomaviruses are dsDNA viruses that are characteristically species-specific (Rector & Van Ranst, 2013). These viruses have small genomes with little plasticity to accommodate recombinant DNA

or expand host range. dsRNA viruses are highly segmented, and the number of segments is highly correlated with the size of the genome ( $R^2 = 0.84$ , Fig. 3.5), which allows for a high degree of recombination via reassortment of segments during a coinfection (Pérez-Losada et al., 2015). Gene expression is regulated by translation for ssRNA(+) viruses. This group includes Coronaviruses and is known for high levels of homologous recombination because of frequent template switching during discontinuous transcription. Having the largest RNA viral genomes, Coronaviruses can produce over 25% recombinant progeny with their high rates of recombination (Makino et al., 1986; Pérez-Losada et al., 2015). ssRNA(-) viruses are not known to recombine with high frequency, so their genetic variation relies on high mutation rates alone (McVean et al., 2002). ssRNA-RT viruses are known for maintaining high mutation rates characteristic of RNA viruses as well as high recombination rates for a subset of these viruses, including HIV. Pseudodiploidy in retroviruses can promote recombination due to the physical proximity of RNA strands in the virion as well as partial masking of deleterious mutations (Archer et al., 2008). Large genomes are more likely to contain intergenic, non-coding regions that assist in gene regulation but also serve as hotspots for recombination and mutation due to weaker selection to conserve these regions (Drummond et al., 2005). The strength of the relationship between zoonotic potential and viral genome length varies between Baltimore groups in part because of this diversity of strategies for variability production. Previous studies that have investigated relationships between genome characteristics and zoonotic potential have not grouped viral genomes by

genome structure, which has prevented resolution of this relationship (Olival et al., 2017b; Walker et al., 2018). Future work should evaluate how replication strategy and predominance of recombination over mutation affects the predictive power of genome length in zoonotic potential.

Viral zoonoses can emerge as a result of large genetic changes associated with recombination or significant mutation—both more likely to occur in large genomes. Phenotypic plasticity is hypothesized to be positively related to genome size and may facilitate cross-species transmission, though this notion requires future exploration. The relationship between likelihood of zoonotic transmission and genome size has implications for predicting future zoonoses and for understanding general principles about viral life history. Host specificity may be considered a function of genome reduction. Guiding principles like the relationship between genome size and host range should be explored to broaden scientific understanding of the viral world and its role in ecosystems. Predicting future epidemics requires knowledge of potential zoonotic viruses. Far less than 1% of viruses have been discovered, and the potential host interactions of each viral species are even less well-characterized, making zoonotic risks difficult to estimate (Anthony et al., 2013; Carlson et al., 2019; Carroll et al., 2018). These data demonstrate that viral genome size is an effective indicator of zoonotic risk across viral genome subtypes. In an age of high-throughput genomics and rapid discovery of new viruses, characterization of recombination and mutation rates is far slower and more labor-intensive than genome sequencing, making genome

size an appealing first step in zoonotic risk evaluation. Analysis of viral genome size and structure has the potential to provide deep insights in predicting future pandemics.

## 3.5 Materials and Methods

### 3.5.1 Derivation of Equation 3.2

The accumulation of substitutions in a genome occurs via mutation and recombination, with associated per nucleotide rates  $\mu$  and  $R$ . These rates can be interpreted as the per nucleotide probability of a substitution occurring in a replication event. Assuming mutation and recombination are independent processes, the probability of a substitution (variability) at each nucleotide can be represented as the union of the probability of mutation occurring and the probability of a recombination event resulting in a polymorphic nucleotide:

$$Pr(V) = Pr(mut) \cup Pr(recomb) = \mu \cup R = \mu + R(1 - \mu) \quad (3.5)$$

The probability that a substitution occurs for each nucleotide can be interpreted as a rate of substitution per replication event. Substitution events are assumed to be deleterious. Each event contributes to the reduction in fitness of the viral progeny, and these events accumulate in a genome, creating non-linear declines in fitness. Viral genome size is constrained by this reduction in fitness: larger genomes accumulate more substitutions and

potential for fitness reduction is greater than for small genomes (Bull et al., 2007; Drake & Holland, 1999). Nucleotide-level fitness thus corresponds to genome-level fitness, and successful production of nucleotide variability is constrained by the fitness decay that occurs with increasing genome size. For purposes here, fitness will be synonymous with viability of each replicated genome. Setting the fitness of the parental virion or template genome at 1, fitness of progeny takes values between 0 and 1. Because a mutation and substitution via recombination event at a given nucleotide are mutually exclusive, the probability of each event occurring separately can be calculated as

$$Pr(\mu \setminus R) = \mu(1 - \frac{\mu R}{\mu + R}) \quad Pr(R \setminus \mu) = R(1 - \frac{\mu R}{\mu + R}) \quad (3.6)$$

Across mutational models, fitness (infectivity) due to lethal mutagenesis is given as  $\omega_{mut} = e^{-\mu\gamma(nt)}$  (Bull et al., 2007). The fitness in the event of recombination is demonstrated to be  $\omega_{recomb} = \rho^{\frac{m(d-m)}{d-1}}$  (Drummond et al., 2005), where  $m$  is the number of amino acid substitution events due to recombination and  $d$  is the maximum number of amino acid substitutions possible given the genetic dissimilarity of the co-infecting viral complement. Genome-level fitness for mutagenesis can be calculated as  $\omega_{mut} = e^{-\mu\gamma L}$ . Because the probability of a random nucleotide substitution resulting in an amino acid substitution is  $415/549 \approx 3/4$ , the number of amino acid substitutions in a genome replication event is  $m = 3RL/4$ . Denoting  $D$  as proportional nucleotide dissimilarity in a complementary

strand from another virus,  $d = 3DL/4$ . This yields  $\omega_{recomb} = \rho^{\frac{9rL^2(D-r)}{4(DL-4)}}$ . Because mutation and recombination are components of fitness, the probability of each occurring with exclusion of the other at each nucleotide must be accounted. This can be done by replacing  $\mu$  with  $Pr(\mu \setminus R)$  and  $R$  with  $Pr(R \setminus \mu)$ . Genome-level fitness for substitution events can be calculated as

$$\bar{\omega} = 1 - (1 - e^{-\mu(1-\frac{\mu R}{\mu+R})\gamma L}) - (1 - \rho^{\frac{9r(1-\frac{\mu R}{\mu+R})L^2(D-r(1-\frac{\mu R}{\mu+R}))}{4(DL-4)}}) \quad (3.7)$$

which simplifies to

$$\bar{\omega} = e^{-\mu(1-\frac{\mu R}{\mu+R})\gamma L} + \rho^{\frac{9r(1-\frac{\mu R}{\mu+R})L^2(D-r(1-\frac{\mu R}{\mu+R}))}{4(DL-4)}} - 1 \quad (3.8)$$

The resulting rate of per nucleotide variability produced is the product of variability accumulation and the average fitness of each genome replicated.

$$\begin{aligned} \frac{dV}{dN} = Pr(V)\bar{\omega} = (\mu + R(1 - \mu))(e^{-\mu(1-\frac{\mu R}{\mu+R})\gamma L} + \\ \rho^{\frac{9r(1-\frac{\mu R}{\mu+R})L^2(D-r(1-\frac{\mu R}{\mu+R}))}{4(DL-4)}} - 1) \end{aligned} \quad (3.9)$$

When  $\mu$  and  $R$  are sufficiently small such that  $\mu R \ll \mu, R$  (i.e.  $\lim_{\mu, R \rightarrow 0} \frac{\mu R}{\mu+R} = 0$ ),

then equation 3.9 can be approximated as

$$\frac{dV}{dN} = (\mu + R)(e^{-\mu\gamma L} + \rho^{\frac{9rL^2(D-r)}{4(DL-4)}} - 1) \quad (3.10)$$

This is given by equation 3.2 in the main text.

### 3.5.2 Parameter values for figure 3.1

Figure 3.1 represents a general simulation for ssRNA(+) viruses. To understand the mutation and recombination consequences for this group of viruses, parameter values were approximated from the literature on ssRNA(+) viruses. Because specific estimates for parameter values for each family of viruses within this group is sparse or non-existent, approximate values are given when possible. Qualitative outcomes for each of the panels in figure 3.1 (main text) are similar across a broad range of possible parameter values, but relationships may differ for other groups of viruses. The parameter values used in the simulation are given in table 3.2.  $k$  is the rate constant ( $k_{pol}$ ) for RNA dependent RNA polymerase in nucleotides per second. This is the speed at which the replicase complex can replicate RNA.  $I$  is the product of  $k$  and the amount of additional time (in seconds) required to replicate a strand of RNA outside of the elongation phase plus any additional delay associated with replicating multiple, short strands of RNA rather than fewer, long strands on which multiple replicase complexes could be active simultaneously. The value for  $I$  chosen was simply an order of magnitude smaller than the large Coronavirus genomes, though this



delay has not been quantified in scientific literature. This value is important in determining the genome size at which the maximum rate of production of genetic variability (per second) occurs.  $\mu$  is the mutation rate per nucleotide per replication event by RNA dependent RNA polymerase. This quantity can vary around the value given in table 3.2, but the value chosen is an appropriate approximation for ssRNA(+) viruses.  $R$  is the recombination rate per nucleotide per replication event. This value varies considerably in RNA viruses but tends to be high for many ssRNA(+) viruses.  $\gamma$  gives the proportion of lethal mutations of the total number of mutations. The vast majority of mutations fall into three categories: neutral, deleterious, or lethal. There is likely variation in the respective proportions of each for viruses, though few attempts have been made to quantify these proportions. In the study referenced, one-third of mutations resulted in viral particles that were not capable of infecting (infectivity assay). This constitutes a lethal mutation and is a good estimate of fitness reduction for cross-species transmission events.  $\rho$  represents the effective tolerance of proteins to a single recombination event. Misfolding proteins likely constitute lethality or severe reduction in fitness in viruses, so this measure is used to simulate the reduction in fitness in the event of amino acid substitutions due to recombination.  $D$  is the proportion of dissimilar nucleotides in a co-infecting viral strain or species. Higher dissimilarity results in higher probability of amino acid substitutions due to recombination. 12% dissimilarity is the nucleotide dissimilarity between COVID-19 and SARS-COV.

### 3.5.3 Literature review

Peer-reviewed scientific literature was searched for reports on zoonotic viruses transmitted from animals to humans using the Web of Science electronic library database for published reports through 2019. Keywords were used in the search as specified in the main text. Centers for Disease Control and Prevention and World Health Organization reports as well as published compilations of human infectious diseases were also used as references, but reported viruses were cross-validated as zoonotic with other supporting peer-reviewed literature (C. K. Johnson et al., 2015; Jones et al., 2008; Taylor et al., 2001). Zoonotic viruses were identified as having known non-human and human hosts via molecular or serological assay (seroconversion). Viral infections supported by circumstantial evidence sans assay verification were not included. The list of zoonotic viruses was formed after searching for complete genome sequences on the NCBI database (NCBI, 2020) then extending to other peer-reviewed full genome sequences if the virus was not found in the NCBI database. This final list included 201 unique viral species. Each viral species was classified according to the Baltimore classification system by mRNA production/genome structure. The compiled list is included as table 3.4.

### 3.5.4 Non-zoonotic virus filtering

The entire NCBI database on viral genomes was downloaded and screened for animal-associated viruses. The category of unidentified viruses was excluded from the screening

process. All incomplete sequences were excluded as well. The majority of viruses are labeled with host associations, though in many instances, these labels were found to be inaccurate. Viruses were organized by families and screened initially by known host range. Viral families associated with prokaryotes, archaea, plants, or fungi exclusively were not included. Satellite viruses and viroids were also excluded. Remaining genera and species were manually cross-checked with existing literature to verify the belonging as animal associated. If species were recovered from an animal or had known relatives recovered from an animal, the species was included in the non-zoonotic, animal associated virus list. The animal grouping was assigned due to the difficulty of parsing associations further to vertebrates. Many viruses are recovered from vectors or other invertebrates, yet have known or unknown association with vertebrates. Sometimes viruses have no known association outside of an insect vector, though relatives of the virus are known to infect vertebrate hosts. As an exercise to ensure genome size did not differ substantially between vertebrate and invertebrate-associated viruses, another filtering process was conducted manually whereby each species was cross-validated with existing literature to remove known or likely known to not be associated with a vertebrate host. The total number of non-zoonotic viruses was reduced from 2088 to 1533 in this process. Results are presented in table 3.3 and Fig. 3.3. A subset of human associated viruses was collected using the same filtering approach, resulting in 440 viruses used in the phylogenetic logistic regression analysis.

## 3.6 Acknowledgments

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**Author Contributions** REG contributed to each section of this manuscript. **Competing**

**Interests** REG declares no competing interest.

### 3.7 Tables

| Group | Genome   | Human Virus Genome Size | Zoonotic Virus Genome Size | P-value |
|-------|----------|-------------------------|----------------------------|---------|
| 1     | dsDNA    | 26630 $\pm$ 5169 (n=97) | 160031 $\pm$ 10014 (n=12)  | <0.001  |
| 3     | dsRNA    | NA (n=0)                | 20997 $\pm$ 1105 (n=10)    | NA      |
| 4     | ssRNA(+) | 9275 $\pm$ 665 (n=72)   | 11961 $\pm$ 678 (n=57)     | 0.027   |
| 5     | ssRNA(-) | 3664 $\pm$ 298 (n=68)   | 13034 $\pm$ 242 (n=111)    | < 0.001 |
| 6     | ssRNA-RT | 8990 $\pm$ 483 (n=2)    | 11247 $\pm$ 559 (n=11)     | 0.019   |

Table 3.1: Comparison of viral genome size between known zoonotic viruses and all other human associated viruses. Comparisons are made within Baltimore groups classified according to genomic structure. Mean and standard errors for each group are reported.

### 3.8 Figures

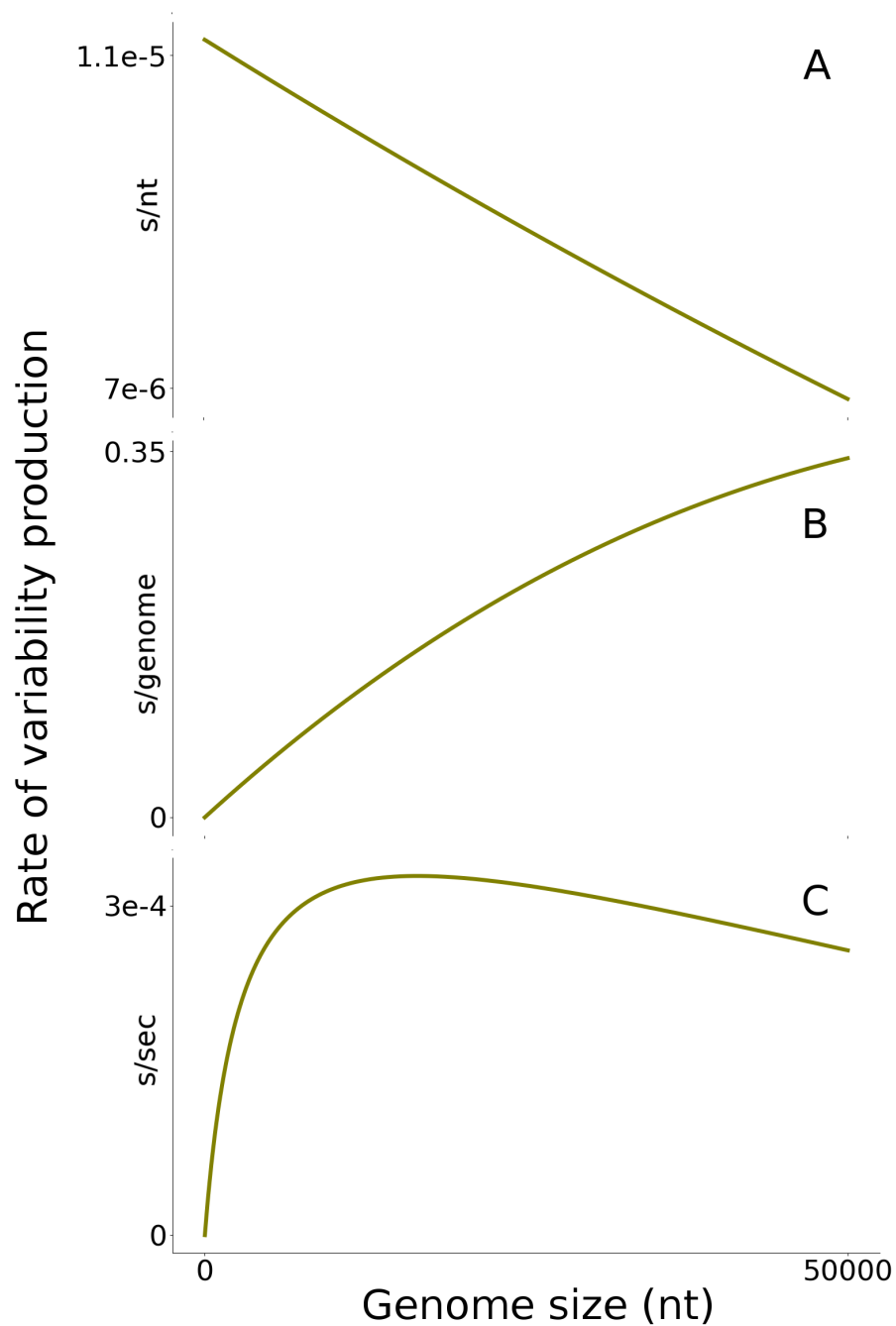


Figure 3.1: The rate at which genetic variability is produced per virion depends on genome size. The relationships are shown as substitutions per nucleotide (A), per genome (B), and per second (C) for a positive sense ssRNA virus.

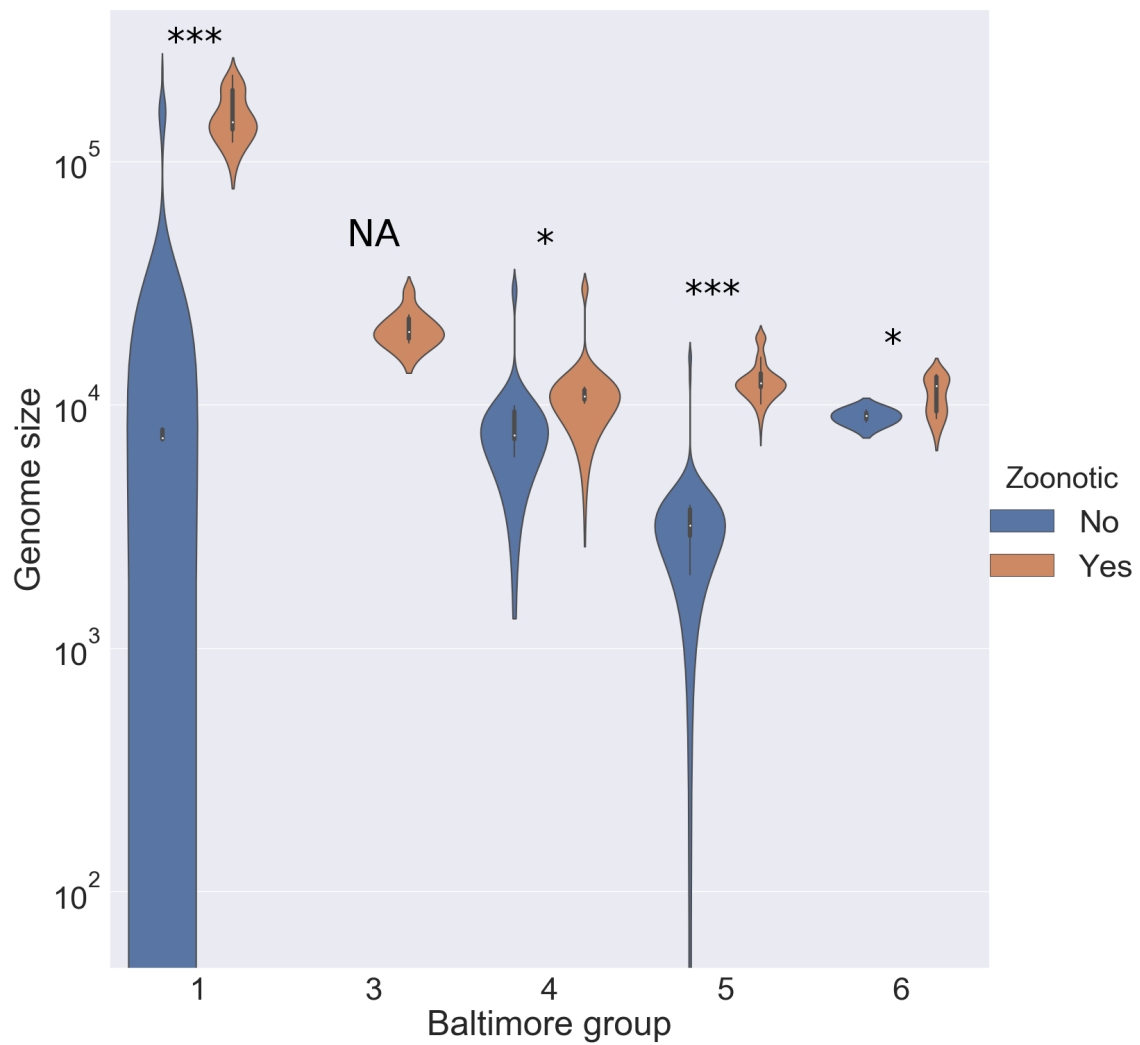


Figure 3.2: Violin plots comparing genome sizes between non-zoonotic animal viruses and zoonotic viruses. Zoonotic virus genome sizes inhabit the upper ranges of values in each group.

## 3.9 Supplemental Information

### 3.9.1 Maximizing genetic variability

The maximum production of genetic variability per unit time is dependent on the virus-specific parameters used in equation 3.4 (main text). For the parameters used, the maximum can be found under the conditions

$$\frac{d}{dL}\left(\frac{dV}{dt}\right) = 0, \quad \frac{d^2}{d^2L}\left(\frac{dV}{dt}\right) < 0 \quad (3.11)$$

Solving this equation yields a value for the length of the genome ( $L$ ) at 16531 nt. This value may not reflect the real maximum for ssRNA(+) viruses, as accurate parameter values are difficult to obtain, particularly for  $I$ . Figure 3.1 (main text) reflects the qualitative behavior of the curve, which is convex and produces a maximum between 0 and the upper range of genome sizes for RNA viruses. To illustrate, replacing  $\mu = 10^{-5}$  with  $\mu = 10^{-7}$ , reflecting the approximate mutation rate of Coronaviruses, which possess unique proofreading capacity (Minskaia et al., 2006), results in a shift from 16531 nt to 22238 nt in the genome size corresponding to the maximum rate of variability production (Figure 3.4).



### 3.9.2 The role of genes and genome size

Mutation and recombination events are more likely to occur in non-coding regions (Drummond et al., 2005; Men et al., 1996). Although these regions are not as widespread in viral genomes, large viral genomes are known to contain over 10% non-coding nucleotides. Non-coding elements are not translated into proteins but serve functions such as gene regulation (Mahmoudabadi & Phillips, 2018; Tycowski et al., 2015). Intergenic regions are among these non-coding elements and have been identified as hotspots for mutation and recombination, presumably for the weaker selection imposed on these elements compared to genes. Substitution there is less likely to be deleterious or lethal. Large genomes across prokaryotic and eukaryotic taxa are typified by expansion of non-coding, regulatory genetic material, and this is anecdotally true for viruses; larger proportions of non-coding nucleotides are found in viruses with large genomes (Konstantinidis & Tiedje, 2004; Mahmoudabadi & Phillips, 2018). Even if the ratio of coding to non-coding nucleotides were fixed as genome size increased, the absolute number of substitution hotspots increases. If the number of genes increases with the size of the genome, the number of intergenic regions increases as well. This says nothing for the size of those intergenic regions, though there are biochemical constraints on the range of sizes these regions can take (Wieringa et al., 1984), and it may be reasonably assumed the size of these regions does not scale inversely with the number of them a genome contains. With this premise, the number of nucleotides associated with intergenic regions is positively related to increased genome size, and the number

of intergenic regions may well be an indicator of the number of substitution hotspots, and therefore potential for genome-wide substitution. Because the number of intergenic regions is one fewer than the number of genes in a non-segmented genome, the number of genes should recapitulate the likelihood of substitution. In segmented genomes, this relationship may not hold. Figure 3.5 shows the relationship between genome size and the number of genes, number of intergenic regions, and number of segments in each genome. The number of intergenic regions is calculated as

$$n_{inter} = n_{seg} \left( \frac{n_{genes}}{n_{seg}} - 1 \right) \quad (3.12)$$

where  $n_{inter}$ ,  $n_{seg}$ , and  $n_{genes}$  denote the number of intergenic regions, the number of genomic segments, and the number of genes, respectively. The number of intergenic regions, thus potential for substitutions, is positively related to genome size for groups 1, 2, 4, and 6. Group 1, dsDNA viruses, shows strong correlation between genome size and intergenic regions. This may explain, in part, why differences in genome size between zoonotic and non-zoonotic viruses in group 1 are so great. Although the number of intergenic regions was not related to genome size for group 3, these dsRNA viruses commonly contain segmented genomes, and this segmentation is strongly related to genome size. This relationship demonstrates a fixed segment size near 2500 nucleotides. Generation of variability is likely related to genome size due to the capacity to reassort in dsRNA viruses rather than substitution events through homologous and non-homologous recombination and mutation.

Groups 5 and 7 show no relationships between genome size and any of the response variables, suggesting that intergenic regions and reassortment play little role in generation of genetic variability as a function of genome size for these viruses.

### 3.9.3 Sampling considerations

Rigorous screening ensured the inclusion of viruses that have known animal hosts and have elicited immune response in humans. Viruses for which there is extensive debate about the host were excluded. An example is human picobirnavirus. Although previously identified in stool samples in rats and humans, this virus shares sequence homology with prokaryotic viruses and is expected to be a prokaryotic virus (Krishnamurthy & Wang, 2018). Further work is needed to determine whether this virus is truly zoonotic or exists in hosts with shared enteric flora. For viruses identified as zoonotic, sequences were retrieved from (NCBI, 2020), and if the sequences were unavailable, complete sequence length was found from other published sources. All sequences in (NCBI, 2020) corresponding to known zoonoses were used in the zoonotic group for analysis. This includes one isolate (England Coronavirus) that may not be considered a separate species. Species-level identification is notoriously difficult for viruses due to rapid mutation and recombination rates, and this likely has resulted in inclusion of instances of species redundancy in the non-zoonotic data set, though these instances are perceived to be rare. The NCBI Viral Genome library is filtered and curated to eliminate redundancies in species identification.

The viral sequences in (NCBI, 2020) encompass a vast array of viruses across host taxa, and considerable effort was given to filter non-animal viruses. All viruses of unknown classification were removed. Viral families tend to cluster with host phyla, so proper classification by viral family was possible in the vast majority of cases. The vast majority of viruses have yet to be detected, and the existing viral sequences are certainly biased toward those associated with human and domestic animal and plant pathology. Viruses were grouped by the Baltimore classification system, which is based on the method of mRNA synthesis and the structure of the viral genome. This level of grouping was chosen over higher level grouping (DNA vs RNA) because there are distinct differences in genome size that occur due to method of mRNA synthesis, and the constraints replication and mRNA synthesis impose on genome structure are best conserved within categories defined by the Baltimore classification system. Lower level grouping was avoided because of the difficulty of taxonomic classification of viruses at lower levels. Additionally, as is evident by the list of zoonotic viruses, some clades of viruses are more likely to exhibit broad host ranges, and parsing genome size effects on the likelihood of cross-species transmission is less reliable within clades than between clades with the available sample sizes.

### 3.9.4 Supplemental Figures

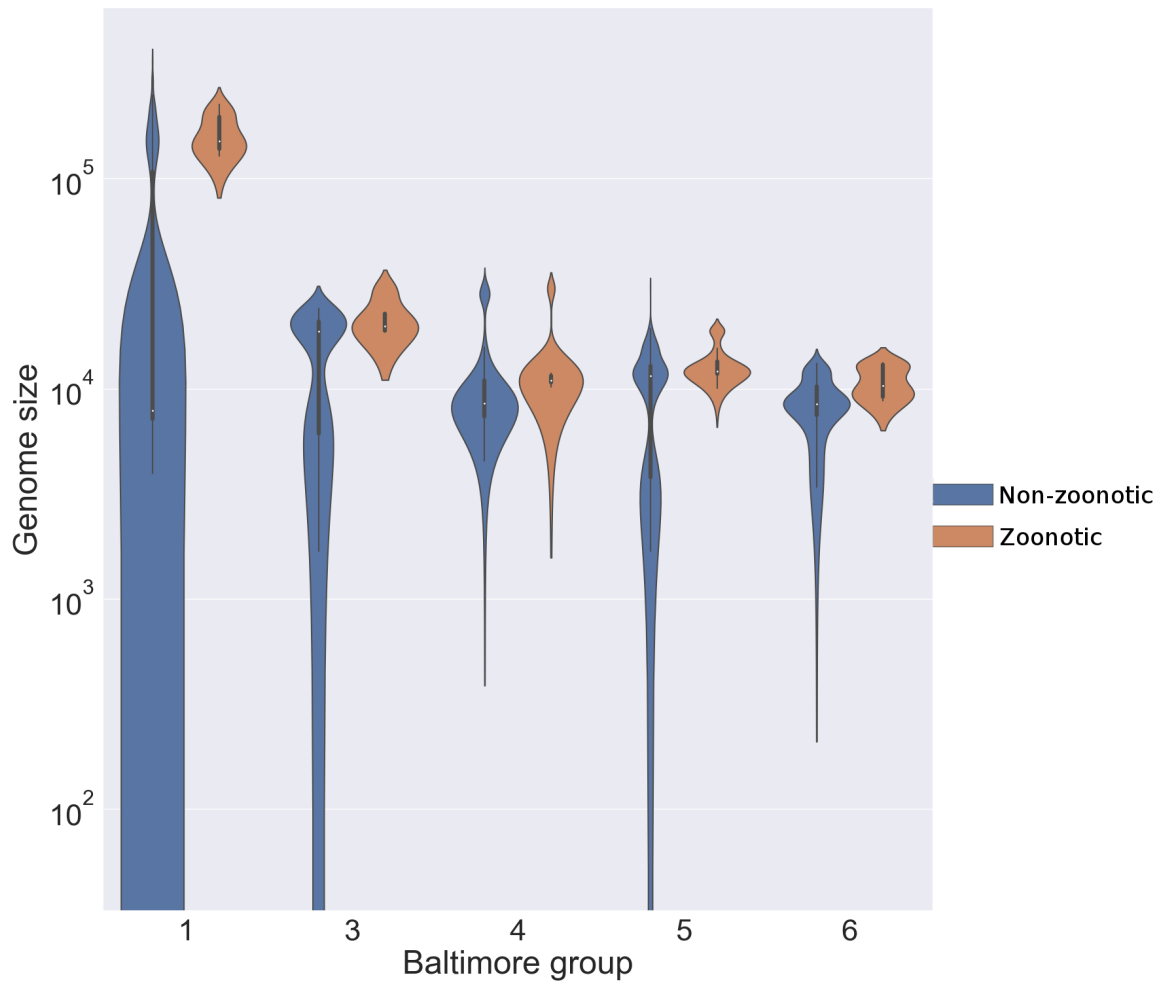


Figure 3.3: Violin plots comparing genome sizes between non-zoonotic vertebrate-associated viruses and zoonotic viruses. Zoonotic virus genome sizes inhabit the upper ranges of values in each group.

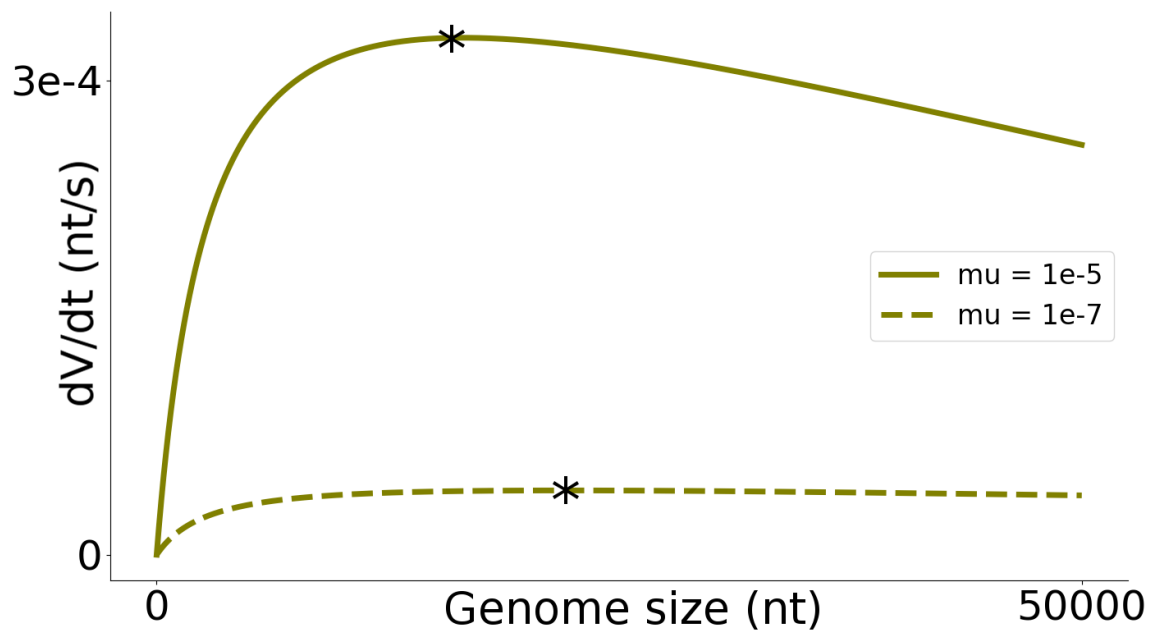


Figure 3.4: Lower rates of mutation favor larger genome sizes for maximum rates of production of variability per unit time per infecting virion. Asterisks indicate maxima for each curve.

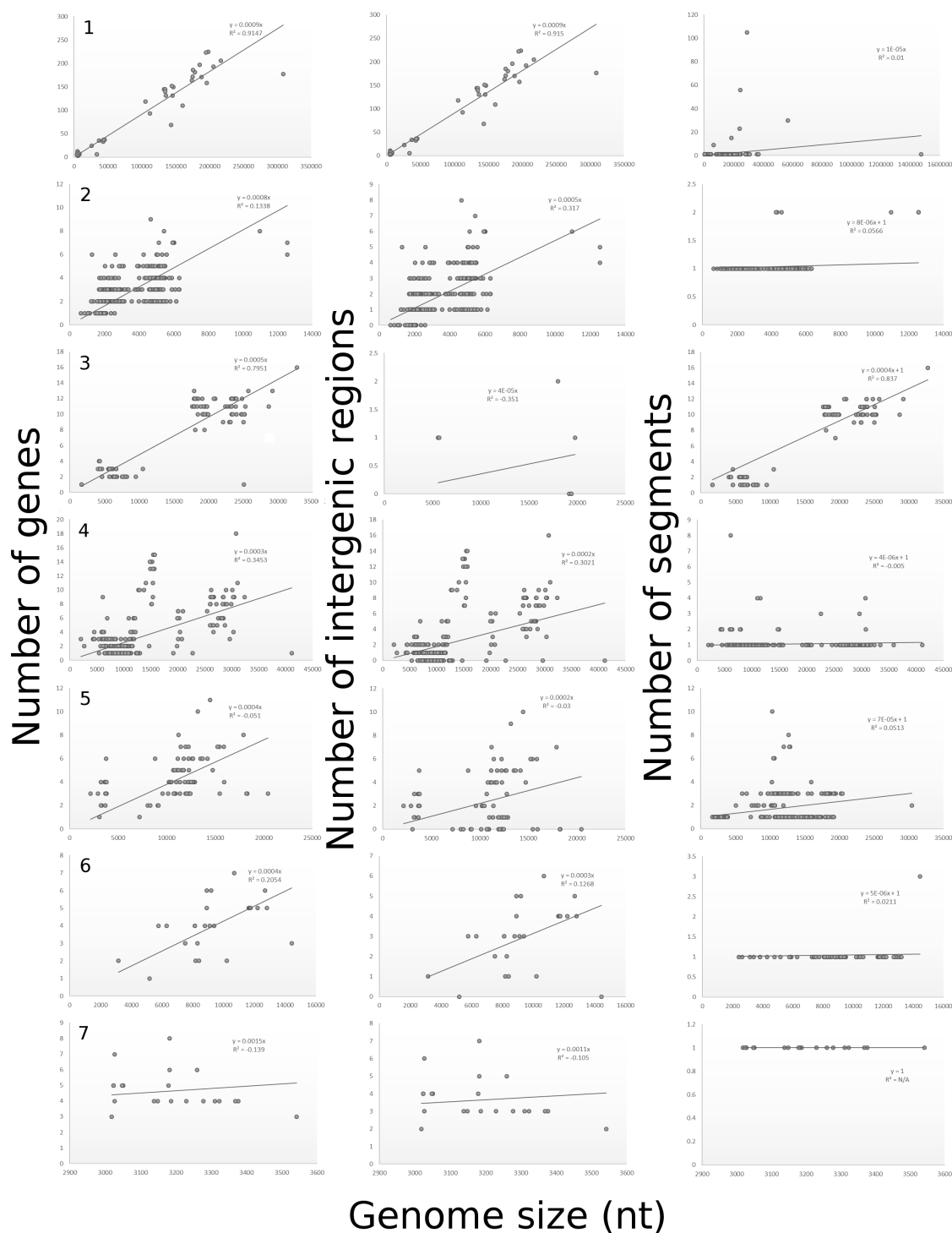


Figure 3.5: Scaling of number of genes, intergenic regions, and genomic segments with genome size. 7 Baltimore groups are presented, one for each row: dsDNA (1), ssDNA (2), dsRNA (3), ssRNA+ (4), ssRNA- (5), ssRNA-RT (6), dsDNA-RT (7). Intercepts of linear regressions are fixed at the origin (or at  $y=1$  for the last column). Negative  $R^2$  values reveal a correlation between the regression line and the data that is weaker than a line with zero slope and fitted intercept.

### 3.9.5 Supplemental Tables

| Parameter | Description                    | Value                     | Reference                      |
|-----------|--------------------------------|---------------------------|--------------------------------|
| k         | RNA polymerization rate        | $40 \text{ nt s}^{-1}$    | (Fitzsimmons et al., 2018)     |
| I         | Initiation time (nt equiv.)    | $3000 \text{ nt}$         | NA                             |
| $\mu$     | Mutation rate                  | $10^{-5} \text{ nt}^{-1}$ | (Drake & Holland, 1999)        |
| r         | Recombination rate             | $10^{-5} \text{ nt}^{-1}$ | (Simon-Loriere & Holmes, 2011) |
| $\gamma$  | Proportion of lethal mutations | 0.33                      | (Bull et al., 2007)            |
| $\rho$    | Tolerance to recombination     | 0.79                      | (Drummond et al., 2005)        |
| D         | Genetic dissimilarity          | 0.12                      | (Lu et al., 2020)              |

Table 3.2: Approximate values for parameters used in figure 3.1 (main text).

| Group | Genome   | Vertebrate Virus Genome Size | Zoonotic Virus Genome Size |
|-------|----------|------------------------------|----------------------------|
| 1     | dsDNA    | $52821 \pm 3235$ (n=535)     | $160031 \pm 10014$ (n=12)  |
| 3     | dsRNA    | $14945 \pm 970$ (n=54)       | $20997 \pm 1105$ (n=10)    |
| 4     | ssRNA(+) | $11433 \pm 338$ (n=453)      | $11961 \pm 678$ (n=57)     |
| 5     | ssRNA(-) | $9765 \pm 253$ (n=343)       | $13034 \pm 242$ (n=111)    |
| 6     | ssRNA-RT | $8482 \pm 310$ (n=69)        | $11247 \pm 559$ (n=11)     |

Table 3.3: Comparison of viral genome size between known zoonotic viruses and all other vertebrate viruses. Comparisons are made within Baltimore groups classified according to genomic structure. Mean and standard errors for each group are reported. Little change is observed between this table and table 3.1 in the main text.



Table 3.4: Zoonotic Viruses

| Viral Genus   | Species Common Name         | Viral Group | Genome Size (ref.)                                         |
|---------------|-----------------------------|-------------|------------------------------------------------------------|
| Centapoxvirus | NY <sub>014</sub> poxvirus* | 1           | 200223 (NCBI, 2020)                                        |
| Orthopoxvirus | Buffalopox virus            | 1           | 195630 (Afrough et al., 2018)                              |
| Orthopoxvirus | Monkeypox virus             | 1           | 196858 (Shchelkunov et al., 2001)                          |
| Parapoxvirus  | Bovine papular stomatitis   | 1           | 134431 (Delhon et al., 2004)                               |
| Parapoxvirus  | Cowpox                      | 1           | 224499 (Hu et al., 1994)                                   |
| Parapoxvirus  | Orf virus                   | 1           | 139962 (Hu et al., 1994)                                   |
| Parapoxvirus  | Pseudocowpox virus          | 1           | 145289 (Hautaniemi et al., 2010)                           |
| Parapoxvirus  | Sealpox                     | 1           | 127941 (Günther et al., 2017)                              |
| Rhadinovirus  | Murid gammaherpesvirus 4    | 1           | 119451 (NCBI, 2020)                                        |
| Simplexvirus  | Herpes virus B              | 1           | 156789 (Perelygina et al., 2003)                           |
| Yatapoxvirus  | Yaba monkey tumor virus     | 1           | 134721 (NCBI, 2020)                                        |
| Yatapoxvirus  | Yaba-like disease virus     | 1           | 144575 (NCBI, 2020)                                        |
| Coltivirus    | Colorado tick fever         | 3           | 29174 (Attoui, Billoir, Biagini, Cantaloube, et al., 2000) |
| Orbivirus     | Lebombo virus               | 3           | 19247 (NCBI, 2020)                                         |
| Orbivirus     | Orungo virus                | 3           | 18894 (Jaafar et al., 2014)                                |
| Orthoreovirus | Mammalian orthoreovirus 3   | 3           | 23176 (NCBI, 2020)                                         |
| Orthoreovirus | Melaka orthoreovirus        | 3           | 23410 (NCBI, 2020)                                         |
| Rotavirus     | Rotavirus strain J19*       | 3           | 17961 (NCBI, 2020)                                         |
| Rotavirus     | Rotavirus A                 | 3           | 18562 (Small et al., 2007)                                 |
| Rotavirus     | Rotavirus B                 | 3           | 17932 (NCBI, 2020)                                         |
| Rotavirus     | Rotavirus C                 | 3           | 17910 (NCBI, 2020)                                         |
| Seadornavirus | Banna                       | 3           | 20682 (Attoui, Billoir, Biagini, de Micco, et al., 2000)   |
| Seadornavirus | Kadipiro virus              | 3           | 20985 (NCBI, 2020)                                         |
| Alphavirus    | Barmah Forest virus         | 4           | 11488 (E. Lee et al., 1997)                                |
| Alphavirus    | Chikungunya                 | 4           | 11826 (Khan et al., 2002)                                  |
| Alphavirus    | Eastern equine encephalitis | 4           | 11675 (V. Volchkov et al., 1991)                           |
| Alphavirus    | Everglades virus            | 4           | 11395 (Kinney et al., 1998a)                               |
| Alphavirus    | Getah virus                 | 4           | 11597 (NCBI, 2020)                                         |
| Alphavirus    | Highlands J virus           | 4           | 11526 (NCBI, 2020)                                         |
| Alphavirus    | Madariaga virus             | 4           | 11624 (NCBI, 2020)                                         |
| Alphavirus    | Mayaro virus                | 4           | 11411 (NCBI, 2020)                                         |
| Alphavirus    | Middelburg virus            | 4           | 11550 (NCBI, 2020)                                         |
| Alphavirus    | Mosso das Pedras virus      | 4           | 11465 (NCBI, 2020)                                         |
| Alphavirus    | O'nyong-nyong virus         | 4           | 11835 (Levinson et al., 1990)                              |
| Alphavirus    | Pixuna virus                | 4           | 11344 (NCBI, 2020)                                         |
| Alphavirus    | Ross River virus            | 4           | 11657 (Faragher et al., 1988)                              |

Table 3.4: Zoonotic Viruses

| Viral Genus     | Species Common Name                  | Viral Group | Genome Size (ref.)                   |
|-----------------|--------------------------------------|-------------|--------------------------------------|
| Alphavirus      | Semliki Forest virus                 | 4           | 11442 (Takkinen, 1986)               |
| Alphavirus      | Sindbis virus                        | 4           | 11703 (Strauss et al., 1984)         |
| Alphavirus      | Tonate virus                         | 4           | 11530 (NCBI, 2020)                   |
| Alphavirus      | Venezuelan equine encephalitis virus | 4           | 11444 (Kinney et al., 1998b)         |
| Alphavirus      | Western equine encephalitis virus    | 4           | 11484 (Netolitzky et al., 2000)      |
| Aphthovirus     | Foot and mouth disease               | 4           | 8201 (Carrillo et al., 2005)         |
| Betacoronavirus | England isolate, MERS Coronavirus    | 4           | 30111 (NCBI, 2020)                   |
| Betacoronavirus | MERS Coronavirus                     | 4           | 30119 (van Boheemen et al., 2012)    |
| Betacoronavirus | Novel Coronavirus                    | 4           | 29882 (Paraskevis et al., 2020)      |
| Betacoronavirus | SARS Coronavirus                     | 4           | 29751 (Marra et al., 2003)           |
| Cardiovirus     | Encephalomyocarditis virus           | 4           | 7835 (Duke et al., 1992)             |
| Flavivirus      | Alkhurma virus                       | 4           | 10775 (Charrel et al., 2001)         |
| Flavivirus      | Cacipacore virus                     | 4           | 10284 (NCBI, 2020)                   |
| Flavivirus      | Dengue fever 1                       | 4           | 10735 (Puri et al., 1997)            |
| Flavivirus      | Dengue fever 2                       | 4           | 10723 (Kinney et al., 1997)          |
| Flavivirus      | Dengue fever 3                       | 4           | 10707 (Peyrefitte et al., 2003)      |
| Flavivirus      | Dengue fever 4                       | 4           | 10649 (Whitehead et al., 2003)       |
| Flavivirus      | Edge Hill virus                      | 4           | 10206 (Grard et al., 2010)           |
| Flavivirus      | European tick-borne encephalitis     | 4           | 11141 (Wallner et al., 1995)         |
| Flavivirus      | Far eastern tick-borne encephalitis  | 4           | 10471 (M. Ecker et al., 1999)        |
| Flavivirus      | Ilheus virus                         | 4           | 10755 (Pauvolid-Corrêa et al., 2013) |
| Flavivirus      | Japanese encephalitis virus          | 4           | 10976 (Sumiyoshi et al., 1987)       |
| Flavivirus      | Kadam virus                          | 4           | 10215 (NCBI, 2020)                   |
| Flavivirus      | Kunjin virus                         | 4           | 10644 (Coia et al., 1988)            |
| Flavivirus      | Kyasanur Forest disease virus        | 4           | 10774 (Grard et al., 2007)           |
| Flavivirus      | Louping ill virus                    | 4           | 10871 (NCBI, 2020)                   |
| Flavivirus      | Murray Valley encephalitis virus     | 4           | 11014 (Melian et al., 2010a)         |
| Flavivirus      | Omsk virus                           | 4           | 10787 (D. Lin et al., 2003)          |
| Flavivirus      | Powassan virus                       | 4           | 10839 (Mandl et al., 1993)           |
| Flavivirus      | Rio Bravo virus                      | 4           | 10140 (NCBI, 2020)                   |
| Flavivirus      | Rocio virus                          | 4           | 10794 (NCBI, 2020)                   |
| Flavivirus      | Spondweni virus                      | 4           | 10290 (NCBI, 2020)                   |
| Flavivirus      | St. Louis encephalitis virus         | 4           | 10940 (Ciota et al., 2007)           |
| Flavivirus      | Usutu virus                          | 4           | 11066 (Moureau et al., 2015)         |
| Flavivirus      | Uganda S virus virus                 | 4           | 10182 (NCBI, 2020)                   |
| Flavivirus      | Wesselsbron virus                    | 4           | 10814 (Volk et al., 2009)            |

Table 3.4: Zoonotic Viruses

| <b>Viral Genus</b>  | <b>Species Common Name</b>     | <b>Viral Group</b> | <b>Genome Size (ref.)</b>           |
|---------------------|--------------------------------|--------------------|-------------------------------------|
| Flavivirus          | West Nile Virus                | 4                  | 10962 (Melian et al., 2010b)        |
| Flavivirus          | Yellow fever virus             | 4                  | 10862 (Rice et al., 1985)           |
| Flavivirus          | Zika virus                     | 4                  | 10807 (Mlakar et al., 2016)         |
| Orthohepevirus      | Hepatitis E                    | 4                  | 7176 (Bi et al., 1993a)             |
| Parechovirus        | Ljungan virus                  | 4                  | 7590 (Bi et al., 1993b)             |
| Salivirus           | Salivirus A                    | 4                  | 7989 (NCBI, 2020)                   |
| Salivirus           | Salivirus NG-J1                | 4                  | 7982 (NCBI, 2020)                   |
| Vesivirus           | Hom-1 vesivirus                | 4                  | 8399 (NCBI, 2020)                   |
| Alphainfluenzavirus | Influenza A virus (2009(H1N1)) | 5                  | 13158 (Laksperet et al., 2014)      |
| Alphainfluenzavirus | Influenza A virus (1996(H5N1)) | 5                  | 13590 (Xu et al., 1999)             |
| Alphainfluenzavirus | Influenza A virus (1999(H9N2)) | 5                  | 13498 (Y. Lin et al., 2000)         |
| Alphainfluenzavirus | Influenza A virus (1968(H2N2)) | 5                  | 13460 (Lindstrom et al., 2004)      |
| Alphainfluenzavirus | Influenza A virus (2004(H3N2)) | 5                  | 13627 (Lindstrom et al., 2004)      |
| Alphainfluenzavirus | Influenza A virus (1934(H1N1)) | 5                  | 13588 (Keller et al., 2018)         |
| Alphainfluenzavirus | Influenza A virus (2013(H7N9)) | 5                  | 13191 (Y. Chen et al., 2013)        |
| Banyangvirus        | Heartland virus                | 5                  | 11567 (NCBI, 2020)                  |
| Banyangvirus        | Huaiyangshan banyangvirus      | 5                  | 11272 (NCBI, 2020)                  |
| Banyangvirus        | SFTS virus HB29                | 5                  | 11490 (NCBI, 2020)                  |
| Orthoavulavirus     | Newcastle disease              | 5                  | 15192 (Liu et al., 2015)            |
| Orthobornavirus     | Borna disease 1                | 5                  | 8910 (Briese et al., 1994)          |
| Orthobornavirus     | Borna disease 2                | 5                  | 8908 (Pleschka et al., 2001)        |
| Ebolavirus          | Bundibugyo Ebola virus         | 5                  | 18940 (NCBI, 2020)                  |
| Ebolavirus          | Reston Ebola virus             | 5                  | 18891 (Groseth et al., 2002)        |
| Ebolavirus          | Sudan Ebola virus              | 5                  | 18875 (Sanchez & Rollin, 2005)      |
| Ebolavirus          | Tai Forest Ebola virus         | 5                  | 18935 (Townner et al., 2008)        |
| Ebolavirus          | Zaire Ebola virus              | 5                  | 18959 (V. E. Volchkov et al., 1999) |
| Henipavirus         | Hendra virus                   | 5                  | 18234 (Wang et al., 2000)           |
| Henipavirus         | Nipah virus                    | 5                  | 18246 (Harcourt et al., 2001)       |
| Jeilongvirus        | J-virus                        | 5                  | 18954 (NCBI, 2020)                  |
| Ledantavirus        | Kumasi rhabdovirus             | 5                  | 11072 (NCBI, 2020)                  |
| Ledantavirus        | Le Dantec virus                | 5                  | 11450 (NCBI, 2020)                  |
| Lyssavirus          | Australian bat lyssavirus      | 5                  | 11822 (Gould et al., 2002)          |
| Lyssavirus          | Duvenhage virus                | 5                  | 11976 (Delmas et al., 2008)         |
| Lyssavirus          | European bat lyssavirus type 1 | 5                  | 11966 (Marston et al., 2007)        |
| Lyssavirus          | European bat lyssavirus type 2 | 5                  | 11930 (Marston et al., 2007)        |
| Lyssavirus          | Lyssavirus Ozernoe             | 5                  | 11980 (NCBI, 2020)                  |

Table 3.4: Zoonotic Viruses

| <b>Viral Genus</b> | <b>Species Common Name</b>              | <b>Viral Group</b> | <b>Genome Size (ref.)</b>       |
|--------------------|-----------------------------------------|--------------------|---------------------------------|
| Lyssavirus         | Mokola virus                            | 5                  | 11940 (Le Mercier et al., 1997) |
| Lyssavirus         | Rabies virus                            | 5                  | 11932 (Tordo et al., 1988)      |
| Mammarenavirus     | Chapare virus                           | 5                  | 10464 (Delgado et al., 2008)    |
| Mammarenavirus     | Guanarito                               | 5                  | 10424 (NCBI, 2020)              |
| Mammarenavirus     | Junin virus                             | 5                  | 10525 (NCBI, 2020)              |
| Mammarenavirus     | Lassa virus                             | 5                  | 10697 (Djavani et al., 1997)    |
| Mammarenavirus     | Lujo virus                              | 5                  | 10352 (Briese et al., 2009)     |
| Mammarenavirus     | Lymphocytic choriomeningitis virus      | 5                  | 10056 (Salvato et al., 1988)    |
| Mammarenavirus     | Machupo virus                           | 5                  | 10635 (NCBI, 2020)              |
| Mammarenavirus     | Sabia virus Brazilian hemorrhagic fever | 5                  | 10499 (NCBI, 2020)              |
| Mammarenavirus     | Wenzhou mammarenavirus                  | 5                  | 10483 (NCBI, 2020)              |
| Mammarenavirus     | Whitewater Arroyo virus                 | 5                  | 10448 (Cajimat et al., 2007)    |
| Mammarenavirus     | Zegla virus                             | 5                  | 12690 (NCBI, 2020)              |
| Marburgvirus       | Marburg virus                           | 5                  | 19114 (Towner et al., 2006)     |
| Metapneumovirus    | Human metapneumovirus                   | 5                  | 13350 (NCBI, 2020)              |
| Orthobornavirus    | Variegated squirrel bornavirus 1        | 5                  | 8798 (NCBI, 2020)               |
| Orthobunyavirus    | Batai virus                             | 5                  | 12270 (NCBI, 2020)              |
| Orthobunyavirus    | Bunyamwera virus                        | 5                  | 12294 (NCBI, 2020)              |
| Orthobunyavirus    | Bwamba orthobunyavirus                  | 5                  | 12587 (NCBI, 2020)              |
| Orthobunyavirus    | Cache Valley virus                      | 5                  | 12283 (NCBI, 2020)              |
| Orthobunyavirus    | California encephalitis                 | 5                  | 12466 (Bowen et al., 1995)      |
| Orthobunyavirus    | Caraparu virus                          | 5                  | 12259 (NCBI, 2020)              |
| Orthobunyavirus    | Catu virus                              | 5                  | 12024 (NCBI, 2020)              |
| Orthobunyavirus    | Fort Sherman virus                      | 5                  | 12294 (NCBI, 2020)              |
| Orthobunyavirus    | Guama                                   | 5                  | 12123 (Shchetinin et al., 2015) |
| Orthobunyavirus    | Guaroa virus                            | 5                  | 12265 (Groseth et al., 2015)    |
| Orthobunyavirus    | Ilesha virus                            | 5                  | 12298 (NCBI, 2020)              |
| Orthobunyavirus    | Jamestown Canyon virus                  | 5                  | 12461 (Bowen et al., 1995)      |
| Orthobunyavirus    | Kairi virus                             | 5                  | 12497 (NCBI, 2020)              |
| Orthobunyavirus    | Keystone virus                          | 5                  | 12455 (NCBI, 2020)              |
| Orthobunyavirus    | LaCrosse virus                          | 5                  | 12490 (NCBI, 2020)              |
| Orthobunyavirus    | Madrid virus                            | 5                  | 12604 (NCBI, 2020)              |
| Orthobunyavirus    | Oropouche virus                         | 5                  | 11985 (NCBI, 2020)              |
| Orthobunyavirus    | Shuni orthobunyavirus                   | 5                  | 12112 (NCBI, 2020)              |
| Orthobunyavirus    | Simbu orthobunyavirus                   | 5                  | 12172 (NCBI, 2020)              |
| Orthobunyavirus    | Tacaiuma orthobunyavirus                | 5                  | 12162 (NCBI, 2020)              |

Table 3.4: Zoonotic Viruses

| <b>Viral Genus</b> | <b>Species Common Name</b>      | <b>Viral Group</b> | <b>Genome Size (ref.)</b>       |
|--------------------|---------------------------------|--------------------|---------------------------------|
| Orthobunyavirus    | Tahyna virus                    | 5                  | 12446 (Quinan et al., 2008)     |
| Orthobunyavirus    | Tataguine virus                 | 5                  | 12052 (NCBI, 2020)              |
| Orthobunyavirus    | Utinga virus                    | 5                  | 12253 (NCBI, 2020)              |
| Orthobunyavirus    | Wyeomyia orthobunyavirus        | 5                  | 12461 (NCBI, 2020)              |
| Orthohantavirus    | Andes virus                     | 5                  | 11909 (Meissner et al., 2002)   |
| Orthohantavirus    | Anjzorobe hantavirus            | 5                  | 11910 (NCBI, 2020)              |
| Orthohantavirus    | Bayou hantavirus                | 5                  | 12189 (NCBI, 2020)              |
| Orthohantavirus    | Dobrava virus                   | 5                  | 11840 (Nemirov et al., 2003)    |
| Orthohantavirus    | Hantaan                         | 5                  | 11845 (Schmauohn et al., 1987)  |
| Orthohantavirus    | Hantavirus Z10                  | 5                  | 11850 (NCBI, 2020)              |
| Orthohantavirus    | Monongahela virus               | 5                  | 12314 (Albariño et al., 2018)   |
| Orthohantavirus    | Muju virus                      | 5                  | 12027 (K.-J. Song et al., 2007) |
| Orthohantavirus    | Prospect Hill orthohantavirus   | 5                  | 11941 (J.-W. Song et al., 2007) |
| Orthohantavirus    | Puumala virus                   | 5                  | 12062 (NCBI, 2020)              |
| Orthohantavirus    | Seoul virus                     | 5                  | 11950 (NCBI, 2020)              |
| Orthohantavirus    | Sin Nombre virus                | 5                  | 12317 (Chizhikov et al., 1995)  |
| Orthohantavirus    | Tula virus                      | 5                  | 12066 (Kukkonen et al., 1998)   |
| Orthonairovirus    | Crimean-Congo hemorrhagic fever | 5                  | 19146 (Kinsella et al., 2004)   |
| Orthonairovirus    | Kasokero virus                  | 5                  | 18380 (NCBI, 2020)              |
| Orthonairovirus    | Nairobi sheep disease virus     | 5                  | 18748 (NCBI, 2020)              |
| Orthonairovirus    | Tacheng Tick Virus 1            | 5                  | 19171 (NCBI, 2020)              |
| Orthopneumovirus   | Human orthopneumovirus 1        | 5                  | 15224 (NCBI, 2020)              |
| Orthopneumovirus   | Human orthopneumovirus 2        | 5                  | 15191 (NCBI, 2020)              |
| Pararubulavirus    | Sosuga virus                    | 5                  | 15480 (NCBI, 2020)              |
| Phlebovirus        | Bhanja virus                    | 5                  | 11511 (Matsuno et al., 2013)    |
| Phlebovirus        | Chandiru virus                  | 5                  | 12495 (NCBI, 2020)              |
| Phlebovirus        | Munguba virus                   | 5                  | 12923 (NCBI, 2020)              |
| Phlebovirus        | Punta Toro virus                | 5                  | 12618 (NCBI, 2020)              |
| Phlebovirus        | Rift Valley fever virus         | 5                  | 11979 (Bird et al., 2007)       |
| Phlebovirus        | Toscana (sandfly) virus         | 5                  | 12488 (NCBI, 2020)              |
| Phlebovirus        | Turkey (sandfly) virus          | 5                  | 12603 (Çarhan et al., 2010)     |
| Phlebovirus        | Uukuniemi virus                 | 5                  | 11372 (NCBI, 2020)              |
| Respirovirus       | Human respirovirus 1            | 5                  | 15600 (NCBI, 2020)              |
| Respirovirus       | Human respirovirus 3            | 5                  | 15462 (NCBI, 2020)              |
| Rubulavirus        | Menangle virus                  | 5                  | 15516 (Barr et al., 2012)       |
| Rubulavirus        | Tioman virus                    | 5                  | 15522 (Chua et al., 2002)       |

Table 3.4: Zoonotic Viruses

| <b>Viral Genus</b> | <b>Species Common Name</b>               | <b>Viral Group</b> | <b>Genome Size (ref.)</b>         |
|--------------------|------------------------------------------|--------------------|-----------------------------------|
| Rubulavirus        | Human parainfluenza virus 2              | 5                  | 15646 (NCBI, 2020)                |
| Rubulavirus        | Human parainfluenza virus 4              | 5                  | 17052 (NCBI, 2020)                |
| Rubulavirus        | Mammalian rubulavirus 5                  | 5                  | 15246 (NCBI, 2020)                |
| Thogotovirus       | Thogoto thogotovirus                     | 5                  | 10461 (NCBI, 2020)                |
| Tibrovirus         | Ekpoma virus 2*                          | 5                  | 12674 (NCBI, 2020)                |
| Vesiculovirus      | Carajas virus                            | 5                  | 11135 (NCBI, 2020)                |
| Vesiculovirus      | Chandipura virus                         | 5                  | 11120 (Gurav et al., 2010)        |
| Vesiculovirus      | Isfahan virus                            | 5                  | 11088 (NCBI, 2020)                |
| Vesiculovirus      | Vesicular stomatitis infection (Alagoas) | 5                  | 11070 (Pauszek et al., 2008)      |
| Vesiculovirus      | Vesicular stomatitis infection (IN)      | 5                  | 11162 (Rodriguez et al., 2002)    |
| Vesiculovirus      | Vesicular stomatitis infection (NJ)      | 5                  | 11123 (Pauszek & Rodriguez, 2012) |
| Deltaretrovirus    | Primate T-lymphotropic virus 1           | 6                  | 9028 (Saksena et al., 1993)       |
| Deltaretrovirus    | Primate T-lymphotropic virus 4           | 6                  | 8791 (NCBI, 2020)                 |
| Lentivirus         | Human immunodeficiency virus 1           | 6                  | 9181 (Martoglio et al., 1997)     |
| Lentivirus         | Human immunodeficiency virus 2           | 6                  | 10359 (Kirchhoff et al., 1990)    |
| Lentivirus         | Simian immunodeficiency virus            | 6                  | 9623 (Fomsgaard et al., 1991)     |
| Spumavirus         | Central chimpanzee simian foamy virus    | 6                  | 13220 (NCBI, 2020)                |
| Spumavirus         | Eastern chimpanzee simian foamy virus    | 6                  | 11954 (NCBI, 2020)                |
| Spumavirus         | Guenon simian foamy virus                | 6                  | 13072 (Rua et al., 2012)          |
| Spumavirus         | Gorilla simian foamy virus               | 6                  | 12258 (Schulze et al., 2011)      |
| Spumavirus         | Rhesus macaque foamy virus               | 6                  | 12983 (NCBI, 2020)                |
| Spumavirus         | Simian foamy virus                       | 6                  | 13246 (Herchenröder et al., 1994) |

Table 3.4: \* = Suspected zoonotic though unconfirmed

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## Chapter 4

# Estimating the Global Infection Fatality Rate of COVID-19

*Background:* Estimating the infection fatality rate (IFR) for COVID-19 has been elusive due to the presence of asymptomatic or mildly symptomatic infections and lack of testing capacity. Estimates of COVID-19 IFR in each country or locality differ due to variable sampling regimes, demographics, and healthcare resources.

*Objective:* To derive the IFR of COVID-19 using global data.

*Design:* We present a simple linear regression framework based on testing capacity and the reported case fatality rate (CFR) of each country, whereby the intercept translates to the global IFR.

*Setting:* Cumulative COVID-19 data from 139 countries.



*Patients:* Inpatients and outpatients tested for SARS-CoV-2 infection.

*Measurements:* GDP, age, and HAQ Index were investigated as additional predictors of a nation's reported CFR through a Bayesian generalized linear model and compared to statistical deviations in each reported CFR and the global estimator in a regression framework to determine whether a higher national CFR indicates a higher underlying IFR.

*Results:* Applying this new statistical approach reveals a global IFR of 0.90% (CI: 0.70%, 1.16%).

Deviation of countries' reported CFR from the estimator does not correlate with demography, per capita GDP, or healthcare access and quality, suggesting variation is due to differing testing regimes or reporting guidelines by country.

*Limitation:* Imprecise estimate of the IFR due to heterogeneous national reporting and testing guidelines and epidemic phase.

*Conclusion:* Estimates of IFR through seroprevalence studies and point estimates from case studies or sub-sampled populations are limited by sample coverage and cannot inform a global IFR, as mortality is known to vary dramatically by age and treatment availability. Our estimated IFR aligns with many previous estimates and is the first global estimate of COVID-19 IFR.

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## 4.1 Introduction

SARS-COV-2 emerged from Wuhan, China at the end of 2019 and has become a global pandemic with almost ten million confirmed infections and half a million associated deaths (J. F.-W. Chan, Yuan, et al., 2020; N. Chen et al., 2020; Guan et al., 2020; C. Huang et al., 2020; Li et al., 2020b). Now spreading in all continents but Antarctica, the disease associated with this novel virus (COVID-19) is a risk to people of all nations (WHO, 2020). Yet, this risk is known to vary widely with access to healthcare and demographic traits (Guan et al., 2020; C. Huang et al., 2020; F. Zhou et al., 2020). Although the extent of sub-lethal impacts of the virus are still not known, deaths attributed to infection are of great concern and are used as a measure of disease burden, being a more reliable metric than the number of confirmed infections as the latter is notoriously dependent on testing capacity (Tinker et al., 2008).

Counter-measures to inhibit the transmission of the virus are in place in most countries, and economic decline has resulted from the closure of businesses and travel restrictions (R. M. Anderson et al., 2020; Atkeson, 2020). With pressure to re-open economies to reduce job losses and downstream repercussions, leaders of countries and localities are weighing the health risks of COVID-19 with economic risks of control measures (Parmet & Sinha, 2020). The linchpin for policymakers is often the risk of mortality upon infection. Reported data from countries reveals a large spread in case fatality rate (CFR) from 0.5-18% (Worldometer, 2020b). This rate is given by the number of deaths per confirmed

infection. The vast difference in the reported CFR by country is primarily a product of testing availability (J. F.-W. Chan, Yip, et al., 2020; Ng et al., 2020). In the early phases of the outbreak and in countries where testing kits were limited, only hospitalized patients with advanced COVID-19 symptoms have been tested. Therefore, the CFR is an inflated estimate of the infection fatality rate (IFR) because many infections in the population are unidentified (Ghani et al., 2005; Lipsitch et al., 2015). This inflation is intrinsic for reported data across all countries, though it is much smaller for countries that have greater testing capacity such that individuals with mild or no symptoms are tested.

To understand how dramatic this inflation is, an increasing number of serological studies has contrasted the number of active infections confirmed with PCR-based tests with the number of individuals with a detectable SARS-COV-2 immune response in sampled populations (Bendavid et al., 2020). Universally, seroprevalence surveys confirm the presence of unreported infections, though the extent of the unreported infections and associated adjustment to IFR is debated due to the non-random nature of population sampling, low sample size, and questioned specificity of some early serological assays. Some countries have conducted larger serological surveys in recent months, though this sampling has not occurred globally. Case studies reporting IFR from whole population sampling, such as the Diamond Princess cruise where all patrons were tested and the calculated IFR is near 2% are not necessarily representative of global demographics, incomes, or healthcare availability (Mizumoto et al., 2020; F. Zhou et al., 2020). In the absence of high resolution information

of case demographics, seroprevalence, or standardization of healthcare and underlying risk factors to COVID-19 morbidity and mortality, there exists no method to assess the global IFR. A robust estimate is essential to inform policy and interventions (Famulare, 2020; Verity et al., 2020a). We present a new and robust statistical approach to estimate the global mean IFR using commonly reported data, despite variability in reporting and risk factors. Specifically, we derive the mathematical relationship between IFR, CFR, and prevalence of infection in the tested population (sample prevalence or test positivity rate), the latter two being parameters that can be computed from readily available data and used to estimate the IFR. This provides a simple linear regression for which the IFR is the intercept. Differences in reported country-level CFR from the regression line are compared to known risk factors for COVID-19 mortality—national demographic, economic, and healthcare profiles—to determine whether these differences convey information regarding a nation’s IFR relative to the global mean. Additionally, these risk factors were evaluated in a Bayesian framework as predictors of national CFR in association with sample prevalence to make recommendations for future studies.

## 4.2 Methods

Reporting standards and testing procedures vary widely across countries, preventing reliable interpretation of the reported (crude) CFR. High CFR are generally associated with restricted access to testing due to low test availability, such that only individuals with marked

COVID-19 symptoms – typically those of older age or with pre-existing conditions, and thus at higher risk of death from COVID-19 – receive testing (R. M. Anderson et al., 2020; Cascella et al., 2020; J. F.-W. Chan, Yip, et al., 2020; Ng et al., 2020). Greater testing capacity allows individuals with mild symptoms or those with suspected exposure to receive testing. Figure 4.1 illustrates how this allows for a broader net of sampling in a population, giving a closer estimate of the number of total infections in the population. As testing captures a greater proportion of the population, the estimated CFR better approximates the IFR because the individuals with mild or no symptoms and more likely to recover are included in the confirmed infections. With increasing testing capacity, the sample prevalence (test positivity rate) decreases. Testing capacity is defined colloquially in several ways, usually as the number of tests performed per unit time or per person in a designated population. Another way to define testing capacity is relative to the number of infections. Empirically, RT-PCR testing only provides an indication of the number of infections in a population, though the number of tests per positively identified infection provides an empirical metric of testing capacity. This is the inverse of sample prevalence (test positivity rate), and low sample prevalence ( $\rho$ ) indicates high testing capacity ( $C$ ).

$$C^{-1} = \rho = \frac{\text{positive tests}}{\text{total tests}} = \frac{P_{test}}{T} \quad (4.1)$$

Provided that testing prioritizes individuals with symptoms, the relationship derived in

equation 4.23 shows that as sample prevalence decreases, a greater proportion of the infected population is captured by testing. In fact, this relationship predicts that when testing capacity approaches universal testing, sample prevalence will decline towards zero. As the number of total tests increases and approaches universal testing of the whole population, the number of positive tests better estimates the active true case load in the population,  $P_{pop}$ . The relation between  $\rho$  and the estimate of the true case load approximates an exponential decay with decay constant  $k$ .

$$\frac{P_{test}}{P_{pop}} \approx e^{-k\rho} \quad (4.2)$$

Despite differences in testing regimes in each country, the approximation in equation 2 becomes closer to exact as testing capacity increases. As  $\rho \rightarrow 0$ ,  $P_{test} \rightarrow P_{pop}$ . From equation 2 the following is true:

$$\frac{P_{pop}}{P_{test}} = e^{k\rho} \quad (4.3)$$

Log transformation yields:

$$\log\left(\frac{P_{pop}}{P_{test}}\right) = k\rho \quad (4.4)$$

Assuming COVID-19 attributed deaths are more reliably estimated than COVID-19 cases (SI 6.11.11), we can derive a relation to the true IFR from the reported CFR.

$$IFR = \frac{deaths}{P_{pop}} \qquad CFR = \frac{deaths}{P_{test}} \qquad (4.5)$$

$$\frac{P_{pop}}{P_{test}} = \frac{CFR}{IFR} \qquad (4.6)$$

Substitution of this relation in equation 4.5 gives

$$\log\left(\frac{CFR}{IFR}\right) = k\rho \qquad (4.7)$$

which is equivalent to

$$\log(CFR) = \log(IFR) + k\rho \qquad (4.8)$$

Equation 4.8 reveals that the y-intercept of a linear regression of  $\rho$  and log-transformed CFR can be exponentiated to yield the true IFR. We compile a comprehensive list of all countries that have recorded COVID-19 associated deaths as of April 21, 2020 using two global data sources (worldometer.org = 139 countries, ourworldindata.org = 80 countries). After calculating the CFR,  $P_{test}$ , and  $\rho$  for each country using cumulative cases, deaths, and tests performed, a weighted linear regression was performed according to equations 4.8 and 4.24. Because ourworldindata.org provides archived data, we are able to compare results achieved at April 21 to the 5 days before and 5 days after to understand how daily

variation in the estimate compares to the confidence intervals of the IFR estimate. April 21 represents a date near the peak in global daily deaths, and therefore provides data prior to changes in the relationship between CFR and  $\rho$  occurring after lock-down measures for most countries (SI 6.11.8). We also obtain estimates using a different approach to data selection where cumulative data for each country was selected as the 50th day since the first recorded death. This approach minimizes the effect of differences in epidemic stage in each country on the IFR estimate.

In the regression performed, countries are inverse variance weighted; in the absence of variance attributed to testing regimes for each country, we assume homoscedasticity and variance of each estimate is inversely proportional to the number of tests performed (SI 6.11.3). Confidence interval calculations are given in SI 6.11.5. Because variance of each country's estimate is not necessarily related to the variance of the global estimate (regression line) at the corresponding sample prevalence, we standardize residuals through a modified procedure that can account for both (unequal) variances. A t-test for unequal variances was performed for each country, comparing its  $\log(CFR)$  to the  $\log(\hat{CFR})$  predicted by the linear regression for the given  $\rho$ . Low p-values indicate a low reported CFR relative to the global mean for a given  $\rho$ , while high p-values indicate a high reported CFR relative to the global mean for a given  $\rho$ . P-values that are exceptionally low ( $p < 0.05$ ) or exceptionally high ( $p > 0.95$ ) indicate significant deviation from the expected global CFR. Through linear regression we investigated whether these deviations might be explained by



per capita GDP, the fraction of the populace 65 years and older, or healthcare access and quality as defined by each country's HAQ Index (Fullman et al., 2018), for these national metrics could correlate with the national IFR. Additionally, these three variables were evaluated as possible predictors for a country's reported CFR, independent of the relationship to testing capacity. Evaluation occurred through model selection via AIC in a GLMM framework using R Stan.

### 4.2.1 Role of the funding source

Excepting the authors, Stanford University had no influence on the study's design, conduct, or reporting.

## 4.3 Results

The transformation of the data from 139 countries according to equation 4.8 yields a linear relationship between sample prevalence ( $\rho$ ) and the logarithm of reported CFR for each country. The weighted regression produces estimates for the y-intercept at  $-4.40 = \log(\hat{IFR})$  and slope at  $8.80 = \hat{k}$ .  $R^2 = 0.5524$ , signaling that the relationship described between testing capacity and reported CFR explains over 55% of the variation in the reported data (Figure 4.2A) (SI 6.11.4). Exponentiating the y-intercept estimate yields a global IFR of 0.0123, or 1.23%. 95% confidence intervals for  $IFR$  and  $k$  are (0.0096, 0.0157) and

(8.1, 9.5), respectively. Because recent evidence suggests a false negative rate for SARS-COV-2 RT-PCR based tests near 30% (Kucirka et al., 2020), we adjust the calculations of capacity and CFR for each country and perform an adjusted regression which yields an intercept at  $-4.76 = \log(\hat{IFR})$ . Additionally, crude CFR estimates for some countries by April 21, 2020 may underestimate the true CFR due to the lag in deaths that may occur after reporting of cases. We calculated this adjustment to  $\log(CFR)$  as 0.005 (SI 6.11.10). The adjusted IFR accounting for false negative tests and time censoring of the CFR is 0.86%. This IFR estimate is cross-validated with a smaller but more transparently curated global dataset provided by ourworldindata.org. 80 countries in this dataset have provided data, and applying the same regression, we achieve an adjusted IFR estimate of 0.95% (Figure 4.2B). Averaging these estimates yields a global IFR estimate of 0.90% (CI: 0.70%, 1.16%). These results support a global IFR between 0.70% and 1.16%. This estimate implicitly accounts for asymptomatic cases and country-level differences in demography and testing regimes, and as such, it represents a global average.

Performing the same procedure for other dates in April yields nearly identical results, as does using different criteria for choosing dates to obtain cumulative data, suggesting the IFR estimate provided above is a robust estimate for the early phase of the pandemic (SI 6.11.8). Variation is small relative to the uncertainty captured by the confidence interval provided. Additional considerations and limitations can be found in SI 6.11.12.

Reported here is a function relating sample prevalence to the CFR estimate, and deviation from that function by a country may convey information of the underlying IFR. Placement above the regression line in panel A of Figure 4.2 could indicate higher national true IFR than the expected global average. Among the most reliable factors to predict IFR from COVID-19 are income, healthcare access and quality, and the age structure of the population, particularly the proportion of individuals 65 and older (Hopman et al., 2020; F. Zhou et al., 2020). We tested whether the reported CFR for each country was higher than the expected global mean at the corresponding sample prevalence.  $p$ -values  $< 0.05$  denote a lower CFR than the global mean, while  $p$ -values  $> 0.95$  denote a higher CFR than the global mean. Figure 4.3 plots these  $p$ -values with World Bank data (2018) on country level per capita GDP and the percent of the populace 65 years or older as well as each country's HAQ Index, which is a derived metric of healthcare access and quality (Fullman et al., 2018; World Bank, 2018a, 2018b). Linear regressions reveal slight trends in favor of a lower CFR with increasing GDP and HAQ Index and higher CFR with increasing age. These trends are marginal, and correlation between deviation from the global estimate and these factors is not substantiated. Rather, deviation is likely due to differences in COVID-19 reporting practices, testing, and stage of the epidemic in each country. Table 4.1 gives a list of the countries deviating significantly from the predicted CFR (all countries listed in SI 6.11.6). Countries with high  $p$ -values have a lower than expected CFR, which could indicate more conservative guidelines for attributing deaths to COVID-19. Conversely,

countries with low  $p$ -values have a higher than expected CFR, which could occur via liberal guidelines for attributing deaths to COVID-19. Alternatively, extreme  $p$ -values could occur when testing does not prioritize at-risk groups (e.g. occurs randomly) or when testing reporting differs substantially from other countries, barring influence from risk factors like age, income, or access to healthcare.

In the weighted regression performed via equations 8 and S16, national per capita GDP, the percent of the populace 65 years and older, and HAQ Index are only weakly informative of a country's reported CFR at the respective sample prevalence, therefore the slope, intercept, and associated uncertainty in these measurements are due primarily to testing differences among countries. However, this does not mean GDP, age, or healthcare access are not important contributors to a nation's CFR. Moreover, these factors may improve the estimate of a country's reported CFR over that provided by sample prevalence alone. Generalized linear mixed models including these three additional predictor variables were compared via AIC to reveal that the model including 3 variables,  $\rho$ , % 65+, and HAQ Index, out-performed other models, including the model with  $\rho$  alone (SI 6.11.9). Results of the model comparison are reported in Table 4.2. Alone, neither GDP, healthcare access, nor age were strong predictors of a nation's reported CFR (Fig. 4.11-4.13). The weak relationships aligned well with the relationships shown in Figure 4.3, where CFR increases with an aging populace and decreases with income and access to healthcare. Due to the weakness of prediction, these models were not included in model comparison. Eight models were

compared, expanding on  $\rho$ . Age, GDP, and HAQ Index marginally improved the model fit, though due to significant correlation between GDP and HAQ Index, GDP is less informative when both are included in the model. These results suggest a small influence of these factors in a country's CFR, confirming that future work investigating factors contributing to a country's CFR should include sample prevalence as defined in this text as well as age and HAQ Index (or, less preferably per capita GDP). We find that sample prevalence is the strongest of the predictors, and future investigation of other correlates may find it important to consider as a covariate.

## 4.4 Discussion

Estimating the infection fatality rate from the case fatality rate is not possible with limited testing capacity, though many policies regarding the COVID-19 pandemic rely on estimates of the severity of the disease. IFR is the primary metric upon which decisions have been based, and estimates range from 0.1% to over 2% (Bendavid et al., 2020; Famulare, 2020; Mizumoto et al., 2020; Verity et al., 2020a; WHO, 2020). The variety in reported CFR values stems from the variation in demography of study groups, income, access to health-care, testing fidelity, or study design. Lack of large sample size or non-random selection of study participants hinders true estimates of the IFR in any locality. The estimate of IFR in one locality will differ from the IFR in another due to differences in underlying health conditions, demography, and medical treatment. Rather than understanding the extent of

the variability these factors can create in IFR measurements, we derive a new approach to IFR estimation using global data. Provided testing has prioritized people at highest risk of SARS-COV-2 infection, the sample prevalence as used in our approach should reflect each country's ability to accurately estimate the true IFR. Despite variation in reporting and testing strategy, we do find a close relationship between sample prevalence and reported CFR. Moreover, the relationship aligns well with the predicted relationship given assumptions regarding the discovery of new cases with changing sample prevalence. Transformation of the data using equation 4.8 results in a linear relationship shown in Figure 4.2, and the weighted regression provides a precise estimate of the global IFR of 0.90%. This estimate will vary by country, and the equations detailed in this study should be used to estimate country level IFR with many independently derived samples of varying sample prevalence as shown in this study. Equation 4.3 approximates global discovery of true positive cases, but it may not be reliable at smaller scales due to variation in testing protocol.

Deviation of reported CFR by each country from the global mean estimated from the regression line does not necessarily reflect true differences in national IFR from the global IFR. There is little to no correlation between these deviations and GDP, age, or health-care access and quality which are considered proxies of risk for mortality from COVID-19 (Hopman et al., 2020; F. Zhou et al., 2020). Instead, deviations likely represent variation in testing and reporting strategies. Table 4.1 gives the countries outside of the 90% confidence interval of the estimated global mean at their reported  $\rho$ . These countries likely

vary from expectation due to testing differences that change estimates of testing capacity or differences in reporting deaths from COVID-19. Additional variation can occur because COVID-19 associated deaths lag diagnosis, so countries early in an epidemic may report a lower than expected CFR.

COVID-19 associated deaths may be positively identified through a PCR-based test or reported if medical diagnostic criteria are met as judged by a physician (Cascella et al., 2020). Due to comorbidities, such as hypertension or diabetes, estimates of COVID-19 deaths have been questioned. Arguments that death coinciding with a positive SARS-COV-2 test does not necessarily meet criteria for a COVID-19 associated death have been made. Given results of this work that indicate the risk of death to be just under 1% from COVID-19, which is higher than the risk of death in the same 2-3 week interval from common chronic comorbidities (Arima et al., 2011; Gregg et al., 2007), the probability that a SARS-COV-2 positive death could be attributed to COVID-19 is higher than the probability the death could be attributed to other chronic comorbidities. In an estimate of 0.1% probability of mortality in a 2 week window due to existing comorbidities, the probability that death of an infected patient with a pre-existing condition is primarily due to the existing condition is 10% (SI 6.11.7). In this case, the reported CFR needs to be adjusted to 90% of its value. In light of this, the vast majority of SARS-COV-2 positive deaths are due in whole or primarily to COVID-19. High reported CFR values are a result of under-counting of positive infections rather than over-counting COVID-19 associated deaths. This contrasts

needed adjustments in deaths associated with influenza, as the lower IFR of active influenza infections leads to a higher coincidence of mortality due to chronic disease and infection relative to the incidence of influenza associated mortality alone (Faust & Del Rio, 2020). In some localities it is estimated that COVID-19 associated deaths are under-reported by 25-50% because these deaths occur outside of a hospital setting (Ciminelli & Garcia-Mandico, 2020). Under-reporting of deaths is likely to outweigh over-reporting due to comorbidities in many localities.

The inflation of reported CFR values can be attributed primarily to testing capacity as it relates to sample prevalence, as we show in this study. The remaining 45% of the variation in the data unexplained by sample prevalence is likely explained by a mixture of variability in national reporting, testing strategies, and stage of the epidemic with select cases explained by demography, underlying health conditions, income, or availability of treatment. The methods provided in this work and the supplement are easy to translate to other studies when high quality data and independent samples are available. We hope this approach can be used to give updated estimates of the global IFR as high quality data becomes available for countries through the pandemic. As more data becomes available for infections and COVID-19 deaths, case fatality rates can be more reliably approximated, which can be translated to a global IFR. Temporal or regional variation in testing strategy affects the relationship between CFR and sample prevalence, therefore cumulative data collected at least 1 month after the first recorded COVID-19 death in each country better represents the



relationship than point estimates (SI 6.11.8). Best estimates rely on standardized global reporting practices, though this method is robust despite differences. Failure to report reliable data impairs resolution of the global IFR, which is crucial in providing a predictive framework for epidemiologists and policy makers to inform appropriate control measures.

The aim of this study was to provide a reliable metric that enables a robust estimate of the global IFR capturing a wide range of variation without accounting for underlying variability that is necessary to give point estimates of the IFR in each locality. Though we provide the first measurement, the estimate of a global IFR of 0.90% is dynamic and can be updated with incoming data. This number is lower than the IFR for the Diamond Princess cruise (2%), whose demography was skewed toward older individuals (Mizumoto et al., 2020). The older age of this sample may be partially counterbalanced by the higher income and access to healthcare of the majority of these individuals than the global average. National surveys performed in Iceland have estimated an IFR below 0.6%, which is among the most randomized testing conducted in any nation, though much of these cases have affected a younger group than is representative of the nation (Stock et al., 2020). A previous estimate of IFR in China of 0.66% aligns well with projections from Iceland (Verity et al., 2020a). Seroprevalence studies have inferred widely different estimates of IFR, including a non-random sample of Santa Clara county residents in CA that suggested an IFR of 0.12-0.2% (Bendavid et al., 2020). NYC seroprevalence of grocery shoppers suggests approximately one of every 5 NYC residents has been exposed. A recent study concluded

the city-wide IFR as 0.86% (Wilson, 2020), while the city-wide IFR for Geneva, Switzerland is estimated at 0.64% (Perez-Saez et al., 2020). A large seroprevalence study in Spain revealed a national IFR estimate greater than 1.1% (Pollán et al., 2020). The variety of IFR estimates reflects study design and underlying risk factors that contribute to COVID-19 mortality. Although randomized seroprevalence surveys will provide the most accurate measurement of local IFR, these surveys cannot be conducted on a global scale, especially if the results of these surveys are to be used proactively to inform control strategies. Much of the current local estimates of IFR are representative of high income areas with high access to treatment. Higher IFR is possible in areas where surveys have not been conducted due to disparity in healthcare access or testing (Hopman et al., 2020). The IFR reported in this study attempts to capture some of the variability associated with these disparities between countries, which has otherwise been neglected in previous studies.

A single metric provided by the IFR reflects only mortality; rising evidence suggests long-term morbidity could be associated with SARS-COV-2 infection (Henry & Lippi, 2020). The IFR presented here represents global mortality in the early stages of the pandemic, and though subsequent shielding of high-risk people from infection may reduce the IFR, the medical community knows little regarding the long-term health consequences of infection in people now considered low-risk for death. Evaluation of chronic and sub-acute conditions associated with COVID-19 will be critical to fully evaluate the impact of this virus.

Most importantly, the realized IFR is dependent on healthcare capacity, which can vary with conditions of the pandemic. High case load can overwhelm hospital staff and resources, leading to high realized IFR. This is a possible explanation for the high departure of Italy's reported CFR from the global estimate for equivalent testing capacity (Onder et al., 2020). We estimate the IFR for Italy as 1.33% (SI 6.11.8), which aligns closely with a recent estimate of 1.31% provided by Rinaldi and Paradisi (Rinaldi & Paradisi, 2020). The IFR for Italy is likely higher than for countries of equivalent GDP and healthcare access due to the spike in cases that occurred in March 2020 that exhausted hospital resources in the northern regions. Proper control measures and testing can minimize infection spikes to levels that hospitals can manage, reducing the IFR.

## **4.5 Contributors**

REG conceived the study, extracted and analyzed data, developed the methods and code, and wrote the manuscript. GDL edited the manuscript and contributed to the analysis and final draft.

## **4.6 Declaration of interests**

Authors declare no competing interests.

## 4.7 Data sharing

All data was collected from publicly available repositories:

<https://www.worldometers.info/coronavirus/>,  
<https://www.worldbank.org/>, <https://www.ourworldindata.org/>,  
<https://github.com/pcm-dpc/COVID-19/tree/master/dati-regioni>,  
and (Fullman et al., 2018). P-values are available in the supplement, and code is available  
at <https://github.com/grewelle/globalCovidIFR>.

## 4.8 Acknowledgements

Many thanks go to the De Leo lab for support and to Kalin Wilson for additional comments on drafts of this manuscript. REG is funded by the Stanford Graduate Fellowship and Stanford Biology Department.

## 4.9 Tables

| Country    | P-value |
|------------|---------|
| Italy      | 2.27E-6 |
| Venezuela  | 0.013   |
| Canada     | 0.023   |
| Hong Kong  | 0.950   |
| Uzbekistan | 0.972   |
| Russia     | 0.994   |
| UAE        | 0.994   |
| Qatar      | 0.998   |
| Singapore  | 0.999   |

Table 4.1: Countries that deviate substantially from the global trend in reported CFR. Countries are ordered by P-value (low to high). The dashed line separates countries with significantly higher ( $\alpha < 0.05$ ) reported CFR from countries with significantly lower ( $\alpha > 0.95$ ) reported CFR.

| Model                                               | WAIC  | dWAIC | Weight |
|-----------------------------------------------------|-------|-------|--------|
| $\rho + \text{Age} + \text{HAQ}$                    | 333.6 | 0.0   | 0.62   |
| $\rho + \log(\text{GDP}) + \text{Age} + \text{HAQ}$ | 335.0 | 1.4   | 0.31   |
| $\rho + \log(\text{GDP}) + \text{Age}$              | 338.1 | 4.5   | 0.07   |
| $\rho + \log(\text{GDP})$                           | 348.1 | 14.6  | 0.00   |
| $\rho + \text{HAQ}$                                 | 350.0 | 16.4  | 0.00   |
| $\rho + \log(\text{GDP}) + \text{HAQ}$              | 350.2 | 16.6  | 0.00   |
| $\rho$                                              | 351.0 | 17.4  | 0.00   |
| $\rho + \text{Age}$                                 | 352.0 | 18.4  | 0.00   |

Table 4.2: Ranked model comparison demonstrating national CFR predictions are best informed by  $\rho$ , % of the nation 65+ in age, and healthcare access and quality. Per capita GDP is a relevant predictor, though it strongly correlates with HAQ Index, which is a better predictor. Lower WAIC values correspond to better model performance, and the reported weight is the proportional explanatory value of each model.

## 4.10 Figures

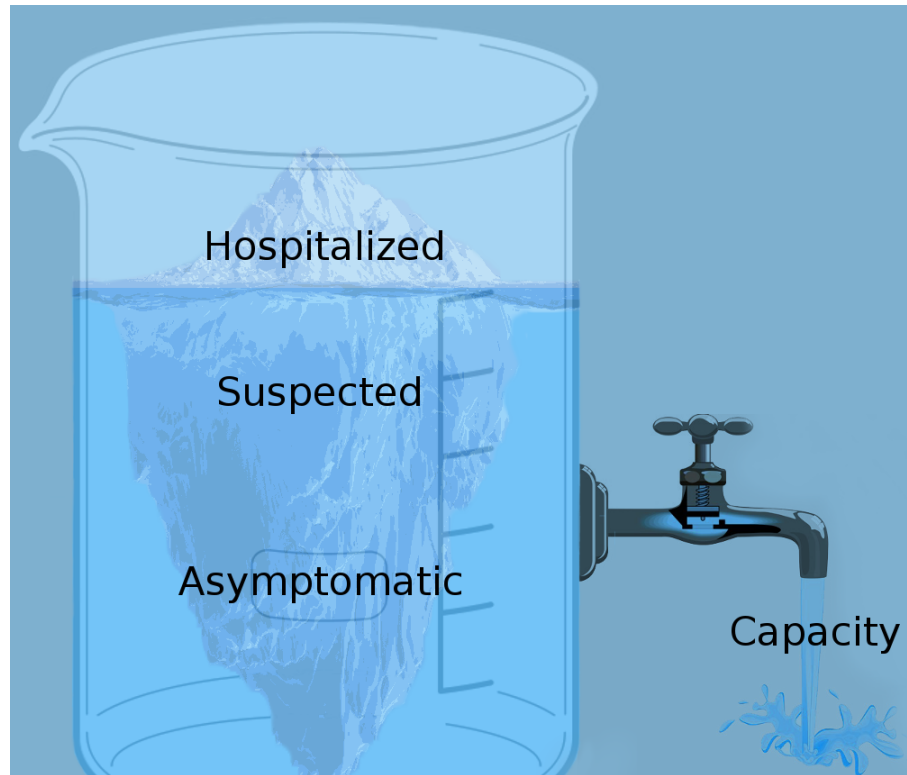


Figure 4.1: Increasing testing capacity uncovers a broader group of individuals, reducing estimates of the CFR for each country. Full capacity allows for estimation of the IFR.

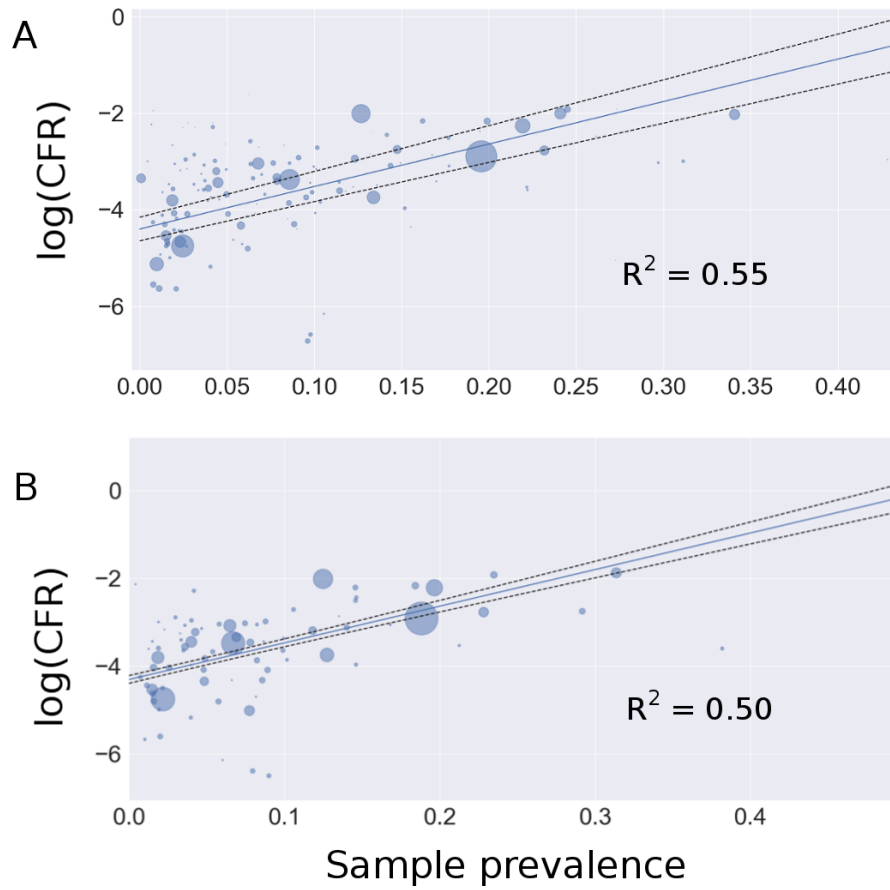


Figure 4.2: Weighted regression of reported national CFR and testing capacity. Circle area is proportional to each country's weight. The intercept corresponds to the logarithm of the estimated global IFR. Panel A: analysis of 139 countries given by worldometer.org. Panel B: analysis of 80 countries given by ourworldindata.org. Black dashed lines represent 95% confidence intervals of the mean.



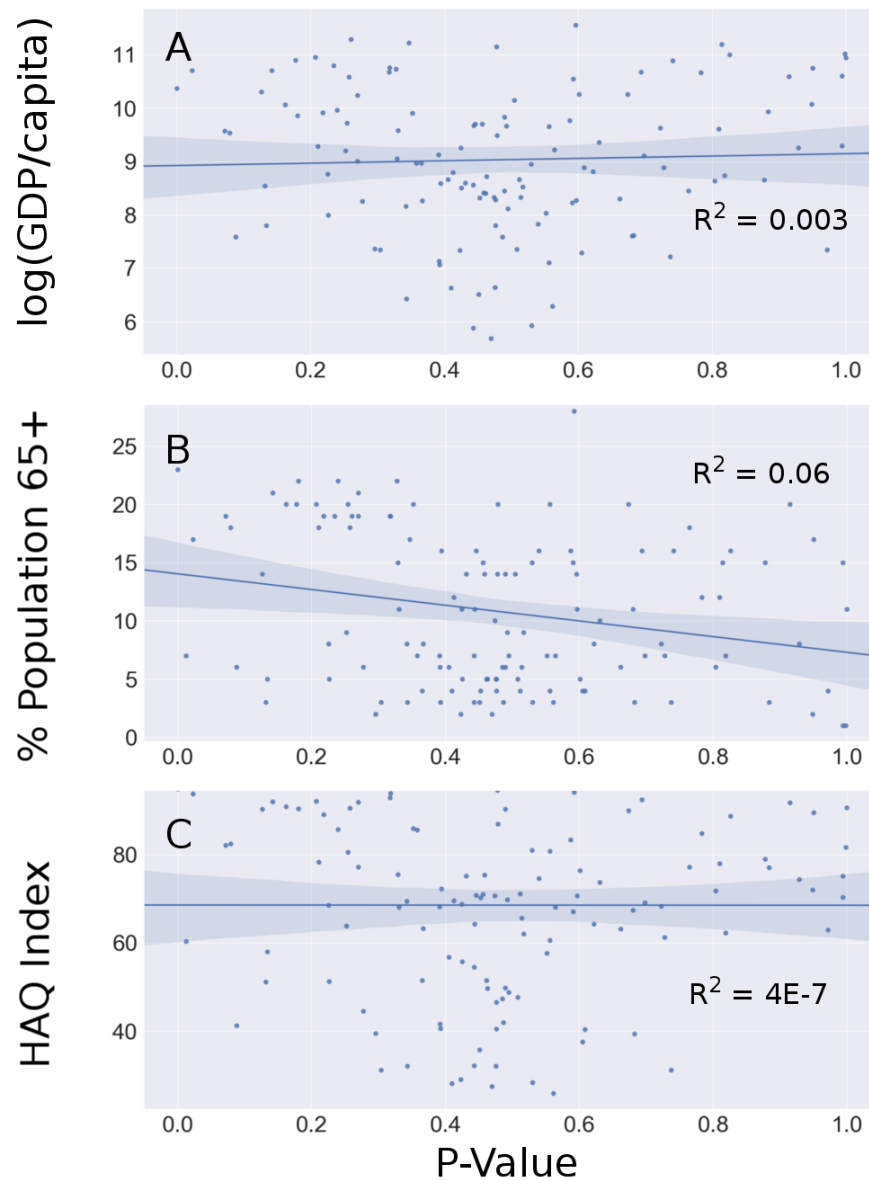


Figure 4.3: Relationships between p-values of the deviation of each country's reported CFR from the global mean and risk factors for mortality from COVID-19. Larger p-values correspond to lower reported IFR relative to the global mean. Panel A: per capita GDP serves as a proxy for income, with lower income being a known risk for COVID-19 mortality. Per capita GDP is not predictive of differences in national CFR relative to the global mean. Panel B: the proportion of individuals 65 and older in the national population is also not predictive of national deviation from global CFR estimates. Panel C: healthcare access and quality, given by each country's HAQ Index also does not provide additional information regarding a country's position relative to the global mean represented by the regression line.

## 4.11 Supplemental Information

### 4.11.1 Equation 4.3

Equation 4.3 in the main text can be derived from simple principles given a finite population of size  $n$ . In this population there is an underlying prevalence of COVID-19 infection such that this true prevalence ( $\overline{prev}$ ) is represented by

$$\overline{prev} = \frac{P_{pop}}{n} \quad (4.9)$$

Discovery of the true number of cases,  $P_{pop}$  in the finite population with increased sampling depends on the information available on the likelihood of infection with COVID-19. For a discrete process whereby sampling of the population for COVID-19 cases occurs iteratively, such that a defined subsample of the population is tested in each iteration, the proportion of the population sampled can be denoted  $z$ . Each subsample,  $s$ , changes the available prevalence of cases in the finite population when information is available to bias sampling in favor of testing COVID-19 positive individuals. This information exists because of the presence of symptoms or suspicion of exposure to infection. The bias associated with the information is denoted  $\lambda > 1$ . When  $\lambda = 1$ , sampling is random. Bias can be defined as the fold increase in likelihood of sampling an infected individual relative to sampling an uninfected individual. The prevalence of COVID-19 positive individuals in the unsampled population can be represented by  $r_s$  where  $r_0 = \overline{prev}$  is the true prevalence

(prior to sampling without replacement) in the population. Each iteration,  $s$ , is not by definition a time-dependent index. Rather, it is related to the number of tests conducted on the finite population, and is thus associated with sampling effort. When the population size is fixed,  $s$  can be indirectly related to time by way of the time taken to conduct  $s$  rounds of tests. The following relation can be found:

$$r_{s+1} = \frac{r_s - \lambda r_s z}{1 - z} = r_s \left( \frac{1 - \lambda z}{1 - z} \right) \quad (4.10)$$

where  $z \ll 1$ . It follows that

$$r_{s+\delta} = r_s \left( \frac{1 - \lambda z}{1 - z} \right)^\delta \quad (4.11)$$

Therefore, the following relation is true:

$$r_\delta = r_0 \left( \frac{1 - \lambda z}{1 - z} \right)^\delta \quad (4.12)$$

The sample prevalence can be represented as

$$\rho_\delta = \lambda r_\delta \quad \rho_0 = \lambda r_0 \quad (4.13)$$

Substitution yields

$$\frac{\rho_\delta}{\lambda} = \frac{\rho_0}{\lambda} \left( \frac{1 - \lambda z}{1 - z} \right)^\delta \quad (4.14)$$

Rearrangement gives

$$\frac{\rho_\delta}{\rho_0} = \left( \frac{1 - \lambda z}{1 - z} \right)^\delta \quad (4.15)$$

The total number of positive test results achieved can be calculated as

$$P_{test} = \int_0^\delta r_0 n \left( \frac{1 - \lambda z}{1 - z} \right)^s ds = r_0 n \frac{\left( \frac{1 - \lambda z}{1 - z} \right)^\delta - 1}{\log\left(\frac{1 - \lambda z}{1 - z}\right)} \quad (4.16)$$

From equation 4.9, the number of cases in the population is

$$P_{pop} = r_0 n \quad (4.17)$$

Combining equations 4.16 and 4.17 yields

$$\frac{P_{test}}{P_{pop}} = \frac{\left( \frac{1 - \lambda z}{1 - z} \right)^\delta - 1}{\log\left(\frac{1 - \lambda z}{1 - z}\right)} \quad (4.18)$$

From equation 4.15 we find

$$\frac{P_{test}}{P_{pop}} = \frac{\frac{\rho_\delta}{\rho_0} - 1}{\log\left(\frac{\rho_1}{\rho_0}\right)} \quad (4.19)$$

As  $\rho_0$  is dependent on the true prevalence in the population and is the maximum in the

set  $\{\rho_s\}$ , the sample prevalence at  $\delta$  can be adjusted to a reported sample prevalence  $\rho$  that can vary between 0 and 1 rather than 0 and  $\rho_0$ .

$$k\rho_\delta = \frac{\rho_\delta}{\rho_0} \quad (4.20)$$

Denoting  $\Delta$  as the quotient of  $\rho_1$  and  $\rho_0$  gives a simplified equation

$$\frac{P_{test}}{P_{pop}} = \frac{k\rho_\delta - 1}{\log(\Delta)} \quad (4.21)$$

Given  $\lambda > 1$ ,  $P_{test}$  must converge to  $P_{pop}$  as  $\rho_\delta \rightarrow 0$ . Therefore,  $\log(\Delta)$  has a unique real valued solution at -1, and the equation becomes

$$\frac{P_{test}}{P_{pop}} = 1 - k\rho_\delta = 1 - e^{-\delta} \quad (4.22)$$

The Taylor expansion of  $e^{-k\rho_\delta}$  at  $\rho_\delta = 0$  is  $1 - k\rho_\delta + O(\rho_\delta^2)$ , therefore supplemental equation 4.22 can be approximated as

$$\frac{P_{test}}{P_{pop}} \approx e^{-k\rho_\delta} \quad (4.23)$$

$\rho_\delta$  is the sample prevalence at a given iteration, and is the same as  $\rho$  given in the main text. Equation 4.22 is an exact relation that represents a closed system in which sampling

occurs much more quickly than the accumulation of new infections. Even under slow sampling, convergence to the true population case load occurs at a constant rate. However, when new cases occur at a similar rate to testing, convergence should occur more slowly. Equation 4.23 has the capacity to capture this property more accurately at high  $\rho_\delta$  (slow sampling) than equation 4.22 while maintaining the same properties at low  $\rho_\delta$  (high sampling).

Equation 4.3 in the main text reports the relationship between testing capacity and the convergence of the positively identified COVID-19 cases and the true number of cases in the population, which is the sum of the identified and unidentified active cases. This relationship will differ with testing regime, as in each locality procedures for administering tests vary, including the frequency of re-testing that occurs. However, the function should approach 1 as  $\rho \rightarrow 0$  and  $C \rightarrow \infty$ . With a higher proportion of symptomatic patients, more information is available to prioritize testing, so convergence of the estimate of cases occurs more rapidly with increasing capacity.  $\frac{P_{test}}{P_{pop}} = e^{-k\rho}$  provides a simple function with the desired property of approaching 1 as  $\rho \rightarrow 0$ . Use of this equation provides the derivation of equation 4.8 in the main text, which gives the relationship between CFR and  $\rho$ . Transforming the data by inverting the x-axis and log-transforming the y-axis produces a linear relationship as predicted by equation 4.22. In this way, the use of equation 4.3 is supported, though because it is not otherwise empirically verified, its use should not be extended to approximate local case loads. As mentioned in the main text and here, variation

in testing protocol invalidate use of equation 4.3 at small scales.

### 4.11.2 Parameter definitions

| Parameter               | Definition                                                                  |
|-------------------------|-----------------------------------------------------------------------------|
| $C$                     | Testing capacity                                                            |
| $T$                     | No. SARS-COV-2 tests conducted in country                                   |
| $P_{test}$              | No. positive test results in country                                        |
| $\rho$                  | Sample prevalence at given testing effort $\delta$ (same as $\rho_\delta$ ) |
| $P_{pop}$               | No. cases in country at a given time                                        |
| $k$                     | Slope of linear regression and decay constant                               |
| $CFR$                   | No. of deaths per positive test result                                      |
| $IFR$                   | No. of deaths per infection                                                 |
| $N$                     | No. of countries                                                            |
| $\overline{prev} = r_0$ | Infection prevalence in population                                          |
| $n$                     | Size of population                                                          |
| $z$                     | Proportion of population tested per iteration $t$                           |
| $s$                     | Index of sampling (testing) effort                                          |
| $\delta$                | Total observed testing effort                                               |
| $\lambda$               | Sampling bias in favor of infected individuals                              |
| $r_s$                   | Infection prevalence after $\delta$ testing effort                          |
| $\Delta$                | $\rho_1/\rho_0$                                                             |
| $\sigma_i^2$            | Sample variance for country $i$                                             |

Table 4.3: List of parameters in order of appearance. The dashed line separates parameters defined in the main text from those defined in the supplement.

### 4.11.3 Linear regression

The linear regression reported in Figure 4.2 (main text) was performed according to equation 4.24 with inverse variance weights.

$$\hat{\beta} = \min\left\{\sum_i w_i (\log(\hat{IFR}) + \hat{k}\rho_i - \log(CFR_i))^2\right\} \quad (4.24)$$

Variance in reported CFR values for each country is a function of the sample variance  $\sigma_i^2$  due to testing protocol, specificity, and accuracy, and the number of tests performed  $T_i$ .

$$\hat{Var}_i = \frac{\sigma_i^2}{T_i} \quad (4.25)$$

Because sample variance has not been measured for each country, we assume  $\{\sigma^2\}$  is homoscedastic across countries. The estimate of variance is therefore inversely proportional to the number of tests performed by each country. Weights are then assigned as

$$w_i = \frac{T_i}{\sum_i T_i} \quad (4.26)$$

These weights are used as the loss function on the squared residuals. Equation 4.24 is minimized with a ‘BFGS’ optimization algorithm provided by the Python `scipy.optimize.minimize` routine. Parameters  $\log(\hat{IFR})$  and  $\hat{k}$  are estimated through minimization of the weighted residuals, and the Hessian is numerically estimated and inverted. The variance-covariance matrix (**V**) of the parameter estimates,  $\log(\hat{IFR})$  and  $\hat{k}$ , is calculated as



$$\mathbf{V} = \frac{\mathbf{H}^{-1}(RSS)}{N - 2} \quad (4.27)$$

where  $\mathbf{H}^{-1}$  is the inverted numerically estimated Hessian,  $RSS$  is the weighted residual sum of squares minimized in equation 4.24, and  $N$  is the number of countries. This provides a scaled variance-covariance matrix that is multiplied by the  $RSS$  and divided by the degrees of freedom to give the variance-covariance matrix,  $\mathbf{V}$ .

The standard error of each parameter is

$$SE_i = \sqrt{\mathbf{V}_{ii}} \quad (4.28)$$

The covariance of  $\log(\hat{IFR})$  and  $\hat{k}$  is reported in the two off-diagonal entries of  $\mathbf{V}$ .

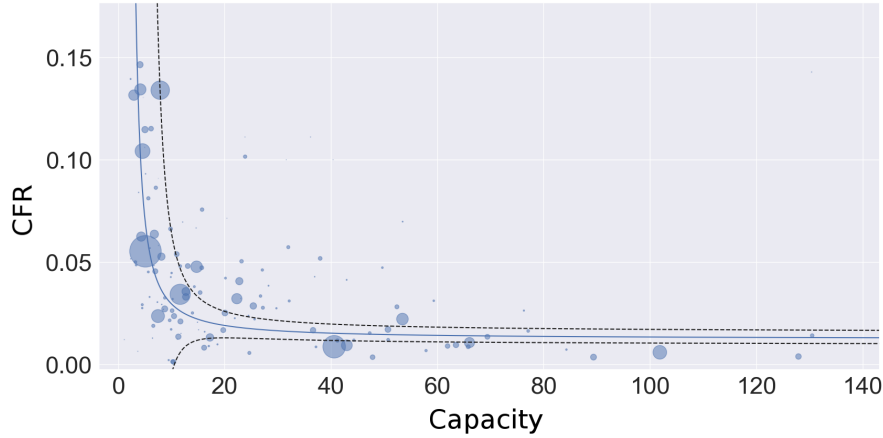


Figure 4.4: Plot of capacity vs CFR by country with superimposed estimated regression. In contrast to Figure 4.2 in the main text, the horizontal axis reports capacity (inverse of sample prevalence). Venezuela is not plotted due to its high capacity, though it resides near  $CFR = 0.04$

#### 4.11.4 $R^2$ calculation

The  $R^2$  reported from the linear regression in Figure 4.2A of the main text is given as follows:

$$R^2 = 1 - \frac{RSS}{TSS} \quad (4.29)$$

RSS is defined as:

$$RSS = \sum_i w_i ((\log(\hat{IFR}) + \hat{k}\rho_i) - \log(CFR_i))^2 \quad (4.30)$$

TSS is defined as:

$$TSS = \sum_i w_i (w_i \sum_i \log(CFR_i) - \log(CFR_i))^2 \quad (4.31)$$

#### 4.11.5 Confidence interval estimation

The linear regression on the log-transformed data provides a estimated line with normally distributed errors, and the 95% confidence intervals for  $\log(\hat{IFR})$  is easily approximated as  $\log(\hat{IFR}) \pm 1.96(0.124)$ , where 0.124 is the standard error of the estimated parameter. The same calculation provides a 95% confidence interval for  $\hat{k}$ , approximated as  $\hat{k} \pm 1.96(0.353)$ , where 0.353 is the standard error for  $\hat{k}$ . A confidence interval for  $\hat{IFR}$  requires exponentiating  $\log(\hat{IFR})$  to produce a log-normal distribution. Letting

$\hat{\alpha} = \log(\hat{IFR})$ , the confidence interval becomes  $(e^{\hat{\alpha}-1.96(SE_{\hat{\alpha}})}, e^{\hat{\alpha}+1.96(SE_{\hat{\alpha}})})$ . This interval can be used to propagate error in the nonlinear regression. To do so, we define values which represent the standard difference between the estimate and the 95% bounds:

$$\sigma_{IFR,0.975} = e^{\hat{\alpha}+1.96(SE_{\hat{\alpha}})} - e^{\hat{\alpha}} \quad \sigma_{IFR,0.025} = e^{\hat{\alpha}} - e^{\hat{\alpha}-1.96(SE_{\hat{\alpha}})} \quad (4.32)$$

The 95% confidence intervals for the linear regression can be calculated as

$$\begin{aligned} \log(\hat{CFR}) \pm 1.96 \left( \frac{\partial \log(CFR)}{\partial \log(IFR)} \right)^2 SE_{\hat{\alpha}}^2 + \frac{\partial \log(CFR)}{\partial k} SE_{\hat{k}}^2 + \\ 2 \frac{\partial \log(CFR)}{\partial \log(IFR)} \frac{\partial \log(CFR)}{\partial k} Cov(\hat{\alpha}, \hat{k}) \right)^{\frac{1}{2}} \end{aligned} \quad (4.33)$$

Partial derivatives are calculated as

$$\frac{\partial \log(CFR)}{\partial \log(IFR)} = 1 \quad \frac{\partial \log(CFR)}{\partial k} = C^{-1} \quad (4.34)$$

Therefore, the confidence interval simplifies to

$$\log(\hat{CFR}) \pm 1.96 (SE_{\alpha}^2 + C^{-2} SE_{\hat{k}}^2 + 2C^{-1} Cov(\hat{\alpha}, \hat{k}))^{\frac{1}{2}} \quad (4.35)$$

These intervals are reported as the dashed lines in Figure 4.2A (main text). Nonlinear

95% confidence intervals are estimated using the values defined in equation 4.33. Upper and lower bounds are described as

$$\begin{aligned}
 Upper : C\hat{F}R + & \left( \frac{\partial CFR^2}{\partial IFR} \sigma_{IFR,0.975}^2 + \frac{\partial CFR^2}{\partial k} (1.96(SE_{\hat{k}}))^2 + \right. \\
 & \left. 2(1.96) \frac{\partial CFR}{\partial IFR} \frac{\partial CFR}{\partial k} Cov(I\hat{F}R, \hat{k}) \right)^{\frac{1}{2}} \quad (4.36)
 \end{aligned}$$

$$\begin{aligned}
 Lower : C\hat{F}R - & \left( \frac{\partial CFR^2}{\partial IFR} \sigma_{IFR,0.025}^2 + \frac{\partial CFR^2}{\partial k} (1.96(SE_{\hat{k}}))^2 + \right. \\
 & \left. 2(1.96) \frac{\partial CFR}{\partial IFR} \frac{\partial CFR}{\partial k} Cov(I\hat{F}R, \hat{k}) \right)^{\frac{1}{2}} \quad (4.37)
 \end{aligned}$$

Partial derivatives are calculated as

$$\frac{\partial CFR}{\partial IFR} = e^{\frac{k}{C}} \quad \frac{\partial CFR}{\partial k} = \frac{I\hat{F}R}{C} e^{\frac{k}{C}} \quad (4.38)$$

Therefore, the confidence interval simplifies to

$$\begin{aligned}
 Upper : C\hat{F}R + & e^{\frac{k}{C}} (\sigma_{IFR,0.975}^2 + \frac{I\hat{F}R}{C} (1.96(SE_{\hat{k}}))^2 + \\
 & 2(1.96^2) \frac{I\hat{F}R}{C} Cov(I\hat{F}R, \hat{k}))^{\frac{1}{2}} \quad (4.39)
 \end{aligned}$$

$$\begin{aligned}
Lower : C\hat{F}R - e^{\frac{k}{C}}(\sigma_{IFR,0.025}^2 + \frac{I\hat{F}R}{C}(1.96(SE_{\hat{k}}))^2 + \\
2(1.96^2)\frac{I\hat{F}R}{C}Cov(I\hat{F}R, \hat{k}))^{\frac{1}{2}}
\end{aligned} \tag{4.40}$$

The covariance of the parameters in the linear regression,  $Cov(\hat{\alpha}, \hat{k})$ , is given by the Hessian-derived variance-covariance matrix,  $\mathbf{V}$ . However, because of the exponentiation of  $\alpha$  to derive  $I\hat{F}R$ , the covariance between the parameters no longer applies.  $Cov(I\hat{F}R, \hat{k}) \neq Cov(\hat{\alpha}, \hat{k})$ .  $Cov(\hat{\alpha}, \hat{k})$  requires derivation. Let  $\xi$  be the log-normally distributed random variable representing  $IFR$  with median at  $I\hat{F}R$ . Let  $\alpha$  be the normally distributed random variable representing  $\log(IFR)$  with mean at  $\hat{\alpha}$ .  $k$  is a normally distributed random variable with mean at  $\hat{k}$ .  $\xi = e^\alpha$

$$\begin{aligned}
Cov(I\hat{F}R, \hat{k}) &= E[(\xi - E[\xi])(k - \hat{k})] \\
&= E[(e^\alpha - e^{\hat{\alpha} + \frac{1}{2}\sigma_\alpha^2})(k - \hat{k})] \\
&= E[e^{\sigma_\alpha^{-1}(2\pi)^{-1/2}e^{-1/2((t-\hat{\alpha})\sigma_\alpha^{-1})^2}} - e^{\hat{\alpha} + \frac{1}{2}\sigma_\alpha^2})(k - \hat{k})] \\
&= E[\epsilon(\alpha - \hat{\alpha})(k - \hat{k})] \\
&= \epsilon Cov(\hat{\alpha}, \hat{k})
\end{aligned} \tag{4.41}$$

$$\epsilon \approx I\hat{F}R \quad (4.42)$$

$$Cov(I\hat{F}R, \hat{k}) \approx (I\hat{F}R)Cov(\hat{\alpha}, \hat{k}) \quad (4.43)$$

This approximation was verified by monte carlo simulation at  $10^7$  replicates.

#### 4.11.6 Statistical test for deviation

Statistical tests for outliers are routine in linear regression frameworks, and the approach usually involves standardizing, or normalizing, residuals. The standardization process incorporates knowledge of the expected variance of along the regression to report a statistical interpretation of the outlying behavior of points. This standardization procedure incorporates one form of variance, as it is used in unweighted regressions. In a weighted regression, whereby points are weighted inversely to the variance attributed to each point, this second form of variance becomes crucial in determining outlying behavior. We use both the variance calculated at each respective value,  $\rho$ , for the regression estimate as well as the variance of each point to perform a Welch's one-way T-test for unequal variances. This process produces p-values, and in doing so, maps the residuals to a uniform distribution of values between 0 and 1. The p-values represent the significance of the deviation of national CFR values from the expected regression estimate. A uniform mapping of deviations from the

expected regression allows a simple linear regression to be performed to determine whether the deviations align with known predictors of COVID-19 mortality, and are therefore informative of an underlying country-specific IFR. The p-values are determined specifically as follows:

The mean and standard error for the regression estimate are available for each value of  $\rho = C^{-1}$  corresponding to each nation's data. The standard error for reported national CFR values was calibrated to the confidence interval reported in the regression such that 95% of p-values fell within  $0.025 < p < 0.975$ . Calibration occurred assuming homoscedasticity in the sampling variance, and denominator of the variance estimate was given as the number of tests administered by each country. The p-values were used in the linear regressions of Figure 4.3 in the main text. Extreme p-values were reported in Table 4.1 of the main text. Table 4.4 of the supplement gives the full list of p-values by country.

Table 4.4: P-values for deviation analysis. Countries are ordered by caseload as of April 21, 2020. Higher p-values indicate lower than expected reported CFR. Lower p-values indicate higher than expected reported CFR.

| Country | P-value     |
|---------|-------------|
| USA     | 0.826336186 |
| Spain   | 0.270157541 |
| Italy   | 2.26846E-06 |

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| <b>Country</b> | <b>P-value</b> |
|----------------|----------------|
| France         | 0.914576609    |
| Germany        | 0.142246921    |
| UK             | 0.257076874    |
| Turkey         | 0.92898805     |
| Iran           | 0.803861253    |
| Russia         | 0.994236252    |
| Brazil         | 0.252094768    |
| Belgium        | 0.317549238    |
| Canada         | 0.022982151    |
| Netherlands    | 0.234649182    |
| Switzerland    | 0.260507996    |
| Portugal       | 0.23950239     |
| India          | 0.087766819    |
| Peru           | 0.621884336    |
| Ireland        | 0.477440109    |
| Sweden         | 0.177677078    |
| Austria        | 0.317627288    |

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Table 4.4 – *Continued from previous page*

| <b>Country</b> | <b>P-value</b> |
|----------------|----------------|
| Israel         | 0.783275514    |
| Saudi Arabia   | 0.883889659    |
| Japan          | 0.592299578    |
| Chile          | 0.81015236     |
| S. Korea       | 0.126123919    |
| Ecuador        | 0.818784914    |
| Poland         | 0.079106717    |
| Pakistan       | 0.60501945     |
| Romania        | 0.210758118    |
| Singapore      | 0.999855755    |
| Mexico         | 0.391216727    |
| UAE            | 0.993970499    |
| Denmark        | 0.207061155    |
| Norway         | 0.34702921     |
| Indonesia      | 0.277136975    |
| Czechia        | 0.21803765     |
| Serbia         | 0.764975613    |

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Table 4.4 – *Continued from previous page*

| <b>Country</b>     | <b>P-value</b> |
|--------------------|----------------|
| Belarus            | 0.877870455    |
| Australia          | 0.741042416    |
| Philippines        | 0.226028066    |
| Qatar              | 0.998365991    |
| Ukraine            | 0.539827104    |
| Malaysia           | 0.564438229    |
| Dominican Republic | 0.727902637    |
| Panama             | 0.722696067    |
| Colombia           | 0.225764624    |
| Finland            | 0.327381656    |
| Luxembourg         | 0.596243094    |
| Egypt              | 0.133888707    |
| South Africa       | 0.462760453    |
| Bangladesh         | 0.508061431    |
| Morocco            | 0.551665026    |
| Argentina          | 0.330781152    |
| Algeria            | 0.661886034    |

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| <b>Country</b>         | <b>P-value</b> |
|------------------------|----------------|
| Thailand               | 0.412721477    |
| Moldova                | 0.680310801    |
| Greece                 | 0.180227219    |
| Hungary                | 0.071451087    |
| Kazakhstan             | 0.698204121    |
| Bahrain                | 0.949006252    |
| Croatia                | 0.478666965    |
| Iceland                | 0.814416624    |
| Uzbekistan             | 0.972284664    |
| Iraq                   | 0.131697771    |
| Estonia                | 0.352250183    |
| Azerbaijan             | 0.514334326    |
| New Zealand            | 0.693552243    |
| Armenia                | 0.596890773    |
| Lithuania              | 0.254472221    |
| Slovenia               | 0.161957569    |
| Bosnia and Herzegovina | 0.393935057    |

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Table 4.4 – *Continued from previous page*

| <b>Country</b>  | <b>P-value</b> |
|-----------------|----------------|
| North Macedonia | 0.431056746    |
| Slovakia        | 0.586887575    |
| Cuba            | 0.329250092    |
| Afghanistan     | 0.561044649    |
| Ghana           | 0.682890952    |
| Hong Kong       | 0.950311445    |
| Bulgaria        | 0.270178118    |
| Djibouti        | 0.831238788    |
| Tunisia         | 0.342506553    |
| Cyprus          | 0.489822576    |
| Nigeria         | 0.486822923    |
| Latvia          | 0.556187427    |
| Andorra         | 0.640812433    |
| Lebanon         | 0.358088409    |
| Costa Rica      | 0.63092592     |
| Niger           | 0.530106254    |
| Albania         | 0.458448083    |

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| <b>Country</b>  | <b>P-value</b> |
|-----------------|----------------|
| Bolivia         | 0.494696456    |
| Kyrgyzstan      | 0.556198556    |
| Uruguay         | 0.456515469    |
| Channel Islands | 0.49501435     |
| Honduras        | 0.476357048    |
| San Marino      | 0.524252782    |
| Palestine       | 0.615918068    |
| Malta           | 0.673737286    |
| Jordan          | 0.452787797    |
| Taiwan          | 0.474240598    |
| Senegal         | 0.737245538    |
| Georgia         | 0.590654083    |
| Mauritius       | 0.424734372    |
| Montenegro      | 0.529807476    |
| Mayotte         | 0.588310991    |
| Sri Lanka       | 0.474131866    |
| Isle of Man     | 0.515523712    |

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| <b>Country</b>      | <b>P-value</b> |
|---------------------|----------------|
| Kenya               | 0.295867433    |
| Guatemala           | 0.461236542    |
| Venezuela           | 0.0125414      |
| El Salvador         | 0.366511736    |
| Jamaica             | 0.516605842    |
| Paraguay            | 0.404899712    |
| Gabon               | 0.608519643    |
| Brunei              | 0.601341629    |
| Myanmar             | 0.391689589    |
| Trinidad and Tobago | 0.443444898    |
| Ethiopia            | 0.409772408    |
| Aruba               | 0.504099989    |
| Togo                | 0.343416797    |
| Bermuda             | 0.484882477    |
| Liechtenstein       | 0.535481231    |
| Barbados            | 0.445650504    |
| Zambia              | 0.42286905     |

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Table 4.4 – *Continued from previous page*

| <b>Country</b>      | <b>P-value</b> |
|---------------------|----------------|
| Sint Maarten        | 0.498089094    |
| Guyana              | 0.489465653    |
| Cayman Islands      | 0.522498783    |
| Libya               | 0.511674277    |
| Haiti               | 0.475863784    |
| Eswatini            | 0.47646627     |
| Zimbabwe            | 0.303789686    |
| Antigua and Barbuda | 0.492345939    |
| Botswana            | 0.365663737    |
| Belize              | 0.425067406    |
| Malawi              | 0.442945877    |
| Curacao             | 0.466133317    |
| Turks and Caicos    | 0.488545444    |
| Gambia              | 0.451386448    |
| Suriname            | 0.443242425    |
| Mauritania          | 0.392601151    |
| Burundi             | 0.469897304    |

#### **4.11.7 Comorbidities and assignment of death to COVID-19**

Estimating the mortality due to COVID-19 is not clear for many cases because infection can co-occur pre-existing conditions that put patients at risk of death upon infection. There is an argument to be made for accurately assigning cause of death, and infection with COVID-19 may not be sufficient. Analogies have been made to estimates of mortality due to influenza, which are notoriously variable depending on the criteria used to assign mortality. The vast majority of patients that die from influenza have pre-existing conditions, much like those that die from COVID-19. Therefore, many influenza associated deaths could be attributed to pre-existing conditions. Although for both influenza and COVID-19 infections, pre-existing conditions may be associated with higher risk of mortality, there are major differences in the responsibility comorbidities share in death between these infectious diseases. The primary difference is due to the greater IFR for COVID-19 and influenza. For symptomatic infections, the IFR-S for influenza is 0.1%. Accounting for asymptomatic patients, which may be between 15 and 85% of cases (we will assume a conservative estimate of 20%) (Furuya-Kanamori L et al., 2016), the IFR is approximately 0.08%. The estimated IFR for COVID-19 from this study is approximately 0.9%. There is mounting evidence suggesting overlapping comorbidities between influenza and COVID-19. Assuming the window of mortality due to infection from influenza or COVID-19 is



approximately 2 weeks, the probability of death from one of the most likely comorbidities like heart disease or diabetes in the two week window is approx.  $10^{-3}$  (after diagnosis and 50+ yrs of age, USA). The harmonic mean of the life expectancy for men (6.7 yrs) and women (7.9 yrs) over 50 diagnosed with heart disease in the USA is 7.25 yrs (Crimmins et al., 2008). The probability of death in a two week window assuming constant death rate is

$$Pr(c) = \frac{-\log(\frac{1}{2})}{7.25(26)} = 0.0037 \quad (4.44)$$

This equation gives the expected value of death in a two week window (26 windows of two weeks in a year). Although heart disease is the highest cause of death world-wide, performing the same calculations for diabetes, hypertension, or other pre-existing conditions associated with COVID-19 deaths yields  $Pr(c) < .001$ . Assuming contracting an infectious disease is independent of pre-existing conditions, the probability that the death of a patient with both a pre-existing condition and infection can be assigned primarily to the infection can be calculated. Given a death of a patient with a pre-existing condition ( $c$  = death by condition) and positive infection ( $i$  = death by infection), the probability the infection can be assigned the primary contributor to death is:

$$Pr(\bar{i}) = \frac{Pr(i \setminus c)}{Pr(i \cup c)} = \frac{Pr(i)(1 - \frac{Pr(i)Pr(c)}{Pr(i) + Pr(c)})}{Pr(i) + Pr(c) - Pr(i)Pr(c)} = \frac{Pr(i)}{Pr(i) + Pr(c)} \quad (4.45)$$

Given  $Pr(c) = 0.001$  and  $Pr(i) = IFR$ , the probability an influenza associated death is due to influenza is:

$$Pr(\bar{i}) = \frac{.0008}{.0008 + .001} = 0.44 \quad (4.46)$$

The probability a COVID-19 associate death is due to COVID-19 is:

$$Pr(\bar{i}) = \frac{.009}{.009 + .001} = 0.90 \quad (4.47)$$

Although these calculations are simplifications of a more complex process (e.g. death rates are not constant with age), the magnitude of the IFR has a dramatic influence on the adjustment needed to assignment of cause of death. Adjustments required for estimates of an IFR for COVID-19 are far smaller than the adjustments needed for influenza. Likely, these adjustments will be less than 10% for countries with an IFR above 0.9%. Lower adjustments are needed with fewer cases with comorbidities, and higher adjustments are needed when more than one comorbidity affect an individual. Assuming a 10% reduction in COVID-19 associated deaths would give a global IFR of 0.81%.

#### 4.11.8 Timing of regression estimation

The regression estimate provided in the main text is adjusted for temporal variation in the crude CFR, though it does not explicitly account for temporal variation in testing strategies

for each country. Variation in testing strategy can result in fluctuations in testing capacity (and sample prevalence). These variations can shape the relationship between sample prevalence and CFR, though the extent to which this relationship varies is particular to each country. Moreover, the largest changes in this relationship occur because of lockdown measures that can sharply change the trajectory of the pandemic. Figure 4.5 shows how the relationship between CFR and capacity can change over time with cumulative data for Italy.

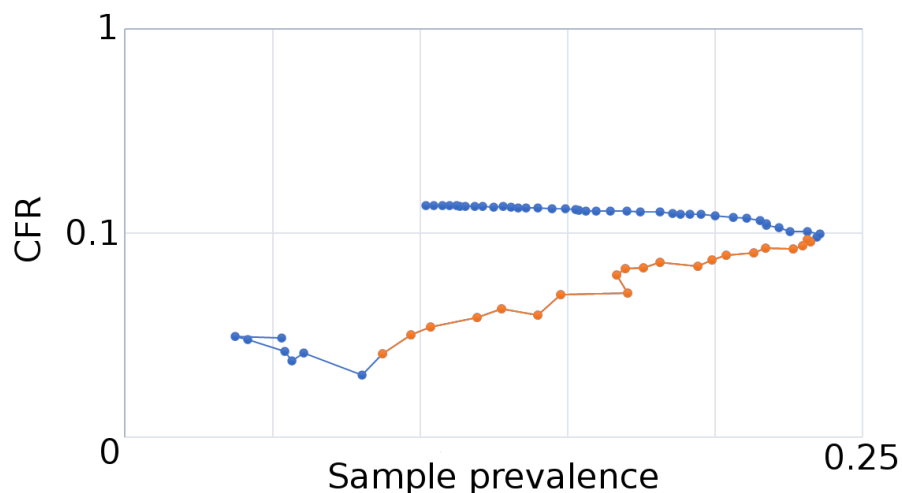


Figure 4.5: The relationship between CFR and capacity changes through the course of the epidemic in Italy. Orange points represent the rise in the epidemic (last orange point = March 22, 2020), demonstrating a close relationship between the scaling of testing and the rise in cases and fatalities. When new infections subside (as a consequence of lock-down measures), testing capacity outpaces the accumulation of new cases relative to the previous weeks, changing the relationship. The estimator works best near the peak of the epidemic. Y-axis is reported as log-scale.

Using cumulative data from any day in March would yield similar results due to the linear nature of the relationship between sample prevalence and  $\log(\text{CFR})$  in this period.

It becomes important to therefore, sample cumulative data on dates corresponding to similar phases of the pandemic for each country, such that the relationship between the CFR and sample prevalence are produced under similar conditions across countries. There is no precise way to do this because lock-down measures occurred on different dates for different countries, and some countries instituted these measures in earlier epidemic stages than other countries. We choose April 21, 2020 as a reasonable date because most countries had experienced several weeks of COVID-19 cases, so crude CFR values approximated the true CFR. This is evidenced by an average adjustment of just 0.005 for  $\log(\text{CFR})$  when compared to  $\log(\text{CFR})$  values on May 31, 2020 (SI 6.11.10). April 21st is just after the peak of global daily deaths from COVID-19, and therefore reflects a period shortly after effects of lock-down and social distancing measures were evident in many countries. Even before this date, sample prevalence for many of these countries declines, but at different rates depending on control measures. Analyzing data before April 21 would arguably produce the best estimates for sample prevalence globally, as the differences in control strategies would not weigh on the resulting changes in the sample prevalence in each country. Likewise, using data before April 21 best standardizes the test used. By mid-April, some countries, including the USA, reported data from both RT-PCR and serological tests. These changes become more pronounced after April, as a greater proportion of serological tests are incorporated in national data. Given these data limitations, April 21 represents a date that is late enough accurately reflect true CFR values while minimizing changes in testing capacity

and procedures that occur after the first weeks of April.

To minimize the possibility of erroneous IFR estimates, we compare two global COVID-19 datasets: worldometer and ourworldindata. The results are strikingly similar for April 21 (adjusted IFR = 0.85% and 0.94%), though data maintenance and recording differ between these databases and one dataset includes 139 countries, while the other includes 80.

The average estimate we provide in the main text is 0.9%. Choosing a single date is a reasonable approach given the approximate alignment of lock-down measures globally, though nations are at different stages of the pandemic. We test whether using a different criterion, namely standardizing by epidemic onset in each country, produces markedly difference results. A priori, we set this point for each country as 50 days after the first recorded COVID-19 associated death. Because the ourworldindata.org dataset is archived, we were able to curate a dataset using data sampled at 50 days from the first recorded death in each country, or the closest date with available data. For the 80 countries, these dates varied from April 2 to May 29, with a median date of May 8. The adjusted IFR estimate for these 80 countries is 0.99%. However, when 2 outliers with high leverage due to unreasonably high sample prevalence (when adjusted by false negative test result, both are greater than 1) are excluded, a regression of the remaining 78 countries provides an adjusted IFR estimate of 0.85%. Despite the use of much different sampling criteria and a different median date, the resulting IFR estimate is very similar to that provided by the April 21 estimate. Importantly, any estimate with or without high leverage outliers falls well within the 95%

confidence intervals given in the main text.

We additionally tested the sensitivity of the IFR in the dates around April 21, as these dates should arguably produce similar results and are similarly good candidate dates for the reasons outlined above. Applying the same regression and adjustments for the five days before and five days after April 21 produces IFR estimates given in Fig. 4.6.

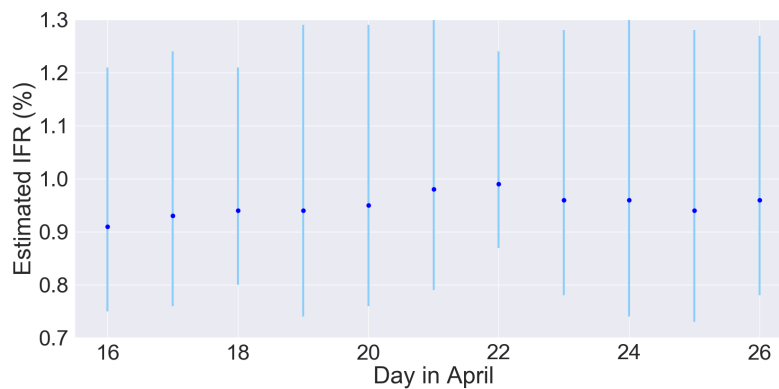


Figure 4.6: Adjusted IFR estimates produced from the 10 days surrounding April 21. For each country, the corresponding date or, when no data was available, the following date was used in the regression. For April 21, the estimate is higher (0.98% vs 0.95%) than the estimate provided in the main text because criteria differed for choosing data when unavailable for April 21. Here, the following date was chosen, in the main text, the closest date with available data (preceding or following) was chosen.

A small increase in the estimate occurs in mid-April due to a still increasing crude CFR. By April 21, however, the difference between  $\log(\text{CFR})$  values on this date and the end of May is near zero as discussed in SI 6.11.10. Importantly, the deviations in the daily IFR estimate are far smaller in magnitude than the confidence intervals established, suggesting that uncertainties in the global IFR estimate stem primarily from testing and reporting guidelines by country and the uncertainty introduced from the approximated false

negative rate (SI 6.11.10).

Applying this method to individual countries removes some of the challenges of establishing a criterion for picking a suitable date unless there are strong regional differences in the course of the epidemic (e.g. differing control measures or epidemic peak). For most countries, the preferred date should coincide with the epidemic peak, as the crude CFR corresponds closely with the true CFR, and no dramatic swings in testing capacity have occurred.

Italy illustrates this concept well due to the easily measured peak in the epidemic near the end of March. Once deaths began to accumulate in each region in the beginning of March, the relationship between  $\log(\text{CFR})$  and  $\rho$  is linear until the peak of the epidemic where testing capacity outpaced new cases, and the number of positive cases per test drastically fell. Because cumulative data from Italy used in the global analysis presented in the main text was taken on April 21, 2020, this may explain the deviation of Italy from the expected curve. 4 weeks past the peak, data for Italy at the end of April is sub-optimal and predicts a higher CFR relative to capacity compared to countries that have yet to peak. For some countries, such as the USA, a peak is not clearly defined due to regional heterogeneity in the course of the epidemic and variation in control strategies. Italy provides a good example of the use of the approach developed in this study, as the epidemic growth occurred quickly, such that a peak is easily defined, and a range of dates near the peak is optimal for obtaining cumulative data and performing a regression. Doing so for Italy (at

March 23, 2020), produces Figure 4.7.

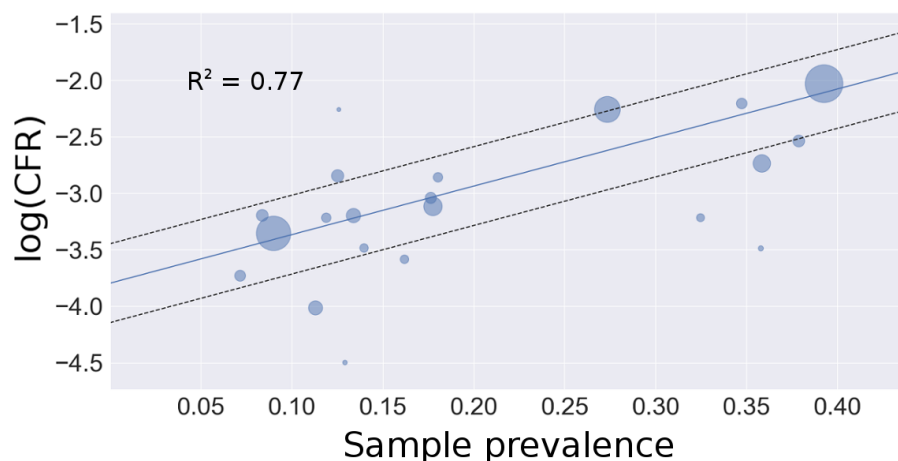


Figure 4.7: Regression estimate of Italy's IFR using 20 Italian regions. The intercept corresponds to an unadjusted IFR of 2.24% (CI: 1.58%, 3.18 %).

Adjustment for false negatives in testing results in an adjusted IFR estimate for Italy at 1.33% (CI: 1.08%, 1.66 %). This estimate is 50% higher than the global estimate, which is not unexpected given the high proportion of older individuals and the spike in cases that overwhelmed the healthcare system in Italy in March 2020.

#### 4.11.9 Bayesian model selection

The generalized linear mixed model run to determine whether national per capita GDP, age (% 65+), or healthcare availability and quality (HAQ Index) are reliable predictors of a country's crude CFR alongside  $\rho$  revealed marginal improvement in the model fit as evaluated by AIC. The best fitting model included three of the four variables ( $\rho$ , age, and HAQ). 8 models were compared using every permutation of the three supporting predictors



in addition to  $\rho$ . Here we report the estimates provided by these models as well as raw output to demonstrate model convergence.

Model convergence is shown below

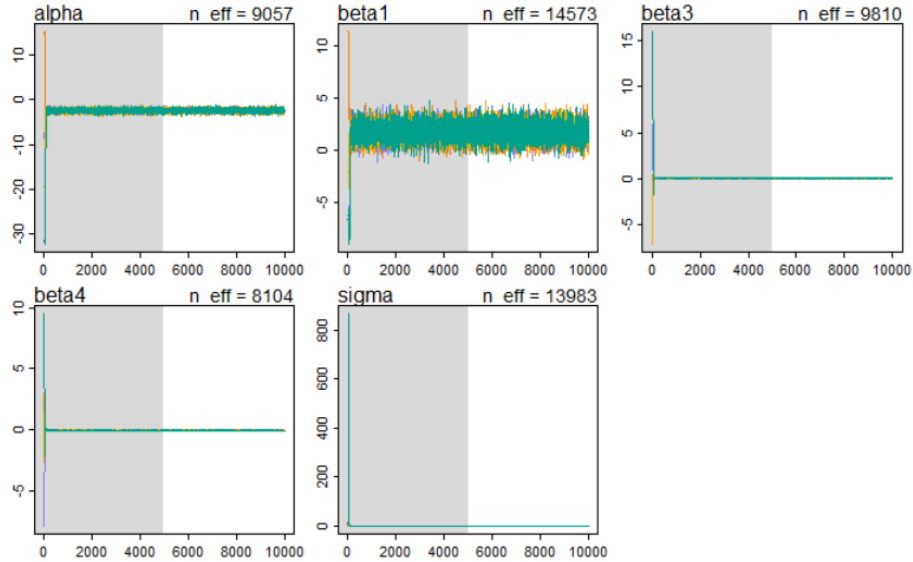


Figure 4.8: Plots of MCMC iterations, showing convergence for all parameter estimates. The shaded region gives the warmup period.  $\alpha$  = intercept,  $\beta_1 = \rho$ ,  $\beta_3$  = age,  $\beta_4$  = HAQ Index,  $\sigma$  = random error of model. Colors represent each of 4 chains.

```
Inference for Stan model: logCFR ~ dnorm(mu, sigma).
4 chains, each with iter=10000; warmup=5000; thin=1;
post-warmup draws per chain=5000, total post-warmup draws=20000.
```

|       | mean   | se_mean | sd   | 2.5%   | 25%    | 50%    | 75%    | 97.5%  | n_eff | Rhat |
|-------|--------|---------|------|--------|--------|--------|--------|--------|-------|------|
| alpha | -2.37  | 0.00    | 0.33 | -3.01  | -2.58  | -2.36  | -2.14  | -1.72  | 9057  | 1    |
| beta1 | 1.72   | 0.01    | 0.74 | 0.28   | 1.22   | 1.72   | 2.22   | 3.21   | 14573 | 1    |
| beta3 | 0.09   | 0.00    | 0.02 | 0.05   | 0.08   | 0.09   | 0.11   | 0.13   | 9810  | 1    |
| beta4 | -0.03  | 0.00    | 0.01 | -0.05  | -0.04  | -0.03  | -0.03  | -0.02  | 8104  | 1    |
| sigma | 0.88   | 0.00    | 0.06 | 0.77   | 0.84   | 0.88   | 0.91   | 1.00   | 13983 | 1    |
| dev   | 321.54 | 0.04    | 3.22 | 317.30 | 319.16 | 320.87 | 323.22 | 329.51 | 8190  | 1    |
| lp__  | -46.65 | 0.02    | 1.61 | -50.64 | -47.49 | -46.32 | -45.46 | -44.53 | 8190  | 1    |

Figure 4.9: Raw output for model parameters.

The relationships among the model parameters are reported below in Figure 4.10.

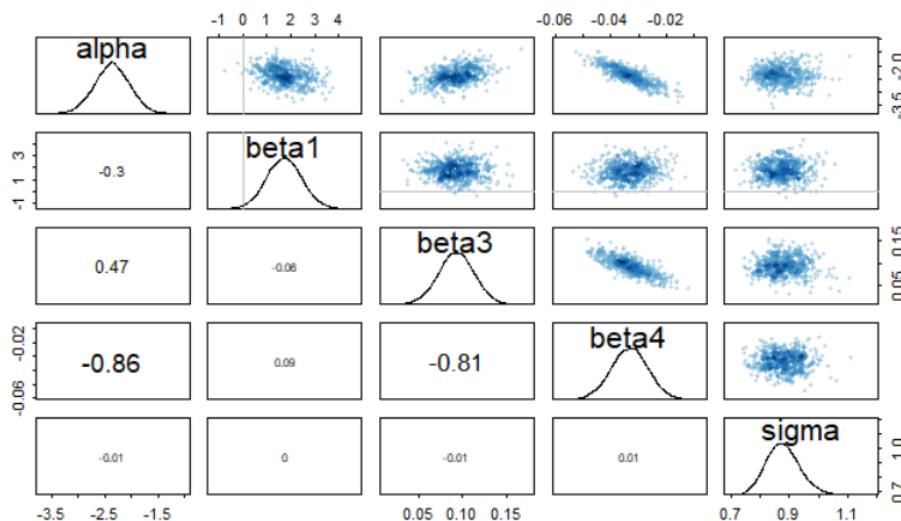


Figure 4.10: Distributions and relationships reported among model parameters. 2D density plots are given in the upper right triangle, while correlation coefficients are available in the corresponding bottom triangle. The diagonal reports the estimated distribution of each parameter and provides column and row labels.  $\alpha$  = intercept,  $\beta_1 = \rho$  coefficient,  $\beta_3$  = age coefficient,  $\beta_4$  = HAQ Index coefficient,  $\sigma$  = random error of model.

The best performing model is specified as

$$\log(CFR) \sim N(\alpha + \beta_1(\rho) + \beta_3(age) + \beta_4(HAQ), \sigma) \quad (4.48)$$

Priors were set as the following:

$$\alpha \sim N(0, 10)$$

$$\beta_1 \sim N(0, 10)$$

$$\beta_2 \sim N(0, 10)$$

$$\beta_3 \sim N(0, 10)$$

$$\beta_4 \sim N(0, 10)$$

$$\sigma \sim \text{Cauchy}(0, 1) \tag{4.49}$$

The estimate of  $\alpha$  provides the intercept, which markedly differs from the weighted regression estimate of  $-4.4$  provided in the main text. The difference here is primarily attributed to the effects of the other predictor variables on the intercept. For these variables, low correlation as demonstrated below in Figures 4.11-4.13 supports an intercept near the mean of the  $\log(\text{CFR})$  values. Because of this effect, incorporation of these variables in the IFR estimation framework is inappropriate. Other residual effects may be due to lack of weighting in the Bayesian GLMM approach to compare variables. A weighting scheme was not appropriate for GDP, age, or HAQ Index data, and to compare  $\rho$  with the same framework to recover underlying relationships, this data was also unweighted. The weighted regression is necessary to account for high uncertainty in locations with fewer performed tests, an unweighted regression performed with  $\rho$  and  $\log(\text{CFR})$  will produce

less reliable intercept values. For April 21, an unweighted regression leads to an intercept of -3.8 rather than -4.4, which suggests a substantially higher IFR.

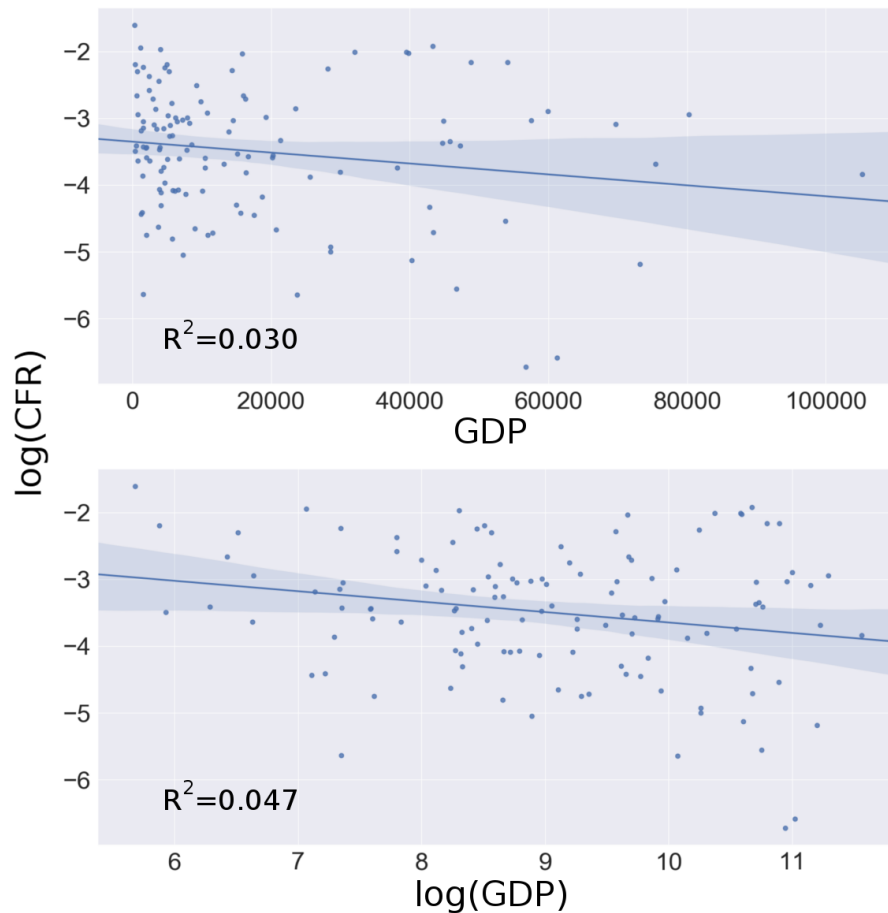


Figure 4.11: Linear regression of per capita GDP and  $\log(\text{CFR})$  reveal little correlation alone, though inclusion in the Bayesian model with sample prevalence improved the model fit, suggesting a relationship between per capita GDP and CFR. The bottom panel differs from the top using a log-transformed horizontal axis to spread the data evenly.

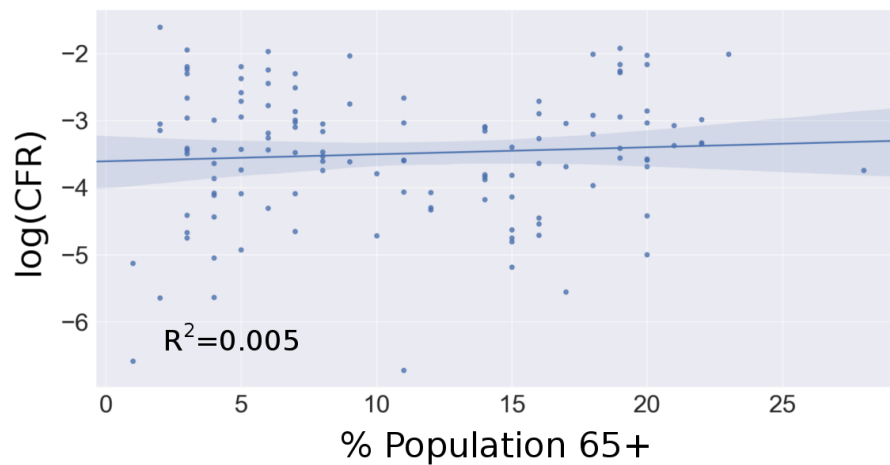


Figure 4.12: Linear regression of % of the population 65 and over with  $\log(\text{CFR})$ . Little to no correlation is supported alone, though the Bayesian analysis supports the use of this variable in the joint model.

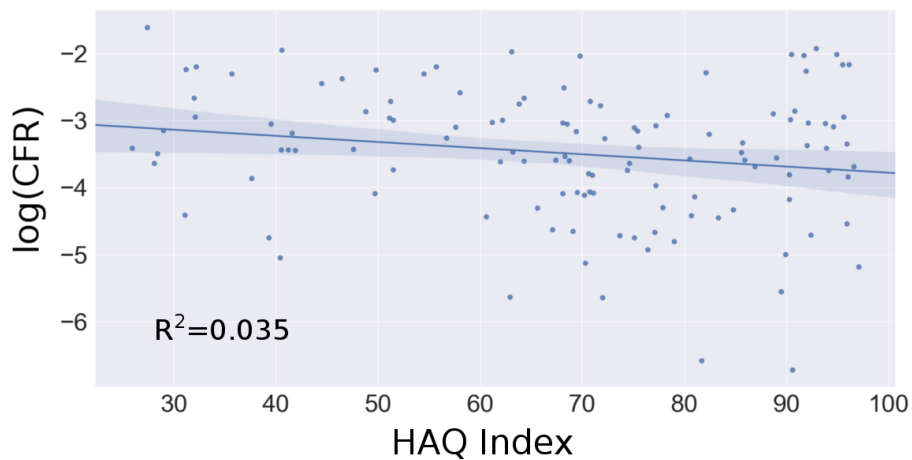


Figure 4.13: Linear regression of HAQ Index, an index of healthcare access and quality, with  $\log(\text{CFR})$  reveals weak correlation.

#### **4.11.10 Adjusting for false negatives, crude CFR, and excess mortality**

There are three potentially significant ways in which this method is susceptible to systematic bias, through which the IFR estimated is higher or lower than the true value. National differences in reporting, testing, and healthcare introduce random error and are unlikely to introduce systematic bias in the IFR estimate. The first and most important source of systematic bias is the predominance of false negative over false positive test results. The specificity of RT-PCR based assays is reportedly much higher than the accuracy. By most accounts the specificity of these tests is greater than 97.5%, whereas the accuracy varies from 33-80% through the duration of infection in symptomatic patients. Detection success is strongly dependent on the timing of the infection, indicating a dose-dependent relationship between viral shedding and detection. Failure of detection during low shedding periods produces false negative results, thereby under-counting the true number of infected persons. At the lowest (3 days after symptom onset) the probability of a false negative result is 20%. This increases to 67% near the end of the infectious period (Kucirka et al., 2020). A mean or median value for the false negative rate worldwide can only be speculated, as testing procedures may capture patients at different times along this period of infection. However, it can be reasonably assumed that globally testing is more likely to occur within the first week or two of symptom onset than in the weeks following. As such, a false negative rate between 20 and 40% is likely given the time-dependent false negative rate curve defined by Kucirka et al. Considering the much greater rate of false negatives to

false positives, we choose an approximate value of 30% under-counting of active infections through RT-PCR methods. This value is used to correct for the number of cases detected in each nation, and incorporation of this adjustment to the calculation of  $\rho$  and CFR produces the adjusted regression estimates. Correct estimation of this value is important to determine IFR, as it introduces the greatest systematic error in the IFR measurement. Due to the uncertainty in this approximation we assume wide estimates of standard error at 10%, which is incorporated into the confidence interval calculation of the IFR in the main text.

Secondly, in this analysis the crude CFR is used to derive the IFR. However, in a growing epidemic the crude CFR underestimates the true CFR due to a lag between reported cases and associated deaths. Therefore, using the crude CFR for each nation may systematically underestimate the IFR. It becomes imperative to account for the difference using the crude CFR may make in outcome of the analysis. To do so, we compared the crude CFR of each nation from April 21, 2020 to the corresponding crude CFR from May 31, 2020 for the 80 nations with reported deaths using archived data from [ourworldindata.org](https://ourworldindata.org). The additional six weeks provides ample time for cumulative data to display convergence toward the true CFR. In fact, the predicted relationship where the crude CFR from April 21 underestimated the CFR reported six weeks later was not substantiated. Although this notion was true for some countries, other countries saw a decline in the crude CFR during this time. For each country we calculate the difference in  $\log(\text{CFR})$  between the two dates to find and average difference of just 0.005. This value is added to the intercept in the main

text to account for the average underestimate that occurs using the crude CFR on April 21, 2020. Although the net adjustment is low, the individual differences in crude CFR for each country are not consistently low, leading to a standard error of 0.053.

Thirdly, mortality not captured by the CFR measurements because deaths are unrecorded could lead to under-estimation of the global IFR. Given the existing framework, the intercept is affected if there is systematic bias such that the percent of unrecorded deaths is invariant across testing capacity. However, this is unlikely, as the percent unrecorded deaths is reasonably a function of testing capacity; at high capacity a higher fraction of COVID-19 associated deaths are recorded as such than at low capacity. Given this assumption, we make a general argument that regardless of testing capacity, the percent of unrecorded deaths is lower than the percent of unrecorded cases. For example, it has been suspected in some localities that up to 25-50% of COVID-19 deaths go unrecorded, while over 90% of cases go unrecorded. Represented above the fraction of cases recorded can be denoted as  $P_{test}/P_{pop} = \theta(\rho)$ . The fraction of deaths recorded is denoted as  $deaths_{test}/deaths_{pop} = \phi(\rho)$ . We assume that for all  $\rho$  the following is true:

$$\frac{\theta(\rho)}{\phi(\rho)} \ll 1 \quad (4.50)$$

Moreover, because the magnitude of undetected cases is far greater than the magnitude of undetected deaths, and detection for each is a function of sample prevalence, it can be assumed that  $d\theta/d\rho > d\phi/d\rho$ . In this way, as sample prevalence changes, the relationship



between CFR and sample prevalence is governed by the much faster convergence of  $\theta$  than  $\phi$  towards unity. In simpler terms, the error in recorded death can be neglected in its contribution to the linear regression because the slope and intercept are a product of the change in detection of cases. To make this argument explicit in the framework outlined in the main text, we again show equation 4.5 (main text):

$$IFR = \frac{deaths}{P_{pop}} \quad CFR = \frac{deaths}{P_{test}} \quad (4.51)$$

In the main text, the fraction of unrecorded deaths was assumed small relative to the fraction of unrecorded cases, and deaths as a variable was removed in equation 4.6:

$$\frac{P_{pop}}{P_{test}} = \frac{CFR}{IFR} \quad (4.52)$$

However, explicitly differentiating the true number of deaths,  $deaths_{pop}$ , from the recorded deaths,  $deaths_{test}$ , yields an alternative to equations 5 and 6:

$$IFR = \frac{deaths_{pop}}{P_{pop}} \quad CFR = \frac{deaths_{test}}{P_{test}} \quad (4.53)$$

$$\frac{P_{pop}}{P_{test}} = \frac{CFR}{IFR} \phi(\rho) \quad (4.54)$$

Following is an alternative to equation 4.8 in the main text:

$$\log(CFR) = \log(IFR) - \log(\phi(\rho)) + k\rho \quad (4.55)$$

There is not a simple argument that allows derivation of an expression for  $\phi(\rho)$ , as recording of COVID-19 associated deaths is likely not based on available information, but rather accessibility of tests and proximity to a hospital when a death occurs. However, at infinite testing capacity,  $\log(\phi(\rho)) = 0$ , as all deaths are recorded, and equation 4.53 simplifies to equation 4.5 in the main text. Therefore, the intercept remains the IFR despite unrecorded deaths. The expression chosen for  $\phi(\rho)$  does affect the slope of the regression line, however, as it is a function of  $\rho$ . In the particular case that we assume this expression takes the same form as  $\theta(\rho)$  (equation 4.23), then

$$\phi(\rho) = \frac{deaths_{test}}{deaths_{pop}} = e^{-\kappa\rho} \quad (4.56)$$

where  $\kappa$  is the decay constant relating the fraction of COVID-19 deaths recorded to testing capacity. Substitution of this expression in equation 4.53 yields:

$$\log(CFR) = \log(IFR) + (k + \kappa)\rho \quad (4.57)$$

The slope of the regression line is therefore  $\kappa + k$ , revealing that although the regression remains unchanged, the interpretation of the slope of the regression does change. The slope is the sum of the two decay constants used to represent the relations of both recorded deaths

and recorded cases to testing capacity. Alternative formulations of  $\phi(\rho)$  could be explored in future work, as there is potential for different expressions to yield minor deviations from the linear relationship between sample prevalence and CFR as shown in this study.

#### 4.11.11 Total uncertainty of the IFR

As previously introduced, the error around the intercept is normally distributed, therefore the error around the IFR estimate is lognormally distributed. The numerically derived Hessian is used to derive the variance-covariance matrix from which the standard error of the  $\log(\hat{IFR})$  is calculated for both datasets: worldometer and ourworldindata. The standard error of these measurements reflects the random error associated with national CFR measurements and does not capture the error intrinsic in the assumption of the systematic adjustments made for 30% under-counting of cases due to false negative results. Above we set a wide standard error of this assumption of 10%. With this assumption, we generate bootstrapped standard deviations for the resulting intercept around the 30% adjustment. Doing so for both datasets yields standard deviations for both intercepts of approximately 0.145. The third remaining error of the intercept is the above stated error of the CFR adjustment when accounting for the presumed under-counting of deaths relative to cases as of April 21, 2020. This standard error was found to be 0.053. Giving equal value to the estimation of the intercepts from both data sets, then we can average the two intercepts after adjustments. The standard error of the averaged intercepts is calculated as

$$SE_{\overline{intercept}} = \sqrt{\frac{1}{4}(SE_{intercept_1})^2 + \frac{1}{4}(SE_{intercept_2})^2} \quad (4.58)$$

Where the standard error of each intercept is given as the combination of the three associated errors (random error of regression, approximated error of adjustment due to false negatives in testing, and error in the CFR estimate for the given date). For example, intercept for worldometer data has an associated error calculated as

$$SE_{intercept_1} = \sqrt{0.124^2 + 0.145^2 + 0.053^2} \quad (4.59)$$

The standard error of the averaged intercepts is

$$SE_{\overline{intercept}} = \frac{1}{2}\sqrt{0.198^2 + 0.161^2} = 0.128 \quad (4.60)$$

#### 4.11.12 Summary of limitations and best practices

Based on the set of uncertainties examined in this work and the potential for application of the method in other areas where data is available, a set of limitations and best practices is warranted to provide reliable IFR estimates. Although much of the following is covered in the previous text, we summarize the points here.

The most variable, and therefore, the most important quantity to control in estimating the IFR with this approach is the sample prevalence. With enough data from a variety of

independently derived samples, variability in sample prevalence that occurs in each location through the course of the epidemic may not dramatically affect the results of the IFR estimate. However, large swings in the sample prevalence (e.g. after lockdown measures) could lead to significant movement in the location of a point on the sample prevalence –  $\log(\text{CFR})$  graph. When this movement is coordinated, such that there is correlation in these swings between some of these points, this can result in poorer correlation in the predicted relationship between  $\rho$  and  $\log(\text{CFR})$  and less reliable IFR estimates. This becomes particularly apparent when the points with the most movement are heavily weighted points. For example, for European countries like Spain, France, and Italy which experienced large caseloads and performed large numbers of tests in March and April, coordinated lockdown measures of these countries results in a decline in sample prevalence that is more dramatic than for many other countries during this period. As this handful of countries deviates from the epidemic trajectory of other countries, the deviation of these points can reduce correlation in the predicted relationship. Importantly, when all points are coordinated in movement (e.g. if all countries employ lockdown measures), little issue arises, and the predicted relationship is maintained. Therefore, **best estimates occur when countries or localities measured are in similar epidemic phases.**

The recovery of the relationship between the true number of cases and those identified through testing as given by equation 4.3 in the main text is made through a consistent, though simplified, sampling process. In reality sampling is not consistent and  $\lambda$  is not

constant. Variation in  $\lambda$  through time does not necessarily pose a significant issue, as cumulative data represents a temporal average. Additionally, convergence is certain provided  $\lambda > 1$ , which is necessarily true in the presence of any symptoms in the population. What is most important is that the majority of testing occurring in a single measured point prioritizes patients with symptoms before prioritizing others for testing. There are likely many localities in which those with symptoms do not have access to, or refrain from seeking, SARS-COV-2 testing, while hospital employees or other interested parties are repeatedly tested. When this occurs, the sample prevalence will be low relative to the crude CFR (compared to the predicted relationship). When a significant fraction of points used in the analysis are affected in this way, it may be possible to lose the spread in the sample prevalence that is required to obtain a robust regression. Two ways to treat this issue could be: 1) isolate a time period where case loads are high, so the majority of tests completed are for patients with symptoms, or when this is not possible, 2) exclude the point from the analysis. In the context of this work, the date chosen minimizes this effect because many countries were escalating or near the peak in case load. Importantly, there is significant spread across sample prevalence to give a slope significantly different from zero and a strong correlation between sample prevalence and  $\log(\text{CFR})$ .

When estimating the IFR of locations for which the epidemic has started at much different times, one approach that minimizes the effect of the associated differences in the reported CFR is to standardize by day since the first death as we show above. Although

we chose 50 days since the first death in each country, this timeframe may not be suitable for areas in which large swings in the sample prevalence interfere with capturing reliable data. For the global dataset, this approach did not produce significantly different results than those in the last two weeks of April, though differences may be apparent for other datasets.

In the case of clear outliers with high leverage, one may choose to exclude the outliers or incorporate a loss function to the regression that minimizes the influence of outliers, as is commonly done for nonlinear regression frameworks.

An important uncertainty in this work is the estimate of the false negative rate of RT-PCR tests. Although we estimate the average to be approximately 30% and provide generous accompanying uncertainty, the average false negative rate for those seeking testing is elusive yet important in producing the most reliable IFR estimates using this approach. Because this rate depends on the viral load and timing of testing relative to the course of infection, an understanding of the average period in the course of infection that patients seek testing would be useful in improving the certainty of the conclusions here and for future work. Despite this uncertainty, the large confidence intervals provided for this estimate should capture the true global IFR within.

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## **Chapter 5**

# **Modeling the Efficacy of CRISPR Gene Drive for Schistosomiasis Control**

### **5.1 Abstract**

CRISPR gene drives could revolutionise the control of infectious diseases by accelerating the spread of engineered traits that limit parasite transmission in wild populations. Gene drive technology in molluscs has received little attention despite the role of freshwater snails as hosts of parasitic flukes causing 200 million annual cases of schistosomiasis. A successful drive in snails must overcome self-fertilisation, a common feature of host snails which could prevent a drive's spread. Here we developed a novel population genetic

model accounting for snails' mixed mating and population dynamics, susceptibility to parasite infection regulated by multiple alleles, fitness differences between genotypes, and a range of drive characteristics. We integrated this model with an epidemiological model of schistosomiasis transmission to show that a snail population modification drive targeting immunity to infection can be hindered by a variety of biological and ecological factors; yet under a range of conditions, disease reduction achieved by chemotherapy treatment of the human population can be maintained with a drive. These results indicate that gene drives, in coordination with existing public health measures, may become a useful tool to reduce schistosomiasis burden in selected transmission settings with effective CRISPR construct design and evaluation of the genetic and ecological landscape.

Keywords: *CRISPR-Cas9* | *epidemiology* | *population genetics* | *immunity* | *self-fertilisation*

## 5.2 Introduction

Gene drive technology is rapidly expanding since the discovery of CRISPR-Cas9 (Burt, 2003; Cong et al., 2013; Jinek et al., 2012). Its potential uses include controlling diseases, invasive species, and pests by spreading targeted genes through a population faster than traditional Mendelian inheritance allows (Oye et al., 2014). For example, there are currently large efforts to harness genetic technology targeting mosquito species that are vectors of malaria and other vector-borne diseases (Connolly et al., 2021; Eckhoff et al., 2017; Gantz et al., 2015; Marshall & Taylor, 2009; Windbichler et al., 2011). Similar efforts could be on the horizon for schistosomiasis, a debilitating disease of poverty caused by blood flukes of the genus *Schistosoma* (Maier et al., 2019).

The battle to eliminate schistosomiasis has been waged for more than a century, and despite local successes, the disease remains widespread (Sturrock, 2001). Globally over 200 million individuals are actively infected. With 800 million people at risk of infection, schistosomiasis is second only to malaria in the breadth of its health and economic impact as an infectious tropical disease (King & Galvani, 2018; Organization et al., 2016). The disease manifests as a complex suite of symptoms stemming primarily from the inflammatory processes the body mounts in response to the schistosome eggs that embed in tissue (Barron & Wynn, 2011). Abdominal pain, release of blood in urine or stool, fever, enlargement of liver or spleen, and accumulation of fluid in the peritoneal cavity are acute symptoms, while fibrosis and lesions of vital organs, infertility, and several forms of cancer



are lasting consequences of infection (Andrade, 2009; Mostafa et al., 1999).

Transmission of schistosomes to intermediate, obligate snail hosts occurs when eggs shed in urine or feces from infected people contact freshwater and emerge as free-swimming miracidia. Once established within the snail, the parasite reproduces asexually and cercariae are released 3-5 weeks after the onset of infection. In this stage, the parasites castrate the freshwater snails, severely reducing reproduction (Faro et al., 2013). Released cercariae can penetrate the skin of humans in contact with infested water bodies and cause infection (Fig. 5.1) (Colley et al., 2014).

Rapid advancements in genomics for the intermediate snail host species provides a mechanistic understanding of innate, genetically-based snail immunity to schistosome infection (Tennessen et al., 2020b). Genes responsible for immunity could be candidates for gene drive mediated spread through snail host populations. Promisingly, selection experiments reveal rapid evolution of immune phenotypes, demonstrating high immunity can be achieved under laboratory conditions within a few snail generations (Tennessen et al., 2015a; J. Webster & Woolhouse, 1998) (Fig. 5.5). Overall, there is good reason to expect that a population modification CRISPR gene drive designed to provide greater immunity in the snail population could soon be developed. However, whether such a gene drive could provide the sustained reduction in transmission necessary to eliminate schistosomiasis in realistic settings laden with barriers to the spread of a drive remains unknown.

Previous theoretical work using classical population genetic models has explored how

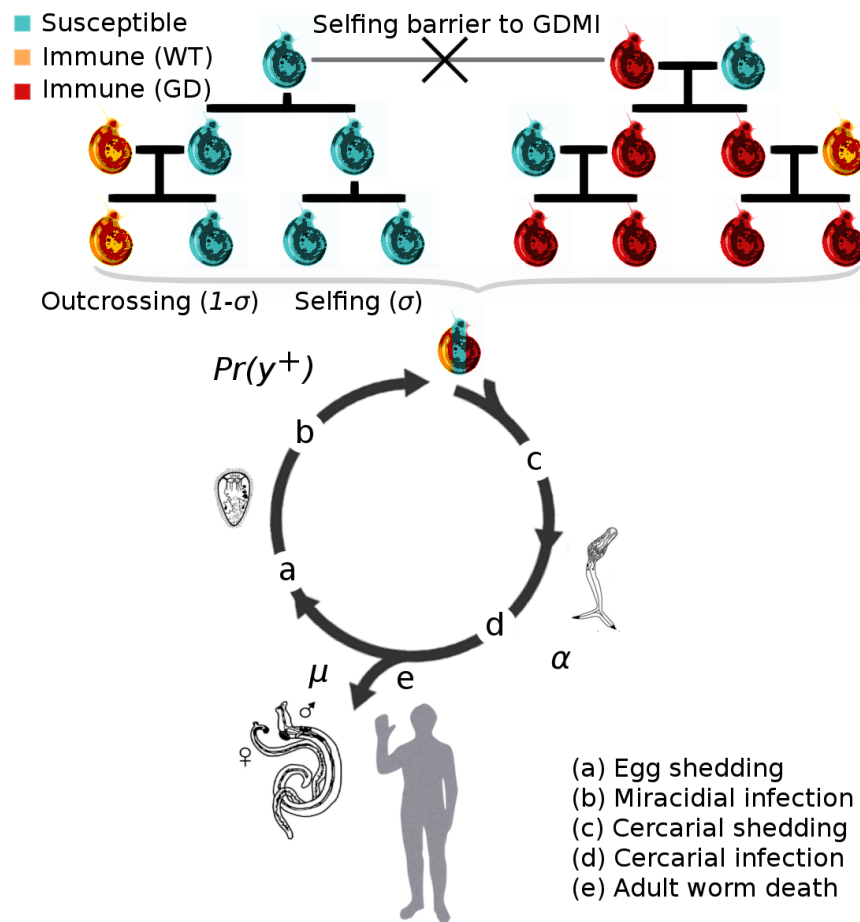


Figure 5.1: Conceptual diagram of the integrated epidemiological and population genetic model describing the evolution of immunity to schistosome infection in the snail population. (a) High worm burden in the human population increases the force of infection on the snail population, which positively selects for immune snail genotypes. (b) Miracidial infection of susceptible snails is density-dependent. (c) Evolution of immunity in the snail population reduces cercarial transmission to humans, thereby regulating parasite densities at an endemic equilibrium. GDMI is inherited more rapidly than natural immunity only when outcrossing occurs. (d) Infection of humans is proportional to cercarial output, and a negative binomial distribution of adult worms in the human population influences mating success and egg production. Immunity in the human population is assumed constant due to accumulated evidence that immunity acquisition occurs over decades and likely varies little relative to immunity in the snail population in a 10 year window in endemic conditions. (e) Mortality of adult worms occurs via constant natural mortality and MDA treatment. Three snail genotypes are modeled: susceptible to infection, innately immune (wild type), and gene drive mediated immune. Iteroparitive reproduction and mortality of these genotypes is modeled with explicit fecundity and viability components of fitness (see SI).

fitness, homing efficiency, selfing, resistance allele formation, gene flow, and other forms of population structure influence invasion success and peak frequency of a drive in general contexts (Diekmann et al., 2010; Noble et al., 2018; Noble et al., 2019; Noble et al., 2017; Unckless et al., 2017a). Stochastic Moran models or discrete deterministic models with non-overlapping generations do not incorporate population dynamics on which the tempo of evolution is highly dependent. Snail populations are iteroparous, reproducing several times within a lifetime, and exhibit density dependent recruitment. This form of reproduction is not modeled in the simplified evolutionary models developed for gene drives to date. Accuracy of gene drive models hinges on realistic assumptions of the target population.

The success of gene drive mediated immunity (GDMI) in natural snail populations is determined by features intrinsic to the design of the drive construct and its deployment – homing efficiency, fitness cost of the payload, evolution of resistance to the drive, and number of releases – and by extrinsic properties of the environment in which GDMI is deployed, such as the size of the focal snail population, transmission rates, and gene flow and standing genetic variation for immunity in snail populations. Importantly, all snail species that serve as intermediate hosts to schistosomes, except for *Oncomelania spp.*, are simultaneous hermaphrodites capable of self-fertilisation (selfing). In contrast to mosquito and fruit fly models for which gene drives have been designed, selfing snail species may be incapable of propagating a drive construct. Gene drive relies on an encoded endonuclease, such as

Cas9, which introduces a double strand break in the homologous chromosome that is repaired using the gene drive allele as a template, thereby copying the gene drive allele to the homologous chromosome (Gantz et al., 2015; Oye et al., 2014). Sexual reproduction (outcrossing) is necessary for gene drive to spread a target allele in a population through pairing and reassortment of gene drive and wild type alleles, facilitating gene conversion. Because the propensity to self-fertilise varies by species and environmental conditions (Jarne et al., 1993), it is imperative to understand how selfing interacts with the variety of intrinsic and extrinsic factors that may influence the establishment of GDMI in natural snail populations.

The impact of GDMI is determined by public health outcomes and not by establishment alone. Local success in schistosomiasis reduction can be achieved through sustained non-pharmaceutical intervention, but such approaches are often resource intensive (e.g. sanitation) or cause collateral damage to the environment (e.g. molluscicides) (Bergquist et al., 2017; Organization et al., 2017). Praziquantel (PZQ) emerged in the 1980s as the drug of choice for mass drug administration (MDA) (Andrews et al., 1983; King & Mahmoud, 1989), and while cheap and effective in removing mature parasites from infected people and temporarily reducing morbidity, PZQ does not prevent reinfection, and extensive MDA campaigns have been unable to locally eliminate the disease in high transmission regions (Burgess et al., 2020; Kittur et al., 2019). For this reason, in recent years there has been a renewed interest in complementing MDA with environmental interventions aimed at targeting the environmental reservoirs of the disease (Hoover et al., 2019; Lo et al., 2018;

Sokolow et al., 2015; Sokolow et al., 2018). GDMI has the potential to augment environmental interventions as a means toward cost-effective and sustainable schistosomiasis elimination, especially when paired with existing anthelmintic treatment of humans.

We investigate the role of selfing and its interaction with other factors influencing GDMI establishment to infer the challenges and opportunities for GDMI in a natural context. We hypothesised that a high selfing rate would incapacitate a gene drive, but a lower selfing rate could be compatible with a drive in certain conditions. To test these ideas we developed a biologically realistic mathematical model incorporating both genetic and environmental factors. This model is integrated in an epidemiological framework to evaluate the reduction of disease burden in humans with and without coincident MDA treatment. This study can be used as an informative first step for scientists, stakeholders, and policy makers looking to address the large human health crisis of schistosomiasis in conjunction with the principles for responsible use of gene drives proposed by the National Academies of Science, Engineering, and Medicine (NASEM) (National Academies of Sciences, Medicine, et al., 2016).

## 5.3 Results

We developed a population genetic model that accounts separately for fecundity and viability components of fitness as well as for density dependent dynamics of the snail host

population. We expand the wild type - gene drive, 2 allele model to separate the naturally occurring alleles into immune and susceptible types. The resulting six genotypes are formed from three alleles (susceptible, innately immune, gene drive mediated immune) and incorporated into a Markov model modified to include overlapping generations and population dynamics of susceptible and infected snails. Finally, we integrated the population genetic and ecological model with an epidemiological model to describe the dynamics of infection in the human population. Parameter values for the genetic model are derived from literature (Table 1) or otherwise explored in sensitivity analyses in the resulting figures, and under default conditions, simulated evolution recapitulates challenge experiments (Fig. 5.5). We examine the impact that the self-fertilisation (selfing) rate of a focus population has on the establishment of gene drive in 10 years.

To represent the two species clusters, we depict results from both ends of the range of observed selfing rates among snail hosts. At high rates ( $\sigma = 0.8$ ), self-fertilisation undermines the gene drive and prevents establishment. However, GDMI is able to overcome low rates of self-fertilisation ( $\sigma = 0.2$ ) and establish at high frequencies (Fig. 5.2A). Self-fertilisation is largely species dependent and may vary with local conditions with higher propensities to self-fertilise observed at low population densities (Escobar et al., 2011a). To simulate this range of conditions, we perform a sensitivity analysis at the 10 year endpoint, so chosen as the likely window in which the efficacy of targeted treatments are evaluated in human populations. Especially for predominantly outcrossing species, reduced

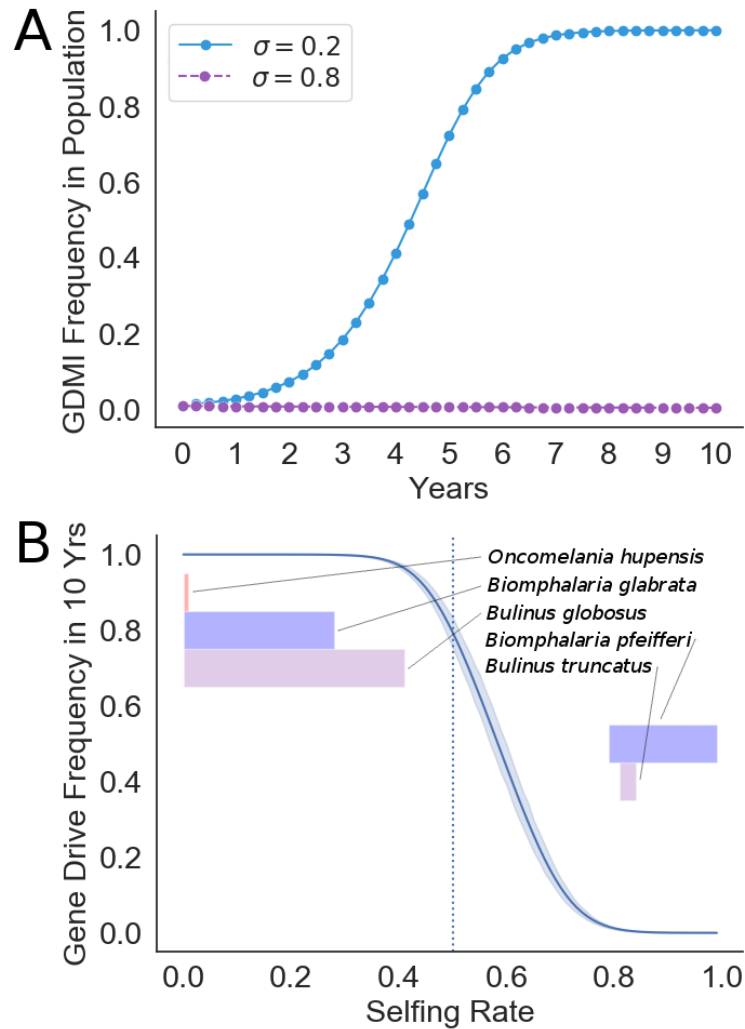


Figure 5.2: Self-fertilisation rate strongly affects establishment of gene drive in a 10 year window. (A) Simulation of gene drive invasion under default conditions when self-fertilisation rate is low at 0.2 and high at 0.8. (B) Endpoint sensitivity analysis depicting the gene drive frequency in the population after 10 years under variable self-fertilisation rates ( $\sigma$ ) from 0 to 1. 95% confidence intervals are reported on the range of results when the fecundity cost of inbreeding varies on the uniform distribution [0,0.6]. The vertical dotted line designates  $\sigma = 0.5$ , which is the value used in future simulations to represent an intermediate selfing rate from those observed. Shaded bars colored by genus display ranges (mean  $\pm$  1 s.d.) of observed selfing rates for each host snail species for which empirical measures exist (Escobar et al., 2011a). Vertical positioning of the bars is ordered by minimum selfing rate according to the displayed ranges.

offspring viability is associated with self-fertilisation (Escobar et al., 2011a). We provide a confidence interval around the endpoint sensitivity analysis based on a range of inbreeding costs to fecundity. Gene drive success in 10 years is highly dependent on low selfing rate, though slower establishment is possible at moderate selfing rates. The inflection point near  $\sigma = 0.6$  gives the value over which gene drive success is improbable in a 10 year window (Fig. 5.2B). These results indicate that for species with a lower rate of selfing, including *Oncomelania hupensis*, *Biomphalaria glabrata*, and *Bulinus globosus*, gene drive could establish rapidly in focus populations (Jarne et al., 1991). Conversely, for species like *Biomphalaria pfeifferi* or *Bulinus truncatus*, which have been observed to self-fertilise at rates higher than 0.6, gene drive will likely be ineffective in increasing immunity in the snail population (Nguema et al., 2013).

Drive success depends on features in the snail-human-schistosome system beyond selfing. Features intrinsic to the design and deployment of the drive like homing efficiency, fitness cost of the payload, resistance evolution, and the number of releases of GDMI individuals are more easily modified than extrinsic factors which are dependent on the ecological and environmental conditions – size of the snail population, force of infection from the human population, gene flow, and standing genetic variation. Yet the success of GDMI may be sensitive to any of these factors. We explore via endpoint sensitivity analyses how variation in these factors alters the frequency of GDMI after 10 years.



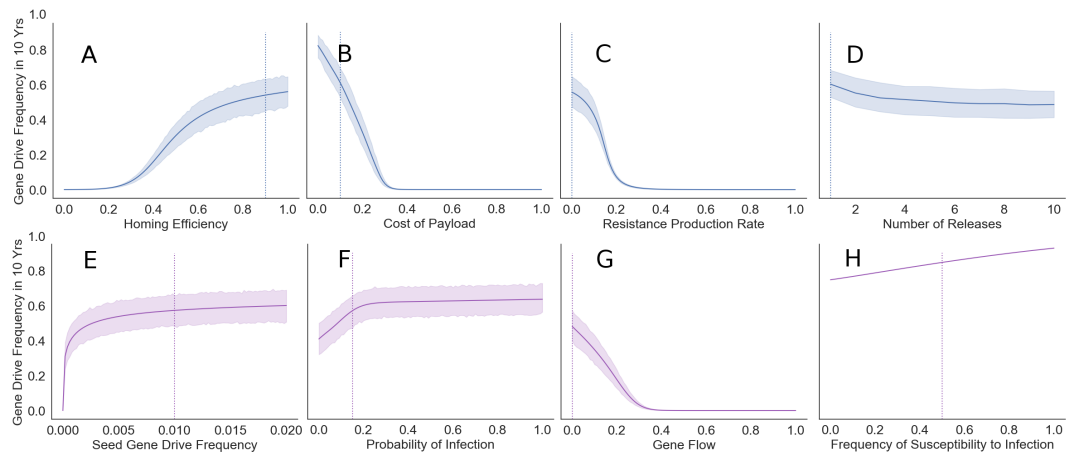


Figure 5.3: GDMI efficacy across a wide range of endemic conditions and genetic design. Bootstrapped 95% confidence intervals of the mean are reported on the range of results when the self-fertilisation rate varies on the uniform distribution  $[0,1]$ . Intrinsic factors to the design and release that may be modified are in the top row in blue: (A) Gene drive homing efficiency (homing success per meiotic event), (B) fecundity cost of the genetic payload (relative fitness loss), (C) rate of production of gene drive resistant mutants (resistant mutants per meiotic event), (D) number of annual GDMI partial releases needed to achieve the size of a single maximum release (e.g. 5 = 1/5th release in each of the first 5 years). Extrinsic factors that are not readily modified are in the bottom row in purple: (E) seed frequency (i.e. proportion introduced in a single release at  $t = 0$ ) of the gene drive engineered snails in the population, (F) the probability a susceptible snail is infected in a generation, (G) bidirectional gene flow as the proportion of the focus population that is replaced by an external source each generation, and (H) starting frequency of snails susceptible to infection in focus the population. Because the frequency of the susceptible genotype is a function of selfing rate, bootstrapping is not appropriate for panel H. Vertical dotted lines depict the default parameter values used in the other figures. Note that the solid line represents a mean across uniformly distributed selfing values,  $\sigma \in [0, 1]$ , rather than the predicted results at  $\sigma = 0.5$ .

Like results from previous modeling and laboratory studies, we find that homing efficiency has a dramatic impact on the outcome of the gene drive release in a focus population. Under the range of selfing scenarios, low homing efficiency leads to minimal gene drive establishment. Laboratory work in mosquitoes and mice shows homing efficiency above 0.4 is achievable and often exceeds 0.9 (Gantz & Bier, 2015a; Gantz et al., 2015). In this range, diminishing returns are observed when  $H > 0.5$  (Fig. 5.3A). The fitness cost of the genetic payload is not often empirically measured, though for this modeled system, gene drive success is highly sensitive to this parameter: GDMI can establish only when the cost is below 0.4 per gene drive copy in the genome (Fig. 5.3B). Results improve nearly linearly below a fitness cost of 0.3 per copy. In natural and laboratory populations, resistance to the gene drive mechanism can evolve quickly without the presence of multiple gRNA or selection against resistance formation (S. E. Champer et al., 2020). Resistance can evolve more quickly when associated fitness costs of the gene drive phenotype are high. The reported mechanisms of resistance are spontaneous mutation and non-homologous end joining which render the Cas9 cleavage site unrecognisable (J. Champer et al., 2018). We combine these associated mechanisms and display the scenarios for the likely range of summed rates of both processes. In a 10-year time frame, a rate of resistance formation greater than 0.2 per meiotic event makes GDMI establishment infeasible (see also SI Fig. 5.11). With the exception of the deployment strategy, in which the number of releases does not significantly alter establishment drive success, intrinsic factors to the design of the gene

drive construct bear heavily on the outcome of GDMI in 10 years.

We also investigated four extrinsic determinants of GDMI establishment: seed gene drive frequency, force of infection, gene flow, and the standing innate immunity in the snail population. Seed frequency is critical to gene drive spread when only low seed frequencies are possible (Fig. 5.3E). Seeding greater than 1% gives strong diminishing returns. As focus snail populations can vary between hundreds and hundreds of thousands of individuals, this implies that anywhere from one to thousands of snails will need to be raised for a successful introduction, and the size of the focus population will determine the feasibility of release. Similarly, diminishing returns are seen as the probability of infection in a generation increases past 0.2 (Fig. 5.3F). This indicator of endemicity provides the positive selection necessary to propagate the drive in the snail population, as susceptibility to infection is disadvantageous. These results suggest that success is similar for localities experiencing moderate or high burden of disease. Loss of drive alleles from the focus population due to migration inhibits establishment of the drive (Fig. 5.3G). Levels of gene flow greater than 40% (i.e. 40% of focus population alleles are replaced by alleles from a non-evolving background population each generation) bring the drive alleles to undetectable levels assuming immigrants to the focus population lack gene drive immunity. Importantly, GDMI to schistosome infection acts by elevating the level of naturally occurring innate immunity in the snail population. This co-occurring immunity is positively selected under the same conditions as GDMI. Susceptibility is positively selected with weak force of infection

due to the fitness costs via reduced egg viability associated with immunity, and immunity is positively selected with moderate to high force of infection due to fitness costs via parasitic castration and reduced lifespan in infected snails (Crews & Yoshino, 1989; J. Webster & Woolhouse, 1999a; Woolhouse, 1989a). High levels of natural immunity will slow the growth of gene drive through direct competition, and therefore, higher susceptibility to infection in a population favors gene drive establishment (Fig. 5.3H). Natural immunity is inherited more slowly, though fecundity for naturally immune snails is assumed higher than GDMI due to added costs of maintaining the genetic payload of the drive.

Although mass drug administration (MDA) is capable of temporary reduction in morbidity, MDA alone is incapable of local elimination at high transmission sites. In these conditions, gene drive offers a potentially promising avenue for coincident MDA and environmental treatment of schistosomiasis. We evaluate the consequences of applying GDMI snails to a community with concurrent annual MDA treatment. We compare the observed reduction in mean worm burden (MWB) between three treatment regimes: gene drive immunity, MDA, and concurrent application of both (Fig. 5.4). Simulations are conducted under the same default conditions evaluated above with the difference that human-to-snail force of infection is a variable that is determined by the number of mated worm pairs in the human population (SI table 2). The pre-treatment prevalence of infection is assumed to be 80%. The snail-to-human force of infection is a function of the number of infected snails at a water access site, a quantity that diminishes as immunity to infection increases in the

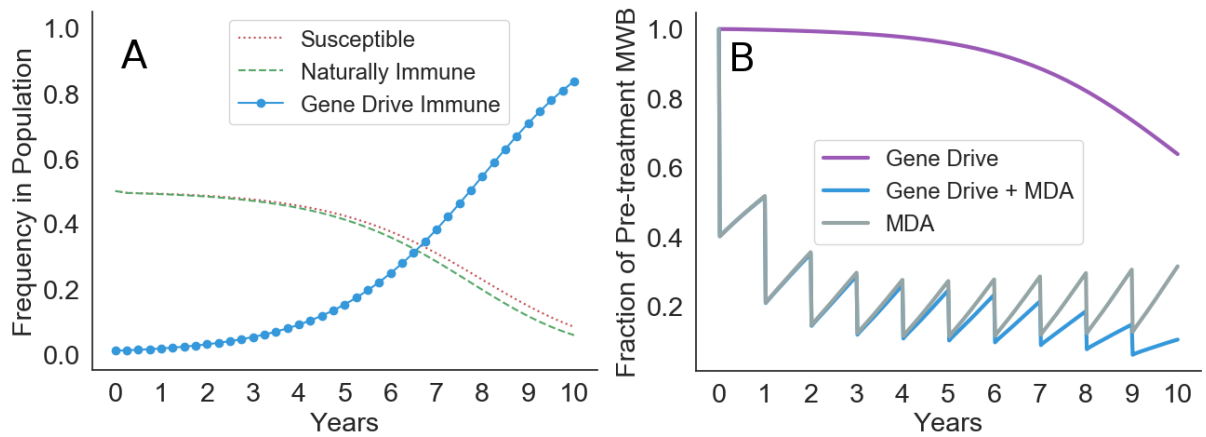


Figure 5.4: Combining gene drive with mass drug administration. (A) 35% reduction in MWB is observed in 10 years with gene drive alone. (B) Targeted administration of MDA at 60% annual reduction (efficacy\*coverage) in MWB results in more rapid but temporary reduction than the use of gene drive. Sustained reductions are achieved with coincident MDA and gene drive treatment.

snail population.

With gene drive treatment alone a 35% reduction in MWB is observed due to the reduced establishment of new worms in humans and natural mortality of existing adult worms with average lifespan of nearly 5 years (Goddard & Jordan, 1980a). Elimination could be achieved with successful gene drive treatment alone, though the lifespan of adult schistosomes precludes rapid elimination (30 years required for 99% reduction, Fig. 5.18). With annual reduction of MWB of 60% through targeted MDA, alleviation is possible, but elimination is infeasible due to the persistence of infected snails in nearby water access sites. Moreover, immunity in snails wanes due to decreased force of infection on the snail population, resulting in an upward trend in MWB from year 3 onward. Concurrent treatment targeting both snail and human hosts leads to sustained elimination provided resistance

formation is low. This is true even when MDA is ended after 10 years because GDMI has reached fixation in the focus snail population (Figs. 5.16-5.18).

## 5.4 Discussion

Our results demonstrate that successful establishment of immunity within a 10 year evaluation period is possible for species of snails with low to moderate selfing rates. Snails species like *B. Pfeifferi* and *B. truncatus*, which are known to self-fertilise at high rates, are likely not desirable targets for GDMI. Many other snail species self-fertilise at lower rates, providing more opportunity for GDMI control of schistosomiasis (Jarne et al., 1993). Likewise, propensity to self-fertilise can also vary by environmental condition. Panmictic and stable snail populations favor out-crossing, which increases the rate of inheritance of GDMI. This work indicates that the potential for success of GDMI could be evaluated prior to programme implementation through genetic studies quantifying selfing rates (e.g. with F-statistics) in intervention areas. In areas with sympatric snail species with differing selfing rates, quantifying the relative abundance of each species and their respective contributions to schistosomiasis burden will inform the potential for success of GDMI locally.

GDMI establishment is sensitive to genetic design and less sensitive to standing genetic variation for immunity. Low payload fitness costs and homing efficiency greater than 50% are essential. Reducing the evolution of resistance to the drive with multiple gRNAs (S. E. Champer et al., 2020) or through other techniques can moderately improve success in a 10

year window and has stronger implications for success after 10 years. Alternative designs incorporating ‘daisy chain’ inheritance or other drive decay mechanisms can provide safeguards to gene drive release in natural ecosystems, and peak GDMI frequency would be contingent on the strength of this decay, which occurs more quickly with fewer loci in the chain (Noble et al., 2019). Selfing requires more loci in a daisy chain to achieve high peak frequencies of GDMI prior to decay, therefore this technology also will perform best for preferentially outcrossing species but will be ineffective for large snail populations (Fig. 5.7). Other genetic features like dominance, penetrance, and epistatic interactions are significant considerations for choosing appropriate gene targets (Figs. 5.8-5.10). Although optimising genetic designs is not trivial (Tycko et al., 2016), because modifying snail habitat on a large scale is more challenging, efforts to improve drive construct designs will yield higher returns in successful establishment of GDMI.

Ecological factors that dilute the frequency of gene drive in a focus population (e.g. at a water access site), such as high gene flow due to snail migration or a large snail population size, inhibit timely establishment of gene drive mediated immunity (see also Fig. 5.12). These results indicate that success is most easily achieved in isolated water bodies with smaller snail populations. Snail population sizes in many areas fluctuate dramatically by season (Perez-Saez et al., 2016), therefore introduction of GDMI snails may be best timed when population sizes are at their lowest and have maximum growth potential. Otherwise,

GDMI will establish slowly in populations experiencing high seasonality (Fig. 5.14). Populations with shorter generation times will achieve greater GDMI frequencies within 10 years (Fig. 5.11). Future studies should build on this foundational simulation by considering snail migration and water flow between locations to assess whether GDMI snails would be effective in a wider range of scenarios.

There are some caveats and complexities that we have not addressed here. This model was built on the assumption that the gene drive works, i.e. that the gene drive is effective in producing snails that are immune to schistosome infection. While immune alleles associated with the PTC I and II gene clusters in *B. glabrata* can be rapidly selected in experimental conditions, these alleles have not yet been successfully deployed in a gene drive construct. Further genetic work is required to discover gene drive targets in other snail host species. Due to the flexibility of the modeling framework, model results can be updated to reflect qualities of constructs in development and the species targeted. In addition, we have not considered potential interactions with other trematode species, as snails can be the intermediate hosts to species other than schistosomes (Laidemitt et al., 2019). Interactions between schistosomes, other trematode species, and immunity can shape the fitness landscape in which GDMI operates, and therefore require further investigation to gauge whether the efficacy of this gene drive approach is sensitive to these interactions. This equally applies to interactions that involve schistosome subtypes that may evade a single GDMI design. Field work to identify sympatric schistosome subtypes will be necessary



to evaluate local deployment of GDMI. Although we evaluate the outcomes of GDMI over a range of sensitive conditions, the strength of this model is its flexibility to incorporate emerging data to simulate local conditions.

These results indicate that the use of GDMI together with MDA could contribute to a longer-lasting reduction of worm burden than either GDMI or MDA alone. This emphasises that gene drive is one potential tool among several that are currently available, and optimal use would likely be in conjunction with current control methods. GDMI is much more targeted than molluscicides, as it does not destroy the populations of snails and other aquatic life, and thus may be preferred by many stakeholders. Moving forward, it will be necessary to model how gene drives could interact with the variety of other control methods, to assess the optimal combination of methods and timing that would result in sustained elimination.

Modeling is crucial to understand the feasibility of implementing a new technology like gene drive, particularly in a natural system. Although this technology represents a new frontier for controlling disease, pests, and invasive species, the spread of designed genes in a natural setting can carry serious ethical and practical implications (Famakinde, 2020; National Academies of Sciences, Medicine, et al., 2016). It is therefore prudent to begin any considerations with *in silico* and *in vitro* studies, before proceeding to *in vivo*, with earlier steps informing the next. Further, modeling can ground critical deliberations amongst stakeholders by providing realistic predictions for the effects of a gene drive

project and provide a useful ability to rapidly perform new simulations to address questions that stakeholders might have for gene drive developers (Kofler et al., 2018). This model is an advancement towards a biologically realistic simulation which integrates population genetics, epidemiology, and population dynamics, and can serve as a template for future work in gene drive feasibility analysis.

## 5.5 Methods

### 5.5.1 Model Summary

We present a model of mixed mating strategy and explore a range of observed selfing rates to understand how reproduction strategy influences success of gene drive technology in a natural population. The gene drive model developed embeds a non-stationary Markov process that accounts for natural inheritance patterns as well as gene drive inheritance and fitness differences among genotypes. In contrast to previous gene drive models, which consider a wildtype allele and gene drive allele, we consider an expanded model with two wildtype alleles: susceptible ( $A$ ) and immune ( $B$ ). A third allele,  $B_g$ , represents engineered immunity to infection in the form of a gene drive construct. The set of six genotypes formed by these three alleles is  $\Omega = \{AA, AB, BB, AB_g, BB_g, B_gB_g\}$ . Let  $P_i$  be the frequency of each genotype where  $i \in \Omega$ . Let  $\mathbf{P}_t$  be the row vector composed of genotype frequencies at time (in generations)  $t$ . We describe the mixed mating system of genetic inheritance

with two transition matrices,  $\mathbf{S}$  (self-fertilisation) and  $\mathbf{T}$  (out-crossing), to describe the transitional probabilities from generation  $t$  to  $t + 1$ .

$$\mathbf{S} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ \frac{1}{4} & \frac{1}{2} & \frac{1}{4} & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ \frac{1}{4} & 0 & 0 & \frac{1-H}{2} & 0 & \frac{H}{2} + \frac{1}{4} \\ 0 & 0 & \frac{1}{4} & 0 & \frac{1-H}{2} & \frac{H}{2} + \frac{1}{4} \\ 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \quad (5.1)$$

$$\mathbf{T} = \begin{pmatrix} P_A & P_B & 0 & (1-H)P_{Bg} & 0 & HP_{Bg} \\ \frac{P_A}{2} & \frac{P_A+P_B}{2} & \frac{P_B}{2} & \frac{(1-H)P_{Bg}}{2} & \frac{(1-H)P_{Bg}}{2} & HP_{Bg} \\ 0 & P_A & P_B & 0 & (1-H)P_{Bg} & HP_{Bg} \\ \frac{P_A}{2} & \frac{P_B}{2} & 0 & \frac{(1-H)(P_{Bg}+P_A)}{2} & \frac{(1-H)P_B}{2} & \frac{H+P_{Bg}}{2} \\ 0 & \frac{P_A}{2} & \frac{P_B}{2} & \frac{(1-H)P_A}{2} & \frac{(1-H)(P_B+P_{Bg})}{2} & \frac{H+P_{Bg}}{2} \\ 0 & 0 & 0 & (1-H)P_A & (1-H)P_B & P_{Bg}(1-H)+H \end{pmatrix} \quad (5.2)$$

Homing efficiency  $H$  takes values between 0 (Mendelian inheritance) and 1 (complete fidelity of gene drive mechanism). A matrix  $\mathbf{Q}$  can be formed to represent the mixed mating system with self-fertilisation rate and cost of inbreeding given by  $\sigma, \xi \in [0, 1]$ , respectively.

$$\mathbf{Q} = \sigma(1 - \xi)\mathbf{S} + (1 - \sigma)\mathbf{T} \quad (5.3)$$

In the absence of population dynamics and fitness differences between genotypes, equation

5.4 would suffice to describe genotype frequency changes over time.

$$P_{t+1} = P_t Q \quad (5.4)$$

To accurately represent the evolution of traits in the snail-schistosome system, we relax these simplifying assumptions by incorporating fitness differences in response to viability and fecundity selection. We also introduce overlapping generations with density dependent recruitment in the snail population (SI Eqns. 5.8-5.51). Model simulations recapitulate laboratory results under the same conditions (Fig. 5.5). We derive analytical solutions at long term equilibrium (SI Eqns. 5.52-5.62) and analyze the evolution of resistance to the gene drive mechanism (SI Eqns. 5.63-5.65). The model is expanded to simulate the effects of ‘daisy chain’ drive (Fig. 5.7, SI Eqns. 5.66-5.75), and invasion analysis is performed for key variables, while a stochastic model is used to observe extinction conditions (Fig. 5.13, SI Eqns. 5.77-5.78).

The genetic model is integrated with an epidemiological model of schistosomiasis through the fraction of immunity in the snail population,  $\rho$ .

$$\frac{dw}{dt} = \alpha y - \mu w \quad (5.5)$$

$$\frac{dy}{dt} = \Lambda^* (1 - e^{-\beta w}) (1 - y - \rho) - v y \quad (5.6)$$

$\alpha$  and  $\beta$  are transmission rates governing the conversion of snail infection prevalence,  $y$ , to adult worms,  $w$ , in humans and vice versa. Gurarie et al. outlined the transmission from humans to snails is saturating with increasing worm burden (Gurarie et al., 2018). The asymptote is the pre-treatment force of infection,  $\Lambda^*$ .  $\mu$  and  $v$  are the death rates of adult worms and infected snails, respectively.

Equations 5 and 6 are integrated in 3 month intervals, corresponding to the expected generation time of the snail population. The probability of a new infection per susceptible snail in a generation determines the strength of selection for immunity in the genetic model.

$$Pr(y^+) \approx \int_{t=\tau}^{\tau+1} \Lambda^* (1 - e^{-\beta w}) dt \quad (5.7)$$

Parameter values and initial endemic conditions are detailed in SI tables 1 and 2. Calculations for equilibrium values and reproduction numbers are made in SI Equns. 5.80-5.98. The epidemiological model was not used to simulate figures 5.2 and 5.3. Instead, the probability of new infections was held constant at the equilibrium value calculated for endemic conditions with the integrated genetic and epidemiological model (Fig. 5.15). Figure 5.4 incorporates both dynamic models to evaluate single and paired treatment. MDA is modeled as instantaneous annual treatment. Percent reduction in worm burden during each treatment was 60% and is the product of coverage and efficacy.

Python code for simulations is available at

[www.github.com/grewelle/ModelGeneDriveSchisto](https://www.github.com/grewelle/ModelGeneDriveSchisto).

### 5.5.2 Model validation with empirical data

Tennessen et al. 2015 Tennessen et al., 2015b performed selection experiments using two infection conditions: 10 and 30 miracidia per snail. These snails were 10 generations from natural *Biomphalaria glabrata* breeding populations and were kept together to breed during each generation of selection. After challenging each group of snails with miracidia, infected snails were removed from the breeding population. Selection for immunity was evident and genetically based as given by experimental evidence of decline in infection through the 6 generations of challenges. We modify our model to replicate these experimental conditions, first by assuming that removal from the breeding pool only occurs via infection (mortality = 0). We assumed a high probability of infection of 80% for susceptible snails in the 30 miracidia experimental condition. Because the snails are kept in close proximity, and *B. glabrata* are known to outcross frequently, we assume that outcrossing was the exclusive mode of reproduction (i.e. selfing = 0). Initial allele frequencies were calculated on the basis of the frequency of observed infections (approx. 57%) in the 30 miracidia experimental condition for a probability of infection of 80 % for susceptible snails. GDMI was absent and set to a frequency of 0. Otherwise, parameters were unaltered from simulation conditions presented throughout the paper. Initial allele and genotype frequencies were

assumed the same between the two experimental treatments, and the probability of infection of susceptible snails was calculated given the frequency of observed infections in the first challenge (48%). The probability of infection for the 10 miracidia treatment is 70%. The curves produced by the model in Fig. 5.5 of expected infection frequencies given these two calculated probabilities (70% and 80%) reflect the observed data well despite some assumptions (e.g. no self-fertilisation) and experimental variability. We consider this fit qualitatively similar because some unknown experimental conditions are assumed, and therefore represent one of the possible model outcomes. However, empirical evidence suggesting that immunity is a dominant trait and that it is regulated by a gene complex, which is tightly linked, corroborates our use of a one locus, complete dominance ( $h = 1$ ) model. Model parameters used in the simulations are able to generate similar evolutionary dynamics to experimentally achieved evolution, and therefore, their values are further supported by our results in addition to support from literature.

## 5.6 Acknowledgments

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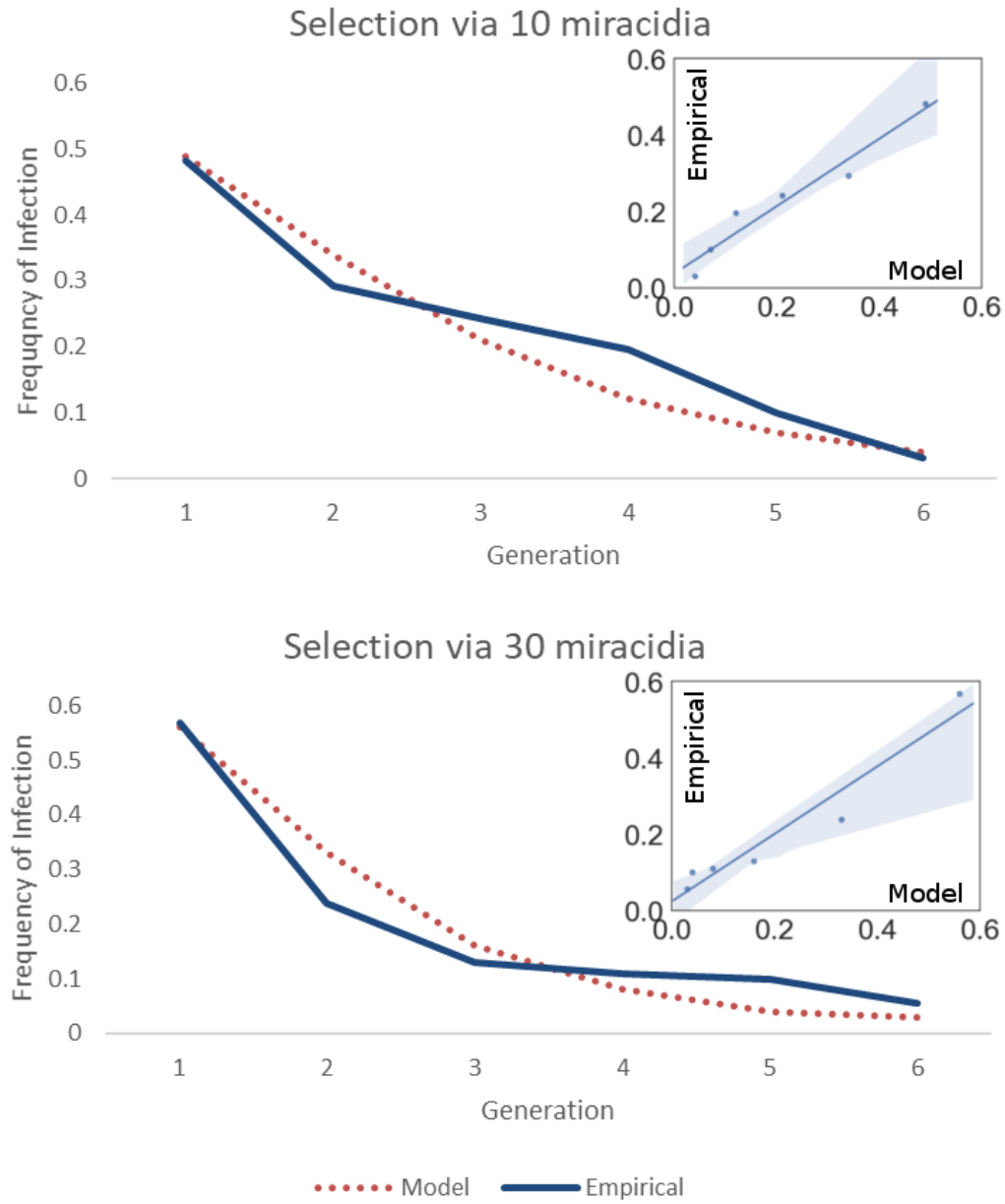


Figure 5.5: Comparison of model results to empirical data from published selection experiments by Tennessen et al. 2015. Two selection experiments were conducted, the first challenging each snail with 10 miracidia (top,  $R^2 = 0.944$ ), and the second challenging each snail with 30 miracidia (bottom,  $R^2 = 0.937$ ). Given initial conditions similar to experimental conditions, both models perform well in recapitulating selection for immunity to infection.



## 5.7 Supplementary Information

### 5.7.1 Population genetic model

The evolution of a focal population seeded with gene drive mediated immune (GDMI) snails is described in rudimentary form in the model summary with transition matrix  $Q$ . This describes a system of inheritance where a susceptible and immune allele are present in the population, and the GDMI allele exhibits the same immunity as the naturally occurring immunity. The three alleles form six distinct genotypes in a diploid species.  $Q$  represents random mating with no selection and full population replacement each generation. In a natural system assortative mating, selection, and iteroparity are known to occur. Because assortative mating as a function of innate immunity to schistosome infection has not yet been demonstrated in host snail species, we maintain this assumption in our population genetic model. However, because several modes of selection are described for this immunity and iteroparity produces overlapping generations, other assumptions for  $Q$  must be relaxed to accurately reflect the evolutionary dynamics of the snails. Viability and fecundity selection are separately accounted in their contribution the fitness of each genotype, as their relative importance in determining the rate of evolution changes with the model of population dynamics and replacement.

### Birth-death process

Snail recruitment is density-dependent. Adult lifespan extends past the mean generation time, allowing for nearly continuous reproduction after sexual maturity. We assume that background mortality is density-independent and that the population replacement rate is modulated by changes in mortality rate provided density-dependent recruitment is sufficient to for full replacement. In contrast to a population model described by reproduction proceeded by culling to a carrying capacity, we model snail population dynamics with culling proceeded by reproduction to a carrying capacity. The sub-population size of genotype  $i$  is described through time with the two step process: (1) death and migration yield the reproducing population of genotype  $i$ ,  $\bar{N}_i(t)$ , which (2) give birth to offspring according to equation 5.9.

$$\bar{N}_i(t) = N_i(t)[1 - \gamma_i(t) + m_i(t)] \quad (5.8)$$

$$N_i(t+1) = \bar{N}_i(t) + \frac{\lambda_i(t)}{\lambda(t)}[G(\bar{N}(t), \lambda(t), t) - \bar{N}(t)] \quad (5.9)$$

where  $N_i(t)$  is the genotype sub-population size in generation  $t$ ,  $\gamma_i(t)$  is the fractional mortality in generation  $t$ ,  $m_i(t)$  is the fractional net migration in or out of the focal population in generation  $t$ ,  $\lambda_i(t)$  is the partial finite growth of genotype  $i$  after mortality and migration, and  $\lambda(t)$  is the maximum total finite growth of the population. Because deaths are

separately accounted in this birth-death process, here  $\lambda(t)$  resembles fecundity (i.e. when mortality is absent, fecundity and finite population growth are equivalent).  $G(\bar{N}(t), \lambda(t), t)$  is the discrete time population growth function which describes total population growth. We use a logistic growth function in the simulations throughout the paper.

$$G(\bar{N}(t), \lambda(t), t) = \frac{\bar{N}(t)K}{\bar{N}(t) + (K - \bar{N}(t))e^{-\lambda(t)}} \quad (5.10)$$

### Viability selection

Viability selection on immunity to schistosome infection is incorporated in the fractional mortality term,  $\gamma_i(t)$ , which is a function of background mortality (neutral) and loss from the reproductive population through infection (directional selection), which has been demonstrated to substantially increase mortality and castrate snails. For these two reasons, we assume that infected snails are removed from the reproducing population. Reproductive compensation has been observed for snails exposed to miracidia, whereby these snails produce offspring at higher rates following exposure. This could counter lost reproduction after infection. However, a genetic link between reproductive compensation and immunity has not yet been shown. Therefore, we assume that the mechanism of reproductive compensation is phenomenological, being linked to exposure but not patent infection, rather than linked to host genetics, and does not produce fitness differences between genotypes. Explicitly, we write  $\gamma_i(t)$  as

$$\gamma_i(t) = d + (1 - d)Pr(y^+)(1 - h\iota) \quad (5.11)$$

$d$  is the adult background mortality per snail in a generation.  $Pr(y^+)$  is the per snail probability of acquiring a new infection.  $h$  is the dominance coefficient, which takes values between 0 and 1, with a value of 0.5 indicating co-dominance and a value of 1 indicating complete dominance of the immune allele over the susceptible allele.  $\iota \in [0, 1]$  is the degree of immunity conferred by the immune allele compared to the susceptible allele. Genetically, this value can be equated to the penetrance of innate immunity. A value of 0 indicates no additional immunity, while a value of 1 indicates full immunity. The loss of reproductive individuals from the population contributed by infection compared to the background mortality determines the strength of viability selection for immunity in the population.

### **Fecundity selection**

Inbreeding and immunity are known to negatively impact reproductive success in host snail populations. Inbreeding can reduce fecundity and egg viability, while immunity is associated with low egg viability (Doums et al., 1996; J. Webster & Woolhouse, 1999b). Because the population model tracks reproductive individuals, and recruitment of offspring to the reproductive class is density-dependent, offspring viability can be treated as a component

of fecundity. Using the broad definition of fecundity as the offspring surviving to adulthood, we institute fecundity costs for inbreeding by self-fertilization and for maintenance of immune alleles. Cost of immune maintenance,  $C$ , is directly related to the phenotype and is dose-independent (i.e. 2 immune alleles are not more costly than 1 immune allele with full dominance). An additional cost,  $C_g$ , is associated with maintenance of the genetic payload in the drive construct. This cost is dose-dependent; gene drive homozygotes carry a two-fold cost compared to heterozygotes. Let the set of alleles  $\{A, B, B_g\}$  be indexed as  $\{1, 2, 3\}$ . The fecundity of each genotype,  $f_i$ , can be represented as:

$$f_{11} = f_{AA} \quad (5.12)$$

$$f_{12} = f_{11}(1 - hC) \quad (5.13)$$

$$f_{22} = f_{11}(1 - C) \quad (5.14)$$

$$f_{13} = f_{11}(1 - hC - C_g) \quad (5.15)$$

$$f_{23} = f_{11}(1 - C - C_g) \quad (5.16)$$

$$f_{33} = f_{11}(1 - C - 2C_g) \quad (5.17)$$

The inbreeding cost is not directly associated with a specific genotype, but rather is incorporated as  $\xi \in [0, 1]$  in the calculation of  $Q$  (equation 5.3):

$$\mathbf{Q} = \sigma(1 - \xi)\mathbf{S} + (1 - \sigma)\mathbf{T} \quad (5.18)$$

Although  $\xi$  is a cost not directly applied to any genotype, because it is a cost of inbreeding due to self-fertilization and self-fertilization produces homozygotes with higher frequency than outcrossing, this cost reduces the fitness of homozygotes relative to heterozygotes when selfing is common in the population. Inbreeding is assumed to only affect the F1 generation of selfing parents, and associated costs are not separately tracked through descent in future generations. This treatment is reasonable for a sufficient degree of outcrossing which mixes lineages in the population. Highly inbred snail populations are shown to be insensitive to inbreeding depression caused by selfing, presumably due to purging of deleterious alleles from the gene pool, and therefore inbreeding costs are low in the absence of outcrossing. Table 1 gives parameter values used in the genetic model, many of which are known to vary by species or even by population and environmental conditions. Values were chosen to be centered in the range of observed values with references given to empirical measurements. Results can differ by system, and the endpoint sensitivity analyses in Figs. 5.2 and 5.3 (main text) provide indication of the most sensitive parameters.

The Markov process described in the model summary with equations 5.3 and 5.4 represents a semelparous population that reproduces once per generation, and adults are completely replaced by offspring with no consideration for fitness differences between genotypes. Instead, however, snail host populations reproduce continuously with overlapping

Table 5.1: Default parameter values for the genetic model

| Parameter     | Description                                       | Value                                                                                  | Ref.                                                                                                         |
|---------------|---------------------------------------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|
| $d$           | background mortality per generation               | 0.5                                                                                    | calculated with reference to <i>B. pfeifferi</i> (Woolhouse, 1989b, 1992)                                    |
| $h$           | dominance coefficient for immune allele           | 1                                                                                      | supported by Fig. 5.5 (main text) and (Tennessen et al., 2015b)                                              |
| $Pr(y^+)$     | probability of infection per generation           | 0.15                                                                                   | derived from epidemiological model (equ. 5.90)                                                               |
| $\iota$       | penetrance of immunity                            | 0.8                                                                                    | (J. Webster & Woolhouse, 1999b)                                                                              |
| $C$           | cost of immunity                                  | 0.2835                                                                                 | model-derived to yield symmetric selection for and against immunity, stabilizing the phenotypic ratio at 1:1 |
| $C_g$         | cost of payload per copy                          | 0.1                                                                                    | (Unckless et al., 2017b) variable/theoretical                                                                |
| $\xi$         | cost of inbreeding                                | 0.3                                                                                    | (Escobar et al., 2011b) highly variable                                                                      |
| $H$           | homing efficiency                                 | 0.9                                                                                    | (Gantz & Bier, 2015b)                                                                                        |
| $\sigma$      | selfing frequency                                 | 0.5                                                                                    | (Escobar et al., 2011b) highly variable                                                                      |
| $f_{AA}$      | per generation fecundity of a susceptible snail   | 20                                                                                     | (J. Webster & Woolhouse, 1999b)                                                                              |
| $P_{AA}(t=0)$ | natural initial frequency of susceptible genotype | 0.5                                                                                    | (Tennessen et al., 2015b; J. Webster & Woolhouse, 1999b)                                                     |
| $P_A(t=0)$    | natural initial frequency of allele A             | $\frac{\sigma - \sqrt{16P_{AA} - 24\sigma P_{AA} + \sigma^2(1+8P_{AA})}}{4(\sigma-1)}$ | calculated for a given $\sigma$                                                                              |
| $P_B(t=0)$    | natural initial frequency of allele B             | $1-P_A$                                                                                | calculated for a given $\sigma$                                                                              |
| $P_{AB}(t=0)$ | natural initial frequency of heterozygote         | $\frac{4P_AP_B(1-\sigma)}{2-\sigma}$                                                   | calculated for a given $\sigma$                                                                              |
| $P_{BB}(t=0)$ | natural initial frequency of immune genotype      | $\frac{P_B^2 + P_AP_B\sigma}{2-\sigma}$                                                | calculated for a given $\sigma$                                                                              |

generations. We devise a modified Markov process to describe these evolutionary dynamics, incorporating the fitness differences detailed above in equations 5.12-5.17. We consider the modified transition matrices:

$$\bar{\mathbf{S}} = \begin{pmatrix} f_{11} & 0 & 0 & 0 & 0 & 0 \\ \frac{f_{12}}{4} & \frac{f_{12}}{2} & \frac{f_{12}}{4} & 0 & 0 & 0 \\ 0 & 0 & f_{22} & 0 & 0 & 0 \\ \frac{f_{13}}{4} & 0 & 0 & \frac{f_{13}(1-H)}{2} & 0 & f_{13}(\frac{H}{2} + \frac{1}{4}) \\ 0 & 0 & \frac{f_{23}}{4} & 0 & \frac{f_{23}(1-H)}{2} & \frac{f_{23}H}{2} + \frac{f_{23}}{4} \\ 0 & 0 & 0 & 0 & 0 & f_{33} \end{pmatrix} \quad (5.19)$$

$$\bar{\mathbf{T}} = \begin{pmatrix} a_{11} & a_{12} & 0 & a_{14} & 0 & a_{16} \\ a_{21} & a_{22} & a_{23} & a_{24} & a_{25} & a_{26} \\ 0 & a_{32} & a_{33} & 0 & a_{35} & a_{36} \\ a_{41} & a_{42} & 0 & a_{44} & a_{45} & a_{46} \\ 0 & a_{52} & a_{53} & a_{54} & a_{55} & a_{56} \\ 0 & 0 & 0 & a_{64} & a_{65} & a_{66} \end{pmatrix} \quad (5.20)$$



$$a_{11} = f_{11}P_{11} + \frac{(f_{11} + f_{12})P_{12} + (f_{11} + f_{13})P_{13}}{4} \quad (5.21)$$

$$a_{12} = \frac{(f_{11} + f_{22})P_{22}}{2} + \frac{(f_{11} + f_{12})P_{12} + (f_{11} + f_{23})P_{23}}{4} \quad (5.22)$$

$$a_{14} = (1 - H)\left(\frac{(f_{11} + f_{33})P_{33}}{2} + \frac{(f_{11} + f_{13})P_{13} + (f_{11} + f_{23})P_{23}}{4}\right) \quad (5.23)$$

$$a_{16} = H\left(\frac{(f_{11} + f_{33})P_{33}}{2} + \frac{(f_{11} + f_{13})P_{13} + (f_{11} + f_{23})P_{23}}{4}\right) \quad (5.24)$$

$$a_{21} = \frac{(f_{11} + f_{12})P_{11}}{4} + \frac{2f_{12}P_{12} + (f_{12} + f_{13})P_{13}}{8} \quad (5.25)$$

$$a_{22} = \frac{(f_{11} + f_{12})P_{11} + 2f_{12}P_{12} + (f_{12} + f_{22})P_{22}}{4} + \frac{(f_{12} + f_{13})P_{13} + (f_{12} + f_{23})P_{23}}{8} \quad (5.26)$$

$$a_{23} = \frac{(f_{12} + f_{22})P_{22}}{4} + \frac{2f_{12}P_{12} + (f_{12} + f_{23})P_{23}}{8} \quad (5.27)$$

$$a_{24} = (1 - H)\left(\frac{(f_{12} + f_{33})P_{33}}{4} + \frac{(f_{12} + f_{13})P_{13} + (f_{12} + f_{23})P_{23}}{8}\right) \quad (5.28)$$

$$a_{25} = (1 - H)\left(\frac{(f_{12} + f_{33})P_{33}}{4} + \frac{(f_{12} + f_{13})P_{13} + (f_{12} + f_{23})P_{23}}{8}\right) \quad (5.29)$$

$$a_{26} = H\left(\frac{(f_{12} + f_{33})P_{33}}{2} + \frac{(f_{12} + f_{13})P_{13} + (f_{12} + f_{23})P_{23}}{4}\right) \quad (5.30)$$

$$a_{32} = \frac{(f_{11} + f_{22})P_{11}}{2} + \frac{(f_{22} + f_{12})P_{12} + (f_{22} + f_{13})P_{13}}{4} \quad (5.31)$$

$$a_{33} = f_{22}P_{22} + \frac{(f_{22} + f_{12})P_{12} + (f_{22} + f_{23})P_{23}}{4} \quad (5.32)$$

$$a_{35} = (1 - H)\left(\frac{(f_{22} + f_{33})P_{33}}{2} + \frac{(f_{22} + f_{13})P_{13} + (f_{22} + f_{23})P_{23}}{4}\right) \quad (5.33)$$

$$a_{36} = H\left(\frac{(f_{22} + f_{33})P_{33}}{2} + \frac{(f_{22} + f_{13})P_{13} + (f_{22} + f_{23})P_{23}}{4}\right) \quad (5.34)$$

$$a_{41} = \frac{(f_{11} + f_{13})P_{11}}{4} + \frac{(f_{12} + f_{13})P_{12} + 2f_{13}P_{13}}{8} \quad (5.35)$$

$$a_{42} = \frac{(f_{13} + f_{22})P_{22}}{4} + \frac{(f_{12} + f_{13})P_{12} + (f_{13} + f_{23})P_{23}}{8} \quad (5.36)$$

$$a_{44} = (1 - H) \left( \frac{(f_{13} + f_{11})P_{11} + 2f_{13}P_{13} + (f_{13} + f_{33})P_{33}}{4} + \frac{(f_{13} + P_{12})P_{12} + (f_{13} + f_{23})P_{23}}{8} \right) \quad (5.37)$$

$$a_{45} = (1 - H) \left( \frac{(f_{13} + f_{22})P_{22}}{4} + \frac{(f_{12} + f_{13})P_{12} + (f_{13} + f_{23})P_{23}}{8} \right) \quad (5.38)$$

$$a_{46} = H \left( \frac{(f_{13} + f_{11})P_{11} + (f_{13} + f_{12})P_{12} + (f_{13} + f_{22})P_{22} + 2f_{13}P_{13} + (f_{13} + f_{23})P_{23}}{4} \right. \quad (5.39)$$

$$\left. + \frac{(f_{13} + f_{33})P_{33}}{4} \right) + \frac{2(f_{13} + f_{33})P_{33} + 2f_{13}P_{13} + (f_{23} + f_{13})P_{23}}{8} \quad (5.40)$$

$$a_{52} = \frac{(f_{11} + f_{23})P_{11}}{4} + \frac{(f_{12} + f_{23})P_{12} + (f_{23} + f_{13})P_{13}}{8} \quad (5.41)$$

$$a_{53} = \frac{(f_{23} + f_{22})P_{22}}{4} + \frac{(f_{23} + f_{12})P_{12} + 2f_{23}P_{23}}{8} \quad (5.42)$$

$$a_{54} = (1 - H) \left( \frac{(f_{11} + f_{23})P_{11}}{4} + \frac{(f_{12} + f_{23})P_{12} + (f_{23} + f_{13})P_{13}}{8} \right) \quad (5.43)$$

$$a_{55} = (1 - H) \left( \frac{(f_{23} + f_{22})P_{22} + (f_{23} + f_{33})P_{33} + 2f_{23}P_{23}}{4} + \frac{(f_{23} + f_{12})P_{12} + (f_{23} + f_{13})P_{13}}{8} \right) \quad (5.44)$$

$$a_{56} = H \left( \frac{(f_{23} + f_{11})P_{11} + (f_{23} + f_{12})P_{12} + (f_{23} + f_{22})P_{22} + (f_{13} + f_{23})P_{13} + 2f_{23}P_{23}}{4} \right. \quad (5.45)$$

$$\left. + \frac{(f_{23} + f_{33})P_{33}}{4} \right) + \frac{2(f_{23} + f_{33})P_{33} + (f_{13} + f_{23})P_{13} + 2f_{23}P_{23}}{8}$$

$$a_{64} = (1 - H) \left( \frac{(f_{11} + f_{33})P_{11}}{2} + \frac{(f_{33} + f_{12})P_{12} + (f_{33} + f_{13})P_{13}}{4} \right) \quad (5.46)$$

$$a_{65} = (1 - H) \left( \frac{(f_{22} + f_{33})P_{22}}{2} + \frac{(f_{33} + f_{12})P_{12} + (f_{33} + f_{23})P_{23}}{4} \right) \quad (5.47)$$

$$a_{66} = \frac{H}{2} \left( (f_{11} + f_{33})P_{11} + (f_{12} + f_{33})P_{12} + (f_{22} + f_{33})P_{22} + \frac{(f_{13} + f_{33})P_{13}}{2} + \frac{(f_{23} + f_{33})P_{23}}{2} \right) \quad (5.48)$$

$$+ \frac{(f_{13} + f_{33})P_{13} + (f_{23} + f_{33})P_{23}}{4} + f_{33}P_{33}$$

Modifying the equation for  $\mathbf{Q}$  in the model summary to reflect the incorporation of demography and fitness differences between genotypes, we achieve:

$$\bar{\mathbf{Q}} = \sigma(1 - \xi)\bar{\mathbf{S}} + (1 - \sigma)\bar{\mathbf{T}} \quad (5.49)$$

The vector of genotype frequencies at time  $t$  is denoted  $\mathbf{P}(t)$ .  $P_i(t)$  represents the frequency of genotype  $i$  at time  $t$ . The vector of partial growth rates is  $\boldsymbol{\lambda}(t)$ :

$$\boldsymbol{\lambda}(t) = \mathbf{P}(t)\bar{\mathbf{Q}}(t) \quad (5.50)$$

and the sum of the elements of this vector is  $\lambda(t)$ :

$$\lambda(t) = \sum_{i=1}^6 \lambda_i(t) \quad (5.51)$$

These values can be substituted in equation 9 to track the evolution of the population.

### 5.7.2 Establishing initial genetic conditions

Prior to deploying GDMI snails in a naive population, the standing background genetic variation for susceptibility to infection has some influence over the success of GDMI. The two forms of genetic variation that are important to consider are: the frequency of susceptibility and the distribution of the susceptible allele across the genotypes. High self-fertilization frequencies favor homozygous populations, which exposes the susceptible allele to selection (assuming it is recessive). Several studies have measured susceptibility empirically through challenge experiments in laboratory conditions. Snail populations that are not far removed from a natural parental lineage demonstrate intermediate levels of susceptibility, though these results vary with miracidial dosing. For simplicity we fix the standing natural frequency of susceptibility at 0.5. The immune phenotype is, therefore, at 0.5 frequency as well in our idealized starting conditions. In a mixed-mating system, which these snails exhibit, the distribution of the  $A$  and  $B$  alleles across the three genotypes is modulated by selfing frequency. The transition matrix describing the evolution of the three naturally occurring genotypes is

$$\mathbf{Q}_{natural} = \begin{pmatrix} \sigma + (1 - \sigma)P_A & (1 - \sigma)P_B & 0 \\ \frac{\sigma}{4} + \frac{(1 - \sigma)P_A}{2} & \frac{\sigma}{2} + \frac{(1 - \sigma)(P_A + P_B)}{2} & \frac{\sigma}{4} + \frac{(1 - \sigma)P_B}{2} \\ 0 & (1 - \sigma)P_A & \sigma + (1 - \sigma)P_B \end{pmatrix} \quad (5.52)$$

Here we denote  $P_A$  and  $P_B$  as the allele frequencies, and  $P_{11}(t)$ ,  $P_{12}(t)$ ,  $P_{22}(t)$  as the genotype frequencies at time  $t$ . To establish initial genetic conditions, we find the equilibrium genotype frequencies given a frequency of self-fertilization. We assume that the genotype frequencies are the result of selection but that otherwise selection is not stronger than the equilibrium behavior of the transition matrix under neutral conditions. Therefore, genotype frequencies can be solved given the frequency of the susceptible genotype. Equilibrium behavior of this transition matrix is strong, with genotype frequencies approaching equilibrium geometrically so that equilibrium is effectively reached within 2 generations. In the absence of imposed selection, the allele frequencies remain constant through each generation despite the changing genotype frequencies. This is the reason genotype frequencies are represented as functions of time above, while allele frequencies are not. From matrix multiplication, we know the frequency of the susceptible genotype in the next generation using  $\mathbf{Q}_{natural}$  is:

$$\begin{aligned}
 P_{11}(t+1) &= P_{11}(t)(\sigma + (1-\sigma)P_A) + P_{12}(t)\left(\frac{\sigma}{4} + \frac{(1-\sigma)P_A}{2}\right) \\
 &= (1-\sigma)P_A^2 + \sigma\left(P_{11}(t) + \frac{P_{12}(t)}{4}\right) \\
 &= P_A^2 + \sigma P_A P_B - \frac{\sigma P_{12}(t)}{4}
 \end{aligned} \tag{5.53}$$

Solving the difference equation yields:

$$P_{11}(t+1) = P_A^2 + \sigma P_A P_B \left(1 - \frac{1-\sigma}{2-\sigma} \left(1 - \left(\frac{\sigma}{2}\right)^t\right)\right) - \frac{\sigma P_{12}(0)}{4} \left(\frac{\sigma}{2}\right)^t \quad (5.54)$$

The limiting distribution of genotype frequencies can be solved as  $t \rightarrow \infty$ . For the susceptible genotype, this gives:

$$P_{11}(\infty) = P_A^2 + \frac{\sigma P_A P_B}{2-\sigma} \quad (5.55)$$

The same derivations can be performed for the other two genotype frequencies from the transition matrix to achieve the limiting distribution.

$$P_{12}(t+1) = P_{11}(t)(1-\sigma)P_B + P_{12}(t)\left(\frac{\sigma}{2} + \frac{(1-\sigma)(P_A + P_B)}{2}\right) + P_{22}(t)(1-\sigma)P_A \quad (5.56)$$

$$\begin{aligned} &= 2(1-\sigma)P_A P_B + \frac{\sigma P_{12}(t)}{2} \\ P_{22}(t+1) &= P_{12}(t)\left(\frac{\sigma}{4} + \frac{(1-\sigma)P_B}{2}\right) + P_{22}(t)(\sigma + (1-\sigma)P_B) \\ &= (1-\sigma)P_B^2 + \sigma(P_{22}(t) + \frac{P_{12}(t)}{4}) \end{aligned} \quad (5.57)$$

The equilibrium values for the heterozygote and immune homozygote are:

$$P_{12}(\infty) = \frac{4P_AP_B(1 - \sigma)}{2 - \sigma} \quad (5.58)$$

$$P_{22}(\infty) = \frac{P_B^2 + P_AP_B\sigma}{2 - \sigma} \quad (5.59)$$

These results differ from the results presented by Karlin (Karlin, 1968) due to a presumed typographical error in his text. A population with 50% susceptible genotype at equilibrium is assumed when GDMI snails are introduced (simulation  $t = 0$ ). Given that  $P_{11}(t = 0) = 0.5$ ,  $P_A$  can be solved:

$$P_B = 1 - P_A \quad (5.60)$$

$$P_{11}(t = 0) = P_A^2 + \frac{\sigma P_A(1 - P_A)}{2 - \sigma} \quad (5.61)$$

$$P_A = \frac{\sigma - \sqrt{16P_{11}(t = 0) - 24\sigma P_{11}(t = 0) + \sigma^2(1 + 8P_{11}(t = 0))}}{4(\sigma - 1)} \quad (5.62)$$

$$P_A|_{P_{11}=0.5} = \frac{\sigma - \sqrt{8 - 12\sigma + 5\sigma^2}}{4(\sigma - 1)}$$

### 5.7.3 Evolution of resistance

Resistance to the drive mechanism can readily develop if the target sequence on the homologous chromosome is mutated so as to be unrecognizable by guide RNA. This occurs primarily through non-homologous end joining (NHEJ), which is an alternative mechanism

of double strand break repair that can occur instead of homology directed repair. Point mutations and standing genetic variation at the target locus can also result in resistance to the drive mechanism. Because NHEJ is a common repair pathway in most organisms, it is the primary producer of resistance in the population, especially as the drive construct increases in frequency in the population. Resistance formation via this pathway occurs due to misrepair after cleavage from the Cas nuclease and occurs proportionally to the number of cleavage events that occur in the population each generation. Homing efficiency is a function of the predominance of homology directed repair over NHEJ, though not every failed drive event is due to NHEJ. Homing efficiency at the population level declines as resistant alleles accumulate. Resistant alleles may represent a spectrum of mutations, and separately accounting for the variety of alleles and their respective fitness requires exponential expansion of the number of genotypes tracked in this genetic model. To simplify, we assume that resistant alleles are equivalent in fitness to their natural counterparts. This gives them a fecundity advantage to the drive allele, which carries an additional cost due to the genetic payload. In a randomly mating population, outcrossing events will randomly pair resistant alleles with each other, natural alleles, or the drive allele. The consequence of non-assortative mating is that the formation of resistant alleles is proportional to the number of gene drive heterozygotes produced due to failed homing. The number of gene drive heterozygotes (hybrids) produced each generation is:



$$N_{hybrids}(t) = \frac{\lambda_4(t) + \lambda_5(t)}{\lambda(t)} [G(\bar{N}(t), \lambda(t), t) - \bar{N}(t)] \quad (5.63)$$

The homing efficiency in generation  $t$  can be calculated from the maximum homing efficiency without NHEJ,  $H_0$ , at  $t = 0$  when resistant allele accumulation is lowest and determined only by background resistance due to standing genetic variation.

$$\begin{aligned} H(t) &= H_0(1 - R(t) - \nu(1 - R(t))) \\ &= H_0(1 - R(t))(1 - \nu) \end{aligned} \quad (5.64)$$

$R(t)$  is the frequency of resistant alleles in the pool of natural and resistant alleles (excluding the drive allele).  $\nu$  is the per homing event rate of production of resistant alleles. If the population is in mutation-selection-drift balance, the rate of production is almost entirely due to NHEJ. The rate of accumulation of resistant alleles is the fraction of the total gene drive heterozygotes (hybrids) produced that are resistant multiplied by the fraction of hybrids produced in the total population each generation.

$$\begin{aligned}
R(t+1) &= R(t) + \frac{\nu(1-R(t))N_{hybrids}(t)}{(1-H(t) + \nu(1-R(t)))N(t)} \\
&= R(t) + \frac{\nu(1-R(t))N_{hybrids}(t)}{(1-H_0(1-R(t))(1-\nu) + \nu(1-R(t)))N(t)} \\
&= R(t) + \frac{\nu(1-R(t))N_{hybrids}(t)}{(1-(1-R(t))(H_0(1-\nu) + \nu))N(t)}
\end{aligned} \tag{5.65}$$

Should mutations leading to resistance be deleterious compared to the respective naturally occurring alleles, resistance is expected to spread more slowly in the population than is depicted in Fig. 5.6. Conversely, should mutations be beneficial, spread will occur more rapidly. The cost of resistance can be modulated by choosing neutral regions for lower cost and regions under strong selection for higher cost (e.g. ribosomal RNA genes). Fitness costs due to off-target CRISPR-induced mutations are possible, but experimental evidence indicates that the frequency of these mutations is low (<2%). Moreover, these mutations do not exhibit drive and are not related to the evolution of resistance. Off-target mutations can be reduced with properly specific gRNA design and restricted expression of the CAS9 protein. The influence of resistance evolution on the establishment of GDMI is explored in Fig. 5.6.

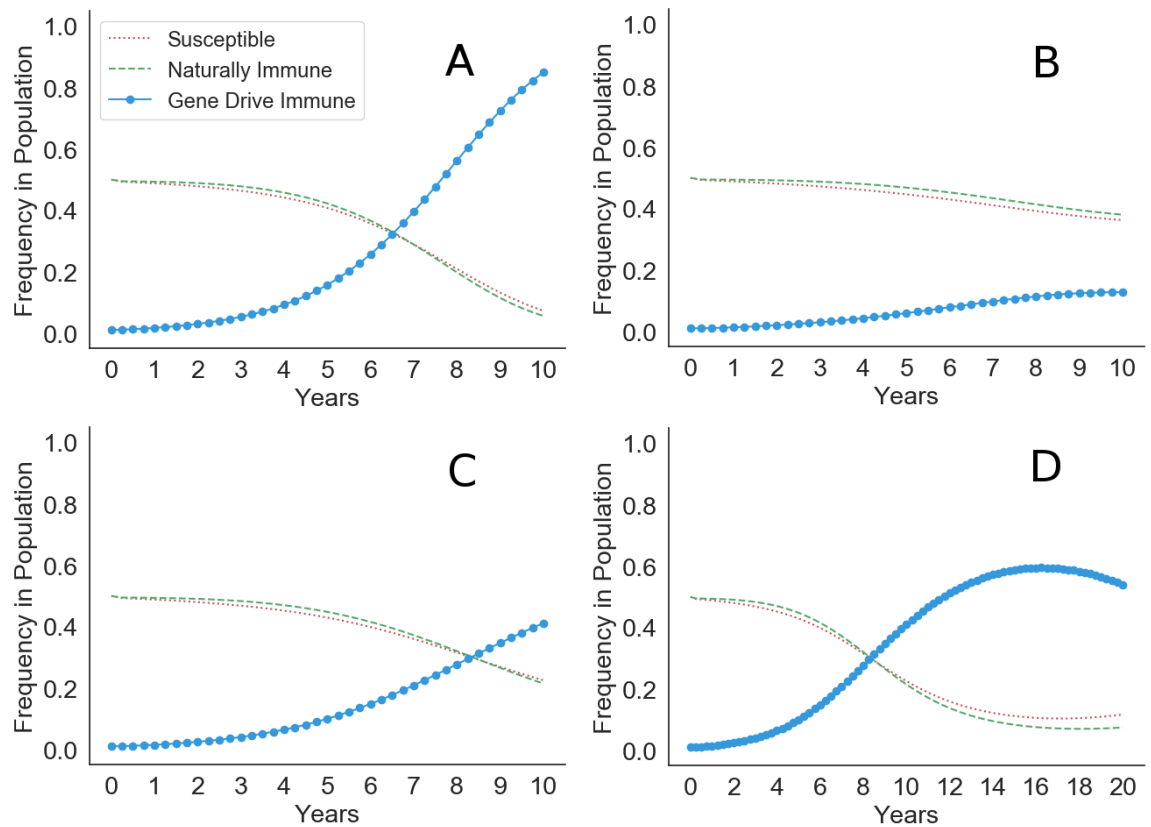


Figure 5.6: Forward simulations under fixed epidemiological conditions of the spread of GDMI with various resistance production rates per homing event. (A) No resistant alleles are produced. (B) Resistant alleles are produced with 20% of homing events. GDMI achieves only low frequency in the population due to rapid evolution of resistance to the drive mechanism. (C) Resistant alleles are produced with 10% of homing events. GDMI rises slowly, achieving half the frequency in the population compared to conditions where resistance does not evolve. (D) Resistant alleles are produced with 10% of homing events as in panel C. In 20 years it is evident that the frequency of resistant alleles outpaces the homing efficiency benefits in inheritance of GDMI, and GDMI declines after reaching intermediate frequency (eventually to negligible frequency).

#### 5.7.4 Daisy chain loci

Previous authors have proposed mechanisms to safeguard the spread and persistence of gene drives in wild populations. One prominent mechanism is known as a daisy drive, which borrows its name from the concept of a daisy chain in which elements are connected in a series. Daisy drives are split drives which split the drive element from the payload by positioning them on separate loci, ideally on different chromosomes for independent inheritance. Daisy drives incorporate multiple splits, with one drive element necessary to produce super-Mendelian inheritance of the next element in the chain. The base of the chain is a non-drive element and the tip of the chain is the genetic payload containing the gene of interest to be inherited in the population. Each element in the chain increases in frequency temporarily and then decays as the preceding elements decline in frequency in the population through natural selection. Each element in the daisy chain is considered haplosufficient, so one copy produces the intended homing efficiency of the proceeding element. Because daisy chain elements (loci) are introduced together in engineered individuals, spread of the payload gene occurs locally within a lineage in association with other loci and cannot be modeled using the same framework so far introduced which describes random pairing of alleles from one locus. The homing efficiency associated with the payload locus is primarily determined by this local lineage-based process of inheritance of daisy chain loci, especially when loci are at low frequency, and secondarily through outcrossing events with other lineages in the population that maintain daisy chain loci. The secondary

process becomes consequential at high frequencies of daisy chain loci in the population. Homing efficiency of the payload corresponds is determined by the frequency of the preceding locus in the daisy chain. We set  $\delta(t)$  as the daisy chain coefficient modulating the homing efficiency. The daisy coefficient is directly proportional to the co-occurrence of the payload and the preceding drive element and can be calculated as follows through time for lineage-specific inheritance (i.e. each outcrossing event occurs with a snail with no drive alleles):

$$\liminf \delta(t) = \begin{cases} 1 & n = 0 \\ 2^{1-t} & n = 1 \\ \frac{2^{n-1} + 2^{n-2}(t-n)}{2^{t-1}} & n > 1 \end{cases} \quad (5.66)$$

$n$  is the number of splits in the drive design, which is equivalent to the number of drive elements in the daisy chain (excluding the payload). This is the lower limit for the value of  $\delta(t)$ , as homing efficiency for the payload increases with the accumulation of the preceding drive element in the population so that with each outcrossing event, the mate outside the primary lineage may carry the preceding drive element. The frequency of the preceding drive element in the population is always lower than the frequency of the payload, and is the frequency of the payload in the prior generation before the peak frequency of the payload is reached. Fitness costs of each drive element are assumed negligible compared to the cost of the genetic payload at the tip of the daisy chain. After the peak frequency is

reached, the frequency of the preceding drive element is lower than the frequency of the payload in the prior generation. We can state the following:

$$\limsup \delta(t) = \begin{cases} 1 & n = 0 \\ 2^{1-t} & n = 1 \\ \frac{2^{n-1} + 2^{n-2}(t-n)}{2^{t-1}} + P_{payload}(t-1) & n > 1 \end{cases} \quad (5.67)$$

$$\delta(t) = \begin{cases} 1 & n = 0 \\ 2^{1-t} & n = 1 \\ \frac{2^{n-1} + 2^{n-2}(t-n)}{2^{t-1}} + P_{drive}(t) & n > 1 \end{cases} \quad (5.68)$$

where  $P_{payload}(t)$  and  $P_{drive}(t)$  are the frequencies of the genetic payload and the associated preceding drive element in the daisy chain, respectively.  $\limsup \delta(t) = \delta(t)$  prior to peak payload frequency. Equation 67 is useful to calculate the peak frequency of the payload in a daisy drive system without the need for a system of equations for each locus, which quickly becomes complex with additional elements in the chain. If we consider  $H_0$  as the maximum homing efficiency without a daisy chain design (1 locus,  $n = 0$ ), then the observed homing efficiency through time can be calculated as:

$$H(t) = \delta(t)H_0 \quad (5.69)$$

The homing efficiency at time  $t$  above represents the calculation for non-overlapping generations where the population is fully replaced with offspring each generation. When generations overlap, this homing efficiency underestimates the observed homing efficiency because younger adults reproduce alongside older adults, which maintain higher loads of daisy chain loci from fewer outcrossing events. Older adults produce more GDMI offspring as a result. The observed homing efficiency at the population level at time  $t$  is a function of the survival of each age class. We calculate the surviving fraction of the payload allele as:

$$S_{B_g}(t) = \frac{\bar{N}_4(t) + \bar{N}_5(t) + 2\bar{N}_6(t)}{2} \quad (5.70)$$

Let  $\mathbf{S}_{B_g}$  be the vector of surviving fractions of the payload allele through time.

$$\mathbf{S}_{B_g} = [S_{B_g}(0), S_{B_g}(1), \dots, S_{B_g}(t)] \quad (5.71)$$

An age distribution,  $\bar{\mathbf{Z}}_{B_g}$ , can be produced by calculating the survival of each age class through time and normalizing the vector by the sum of the elements.

$$\mathbf{Z}_{B_g} = [S_{B_g}(0), \prod_{i=0}^1 S_{B_g}(i), \frac{\prod_{i=0}^2 S_{B_g}(i)}{\prod_{i=0}^1 S_{B_g}(i)}, \dots, \frac{\prod_{i=0}^t S_{B_g}(i)}{\prod_{i=0}^{t-1} S_{B_g}(i)}] \quad (5.72)$$

$$\bar{\mathbf{Z}}_{B_g} = \frac{\mathbf{Z}_{B_g}}{\sum_i Z_{B_g}(i)} \quad (5.73)$$

An adjusted daisy chain coefficient,  $\delta_{adj}(t)$ , can be produced by calculating the dot product of the vector of daisy chain coefficients and the age distribution:

$$\delta_{adj}(t) = \boldsymbol{\delta} \bar{\mathbf{Z}}_{B_g}^T \quad (5.74)$$

Homing efficiency at time  $t$  for a population with overlapping generations is therefore:

$$H(t) = \delta_{adj}(t) H_0 \quad (5.75)$$

This value for the homing efficiency in each generation can be substituted into the existing framework described to calculate the frequency of GDMI through time. Fig. 5.7 shows the trajectory of GDMI with the use of an increasing number of daisy chain loci.

### 5.7.5 Dominance and penetrance of immune allele

The simulation in Fig. 5.5 (main text) demonstrates a strong qualitative fit to empirical data from selection experiments. The frequency of infection, which is the fraction of infected snails out of the total surviving exposed individuals, is a phenotype resulting from immunity to a miracidial strain. The phenotype is a function of the exposure dose (number of miracidia) and the genetic underpinnings of immunity, including the number of loci involved, the dominance of immune alleles over susceptible alleles, and the penetrance of immune alleles. The genetic contributions to this phenotype could be myriad, but some



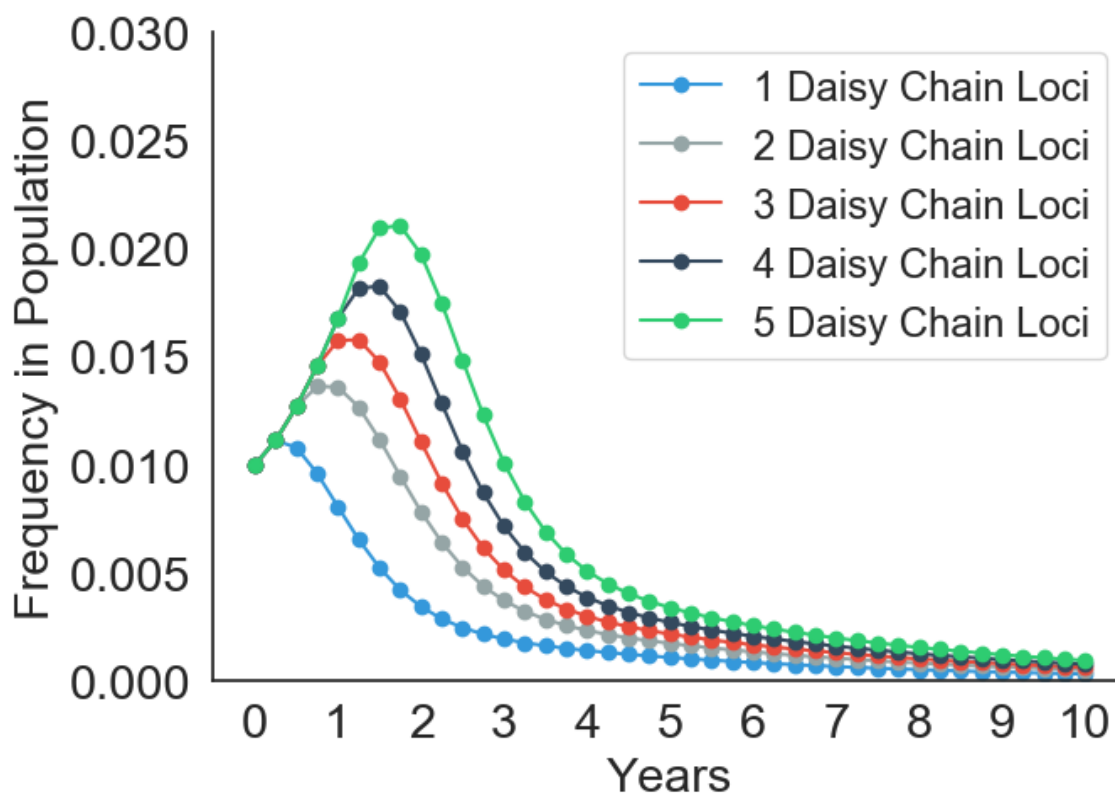


Figure 5.7: Forward simulations of daisy drive systems for the inheritance of GDMI designed with 1-5 daisy chain loci. Decay of the drive occurs after  $n$  generations, therefore more loci produce a longer lasting drive. However, because GDMI spreads slowly in the population compared to a fully outcrossed population, peak frequency of GDMI is low. Nearly 30 daisy chain loci are required to reach peak frequency of 50%, rendering daisy drive infeasible for implementation in this system.

large-effect loci have been identified in model snail species like *Biomphalaria glabrata*. These loci tend to be regions with high genetic variability and are linked to transmembrane proteins and receptors, which suggests a role in epitope recognition of an invading miracidium or sporocyst. One large-effect locus identified in Tennessen et al. 2015 served as a template for the default parameters used in the simulations in this work. We assumed a single locus model to represent this tightly linked gene cluster, and dominance of the immune allele over the susceptible allele was assumed complete as demonstrated in their empirical work. With a penetrance of 0.8, the model closely replicated observed evolution of immunity. However, as genetic work, such as genome wide association studies, identify new regions associated with immunity to schistosome infection in snails, more clarity will exist in the genetic contributions to immunity. Genes conferring immunity to one species or strain of schistosome may not confer immunity to others. These genes may not be conserved across snail species, and it is likely that immunity constitutes a wide array of variable genes. In *B. glabrata* two such polymorphic loci have been identified and described by Tennessen et al. 2015 and Tennessen et al. 2020 (Tennessen et al., 2020c; Tennessen et al., 2015b). Named Polymorphic Transmembrane Cluster 1 and 2 (PTC 1 and 2), these regions are each associated with several fold decreased odds of infection. However, the immune allele within PTC 2 likely has higher penetrance than the immune allele within PTC 1, and although the immune allele in PTC 1 is haplosufficient and completely

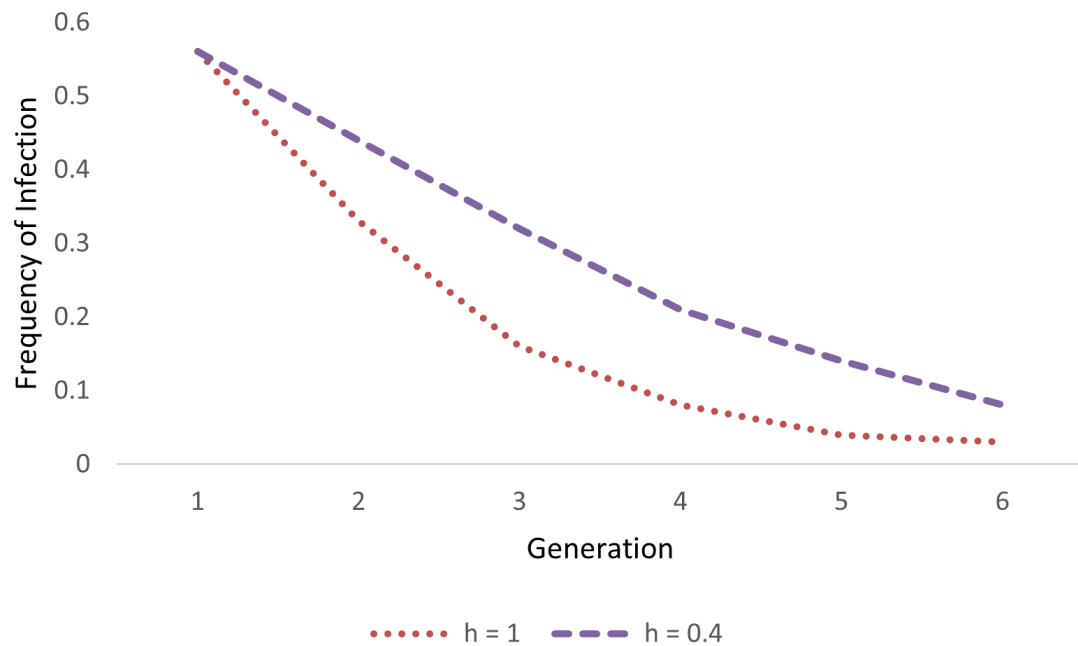


Figure 5.8: The relationship between the immune and susceptible alleles described by the dominance coefficient governs the trajectory of evolution for naturally-occurring immunity. Lower dominance of the immune allele leads to slower evolution of immunity, which could change the speed at which GDMI increases in frequency in a population.

dominant, incomplete dominance is observed for the immune allele within PTC 2. Variation in the genetic mechanisms of immunity can result in altered evolutionary trajectories in the face of the same strength of selection due to infectious miracidia. We show below how variation in dominance and penetrance changes the expected frequency of infection after generations of selection observed in Fig. 5.8.

In contrast to naturally-occurring alleles that whose inheritance is governed by the interaction between selection and dominance, an effective gene drive (high homing efficiency) may not be sensitive to this interaction because gene drive heterozygotes are produced at

low frequencies, and therefore, dominance plays only a small role in determining the fitness of gene drive alleles. We simulate GDMI inheritance under default conditions to test whether GDMI frequency is significantly altered by the strength of dominance of the immune allele over the susceptible allele(s). Dominance coefficients of 1 and 0.4 were chosen as the measured upper and lower bounds for PTC 1 ( $h = 1$ ) and PTC 2 ( $h = 0.4$ ). The results in Fig. 5.9 show that the effect of dominance on the success of gene drive in the 10 year evaluation window is minimal. These results support the notion that an effective drive designed targeting either locus would operate similarly provided other factors are equivalent.

Two caveats may change these results: PTC 2 contains 2 susceptible alleles, therefore a natural heterozygote of both susceptible allele exhibits intermediate immunity compared to natural homozygotes of each susceptible allele, and penetrance of immunity associated with PTC 2 was measured higher than PTC 1 (approx. 2-fold higher odds ratio). In the case of two susceptible alleles displaying a range of immunity across natural susceptible genotypes, independent assortment ensures that relative fitness of immune alleles will depend on the average absolute fitness of the susceptible alleles. The average absolute fitness of susceptible alleles is a byproduct of their interactions to produce a range of susceptible phenotypes. One susceptible allele in PTC 2 is additive: the homozygote is twice as susceptible as the heterozygote (one susceptible allele, one immune allele). The other susceptible allele in PTC 2 is partially additive: the homozygote is less than twice as susceptible as the heterozygote. Barring other epistatic interactions, the measured susceptibility of the

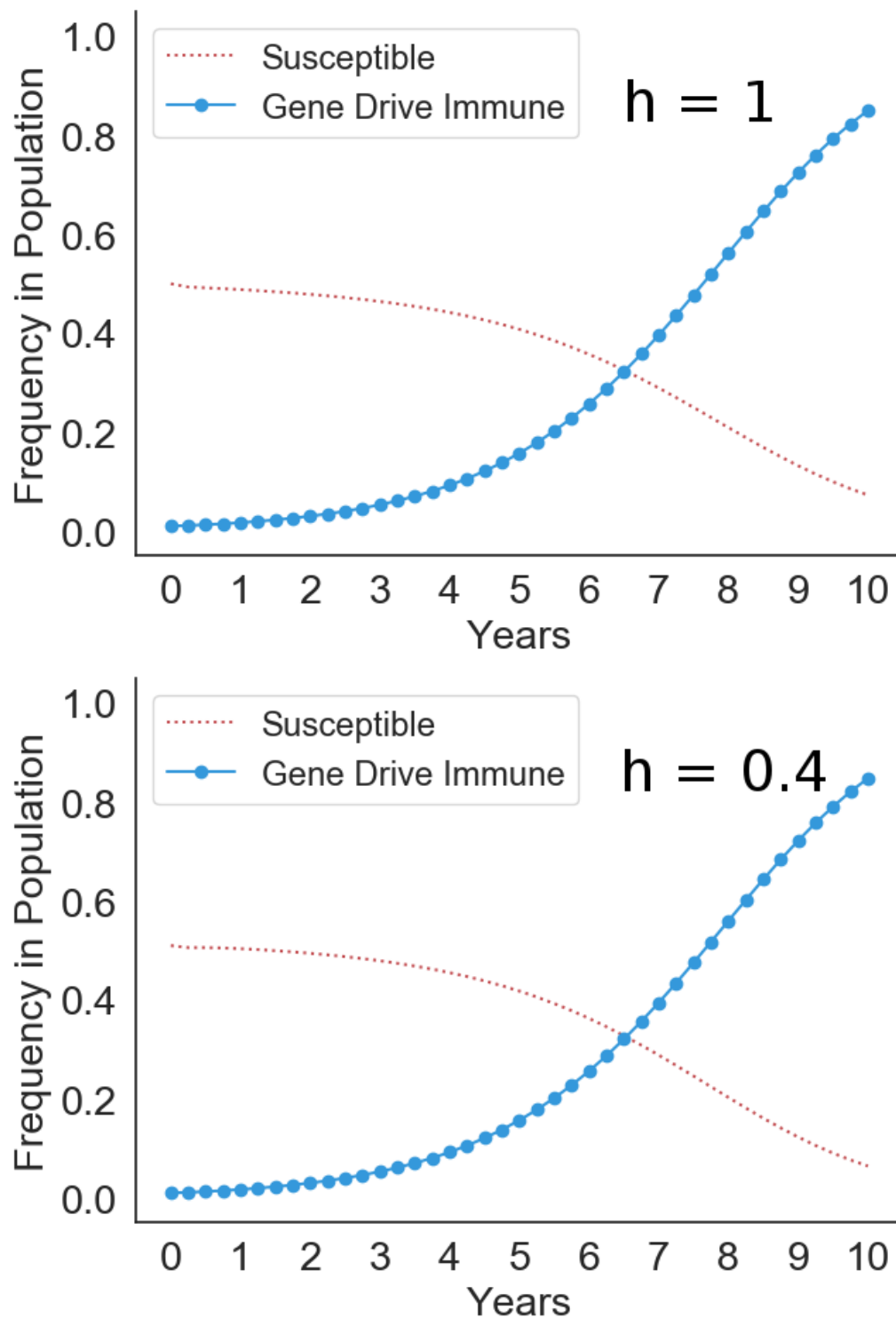


Figure 5.9: High dominance (top panel,  $h=1$ ) representing PTC 1 and low dominance (bottom panel,  $h=0.4$ ) representing PTC 2 do not yield measurably different results under default simulation conditions after 10 years.

genotypes, their frequency, and the force of infection (directional selection) can be used to determine the relative fitness of immunity. However, differences between the alleles, including costs of maintaining each of the susceptible genotypes, is unknown and precludes investigation into differences between the fitness of the susceptible genotypes in the face of selection. This subject will require further empirical investigation to determine whether a spectrum of susceptible alleles may alter the speed of establishment of GDMI for a PTC 2 -like target. Based on results presented in Fig. 5.3 (main text), GDMI establishment is mildly sensitive to standing genetic susceptibility to infection, thus we expect minor differences in the evolutionary dynamics between a single susceptible allele system and a diversified susceptible allele system. A factor that has greater potential for impact on the evolutionary dynamics of GDMI is penetrance. Higher penetrance of immunity results in greater phenotypic variation with a heritable basis, which provides greater evolutionary potential. Immunity associated with PTC 1 is modeled with  $\iota = 0.8$ . Susceptibility associated with PTC 2 represents up to 2-fold greater odds of infection. We simulate with  $\iota = 0.9$  in Fig. 5.10.

Despite the 2-fold greater odds of infection, PTC 2 susceptibility is not a significantly better target for GDMI. Resulting immunity in the population is similar after 10 years. These results change as the fitness advantages of GDMI over natural immunity diminish (e.g. low homing efficiency) and the fitness advantages of immunity over susceptibility strengthen (e.g. high force of infection).

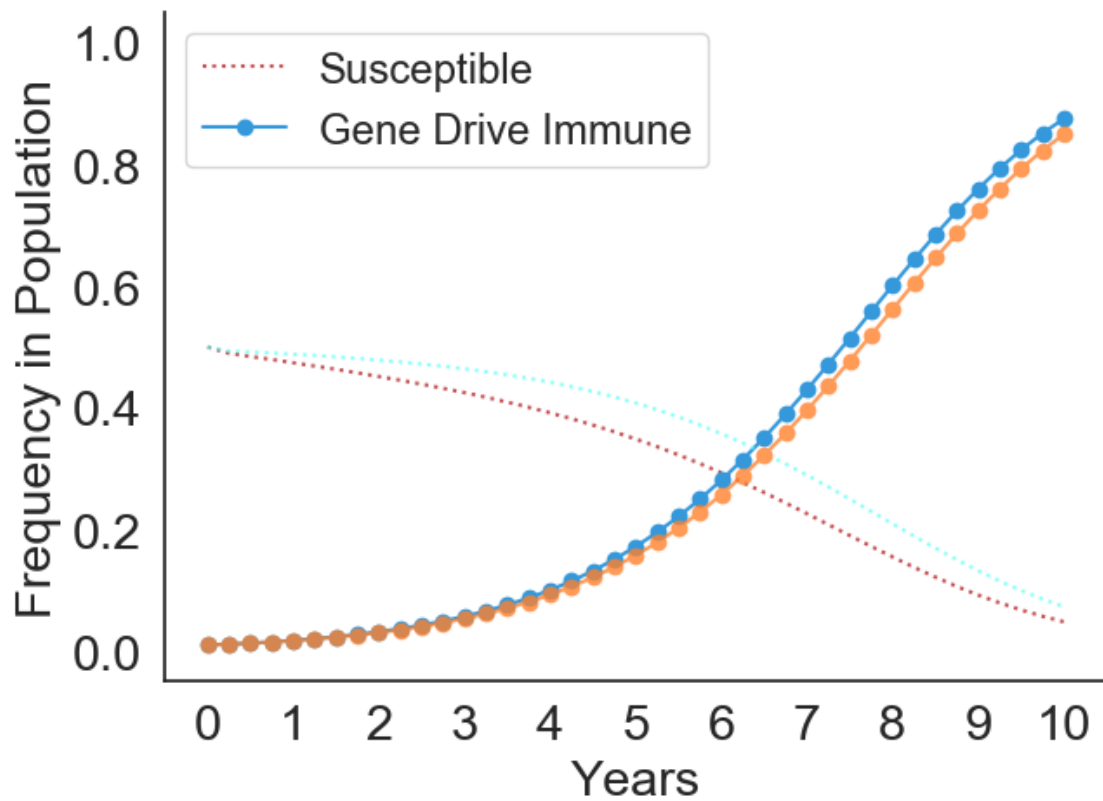


Figure 5.10: The effect of default penetrance ( $\iota = 0.8$ ) compared to higher penetrance ( $\iota = 0.9$ ) in the establishment of GDMI. Higher penetrance produces the blue GDMI and red susceptible lines, while lower penetrance produces the orange GDMI and light blue susceptible lines.

### 5.7.6 Generation time and population turnover

The evolutionary dynamics for GDMI reported here describe conditions in which the average time to reproduction is 3 months and the natural background mortality is half of the adult population in that time. However, when fortuitous environmental conditions prevail, or for snail species with shorter generation times, the establishment of GDMI in a population may happen at a different speed. In ten years, genotype frequencies in the population may be far different for these variable conditions. We demonstrate how variation of two basic life history parameters – natural mortality rate and generation time (mean time to reproduction) – influence the establishment of GDMI in 10 years. Fig. 5.11 displays simulations under default conditions, while these two life history parameters vary.

### 5.7.7 Invasion conditions

Each genotype can be determined to be invading given that it is increasing in frequency at time  $t = 0$ . Invasion of a genotype does not guarantee increasing frequency at any time  $t$ , as conditions may change, even in the deterministic model (i.e. model results are not monotonic). However, invasion criteria are important determinants in understanding the behavior of the genetic system in the early stages of gene drive release or even in a natural but unstable genetic system (e.g. strong directional selection). For GDMI establishment, the relative fitness of the gene drive homozygote must be greater than 1. This can be directly



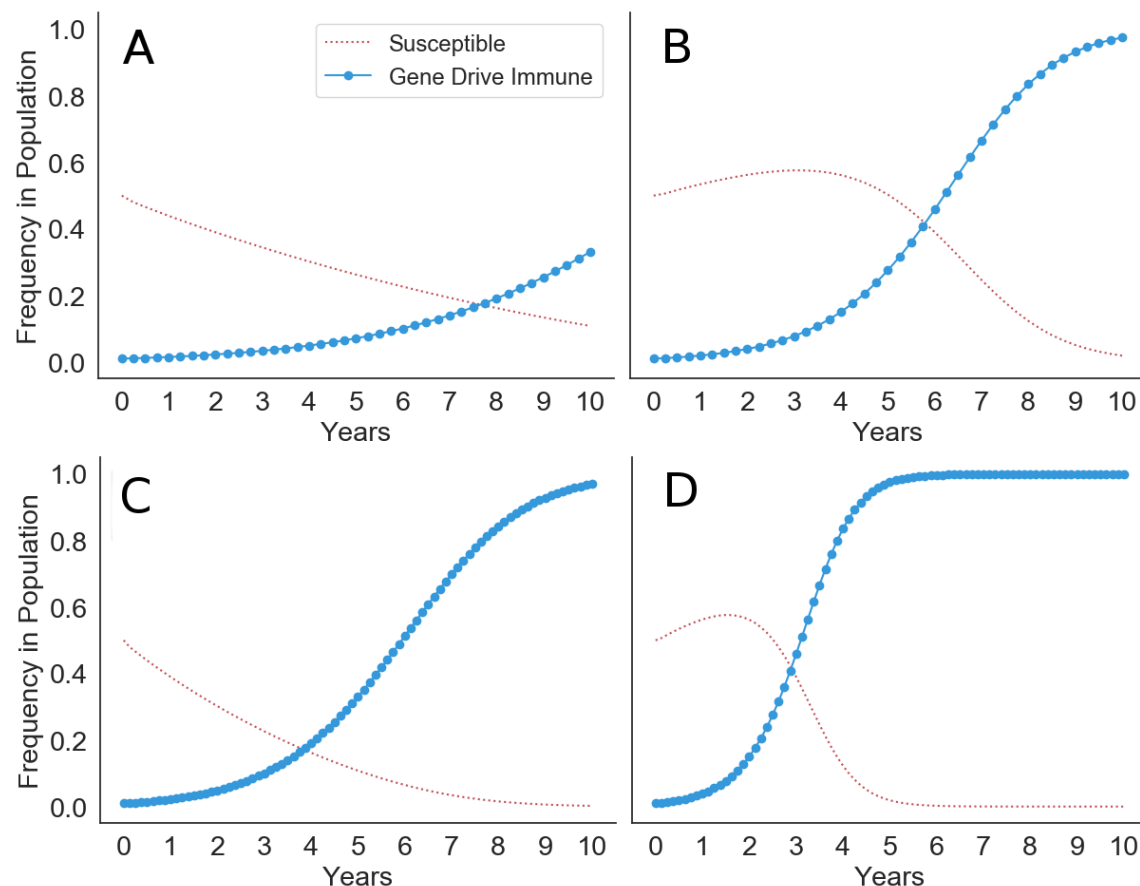


Figure 5.11: Simulations of susceptible and GDMI frequencies under variable life history strategies, namely mean generation time and death rate. Increasing death rate results in more population turnover each generation and more rapid fixation of GDMI. Panel A shows results for  $\mu = 0.25$  while panel B shows results for  $\mu = 0.75$ . Similarly, shorter generations yields more rapid fixation of GDMI in 10 years because more generations occur within the time window. Panels C and D give show results for a mean generation time of 1.5 months (80 generations in 10 years) in contrast to 3 months (40 generations in 10 years). Panel C maintains  $\mu = 0.25$ , and panel D maintains  $\mu = 0.75$ .

determined by ensuring:

$$\frac{\lambda_6(0)}{\lambda(0)} > P_{33}(0) \quad (5.76)$$

$$\implies \frac{\lambda_6(0)}{\lambda(0)P_{33}(0)} > 1 \quad (5.77)$$

Additionally, the total population size must not decline towards extinction for this invasion to be successful. In Fig. 5.12 we calculate invasion thresholds for the variety of model parameters in relation to self-fertilization frequency.

Selfing rate and homing efficiency interact to form a curved region bounding values above  $H = 0.6$  and below  $\sigma = 0.8$ . Homing efficiencies below 0.6 do not produce a viable gene drive under default conditions for any selfing rate. Similarly, selfing rates above 0.8 render a gene drive less fit than the natural population and unable to invade. Also supported by Fig. 5.3 (main text), the cost of the payload is a strong determinant in the success of the invasion of GDMI. Perhaps strongest is the influence of gene flow on the invasion of GDMI, which restricts invasion to a small subset of conditions, rapidly excluding snail species with moderate selfing rates as gene flow increases. This does not capture long-term dynamics where GDMI individuals immigrate to the focus population – a process that may occur if GDMI establishes in the neighborhood of the focus population. Highlighted here is the robustness of invasion across the range of disease conditions. In both low and high transmission areas, invasion of GDMI is possible for some or all snail species. Species

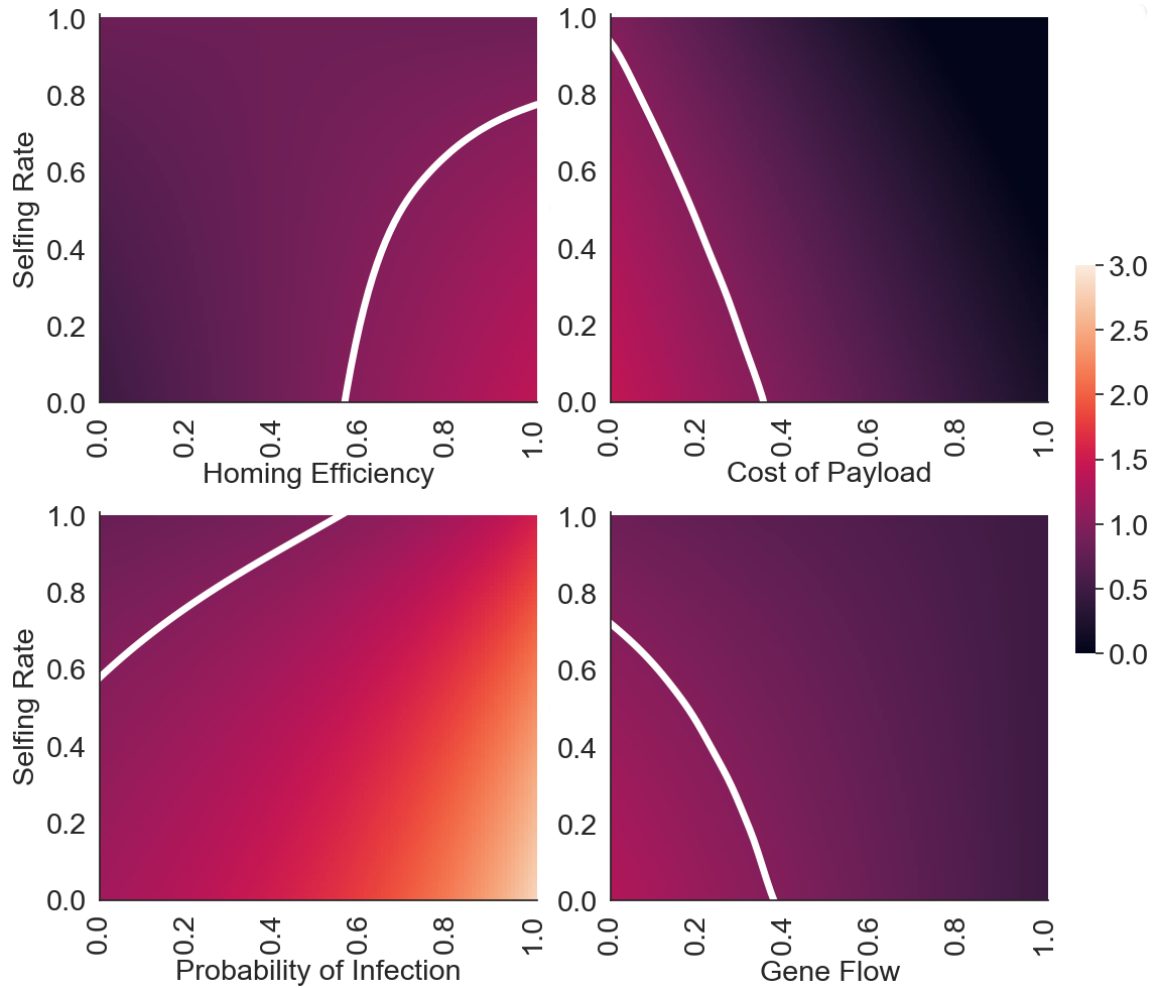


Figure 5.12: Invasion analyses for variables that influence the probability of invasion. Other parameters are held at their default value according to table 1, while the reproduction number is calculated as selfing rate varies. Lighter areas indicate higher reproduction numbers, and white lines represent the isocline at threshold conditions ( $R_0 = 1$ ). The ratio reported in equation 77 and  $R_0$  share a value of 1 under threshold conditions but are otherwise not precisely equal due to the nature of overlapping generations in the model.

exhibiting high selfing rates may be invaded by GDMI in high transmission areas.

### 5.7.8 Extinction risk

Invasion thresholds are valid and provide context for the conditions in which GDMI will proliferate given that the GDMI allele is not lost from the population due to genetic drift. Drift is strongest in generations immediately proceeding introduction (in a homogeneous environment) when the size of the pool of GDMI alleles is small. In contrast to a deterministic invasion process, genetic drift depends on the size of the seed GDMI population. We show how the probability of extinction of GDMI in 10 years (40 generations) varies with the size of the seed population and the absolute fitness of GDMI. We assume the probability of extinction is driven by a stochastic death process contributed through background mortality and infection prior to reproduction in each generation. Default parameters for background mortality and infection for GDMI homozygotes are  $0.5 \text{ gen}^{-1}$  and  $0.03 \text{ gen}^{-1}$ , respectively. Therefore, we model the stochastic death process as  $Pr(X = k \text{ deaths}) = B(n, 0.53)$ , where  $n$  is the number of GDMI homozygotes (excludes heterozygotes for simplicity due to their transiency at  $H = 0.9$ ). Given a geometric mean absolute fitness  $f$  and number of generations from introduction,  $t$ , the cumulative distribution function representing the probability of extinction at time  $t$  is

$$Pr(X \leq (t + 1)) = Pr(X \leq t) + 0.53^{n_{f^{t+1}}} (1 - 0.53^{n_{f^t}}) (1 - Pr(X \leq t)) \quad (5.78)$$

We calculate the probability of extinction within 40 generations ( $t = 40$ ) using this recursive formulation and plot the results in Fig. 5.13 across a range of absolute fitness values and number of seeded GDMI individuals in the focal population.

Results indicate that the probability of extinction depends primarily on the absolute fitness of GDMI and little on the number of seeded individuals. Extinction is guaranteed below geometric mean absolute fitness of 0.9, regardless of the size of the introduced GDMI cohort. At low numbers, the threshold of extinction resides at a fitness of 1. This threshold is stark, with very little intermediate extinction risk in 40 generations. This is due primarily to the number of generations simulated; fewer generations would yield more intermediate extinction probabilities on the same plot. Combined with earlier results, this shows that the size of the introduced cohort is important for rapid fixation of GDMI but less important for persistence of GDMI in the population. This conclusion may not hold when reproduction and death is highly variable due to factors like seasonality. Higher variability will result in higher extinction risk, particularly for smaller seed populations.

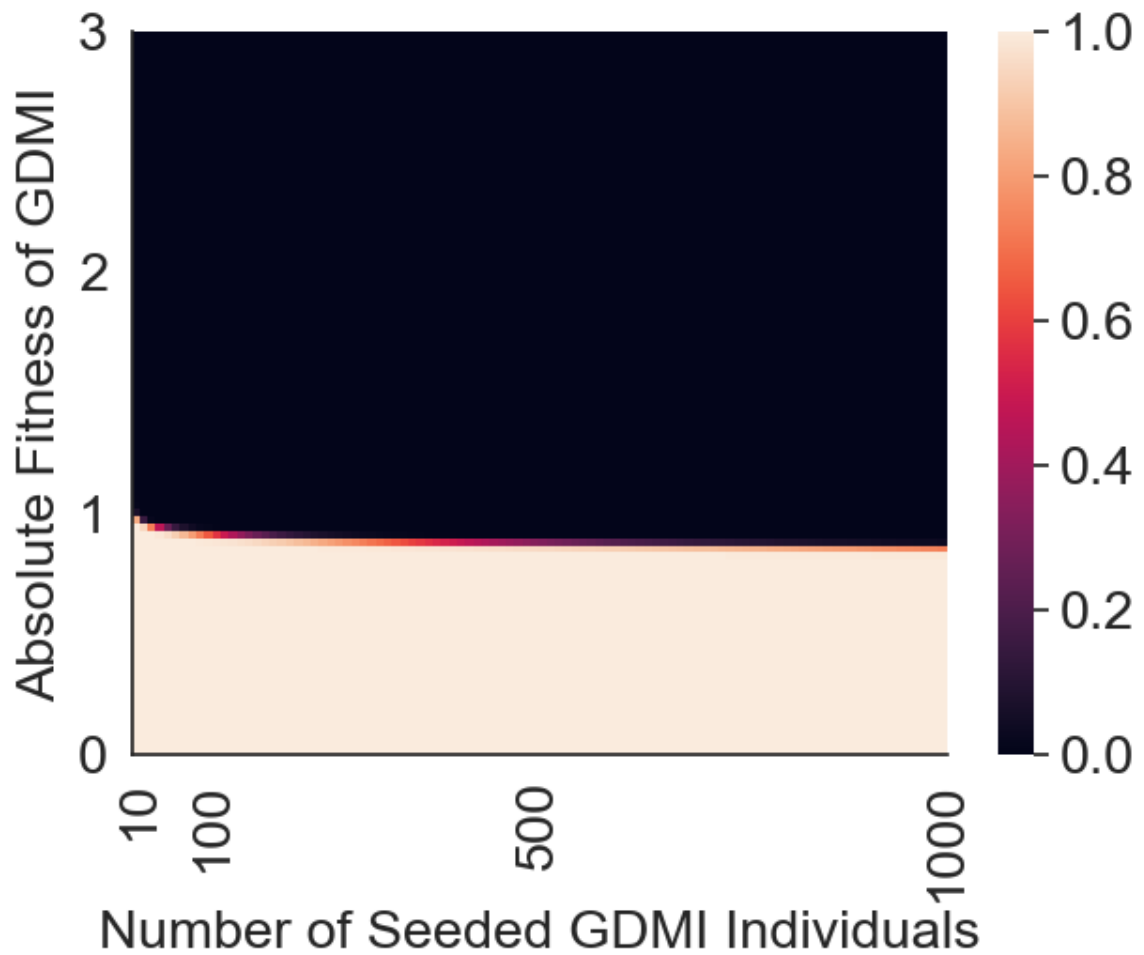


Figure 5.13: The probability of extinction within 40 generations according to absolute fitness and the number of seeded GDMI individuals. Darker values represent low likelihood of extinction.

### 5.7.9 Seasonality

Dramatic variation in available snail habitat due to seasonal changes in precipitation is common in schistosomiasis endemic regions. Highest variation is observed in sites with ephemeral water bodies and agricultural areas. It is unclear how seasonal variation in habitat availability will alter the speed of establishment of GDMI. We compare GDMI establishment with and without seasonality in carrying capacity of the snail population, with a four fold change in carrying capacity simulated in the seasonally variable population. Fig. 5.14 demonstrates that seasonality slows the establishment of GDMI, even in a deterministic model. Although not shown in Fig. 5.14, higher variability in carrying capacity corresponds monotonically to slower establishment of GDMI. These results support the use of GDMI in sites with less seasonal variability in snail population abundance.

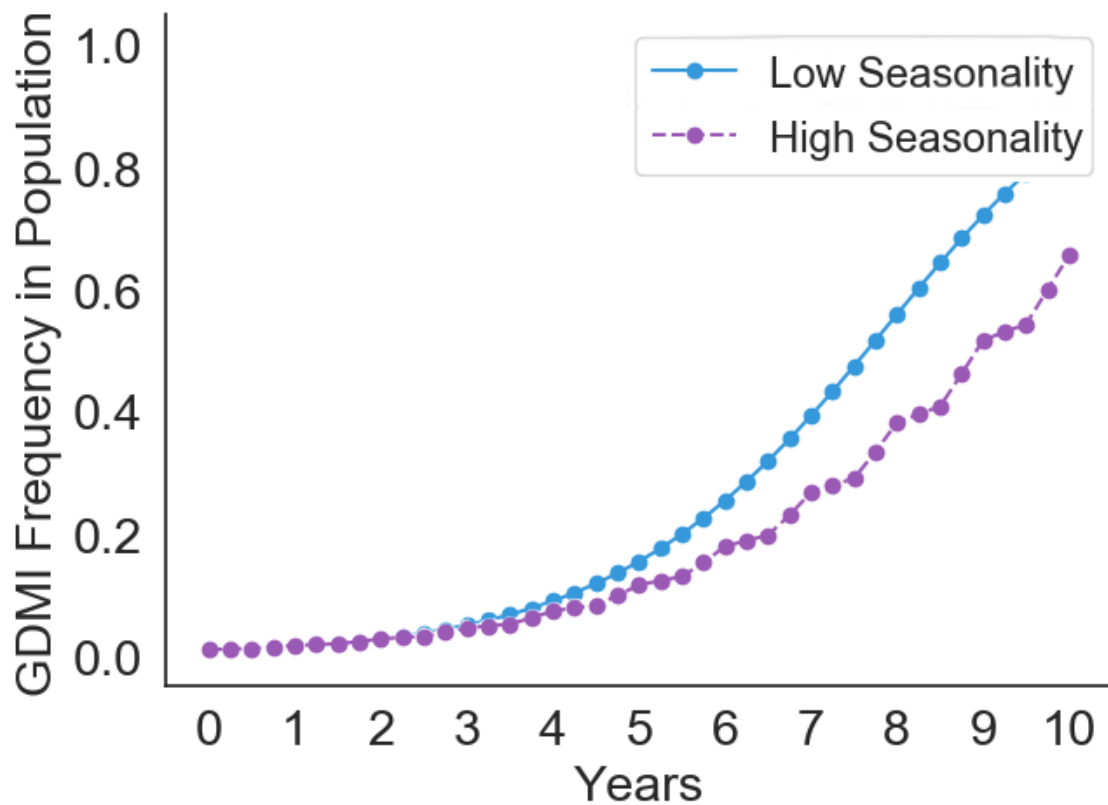


Figure 5.14: The spread of GDMI in a population with fluctuating carrying capacity due to seasonal rainfall and habitat variation. High seasonality assumes at 4 fold change in carrying capacity in 2 generations, with a full cycle occurring in 4 generations (equal to 1 year with default generation time): 200 %, 100 %, 50 %, 100% carrying capacity cycle. Low seasonality assumes no fluctuation in carrying capacity.



### 5.7.10 Epidemiological model

Here we modify the classic MacDonald model for schistosome transmission to include the frequency of resistant snails that occurs in the environment due to introduction and subsequent spread of GDMI in the population. The simplest form this takes is to subtract the frequency of immune snails from the susceptible snail frequency such that the frequency of susceptible snails is given by  $(1 - y - \rho)$ , where  $\rho$  is the frequency of immunity (natural and engineered). The resulting system of coupled ordinary differential equations is given below (equations 5 and 6):

$$\frac{dw}{dt} = \alpha y - \mu w \quad (5.79)$$

$$\frac{dy}{dt} = \Lambda^*(1 - e^{-\beta w})(1 - y - \rho) - vy \quad (5.80)$$

These two equations govern the prevalence of infection in snails,  $y$ , and the mean per capita worm burden in humans,  $w$ . The distribution of adult worms in the human population is assumed to approximate a negative binomial distribution. Parameters and their values are described in table 2.

We evaluate the efficacy of GDMI intervention by comparing mean worm burden after a ten year period with the mean worm burden at equilibrium endemic conditions. We calculate transmission rates  $\Lambda$  and  $\alpha$  at endemic equilibrium. Let  $w^*, y^*$  be nontrivial equilibria

Table 5.2: Parameter values for the epidemiological model

| Parameter   | Description                                                            | Value                                        | Ref.                                                                                                                                          |
|-------------|------------------------------------------------------------------------|----------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| $\alpha$    | transmission rate converting snail infections to adult worms in humans | $142 \text{ wk}^{-1}$                        | calculated based on model equilibrium at $w^* = 710$ , $y^* = 0.02$                                                                           |
| $\mu$       | death rate of adult worms                                              | $0.004 \text{ wk}^{-1}$                      | Harmonic mean of range of $(3 - 7 \text{ yrs})^{-1}$ commonly reported in literature across <i>Schistosoma spp.</i> (Goddard & Jordan, 1980b) |
| $\Lambda^*$ | force of infection from humans to snails at endemic equilibrium        | $0.0104 \text{ wk}^{-1}$                     | derived from epidemiological model                                                                                                            |
| $\rho$      | fraction of immune snails                                              | variable, $\rho^* = 0.5$                     | determined by genetic model                                                                                                                   |
| $v$         | death rate of infected snails                                          | $0.25 \text{ wk}^{-1}$                       | Harmonic mean of death rates of infected <i>Bulinus globosus</i> and <i>Biomphalaria pfeifferi</i> (Woolhouse, 1989b)                         |
| $b$         | per capita worm to snail transmission rate                             | $3 * 10^{-5} w^{-1}$                         | calculated based on model equilibrium at $w^* = 710$ , $y^* = 0.02$                                                                           |
| $\beta$     | human population to snail transmission rate                            | variable, $\beta^* = 0.0147 \text{ wk}^{-1}$ | calculated: $\beta = \frac{1}{2}\phi b$                                                                                                       |
| $m$         | per capita mean number of mated pairs of adult worms                   | variable, $m^* = 348$                        | calculated: $m = \frac{1}{2}\phi w$                                                                                                           |
| $k$         | clumping parameter: $NB(w, k)$                                         | 0.24                                         | fitted to <i>S. mansoni</i> data (Mangal et al., 2008)                                                                                        |
| $w$         | per capita mean worm burden                                            | variable, $w^* = 710$                        | calculated based on model equilibrium at $\Omega = 0.80$                                                                                      |
| $y$         | frequency of patent infections in snail population                     | variable, $y^* = 0.02$                       | field-observed average in endemic regions (R. Anderson & May, 1979)                                                                           |
| $\phi$      | per adult worm mating probability                                      | variable, $\phi^* = 0.98$                    | calculation based on $NB(w, k)$                                                                                                               |
| $\Omega$    | per capita prevalence of at least one mated pair of adult worms        | variable, $\Omega^* = 0.80$                  | field-observed average in endemic and hyperendemic regions (M. Chan et al., 1995; Tchuente et al., 2013)                                      |

for which  $\frac{dw}{dt} = \frac{dy}{dt} = 0$ .

$$w^* = \frac{\alpha y}{\mu} \quad (5.81)$$

$$y^* = \frac{(1 - \rho)\Lambda(1 - e^{-\beta w})}{\Lambda(1 - e^{-\beta w}) + v} \quad (5.82)$$

$y^*$  is frequently estimated in field surveys and may vary across sites or through the year due to seasonal variability in rainfall and human use of aquatic snail habitat like drainage areas, irrigation ditches, or natural water bodies. Despite variation, low infection prevalence (0-5%) in snails is observed, even in hyperendemic areas. Explanations for low prevalence are multifactorial and relate to the duration of patency, increased mortality rate of patent snails, heterogeneous exposure to miracidial infection, partially evolved immunity to infection, and competition with other trematodes.  $w^*$  is not as easily estimated, as measurements rely on quantification of shed eggs in urine and fecal samples. The quantity of eggs is correlated but not linearly related to the number of paired worms, as human immunity leading to granulomatous formation around released eggs as well as potential interactions among adult worms and variability in egg production can obscure the relationship between eggs shed and worm burden. Human autopsies performed on known and suspected schistosomiasis cases reveal differential distribution of eggs and associated pathology with increasing intensity of infestation. Cheever (1968) observed that fewer eggs were present in the rectal

mucosa and feces of *S. mansoni* infected individuals with associated fibrosis of the liver. This demonstrates that pathology, intensity, and egg count are not directly related, and the nature of their relationship requires biological knowledge of both the distribution of worms across tissue and the interactions between worms and the immune system. Despite these limitations, a reasonable heuristic is a 1:1 ratio of adult worm mated pairs and eggs per gram (EPG) in feces (*S. mansoni*). Multiple lines of evidence, including challenge experiments in mice, organ specific autopsies and perfusions, as well as observed distributions of EPG in human populations suggests that per capita mean worm burden (MWB) in highly endemic areas can exceed 1000. We simulate moderate-high endemicity with an infection prevalence of  $\Omega^* = 0.80$ . When the prevalence of infection is  $\ll 1$ , a proportion of adult worms fail to pair with a mate and reproduce. The number of mated pairs can be calculated given the MWB and the distribution of adult worms in the human population. A negative binomial distribution is found to best represent the distribution of adult worms in humans. It is overdispersed, and dispersion increases as prevalence decreases. Prevalence of detectable eggs, and therefore successfully mated pairs is given as

$$\Omega(w, k) = 1 - 2\left(1 + \frac{w}{2k}\right)^{-k} + \left(1 + \frac{w}{k}\right)^{-k} \quad (5.83)$$

and  $w$  is calculated via substitution of the known prevalence of infection,  $\Omega$ , and aggregation parameter,  $k$ , and solving numerically. An equal ratio of male and female schistosomes is assumed in this calculation, as is that the rates of transmission between the two

sexes are equivalent (May, 1977). We also assume that both sexes transmit together, and there is no sex-specific compartmentalization in the human body that would limit pairing of adult schistosomes.  $w^* = 710$  occurs at an endemic equilibrium prevalence,  $\Omega$ , of 80%. Given  $w^* = 710$  and  $y = 0.02$ ,  $\alpha$  can be calculated as

$$\alpha = \frac{w^* \mu}{y^*} = 142 \quad (5.84)$$

In contrast the  $\alpha$ , which holds a constant value,  $\beta$  is a function of the distribution of worms in the local human population. The distribution changes non-linearly with worm burden and prevalence. For simplicity, we assume that  $k$  is invariant as worm burden and prevalence change, although evidence suggests higher aggregation with higher burden in some populations.  $\beta$  takes the form:

$$\beta = \frac{1}{2} b \phi \quad (5.85)$$

in which  $b$  is a transmission constant that relates the per capita number of mated worm pairs,  $m$ , to new infections in snails.

$$m = \frac{1}{2} \phi w \quad (5.86)$$

$\phi$  is the mating probability given by the negative binomial distribution where  $\delta = \frac{w}{w+k}$ .

$$\phi = 1 - \frac{(1 - \delta)^{1+k}}{2\pi} \int_0^{2\pi} \frac{(1 - \cos\theta)d\theta}{(1 + \delta\cos\theta)^{1+k}} \quad (5.87)$$

Given  $w^*$  and  $y^*$ ,  $\Lambda^*$  can be calculated by approximating that  $(1 - e^{-\beta w}) \approx 1$  at endemic equilibrium conditions. Equation 80 simplifies to:

$$\frac{dy}{dt} = \Lambda^*(1 - y - \rho) - vy \quad (5.88)$$

Solving for the nontrivial equilibrium yields:

$$\Lambda^* = \frac{vy^*}{1 - y^* - \rho^*} = 0.0104 \quad (5.89)$$

The probability of infection per generation,  $Pr(y^+)$ , can be approximated from the force of infection and the differential equation for snail infection prevalence:

$$Pr(y^+) \approx \int_{t=\tau}^{\tau+1} \Lambda^*(1 - e^{-\beta w})dt \quad (5.90)$$

This expression represents the per capita number of snail infections expected in a susceptible population in a generation ( $t = \tau$  weeks).

We do not yet have an estimate for  $\beta$  (variable) and therefore no estimate for  $b$  (constant). These values we determine by calibrating the model with known values for  $R_0$  in moderate-high transmission sites. The magnitude of  $R_0$  has never been precisely measured

for schistosomiasis, as doing so would require measurements of innate immunity in the snail population. It is unknown whether genetic immunity provides cross protection for other trematode species, and therefore, even in a previously schistosome-naïve area, pre-existing immunity requires measurement through challenge experiments. With this caveat in mind,  $R_0$  measurements are widely thought to exist in the range of 2-5 for schistosomiasis, likely exceeding 3 in moderate-high transmission sites (Woolhouse et al., 1996). In a fully susceptible snail population, these values will be higher, and in all likelihood empirically measured  $R_0$  values underestimate true  $R_0$  values predicated on a fully susceptible host population. From our system of differential equations we calculate the effective reproductive number  $R_t$  and from it, derive the  $R_0$  under conditions of partial immunity in snails to calibrate  $\beta$ . Linearizing the system of equations with respect to  $w$  and  $y$ , we form the transmission and transition matrices outlined by Diekmann et al. in their next generation matrix (NGM) approach to calculate  $R_0$  (Diekmann et al., 2010). We extend this approach by relaxing the assumption that the populations of snails and humans are fully susceptible and that no disease is present before an index case. Doing so, we calculate transmission and transition matrices for  $R_t$  as:

$$\mathbf{T}_t = \begin{pmatrix} 0 & \alpha \\ (1 - \rho)\beta\Lambda^*e^{-\beta w} & 0 \end{pmatrix} \quad (5.91)$$

$$\Sigma_t = \begin{pmatrix} -\mu & 0 \\ -y\beta\Lambda^*e^{-\beta w} & -(v + \Lambda^*(1 + e^{-\beta w})) \end{pmatrix} \quad (5.92)$$

We calculate the time-varying NGM as:

$$\mathbf{K}_t = -\mathbf{T}_t \Sigma_t^{-1} = \begin{pmatrix} \frac{-\alpha\beta y \Lambda^* e^{-\beta w}}{\mu(\Lambda^*(1+e^{-\beta w})+v)} & \frac{\alpha}{\Lambda^*(1+e^{-\beta w})+v} \\ \frac{(1-\rho)\beta\Lambda^*e^{-\beta w}}{\mu} & 0 \end{pmatrix} \quad (5.93)$$

The expression for  $R_t$ , computed as the spectral radius of  $\mathbf{K}_t$ , is

$$R_t = \frac{\sqrt{\alpha\beta\Lambda^*(\alpha\beta\Lambda^*y^2 + 4\mu(1-\rho)(\Lambda^* + (\Lambda^* + v)e^{\beta w}))} - \alpha\beta\Lambda^*y}{2\mu(\Lambda^* + (\Lambda^* + v)e^{\beta w})} \quad (5.94)$$

A derivation of  $R_0$  would require setting  $\rho = w = y = 0$ . However, because empirical measurements of  $R_0$  have not accounted for variations in  $\rho$ , we calibrate  $\beta$  from an empirical form of this equation. Specifically, we set  $\rho = 0.5$  according to default conditions that are based on empirical measurements of innate immunity in field captured snails. Setting  $w = y = 0$  yields the following expression for an empirical  $R_0$



$$R_0 = \sqrt{\frac{\alpha\beta\Lambda^*(1-\rho)}{\mu(2\Lambda^*+v)}} \quad (5.95)$$

and when  $\rho = 0.5$ , the expression becomes:

$$R_0 = \sqrt{\frac{\alpha\beta\Lambda^*}{2\mu(2\Lambda^*+v)}} \quad (5.96)$$

Solving for  $\beta$  yields

$$\beta = \frac{2\mu R_0^2(2\Lambda^*+v)}{\alpha\Lambda^*} \quad (5.97)$$

Recall that  $\beta$  is a function of the negative binomial distribution of worms, which determines the probability of mating success among adult worms. However, this theoretical construct breaks down for the low numbers assumed in an index case. For at low numbers,  $\phi$  would approximate zero for a stationary  $k = 0.24$ , and extinction is predicted. The concept of  $R_0$  would be irrelevant for schistosomiasis if these theoretical predictions were valid. Instead we assume that early transmission of cercariae are highly clustered and that  $\phi$  remains high for the purposes of estimating the constant  $b$  which scales  $\beta$ . Setting  $\phi = 1$ , we achieve

$$b = \frac{4\mu R_0^2(2\Lambda^*+v)}{\alpha\Lambda^*} \quad (5.98)$$

We set  $b$  to a value with one significant digit so that  $R_0$  is approximately the median of empirically measured values. This gives  $b = 0.03$  and  $R_0 = 3.2$ . In practical terms,  $b$  represents the ‘rebound speed’, which is the pace infections can accrue after chemotherapy treatment. Under certain conditions, our estimate may represent the low end of this rebound speed due to the assumption of 100% mating success in index case infections. Moreover, our estimate of  $R_0 = 3.2$  is calibrated based on prior empirical measurements, which almost certainly underestimate the true  $R_0$  as specified by a fully susceptible host population. We do not explicitly account for adaptive immunity in humans, which has been shown to increase over 10+ years into adulthood. Accounting for evolved innate immunity in snails by setting  $\rho = 0$ , we find that  $R_0 = 4.5$ .

In Fig. 5.15 we show the long-term behavior of the default model without introduction of GDMI. A reproduction number of 3.2 produces a rapid rise of an epidemic past the endemic equilibrium, and as the snail population evolves immunity, an equilibrium is established. Feedback from schistosome transmission produces stabilizing selection on immunity in snails.

In Fig. 5.4 (main text), GDMI was evaluated in comparison to and with coincident annual MDA treatment. 60% reduction in MWB in the population was modeled for each treatment and is a product of coverage and efficacy of the chemotherapy. Although alone GDMI is not capable of eliminating schistomiasis locally within a 10 year evaluation period, it was shown to successfully complement MDA under simulated conditions to produce

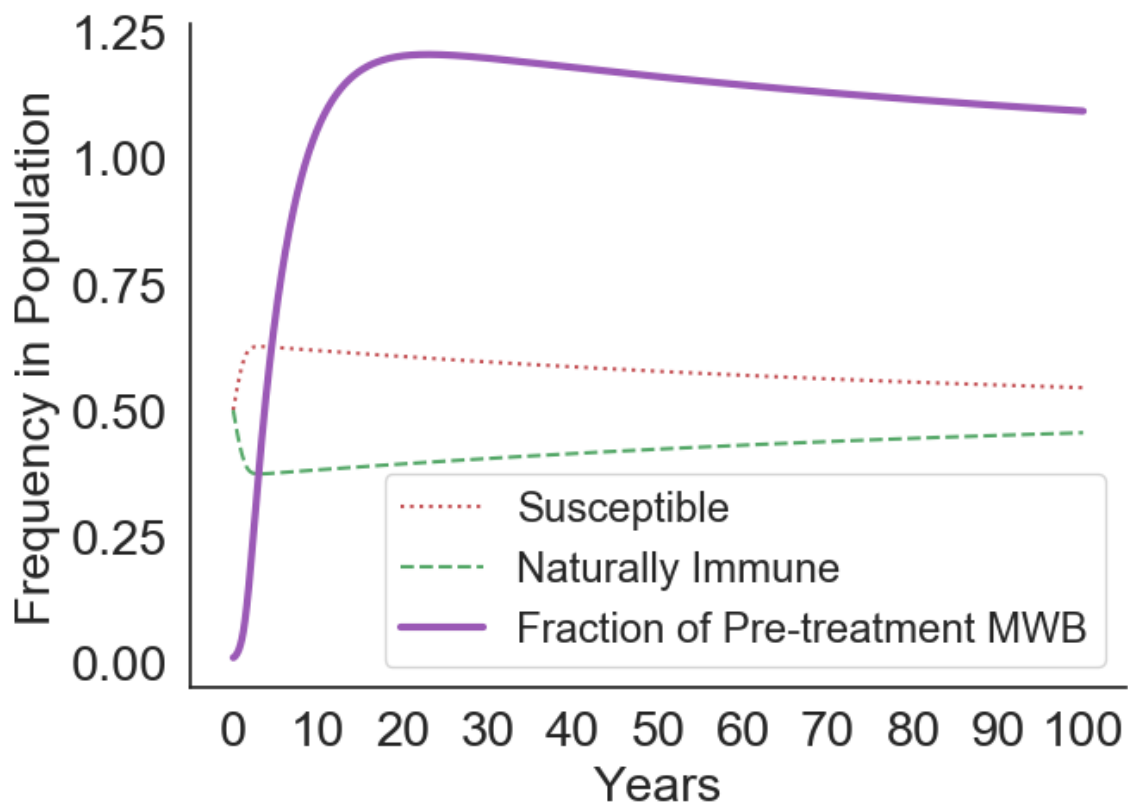


Figure 5.15: Simulation of the emergence of a schistosomiasis epidemic under default conditions. GDMI is not present, and long-term behavior of the model is observed to overshoot endemic equilibrium conditions and return to equilibrium over the course of many years. Susceptibility in snails is advantageous at low levels of infection early in the epidemic and is disadvantageous above equilibrium conditions.

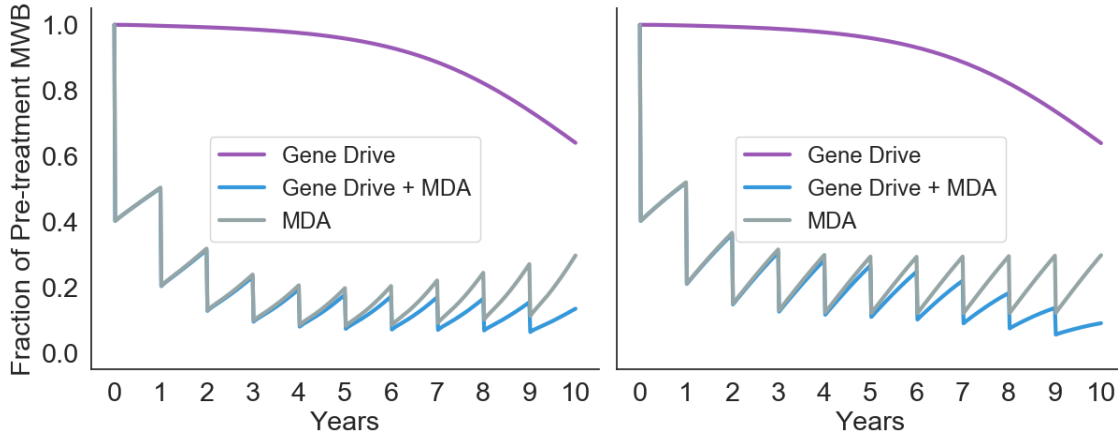


Figure 5.16: Comparative results among three treatment regimes under high and low transmission conditions.  $b$  is half of default conditions (left) and  $R_0 = 2.3$ , producing slower rebounds after annual MDA treatment. More rapid rebounds are observed when  $b$  is twice default conditions and  $R_0 = 4.5$  (right).

greater and more sustained reduction than MDA alone. However, these results may be sensitive to several factors, especially the force of infection to humans which determines how rapidly the human population becomes infected from an infected snail population. Rapid reinfection results in a faster rebound to pre-treatment MWB, and therefore, subsequent treatment is less effective because MWB reduction is not long lasting. We explore high and low transmission conditions by manipulating  $b$ , which in turn, changes  $\beta$ . Fig. 5.16 shows the difference between  $R_0 = 2.3$  and  $R_0 = 4.5$  conditions as MDA and GDMI are applied.

GDMI performs favorably in absolute reduction in joint use with MDA when transmission is higher, indicating that the efficacy of GDMI is enhanced in conditions that are challenging for reduction through MDA alone. Additionally, the intensity of MDA treatment may have a strong effect on the benefits of GDMI, as selection pressures are changed.

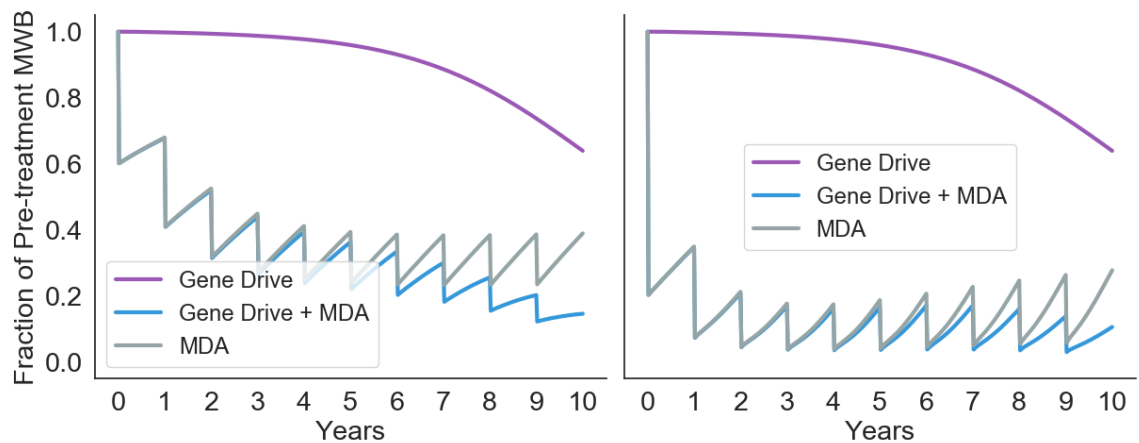


Figure 5.17: Comparative results among three treatment regimes under high and low intensity MDA application in the human population. 40% annual reduction in MWB (left) produces slower elimination across all treatment regimes compared to 80% annual reduction (right). Rebounds are concave down and relatively smaller for lower intensity MDA and concave up for high intensity MDA. This reflects slower loss of immunity, and for joint treatment the faster gain of GDMI, in the snail population due to higher selection pressure in favor of immunity in higher transmission conditions.

Fig. 5.17 displays the difference in reduction of MWB between low and high intensity MDA use under equivalent GDMI application.

These results demonstrate diminishing returns for the application of MDA at higher concentrations as immunity in snails evolves to favor higher transmission conditions when adult worms are eliminated quickly. Success of GDMI is slowed when force of infection on snails, and therefore positive selection on immunity, is reduced.

The treatment window of 10 years is common for evaluating funded public health campaigns, though results of this study will differ using longer treatment windows. We extend this window to 40 years to demonstrate the long-term effects of each of the treatment regimes. Additionally, we show that when MDA is remitted after 10 years, GDMI is able to

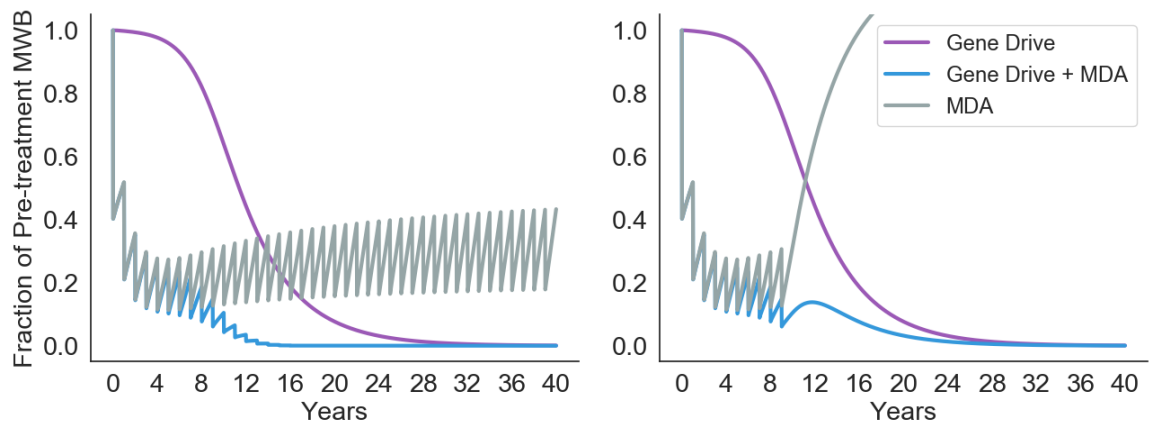


Figure 5.18: Simulations of the three treatment regimes for 40 years. MDA is continued annually for the duration of the simulation (left). MDA is stopped after 10 years of treatment (right).

maintain reductions in MWB, while without GDMI MWB returns to endemic equilibrium conditions (after an overshoot also depicted in Fig. 5.15).

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## Chapter 6

### Conclusions

This dissertation offers six examples in its chapters and appendices of efforts to harness new quantitative metrics to overcome knowledge and data deficits, and optimize efforts to address current and future health risks to humans and wildlife. The resulting practical tools, and the biological insights can inform future ecosystem management policy, species recovery, and infectious disease mitigation. In brief, in each of these chapters we find a few key results that progress our collective understanding. We show that population dynamics of the intermediate host, *Emerita analoga*, is the primary driver of spillover acanthocephalan peritonitis in otters. Because this crab population is sensitive to ocean temperatures and is specific to sandy, wave-swept beaches, ocean warming may contribute to future increased risk of acanthocephalan peritonitis in sea otters due to larger crab host populations. In addition, regions such as San Francisco Bay that do not maintain this crab population offer

opportunities for sea otter reintroduction efforts due to a lower risk of acanthocephalan-associated otter death. We find that zoonotic viral genomes are larger than non-zoonotic viral genomes in each category of viruses classified by genome structure. This suggests a common principle for viral spillover, and that genome size may be a useful indicator of zoonotic potential and future outbreaks in humans and wildlife. We also find that the infection fatality ratio, an important epidemiological metric, can be reliably estimated by a strong fit of our mathematically proven relationship to country level data of COVID-19 deaths, cases, and tests. Early in the pandemic before vaccines and targeted treatments were available, we determined a baseline infection fatality ratio near 1%, which represents the average across all countries. We find that the success of an immunity-modifying drive to reduce schistosomiasis transmission will be largely species-dependent; high selfing snail species are not suitable targets. Meanwhile, for species that represent good targets, optimizing the genetic design will have a strong and positive impact on performance. Under these circumstances, gene drive technology could lead to sustained reduction of disease in localized areas. In the appendices we advance the evaluation of ecosystem health in our oceans. Our revised approach to the Productivity-Susceptibility Analysis demonstrates that many fished and bycatch species are at higher risk of population loss than previously considered. We find that species' risk of population loss can increase in relation to the number of stressors and the magnitude of the stressors evaluated.

Humanity's relationship with the environment and its ecosystems is at a critical point,

and we have the opportunity to remedy some of the health risks facing human and wildlife populations through vigilance and development of technologies that integrate our understanding of epidemiology, ecology, and genetics. Despite the challenges, I would assert that management of these risks is improving due to our collective and innovative efforts. My contribution through this dissertation may only inch us a bit closer toward a healthier planet, but at least it hasn't moved us backward.

# Appendix A

## Redefining Risk in Data-poor Fisheries

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### A.1 Abstract

The Productivity Susceptibility Analysis (PSA) is a widely used method to rapidly assess species risk to fishing activities in data-poor fisheries. A step in ecological risk assessments and used in data-poor assessment for sustainable fisheries certification programs (e.g. MSC) and recommendation lists (e.g. Seafood Watch), the PSA is semi-quantitative,

yet little attention has been given to the theoretical basis of this analysis. Current thresholds designating low, medium, and high risk categories divide the PSA plot by equal area, assuming area corresponds to likelihood. We show that plot area does not correspond to likelihood, however, and existing thresholds need revision due to the non-uniform distribution of vulnerability scores on the PSA plot. The probability of medium risk assignment increases with the number of attributes used to characterize productivity and susceptibility. Here we present a novel and statistically robust method to derive vulnerability, where threshold values between the risk categories are adjusted with the number of attributes used in the assessment. Our comprehensive framework accounts for all variations in the method, including logarithmic scaling of axes, weighting of attributes, and scoring procedures. Simulated results across a range of conditions and comparative evaluation of 302 species in five studies show that one-third of species may be re-categorized with the new PSA approach. Importantly, the existing PSA approach underestimates risk by up to 35% when compared with the new method. These findings have strong implications for management of data-poor fisheries. We recommend adoption of this approach to the PSA to better resolve species' risk.

**Keywords**

*Ecosystem Based Management, Productivity Susceptibility Analysis, Stock Assessments, Vulnerability*

## A.2 Introduction

The turn of the century saw a revolution in fisheries management. From the reinforcement of the Magnuson-Stevens Act in 1996 and 2006 in the U.S., to the introduction of the Australian Environment Protection and Biodiversity Conservation Act (EPBCA) in 1999, to the more recent reformation to the European Common Fisheries Policy in 2013, and adoption of a “Negative Growth” strategy by the Chinese Ministry of Agriculture in 2017, major countries are adjusting their approaches to managing shared marine resources (Day et al., 1999; Hsu & Wilen, 1997; S. Huang & He, 2019; Salomon et al., 2014). Inevitably, time and money to manage these resources is allocated disproportionately to species of great economic interest, neglecting many other species and habitats, especially in low income countries and for small scale fisheries. Due to the ecological value of non-target or bycatch species, some countries, including the U.S. and Australia, mandated monitoring of these otherwise neglected species to minimize bycatch mortality (Dalton et al., 2018; Day et al., 1999; Hsu & Wilen, 1997). Without the capacity to perform full stock assessments on all species of conservation or commercial interest, government agencies and scientists have devised risk assessment frameworks to provide preliminary estimates of vulnerability for species or other managed resources (Hsu & Wilen, 1997; Pilling et al., 2009a).

One of the most used risk assessment frameworks was initially devised by Stobutzki et al. to examine the sustainability of the Australian Northern Prawn Fishery (I. Stobutzki et al., 2001a). Since 1999, this framework has been expanded and incorporated into



ecosystem-based fisheries management (EBFM) programs (Hazen et al., 2016; Townsend et al., 2019a). Pioneered by Hobday et al. in 2004 and 2011, this framework is called the Productivity-Susceptibility Analysis (PSA). Developed by Hobday et al. as part of a hierarchical approach called the Ecological Risk Assessment for the Effects of Fishing (ERAEF), the PSA assigns low, medium, or high vulnerability scores to species at risk to fishing activities (Hobday et al., 2011b; Hobday et al., 2007a). Assignment occurs via scoring of attributes in both productivity and susceptibility categories. In its most popular form there are 7 productivity attributes, each related to the capacity of the species to increase its population size, and 4 susceptibility attributes, each corresponding to potential impact on the species by fishing activities (Hobday et al., 2011b; Hobday et al., 2007a). Subsequent modifications of this approach change or add relevant attributes in the assessment (Fujita et al., 2014; Micheli et al., 2014b; Patrick et al., 2010). Each productivity and susceptibility attribute is valued 1, 2, or 3 as low, medium, or high, respectively. Calculating the mean of each set of values yields two overall scores, one for productivity and one for susceptibility. After adjusting P by subtracting from 4, vulnerability (V) is calculated as the Euclidean distance of the point (4-P, S) from (0, 0) (Hobday et al., 2007a):

$$V = \sqrt{(4 - P)^2 + S^2} \quad (\text{A.1})$$

The adjustment for P occurs because high productivity is associated with low vulnerability. The PSA uses P and S to calculate vulnerability as an index of risk analogous to risk

metrics otherwise defined in the variety of existing risk assessment frameworks in ecology or other fields. Thresholds are given to divide the 2x2 P-S plane into three regions of equal area (Fig. A.1). Species with vulnerability scores less than 2.64 garner a low risk designation, while species with scores above 3.18 are high risk (Hobday et al., 2007a). Anything in between is medium risk (see supplement for additional discussion on threshold derivation). These risk categories serve as rapid, easy-to-use indicators of management priority, and are often used as guidelines for downstream assessment. Those species interpreted as medium or high risk can be assessed in greater detail in the next step of the ERAEF framework or given higher priority in an agency's management plan. The vulnerability scores reflect relative risk and are not necessarily comparable across studies (Hobday et al., 2011b).

The PSA has been used for a wide variety of fisheries by many agencies, scientists, and organizations across the globe in the past two decades. A vulnerability index calculated from 10 productivity and 12 susceptibility attributes is used by NOAA for risk assessment of 166 U.S. fisheries (Patrick et al., 2010; Patrick et al., 2009). The PSA has been used now in dozens of published studies and government reports to evaluate over 1000 fished and bycatch species. It has established itself as a widespread and rapid tool to augment our understanding of data-poor fisheries (Dee et al., 2019; L. M. Duffy et al., 2019; Micheli et al., 2014b; I. Stobutzki et al., 2001a; I. C. Stobutzki et al., 2002). It is used to inform sustainable seafood practices for businesses and the public (Marine Stewardship Council, 2019a; Monterey Bay Aquarium, 2020). Through the years the PSA has been honed and

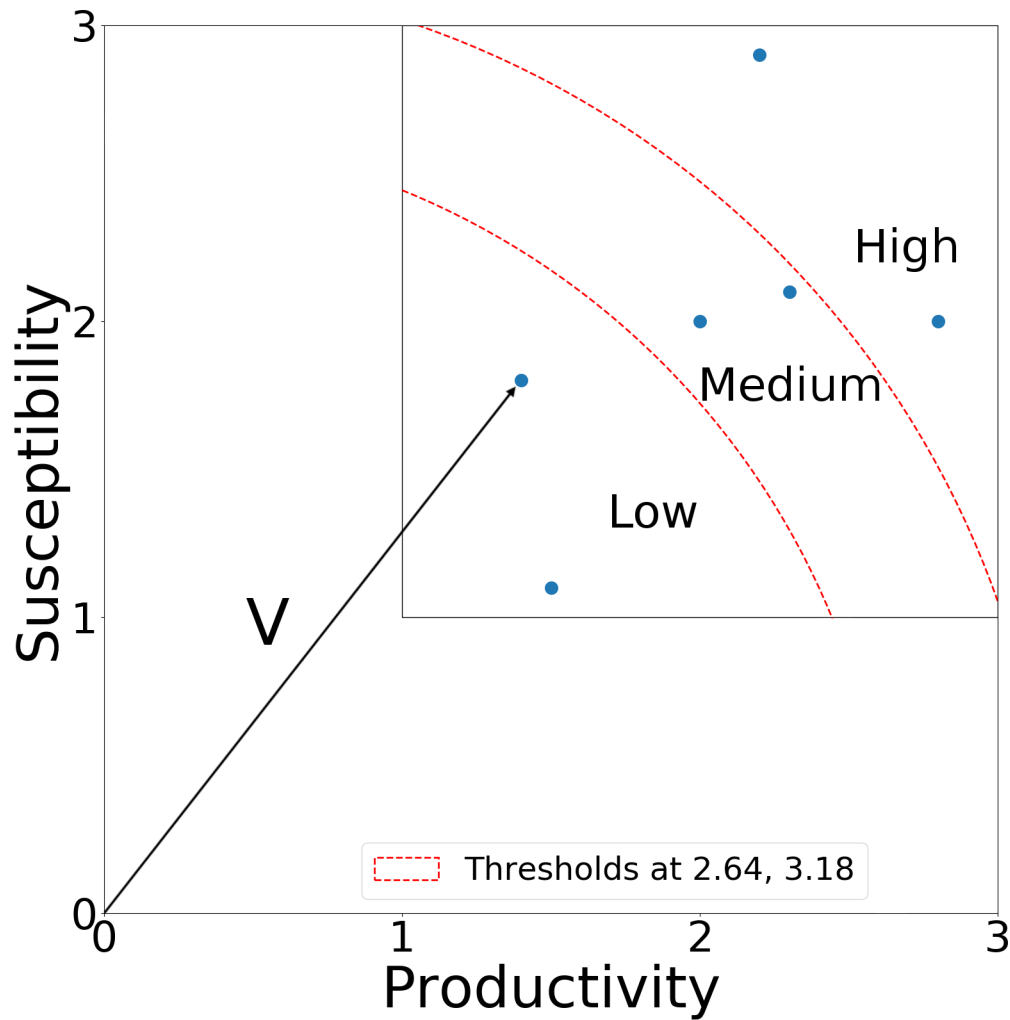


Figure A.1: Vulnerability for each species is calculated as the distance of the plotted point from the origin (0,0). Productivity and susceptibility scores range from 1 to 3, and the 2x2 PSA plot is divided into thirds of equal area at  $V=2.64$  and  $V=3.18$ .

modified to individual purposes, including the weighting of attributes by perceived importance, as in Patrick et al. (Patrick et al., 2010; Patrick et al., 2009). The number of attributes used varies significantly with each study as does the use of the geometric or the arithmetic mean to calculate the susceptibility score.

Given the variety of ways the PSA is applied, the scientific community has scrutinized the degree to which the PSA is able to provide a robust and unbiased assessment of the vulnerability of species. Interpretation of the PSA and related ecological risk assessment results (e.g. CARE, SAFE, Multiple Stressor EBRA, Cumulative Impacts assessment) requires attention to inputs and model assumptions (Battista et al., 2017a; Dowling, Smith, et al., 2019; Halpern et al., 2009a; Samhoury et al., 2019a; S. Zhou et al., 2016). Hordyk and Carruthers evaluated the consistency of the vulnerability scores across interpretations of the PSA and reliability of the PSA to conventional stock assessments (Hordyk & Carruthers, 2018). They found that the PSA performed unfavorably in a variety of conditions, particularly in its ability to accurately assign risk when vulnerability was not extremely high nor extremely low. The failure to rank species accordingly unless risk could be easily identified prior to analysis limits the power of the PSA as a semi-quantitative risk assessment method. The potential utility of the PSA has been limited by several factors. A few of these reasons are mentioned previously or explicitly tested, including correlation among attributes, use of uninformative attributes, use of ineffective weighting schemes, assignment of precautionary scores to species when data is unavailable, or even the quality of the data used

to perform the analysis (L. Duffy & Griffiths, 2017; Hordyk & Carruthers, 2018). Other reasons that deserve attention include the type of mean used (geometric vs arithmetic) for the susceptibility score and the number of attributes used; both factors warrant changing the thresholds used for risk categorization.

Here we show that a major problem with the PSA is how vulnerability and the associated risk categories have been derived. As constructed, the three risk categories are divided by two vulnerability thresholds. According to Hobday et al. 2007, “The divisions between these risk categories are based on dividing the area of the PSA plots into equal thirds. If all productivity and susceptibility scores (scale 1-3) are assumed to be equally likely, then 1/3rd of the Euclidean overall risk values will be greater than 3.18 (high risk), 1/3rd will be between 3.18 and 2.64 (medium risk), and 1/3rd will be lower than 2.64 (low risk)” (Hobday et al., 2007a). This assumption is not true because area does not correspond to probability on the PSA plot. Due to the Central Limit Theorem, productivity and susceptibility scores will cluster around the mean, and therefore plotted fisheries will cluster toward the center of the PSA plot. Independent of the fishery analyzed, the PSA will produce middling vulnerability scores with higher frequency than extremely low or high scores. This effect becomes more dramatic as the number of independent attributes increases (Fig. A.2). For these reasons, the existing risk categories do not represent the likelihood of a species receiving a vulnerability score and therefore are not capable of appropriately assigning risk. This fact may partially explain the inability of the PSA to appropriately discern risk in

mid-range vulnerability scores as found by Hordyk and Carruthers (Hordyk & Carruthers, 2018). To remedy this problem, it is crucial to adjust risk thresholds to reflect the statistical properties of the PSA plot defined by the Central Limit Theorem as well as the assumptions used in each study for which the PSA is used. The number of independent attributes and the type of mean used to score Productivity and Susceptibility can have dramatic effects on risk categories.

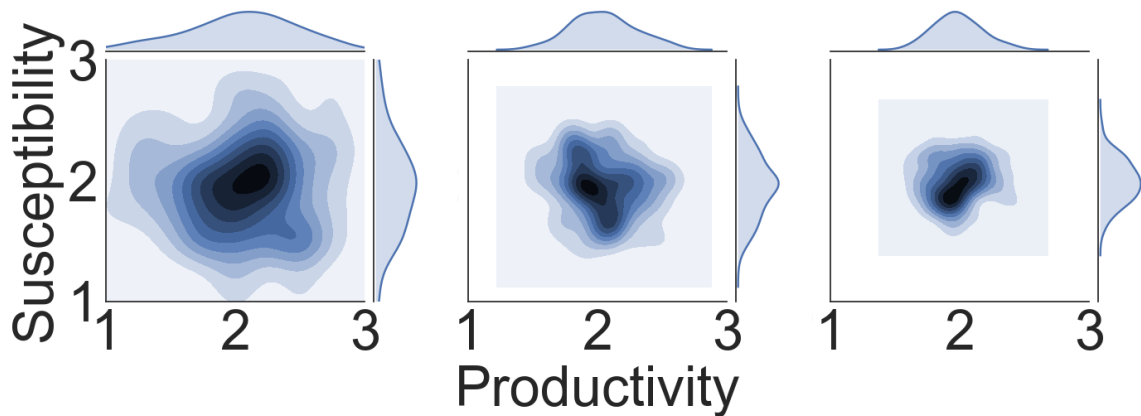


Figure A.2: Kernel density plots showing the bivariate gaussian distribution produced as a result of the scoring of productivity and susceptibility. More attributes used in scoring yields higher density plots. From left to right: 4 attributes each to calculate P and S, 10 attributes each, and 15 attributes each.

The purpose of this paper is to make available a revised PSA with greater discriminatory power as the number of attributes increases and analysis assumptions change, so the PSA can be applied reliably across fisheries. We provide a statistically robust foundation to derive vulnerability values and thresholds for risk categories. This new approach addresses issues with correlation between attributes; handles any weighting scheme, axis

scaling (multiplicative vs additive), and number of attributes; and provides recommendations for scoring procedures. Provided in this paper and the supplement are theoretical and empirical examples of the use of this new method. To illustrate the different outcomes between the standard PSA (sPSA) and our newly proposed “revised PSA” (rPSA), we report the differences achieved between the two approaches when applied to NOAA’s report in 2008 on 166 federally managed species (Patrick et al., 2010; Patrick et al., 2009) and 4 other case studies, together representing 302 fisheries across 4 continents. Full functionality of this method is accessible via Excel, R, and Python software packages accompanying this paper at <https://github.com/grewelle/rPSA>, though conversion tables are provided in the supplement that allow the same procedures to be done manually.

### A.3 Methods

In the PSA, productivity and susceptibility are each calculated as the mean of several attributes that take values 1, 2, or 3, corresponding to low, medium, or high productivity/susceptibility, respectively. These three values correspond to biologically relevant cut-offs for each attribute that are consistent across fisheries (Hobday et al., 2011b; Hobday et al., 2007a). The cut-offs serve to segregate the range of biological values for each attribute into bins standardized by percentile. When bins have equal size, namely,  $1 = 0 - 33\%$ ,  $2 = 33 - 67\%$ ,  $3 = 67 - 100\%$ , then the expected standard deviation for each attribute value is computed as follows:

$$\sigma = \sqrt{\frac{(1-2)^2 + (2-2)^2 + (3-2)^2}{3}} = \sqrt{\frac{2}{3}}$$

If scoring is distributed  $1 = 0 - 25\%$ ,  $2 = 25 - 75\%$ ,  $3 = 75 - 100\%$ , the standard deviation would be

$$\sigma = \sqrt{\frac{(1-2)^2 + 2(2-2)^2 + (3-2)^2}{4}} = \sqrt{\frac{1}{2}}$$

Choosing productivity and susceptibility attributes is a key step in performing a robust analysis. This matter and the matter of weighting of influential attributes to produce a weighted mean are discussed but not statistically treated in previous works (L. Duffy & Griffiths, 2017; Hordyk & Carruthers, 2018; Patrick et al., 2010; Patrick et al., 2009). The method presented here statistically treats both considerations as part of a flexible framework to compute vulnerability under a variety of conditions.

When attributes are judged sufficiently independent, each attribute is an independent and identically distributed random variable. Let each attribute value for productivity be given as  $p_i \in \{p_1, p_2, \dots, p_N\}$ , where  $N$  is the number of productivity attributes assessed in the PSA. Similarly,  $M$  susceptibility attribute values can be given as  $s_j \in \{s_1, s_2, \dots, s_M\}$ . Each productivity attribute value  $p_i$  is calculated by subtracting the initial value from 4 to produce a plot with origin  $(1, 1)$ . The productivity score is calculated as the arithmetic mean of productivity attribute values, while the susceptibility score is calculated either



as the arithmetic (additive) or geometric (multiplicative) mean of susceptibility attribute values. To generalize, presented are methods for which the attributes of both productivity and susceptibility can be additive or multiplicative.

### A.3.1 Additive Scoring

In the additive case, productivity and susceptibility scores for each species can be computed as the arithmetic mean of the corresponding attribute values, and according to the Central Limit Theorem, the distribution of scores approaches Gaussian (normal). We can define random variables,  $P$  and  $S$ ,  $P \sim N(\mu_p, \sigma_p)$  for productivity and  $S \sim N(\mu_s, \sigma_s)$  for susceptibility.  $\sigma_p$  is equivalent to the standard deviations shown above when productivity attributes are scored with the same bin sizes, and likewise for  $\sigma_s$  when the scoring procedure is standardized for all susceptibility attributes. As values for attributes can only take values 1, 2, or 3, each with equal probability, the arithmetic mean is:

$$\mu_p = \mu_s = 2$$

and associated standard error of the mean:

$$SE_p = \frac{\sigma_p}{\sqrt{N}} = \sqrt{\frac{2}{3N}} \quad SE_s = \frac{\sigma_s}{\sqrt{M}} = \sqrt{\frac{2}{3M}} \quad (\text{A.2})$$

The standard error of the mean decreases with increasing number of attributes used to

calculate P and S. Accordingly, P and S scores for a set of species naturally clump toward the middle of the PSA plot (Fig. A.2).

### Additive weighting

Where weighting of each attribute is needed, the mean does not change, but productivity and susceptibility for each species  $k \in \{1, 2, \dots, K\}$  are expressed as

$$P_k = \sum_{i=1}^N w_i p_{i,k} \quad S_k = \sum_{j=1}^M w_j s_{j,k} \quad (\text{A.3})$$

where  $0 \leq w_i, w_j \leq 1$  is the weight given to each attribute, and  $\sum_{i=1}^N w_i = \sum_{j=1}^M w_j = 1$ .

1. When weighting has been applied in the PSA, values of weights took whole integers. To convert whole integer weights to  $w$ , simply divide the whole integers by the sum of all integer weights used for the each set of attributes. The standard error of the weighted mean is:

$$SE_p = \sigma_p \left( \sum_{i=1}^N w_i^2 \right)^{1/2} = \left( \frac{2}{3} \sum_{i=1}^N w_i^2 \right)^{1/2} \quad SE_s = \sigma_s \left( \sum_{j=1}^M w_j^2 \right)^{1/2} = \left( \frac{2}{3} \sum_{j=1}^M w_j^2 \right)^{1/2} \quad (\text{A.4})$$

### A.3.2 Multiplicative Scoring

In the multiplicative case, the mean is geometric and the distribution of values the attributes take is lognormal. This distribution can be transformed to Gaussian given the nature of logarithmic identities that the mean is:

$$GM(S) = \left( \prod_{j=1}^M s_j \right)^{\frac{1}{M}} = e^{\frac{1}{M} \sum_{j=1}^M \log s_j} \quad (\text{A.5})$$

Therefore by taking the natural logarithm of each attribute, the resulting distribution is normal around the arithmetic mean of the log-transformed values. This gives  $\log P \sim N(\log GM(P), \sigma_{\log P})$  and  $\log S \sim N(\log GM(S), \sigma_{\log S})$ . The log-transformation produces a symmetric distribution that allows direct comparison with distributions around an arithmetic mean in the case where one axis in a PSA plot is additive (often productivity) and the other axis is multiplicative (often susceptibility). Let  $\mu_{\log P}$  and  $\mu_{\log S}$  be the arithmetic means of the log-transformed attribute values.

$$\mu_{\log P} = \mu_{\log S} = \frac{\log(6)}{3}$$

Standard error of the mean is:

$$SE_{\log P} = \frac{\sigma_{\log P}}{\sqrt{N}} = \frac{0.4536}{\sqrt{N}} \quad SE_{\log S} = \frac{\sigma_{\log S}}{\sqrt{M}} = \frac{0.4536}{\sqrt{M}} \quad (\text{A.6})$$

### Multiplicative weighting

Where weighting of each attribute is needed, the geometric mean is expressed as

$$GM(P) = \left( \prod_{i=1}^N p_i^{w_i} \right)^{\frac{1}{N}} \quad GM(S) = \left( \prod_{j=1}^M s_j^{w_j} \right)^{\frac{1}{M}} \quad (\text{A.7})$$

Log-transforming yields

$$P_k = \sum_{i=1}^N w_i \log p_{i,k} \quad S_k = \sum_{j=1}^M w_j \log s_{j,k} \quad (\text{A.8})$$

The mean of the data does not change with weighting. The corresponding standard error of the mean is:

$$SE_{\log P} = \sigma_{\log P} \left( \sum_{i=1}^N w_i^2 \right)^{1/2} = (0.2058 \sum_{i=1}^N w_i^2)^{1/2} \quad (\text{A.9})$$

$$SE_{\log S} = \sigma_{\log S} \left( \sum_{j=1}^M w_j^2 \right)^{1/2} = (0.2058 \sum_{j=1}^M w_j^2)^{1/2} \quad (\text{A.10})$$

### A.3.3 The Risk Axis

With calculated mean and standard error or associated log-transformed values for productivity and susceptibility we can define a bivariate Gaussian distribution  $\mathbf{X} \sim N_2(\boldsymbol{\mu}, \boldsymbol{\Sigma})$ , where  $\mathbf{X}$  is a random column vector.  $\boldsymbol{\mu}$  is the mean column vector  $[\hat{\mu}_p, \hat{\mu}_s]^T$  where  $\hat{\mu}_p = \mu_p$  or  $\mu_{\log P}$  and  $\hat{\mu}_s = \mu_s$  or  $\mu_{\log S}$ .  $\boldsymbol{\Sigma}$  is the covariance matrix. Let  $\hat{SE}_p = SE_p$  or  $SE_{\log P}$

and  $\hat{S}E_s = SE_s$  or  $SE_{\log S}$ . The values of  $\hat{\mu}$  and  $\hat{S}E$  are determined by whether attributes are treated as multiplicative or additive. Assuming productivity and susceptibility are statistically independent, the covariance matrix takes the form

$$\Sigma = \begin{pmatrix} (\hat{S}E_p)^2 & 0 \\ 0 & (\hat{S}E_s)^2 \end{pmatrix} \quad (\text{A.11})$$

The probability density of such a bivariate distribution is elliptical, with elliptical isolines representing standard deviations from the mean. The risk axis is the line whose slope is the reciprocal of the slope of the line that divides portion of the ellipse in the 1st and 3rd quadrants equally and passes through the mean  $(\hat{\mu}_p, \hat{\mu}_s)$ . Risk as a function of productivity and susceptibility of a species increases moving along the axis up and to the right. The equation for this axis can be expressed as

$$S - \hat{\mu}_s = \frac{\hat{S}E_p}{\hat{S}E_s} (P - \hat{\mu}_p) \quad (\text{A.12})$$

Let  $\mathbf{r}$  be the risk vector in the first quadrant of a coordinate plane with origin at the mean  $(\hat{\mu}_p, \hat{\mu}_s)$  and defined by equation A.12. Any vector  $\mathbf{x}_k = [P_k - \hat{\mu}_p \ S_k - \hat{\mu}_s]^T$  can be projected along this risk vector to map  $\mathbf{X}$  to one dimension. After mapping all points to the risk vector, the distance of each point  $x_k = (P_k, S_k)$  from the mean is the magnitude of the projection:

$$D(\mathbf{x}_k) = \frac{\mathbf{r} \cdot \mathbf{x}_k}{|\mathbf{r}|} \quad (\text{A.13})$$

Projecting points along the risk axis does not change the mean, but the resulting distribution along the risk axis is Gaussian and has a univariate variance and standard error of the mean. The projection

$$proj_{\mathbf{r}}(\mathbf{X}) = [\mathbf{r}(\mathbf{r}^T \mathbf{r})^{-1} \mathbf{r}^T] \mathbf{X} \quad (\text{A.14})$$

results in a linear transformation of  $\mathbf{X}$ . It can be shown that linear transformation preserves the mean and the standard error is expressed as

$$\hat{SE}_{\mathbf{r}} = \frac{\sqrt{2} \hat{SE}_p \hat{SE}_s}{\sqrt{\hat{SE}_p^2 + \hat{SE}_s^2}} \quad (\text{A.15})$$

### A.3.4 Thresholds for Risk Categories

The cumulative distribution function for this Gaussian distribution is

$$Pr(D(\mathbf{x}_k) \leq T) = \frac{1}{2} + \frac{1}{\sqrt{\pi}} \int_0^{\frac{D(\mathbf{x}_k)}{\hat{SE}_{\mathbf{r}} \sqrt{2}}} e^{-t^2} dt \quad (\text{A.16})$$

where  $T$  is the threshold of interest. One can define  $Pr_1(\cdot) = \frac{1}{3}$  and  $Pr_2(\cdot) = \frac{2}{3}$  to divide the set of points into 3 equal bins by probability. Solving the set of equations yields thresholds  $T_1$  and  $T_2$ . Because  $Z^{-1}(\frac{1}{3}) = -0.431$  and  $Z^{-1}(\frac{2}{3}) = +0.431$ , we can set

$T_1 = -0.431(\hat{SE}_r)$  and  $T_2 = 0.431(\hat{SE}_r)$  units from the mean along the risk axis to divide the possible set of values into equal thirds. A way to define thresholds in two dimensions is by constructing two lines  $L_1$  and  $L_2$  orthogonal to the risk axis that intersect the risk axis at  $T_1$  and  $T_2$ , respectively:

$$L_1 : S = -\frac{\hat{SE}_s}{\hat{SE}_p}P + \hat{\mu}_s + \frac{\hat{SE}_s}{\hat{SE}_p}\hat{\mu}_p - \frac{2(.431)\hat{SE}_s}{\sqrt{2}} \quad (\text{A.17})$$

$$L_2 : S = -\frac{\hat{SE}_s}{\hat{SE}_p}P + \hat{\mu}_s + \frac{\hat{SE}_s}{\hat{SE}_p}\hat{\mu}_p + \frac{2(.431)\hat{SE}_s}{\sqrt{2}} \quad (\text{A.18})$$

Along these two lines and any line parallel to these, any given point has coordinates  $P_k$  and  $S_k$  that satisfy the equation

$$P_k\hat{SE}_s + S_k\hat{SE}_p = V \quad (\text{A.19})$$

where  $V$  is a standardized risk score that represents vulnerability of a species to fishing activities. For  $L_1$  and  $L_2$ ,  $V$  is

$$L_1 : V = -\sqrt{2}(.431)\hat{SE}_p\hat{SE}_s + \hat{\mu}_p\hat{SE}_s + \hat{\mu}_s\hat{SE}_p \quad (\text{A.20})$$

$$L_2 : V = \sqrt{2}(.431)\hat{SE}_p\hat{SE}_s + \hat{\mu}_p\hat{SE}_s + \hat{\mu}_s\hat{SE}_p \quad (\text{A.21})$$

Grouping into low, medium, or high risk is made simple by this relationship. After calculating the standard error and mean for each component, given any point  $(P_k, S_k)$ , grouping is as follows:

$$Low : P_k \hat{SE}_s + S_k \hat{SE}_p \leq -\sqrt{2}(.431) \hat{SE}_p \hat{SE}_s + \hat{\mu}_p \hat{SE}_s + \hat{\mu}_s \hat{SE}_p$$

$$Med : -\sqrt{2}(.431) \hat{SE}_p \hat{SE}_s + \hat{\mu}_p \hat{SE}_s + \hat{\mu}_s \hat{SE}_p < P_k \hat{SE}_s + S_k \hat{SE}_p \\ < \sqrt{2}(.431) \hat{SE}_p \hat{SE}_s + \hat{\mu}_p \hat{SE}_s + \hat{\mu}_s \hat{SE}_p$$

$$High : \sqrt{2}(.431) \hat{SE}_p \hat{SE}_s + \hat{\mu}_p \hat{SE}_s + \hat{\mu}_s \hat{SE}_p \leq P_k \hat{SE}_s + S_k \hat{SE}_p$$

To make this categorization easier, a table is provided in the supplement with a list of values for  $V$  at  $L_1$  and  $L_2$  under various conditions. Those wanting to quickly categorize a set of points can use equation A.35 and the table. The supplement addresses extensions of these methods, including dependency among attributes and among axes. Vulnerability ( $V$ ) is defined relative to the study conditions, and caution should be used to make cross-study comparisons when scoring procedures and input assumptions differ. However, cross-study comparisons can be made with the strict formulation of Vulnerability as a probability:

$$V_p = Pr(D(\mathbf{x}_k) \leq T) \in (0, 1) \quad (A.22)$$



$V$  and  $V_p$  are equally valid statistical metrics of vulnerability, with  $V$  holding an advantage in ease of calculation.  $V_p$  holds values between 0 and 1 and can be translated between studies and compared directly to probabilities corresponding to threshold values (e.g.  $\frac{1}{3}$  and  $\frac{2}{3}$ ).

## A.4 Analysis

The extent to which the results of the rPSA differ from results of the sPSA depends on how dramatically the risk thresholds change. Management priorities are shaped by risk categorization of species, so below we compute vulnerability scores and associated risk categorization using both PSA approaches on (a) synthetic data representing 100 simulated species evaluated across 3-15 productivity and susceptibility attributes and (b) 5 empirical cases studies, including the 2008 NOAA report on 166 commercial stocks by Patrick et al. (Patrick et al., 2010; Patrick et al., 2009). We evaluate the proportion of species that change risk category when the new approach is applied as well as the net shift in average risk. Net shift in average risk is defined as the difference between the proportion of species moved into a higher risk category and the proportion of species moved into a lower risk category with the new approach.

### A.4.1 Sensitivity analysis of a synthetic dataset

100 simulated species were randomly (uniformly) assigned attribute values for every permutation of number of attributes from 3-15 for productivity and susceptibility. Attributes were equally weighted, and both productivity scores were arithmetic means of the associated attribute values. Susceptibility scores were arithmetic means or geometric means, reflecting the use of both additive and multiplicative models for susceptibility scores in PSA studies. Vulnerability scores were calculated for each species for both the sPSA and rPSA. We categorized species according to the respective thresholds for both methods, and for each permutation, computed the mean fraction of species that change categorization under the new method averaged from 1000 bootstrap replicates (standard error < 0.01 for each value). We demonstrate that the number of attributes has a strong effect on categorization as expected from clumping of species toward the center of the plot. Up to 52 percent of species are reassigned to another risk category when the new method is applied (Fig. A.3), with up to 35 percent net increase in risk observed (Fig. A.4). Universally, the rPSA is expected to re-categorize species and do so in a way that favors higher risk assignment than previously recognized through the sPSA. These effects are largely due to lumping of many species in the medium risk category and the curvature of the thresholds in the sPSA. When the geometric mean is used for susceptibility (Fig. A.3a), higher risk assignment occurs more frequently than when the arithmetic mean is used (Fig. A.3b).

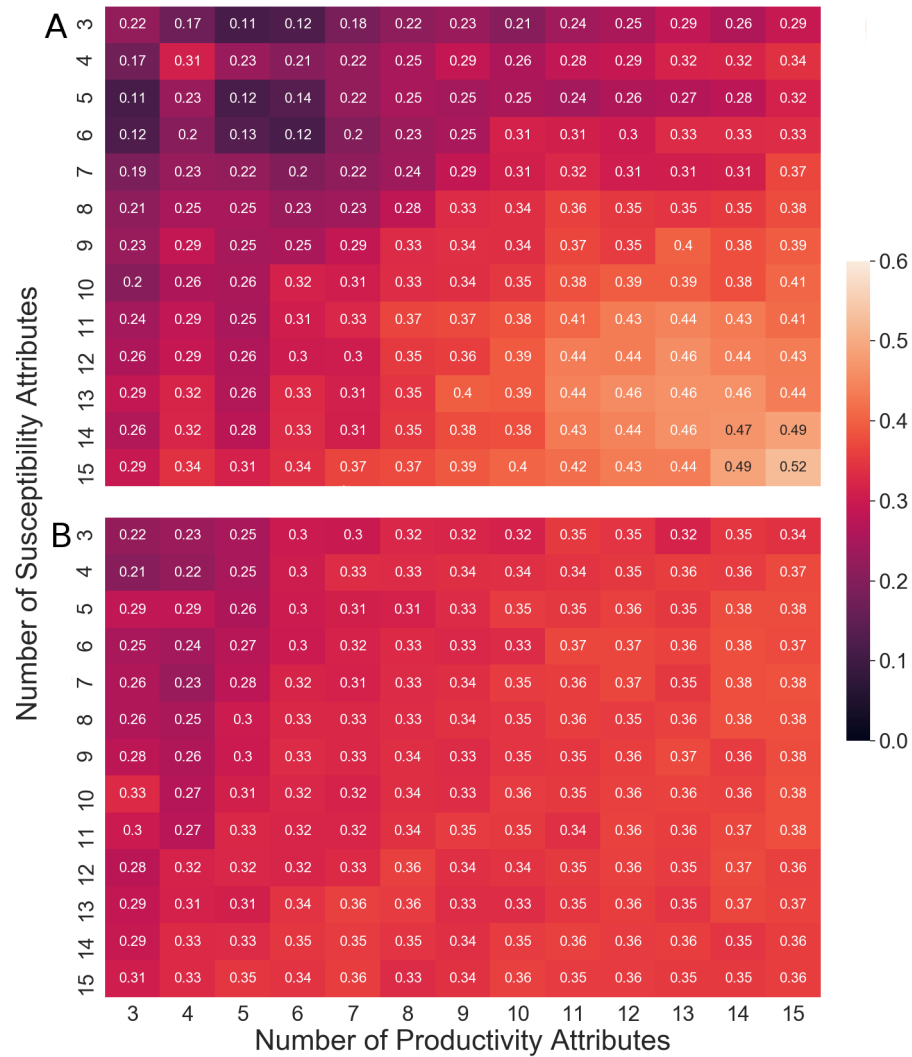


Figure A.3: Heat map representing the proportion of species that change risk category when applying the new PSA approach to 100 simulated species. Values are the average of 1000 bootstrap replicates. (A) P and S are arithmetic means (additive model) of 3-15 attribute values. Re-categorization is more pronounced as more attributes are used in the analysis. (B) P is the arithmetic mean and S is the geometric mean (multiplicative model) of 3-15 attribute values. Re-categorization is pronounced as more P attributes are used, though this trend is weaker as more S attributes are used, reflecting the differing results between the multiplicative and additive models.

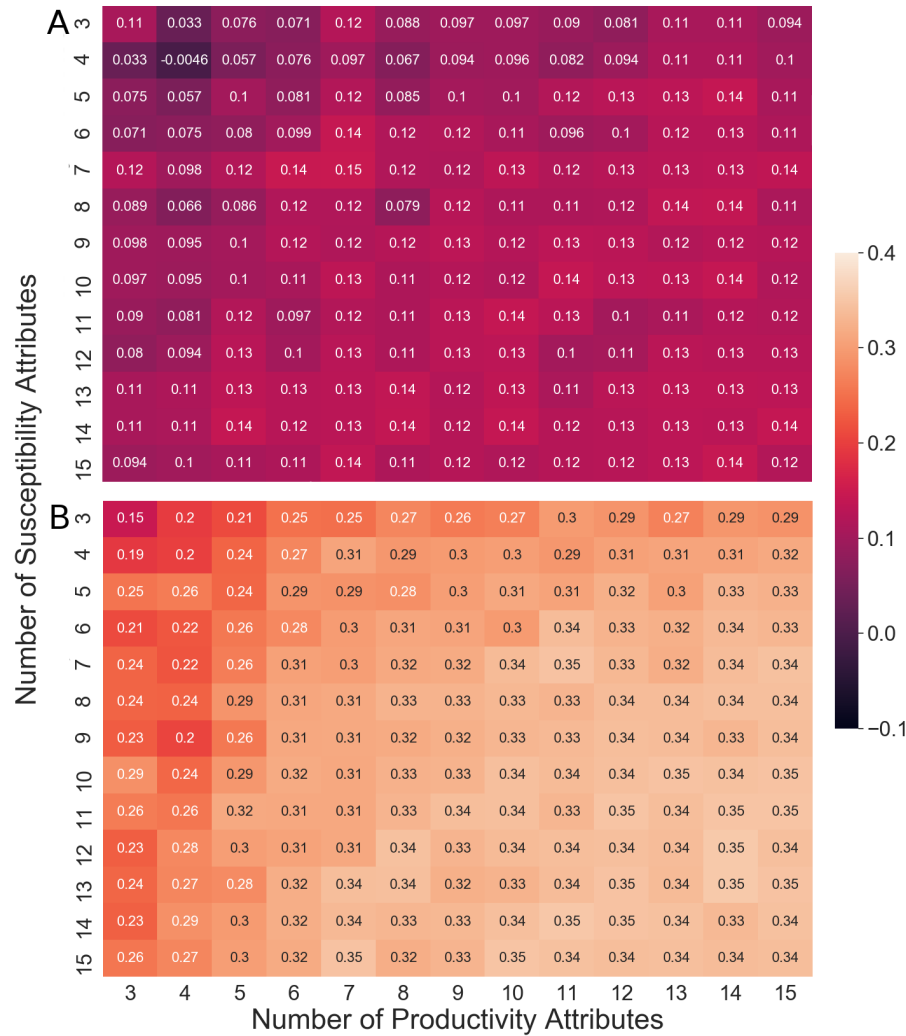


Figure A.4: Heat map representing the net proportional change in risk when applying the new PSA approach to 100 simulated species. Values are the average of 1000 bootstrap replicates. (A) P and S are arithmetic means (additive model) of 3-15 attribute values. In all but one permutation, the new method assessed risk higher than the existing PSA. (B) P is the arithmetic mean and S is the geometric mean (multiplicative model) of 3-15 attribute values. Net proportional change in risk is higher when the multiplicative model is used for S because the lower mean ( $S=1.8$  rather than 2) and shape of the resulting thresholds in the rPSA strongly favors higher risk assignment compared to the sPSA.

### **A.4.2 Empirical case studies**

To illustrate the use of this method on an empirical data set, we applied the existing and new PSA methods to the 2008 NOAA report by Patrick et al. This study includes 166 species in managed North American fisheries. In this study, screening methods were used to remove redundant attributes in the analysis. This resulted in 10 attributes for productivity and 12 for susceptibility (SI 6.11.4). When attributes were not applicable or informative for a species, these attributes were not scored, resulting in fewer attributes used to yield productivity and susceptibility scores. Attributes in this study are weighted according to perceived importance and the productivity score is given as the arithmetic mean of corresponding attribute values. The susceptibility score is given as the arithmetic mean (Fig. A.5a) and the geometric mean (Fig. A.5b) of corresponding attribute values. Scoring of attributes is partitioned in 3 equal sized categories, whereby assignment of values 1, 2, or 3 could occur uniformly. We analyzed the data set using both the standard PSA approach and the new, revised form. Figure A.5 shows the results of the analysis. Size and shading of dots corresponds to the number of species sharing the same coordinates on the PSA plot. Blue species are categorized as lower risk with the new method, red species are now higher risk than previously assessed, and gray species were assigned in the same risk category between methods. When P and S were additive, 10% (n=16) of species were assigned to a lower risk category with the new method, 25% (n=42) of species were assigned to a higher

risk category with the new method, and 65% (n=108) of species retained their risk categorization. An overall reassignment of 35% (n=58) of species and a net increase in risk of 16% with the new method reveals striking changes in the conclusions of the PSA study of these fisheries. These percentages align well with the simulation results, which predict reassignment of 39% of species and net increase in risk of 13% for a PSA study using 10 productivity and 12 susceptibility attributes. When P was additive and S was multiplicative, 1% (n=2) of species were assigned to a lower risk category with the new method, 37% (n=61) of species were assigned to a higher risk category with the new method, and 62% (n=103) of species retained their risk categorization. 38% of species were reassigned with a net increase in risk of 36% with the new method, matching well to the 34% predicted via simulation for both values. Additionally, the sPSA assigns 34 species low risk, 91 species medium risk, and 41 species high risk. There is a disproportionate number of medium risk species due to clumping of species toward the center of the PSA plot as previously discussed. No species are found in lower extremes of the plot, and some species are found in upper extremes of the plot due in part to a precautionary approach to scoring of attributes without sufficient data. With the new method, 34 species are low risk, 32 are medium risk, and 100 are high risk. Species are more evenly distributed in low and medium categories, with a larger bulk of species in the high risk category, representative of the precautionary scoring approach used.

The PSA is used widely to assess data-poor fisheries with different needs and contexts.

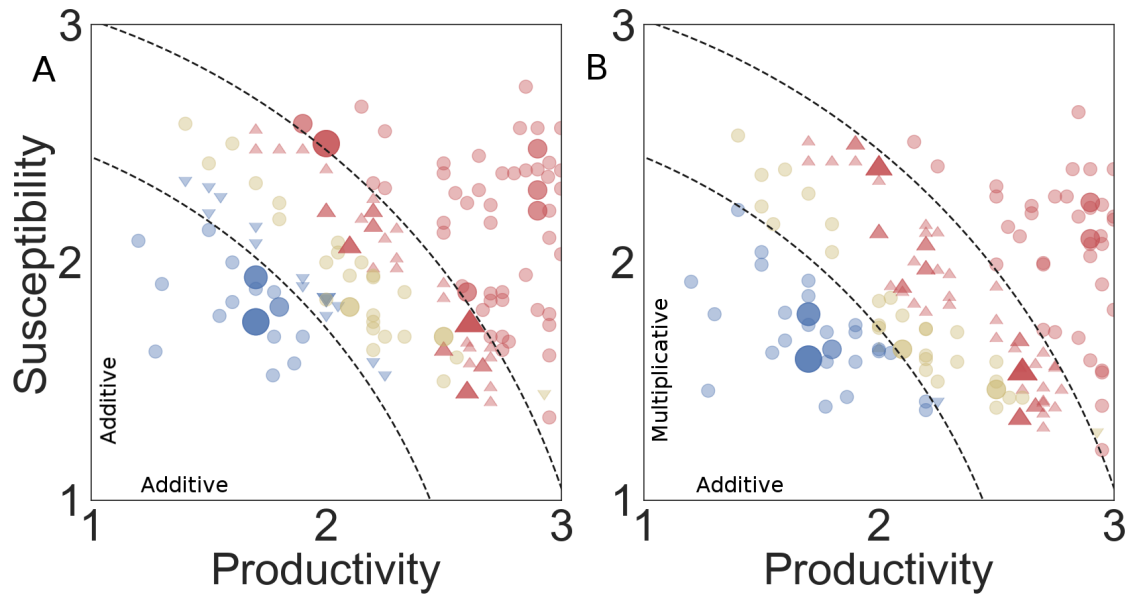


Figure A.5: 166 North American species from Patrick et al. plotted on a PSA plot. Shade and area of the points correspond to the number of species at each coordinate. Down facing arrows represent species that are designated a lower risk categorization with the new PSA method compared to the existing method. Species with up facing arrows are those designated a higher risk categorization. Circles represent species that are designated the same risk categorization. Colors give the risk categorization assigned by the rPSA (blue = low, yellow = medium, red = high). The dotted lines are given to show thresholds of the existing method at  $V=2.64$ ,  $3.18$ . New thresholds vary with each species in this study because not all species received values for all attributes. Therefore, these thresholds are not plotted. (A) P and S are arithmetic means (additive model) of attribute values. (B) P and S are arithmetic and geometric (multiplicative model) means, respectively, of attribute values.

Its use across six continents demonstrates the flexibility of the approach, and the differences in species risk categorization between the sPSA and the rPSA are not equal between studies. The set of attributes used, model assumptions, as well as the species assessed play roles to determine the magnitude of the change observed when applying the rPSA. We compare five previously published studies, conducted across 4 continents and 302 species: Sri Lanka (12 species, 11 attributes) (Cotter & Lart, 2011), aquarium trade (61 species, 22 attributes) (Dee et al., 2019), Taiwan (52 species, 12 attributes) (C.-Y. Lin et al., 2020), North America (166 species, 22 attributes) (Patrick et al., 2009), Galapagos (11 species, 11 attributes) (Pontón-Cevallos et al., 2020a). Figure A.6 demonstrates that despite the range of inputs to the PSA, a significant proportion of species is recategorized with the rPSA. Moreover, in all five case studies, there is an observed net increase in risk assessed with the rPSA. Differences in each study tend to be highest when (1) a wide range of species is analyzed, (2) more attributes are used, (3) a multiplicative model is used for susceptibility (SI 6.11.4). These observations are expected given simulated results in Figure A.4 and empirical results in Figures A.5 and A.10 for the Patrick et al. study, which satisfies all 3 criteria.

## **A.5 Discussion**

Given here is a new approach to the PSA that is broad in scope to account for weighting of attributes, any number of attributes used to calculate productivity and susceptibility, and



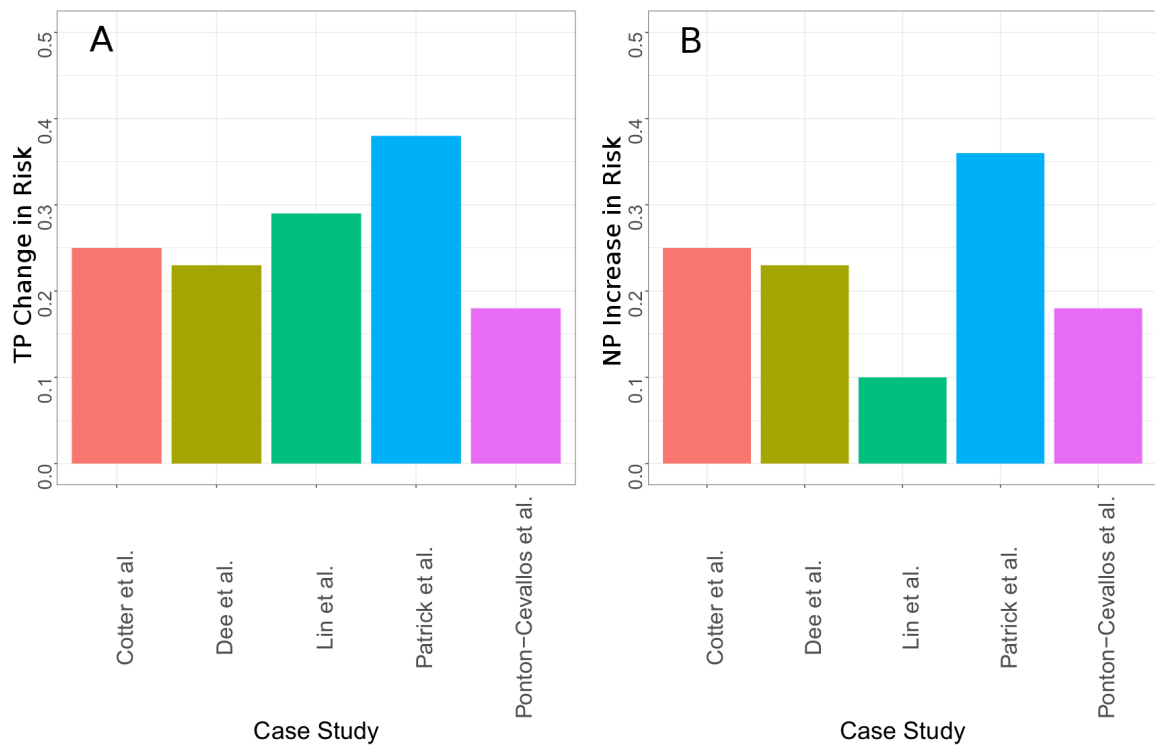


Figure A.6: Compared changes in risk categorization between the sPSA and rPSA applied to five previously published studies. From left to right: Cotter et al. (Sri Lanka), Dee et al. (Aquarium), Lin et al. (Taiwan), Patrick et al. (North America), Ponton-Cevallos et al. (Galapagos). The two panels representing (A) the total proportion of species that changed categories between the methods and (B) the net increase in risk assessed with the rPSA show that across studies of disparate systems, recategorization is significant and leads to an increased assessed risk to species.

use of additive or multiplicative models for productivity and susceptibility. Quality of information can be easily incorporated without loss of application as components of the weights given to each attribute. This revised method is adaptable to any level of mathematical training. Modifications are possible for large analyses with complex assumptions. Those variations are outlined in the methods and supplement, and software packages in Excel, R, and Python accompany this publication. Vulnerability calculations and risk categorization can also be performed manually via the conversion tables provided in the supplement. The tables have simplified the analysis to four values, such that results are achieved rapidly. Vulnerability values ( $V_p$ ) in this method are standardized, so comparisons can be made across studies with shared attributes, and species can be ranked accordingly.

Analyses examining the differences between the results of the sPSA and the new approach proposed (rPSA) here show significantly improved resolution of risk for the species in the middle of the PSA plot. Because there is an increasing probability for species to fall in the middle region of the PSA plot as more attributes are used in the analysis, we derive a statistically robust method to compute vulnerability and define risk categories according to the expected distribution of vulnerability scores rather than dividing the plot into three regions of equal area. With the new method fewer species are now categorized as medium risk. Re-analysis of the Patrick et al. study of 166 North American species demonstrates this phenomenon with the medium risk category downsizing from 91 species to 32. The magnitude of the shift of species to new risk categories with the new method can be large.

In some case studies, more than one-third of species shifted to new risk categories. This aligned well with the expected percent of re-categorization as predicted with simulation, and for a reasonable range of attributes used in a PSA, this can vary from 11% to 52%. The majority of re-categorized species are mid-risk species that are categorized low or high risk with the rPSA. Due to the difference in curvature of the thresholds between the sPSA and rPSA, other species shift categorization, with a net shift to higher risk categories using the rPSA. Compared to the rPSA, the sPSA underestimates overall risk by 0 to 35% across a range of simulated scenarios and does so by 36% specifically for the 166 species in the NOAA vulnerability assessment presented by Patrick et al. Applying the rPSA to four other case studies demonstrated similar patterns, with the magnitude of recategorization depending on study assumptions and analyzed species, and net increase in assessed vulnerability in all studies (Figs. A.6, A.11).

Together our analysis shows that the existing framework to assess vulnerability through the PSA incompletely resolves and under-evaluates risk of species to fishing pressure, and may therefore, jeopardize the utility of the precautionary approach used by most to assign higher risk to species when data is limited for certain attributes (Hobday et al., 2011b; Hobday et al., 2007a). Improper resolution paired with incomplete data clouds prioritization of vulnerable species for downstream analysis or management. We recommend attention be given to the practice of high risk assignment in the absence of data as the field moves toward a more refined means to assess data-poor fisheries. If limited in use, this type 1

error in risk assignment serves to benefit the few species that would have been missed due to lack of data in a vulnerability analysis. However, if applied to many species, this practice may dilute directives and resources to serve species in most critical need. Future work is needed to determine whether agnostic assignment of risk (with medium scores) rather than precautionary assignment of risk (with high scores) leads to improved efficiency in downstream evaluation proceeding the rPSA.

Application of the rPSA in many cases will yield substantially different results than the standard PSA. Better resolution of species' risk will both streamline the next step of the ERAEF and shift management priorities for many marine species. With greater pressure to determine the impact of fisheries, this new framework is a step toward creating a robust, reliable method to assess small-scale or data-deficient systems. Alone this new method represents only a small piece of efforts to assess these fisheries. Efforts to improve scoring through standardization, use of independent and informative attributes, and broad acquisition of available data is key to improving accuracy. Even still, when using the ERAEF framework, analyses only provide recommendations to be used in downstream evaluation. Decisions on mitigation strategies rely on integration of vulnerability analyses with the needs of the communities that rely on the managed fishery. Corroborating vulnerability assessment provided by the PSA with local knowledge, trend analyses, and extrapolation techniques can give a more complete picture of how to best prioritize research and management efforts (Finkbeiner et al., 2017a; Frawley et al., 2019; Mason et al., 2019; Oestreich

et al., 2019a; Schall et al., 2018). Previous studies employing the existing PSA could benefit from re-analysis to discover whether management priorities require shifts. Realistically, this method provides an opportunity to gain insight in the risk of fished species in later analyses and gives a framework that allows more reliable comparison across similar studies. Comparison of this method to sensitivity analyses of traditional stock assessments will inform our confidence in the reliability of the approach and is a recommended future step. Although we compare a the standard form of the PSA (sPSA) devised by Hobday et al. to our revised form (rPSA), other variants of ecological risk assessments exist that share properties with the sPSA. One such variation calculates vulnerability as the distance from (1,1) rather than the origin at (0,0) (equation A.27). Other studies which use attributes to highlight different components of risk like exposure and sensitivity (Samhouri et al., 2019a) can similarly benefit from the methods outlined in this work. The choice of the Euclidean norm by many of these studies (rather than other metrics, such as other members of the p-norms family) implies the improvements we show will be similar in magnitude for these related methods.

There are scenarios when caution is appropriate before applying the revised PSA approach proposed here. Firstly, if some attributes are highly correlated, redundant attributes should be excluded or the number of effectively independent attributes should be estimated as described in the methods presented in the supplement. Secondly, when attributes are weighted, if one of the attributes is weighted far more than others, this method will be less

accurate. The more attributes used, the less this is a problem, as this heavily weighted attribute will contribute less overall to productivity or susceptibility (see Lindeberg's condition for Central Limit Theorems). Often attributes are not scored for all species due to insufficient data or because some attributes are not applicable for all species. This new PSA approach should be applied specific to each species when the weighting structure or number of attributes varies by species within the same study. For example, some species are assessed with 10 attributes while others are assessed with 5, threshold lines must be re-evaluated on a species-by-species basis. Calculated vulnerability values ( $V_p$ ) are directly comparable in this way, and either  $V$  or  $V_p$  can be used for risk categorization. Because the PSA is used to assess data-poor species, attribute scoring is often qualitative. Efforts to standardize this procedure by establishing specific bins for scoring based on percentiles in a biologically realistic range of values is key to making the PSA more robust. There is little use in applying statistics to non-statistical data. This makes for overly precise but inaccurate conclusions. The bins established can be in any chosen percentile range as long as calculations for standard deviation are adjusted as shown above. However, the percentile ranges must be consistent across attributes. For ease, we recommend bins be treated equally so each score (1, 2, or 3) captures one-third of potential values. This standardization process should be considered an essential component of the rPSA. Ecological modeling or, when available, empirical data can inform the choice of bin thresholds, as is commonplace in ecosystem-based management analyses (Samhouri et al., 2010). Choice

of model (additive vs multiplicative) depends on the expected nature of the contributions of attributes to productivity or susceptibility. Generally, a multiplicative model gives greater importance to extremely low values than an additive model. Therefore, its use as a model for susceptibility implies, for example, that a small number of low scoring attributes outweighs the same small number of high scoring attributes. This assumption may not be supported in practice if instead a few high scoring attributes should have a larger than additive effect on the susceptibility of a species. In the most common use of susceptibility, the four attributes (Availability, Encounterability, Selectivity, and Post-capture Mortality) are presumed to interact in a multiplicative fashion because low scoring of any of these attributes reduces the risk associated with the other attributes. From Hobday et al. 2011: 'For example, if a species is available in a fishing area, encounters the fishing gear, is selected by the gear, but is returned to the water unharmed (post-capture mortality low), then the overall susceptibility should be recognized as low.' In contrast, an additive model assumes the magnitude of the effect of each attribute is not contingent on the magnitude of other attributes. Choice of model is distinct from effects that correlation among attributes may have on the representation of the analysis.

Use of the rPSA, as with the sPSA, is best conducted in a risk assessment framework like ERAEF because results are meant to be used as a relative measure of vulnerability of fished or bycatch species to fishing. Adaptation of the rPSA in each local context requires input from scientists, managers, and stakeholders to determine how to appropriately assess

the present risks to fisheries. The framework presented allows flexible choice of attributes while providing the statistical rigor needed to move the PSA and similar risk assessments to a quantitative format. Moreover, the method can be scaled by level of expertise and complexity of assumptions, making practical use widely accessible. The degree to which results of the rPSA and adaptations of this method will change existing management will depend on the downstream evaluation practices of each management community. For communities that rely on unrelated methods to assess data-poor fisheries, adoption of the rPSA could provide a robust alternative, though the practical choice depends on context. Unlike quantitative stock assessments, which rely on empirical data, the PSA is qualitative, and relies on associations between the attributes used and Productivity or Susceptibility. Chosen attributes should, therefore, demonstrate a sufficiently strong and causal relationship to either Productivity or Susceptibility, which are assumed directly related to Vulnerability. Weighting is designed to quantify the strength of these relationships, and efforts should be made to justify weight values with the most applicable empirical data. Future exploration is needed to optimize weighting of attributes in a way that leverages the improvements introduced with the rPSA. Vulnerability as an index of risk is not an absolute indication of stock decline or potential for stock decline and depends on the attributes used in the analysis. Because risk categorization of species using the PSA is best interpreted relative to other species for which the same set of attributes can be applied, caution should be taken to over-interpret results of broad studies where evaluated attributes differ among species.



In these cases it is imperative to conduct further assessment comparing vulnerable species as outlined by frameworks like ERAEF. Nevertheless, the rPSA improves upon an accessible method to assess risk in 90+% of fisheries which are considered data-poor. Without a cohesive framework to garner crucial information about these species, the vast majority of non-commercially fished species would be neglected when establishing management priorities.

## A.6 Conclusion

The PSA provides a standardized means to determine the vulnerability of marine species affected by fisheries. Its structure and simplicity enable its use by broad interest groups, scientists, and stakeholders. Great strides have been made to improve the way we assess data deficient species, and the work presented in this study is intended to be a step in that direction. This new approach to the PSA allows incorporation of all existing modifications that have accumulated over the years and gives a statistically robust means of determining relative risk of species. Light is shed on why the standard PSA needs improvement and how resolution can be gained, especially for mid-risk species, with the new index of vulnerability provided by the new method. Because of its robustness across any variety of conditions and study types, we consider this new approach a revised PSA. We encourage incorporation of the rPSA into the existing ERAEF framework to improve validity of its results.

## **A.7 Acknowledgements**

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## **A.8 Author Contributions**

Conceptualization, REG; Methodology, REG; Software, REG; Analysis, REG & EM; Visualization, REG; Writing – Original Draft, REG; Writing – Review & Editing, REG, EM, FM, GDL; Funding Acquisition, REG, FM, GDL

## **A.9 Conflict of Interest Statement**

The authors declare no competing interests.

## **A.10 Data Availability Statement**

Software associated with this study is available for download and use at

<https://github.com/grewelle/rPSA>. Empirical data evaluated is publicly available in referenced works.

## A.11 Supplemental Information

### A.11.1 Revisiting existing thresholds

This supplement begins with an exposition of the existing thresholds. In no previous works have the derivation of these thresholds been given, and it is worth calculating these thresholds to verify their use as part of previous implementations of the PSA. The equations to follow generalize the thresholds to be used for any number of subdivisions of the PSA plot into  $N$  parts of equal area. Because the area of the plot is fixed at 4 units, setting the threshold equations equal to  $\frac{4n}{N}$  gives the desired threshold values  $T_n \in T_1, T_2, \dots, T_{N-1}$ .

Threshold calculations are given by the piecewise function

$$\frac{4n}{N} = \begin{cases} 1 - \sqrt{T_n^2 - 1} + \frac{T_n^2}{4}(\pi - 4\text{csc}^{-1}(T_n)) & T_n \leq \sqrt{10}, \frac{n}{N} \leq 0.659 \\ T_n^2(\sin^{-1}(\frac{\sqrt{T_n^2 - 9} + 9}{T_n\sqrt{10}}) - \frac{\pi}{4} - \sin^{-1}(\frac{1}{\sqrt{10}})) & \\ + 3\sqrt{T_n^2 - 2(\sqrt{T_n^2 - 9} + 4)} - 2 & T_n > \sqrt{10}, \frac{n}{N} > 0.659 \end{cases} \quad (\text{A.23})$$

PSA plots are traditionally divided by two thresholds into 3 regions of equal area. Vulnerability scores falling below the first threshold signal a stock of low vulnerability, scores between the first and second threshold indicate of stock of medium vulnerability, and scores above the second threshold indicate a highly vulnerable stock. These two thresholds are traditionally given as 2.64 and 3.18, yet no formal calculation exists in the literature. Likely these values were informally approximated through simulation. These approximations are

close to the actual values that can be calculated by solving the piecewise function above.

The first threshold ( $T_1$ ) is found by solving:

$$\frac{4}{3} = 1 - \sqrt{T_1^2 - 1} + \frac{T_1^2}{4}(\pi - 4\csc^{-1}(T_1)) \quad (\text{A.24})$$

$$T_1 = 2.645$$

The second threshold ( $T_2$ ) is found by solving:

$$\frac{8}{3} = T_2^2 \left( \sin^{-1} \left( \frac{\sqrt{T_2^2 - 9} + 9}{T_2 \sqrt{10}} \right) - \frac{\pi}{4} - \sin^{-1} \left( \frac{1}{\sqrt{10}} \right) \right) + 3\sqrt{T_2^2 - 2(\sqrt{T_2^2 - 9} + 4)} - 2 \quad (\text{A.25})$$

$$T_2 = 3.173$$

Upon calculation, we find that only small error exists in original approximations of threshold values. The original thresholds were approximations given that vulnerability ( $V$ ) is calculated as the distance from the origin of points plotted on a PSA plot with axes from 1 to 3 for productivity ( $P$ ) and susceptibility ( $S$ ). The origin here is  $(0, 0)$ , not the origin of the plot  $(1, 1)$ . The equation for the distance of each point  $(S_k, P_k)$  from the origin  $(0, 0)$  is

$$V_k = \sqrt{S_k^2 + P_k^2} \quad (\text{A.26})$$

Calculating vulnerability this way produces thresholds given by circles centered at  $(0, 0)$  rather than thresholds given by circles centered at the plot origin  $(1, 1)$ . This effect is noticed by some, who correctly change the calculation for vulnerability to

$$V_k = \sqrt{(S_k - 1)^2 + (P_k - 1)^2} \quad (\text{A.27})$$

which shifts the values vulnerability can take to  $[0, 2\sqrt{2}]$  rather than  $[\sqrt{2}, 3\sqrt{2}]$ . Here,  $P_k$  is the productivity value for each stock subtracted from 4 to scale axes from 1 to 3. Of course, now the previously calculated thresholds no longer apply if equation A.27 is used to calculate vulnerability. There is no simple transformation to make equations A.26 and A.27 comparable, so provided below are the equations for this formulation of the PSA.

Thresholds for vulnerability can be calculated for equation A.27 by solving the piecewise function

$$\frac{4n}{N} = \begin{cases} \frac{\pi T_n^2}{4} & T_n \leq 2, \frac{n}{N} \leq \frac{\pi}{4} \\ \frac{\pi T_n^2}{4} + 2T_n \sqrt{1 - \frac{4}{T_n^2}} - T_n^2 \cos^{-1}\left(\frac{2}{T_n}\right) & T_n > 2, \frac{n}{N} > \frac{\pi}{4} \end{cases} \quad (\text{A.28})$$

Dividing the newly scaled PSA plot with axes from 0 to 2 into thirds yields two new thresholds. The first threshold ( $T_1$ ) is found by solving:

$$\frac{4}{3} = \frac{\pi T_1^2}{4} \quad (\text{A.29})$$

$$T_1 = 1.303$$

The second threshold ( $T_2$ ) is found by solving:

$$\frac{8}{3} = \frac{\pi T_2^2}{4} \quad (\text{A.30})$$

$$T_2 = 1.843$$

To plot these thresholds on a PSA plot with axes from 1 to 3, an arc of radius  $T_n + 1$  can be drawn. The thresholds calculated above would take the values  $T_1 = 2.303$ ,  $T_2 = 2.843$ .

These two thresholds are shown in Figure A.7.

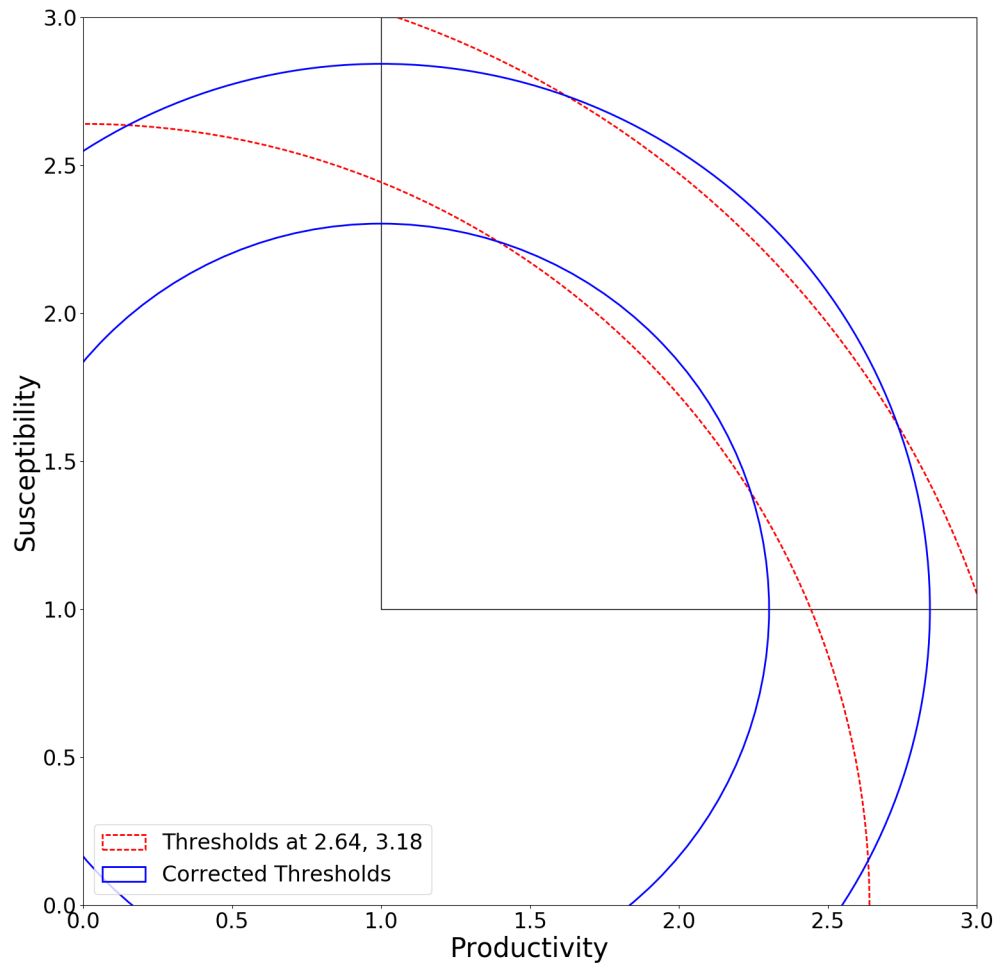


Figure A.7: PSA plot in the upper right corner of a square plot with axes from 0 to 3. The existing thresholds are concentric circles centered at the origin (0,0), though some studies calculated vulnerability as the distance from (1,1). The thresholds at  $V=2.64, 3.18$  no longer apply, and the corrected thresholds are given to show the difference in shape. None of these thresholds are valid in the new method.

### A.11.2 Examples of threshold derivations for the rPSA

Below describes how to use this framework in two example cases to illustrate its use. The first example case applies this method in a simple form where productivity and susceptibility are calculated as arithmetic means for each respective set of attributes. Each attribute is weighted equally, and productivity and susceptibility are composed of equal number of attributes. The second case includes use of weighted attributes, susceptibility and productivity differ in numbers of attributes, and those attributes are assumed multiplicative for susceptibility and additive for productivity.

#### Example 1: a simple case

In this first example, productivity is composed of 10 unweighted attributes and susceptibility is composed of 10 unweighted attributes. Both sets are additive, and their means are arithmetic. Because there should be equal probability of assignment of values 1, 2, or 3 to attributes in the null case for step 1:

$$\hat{\mu}_p = \hat{\mu}_s = 2 \quad SE_p = SE_s = \sqrt{\frac{2}{3 * 10}} = \frac{1}{\sqrt{15}}$$

$$\Sigma = \begin{pmatrix} (\frac{1}{\sqrt{15}})^2 & 0 \\ 0 & (\frac{1}{\sqrt{15}})^2 \end{pmatrix} = \begin{pmatrix} \frac{1}{15} & 0 \\ 0 & \frac{1}{15} \end{pmatrix}$$

The risk axis can be defined by the equation:



$$S - 2 = \frac{\frac{1}{\sqrt{15}}}{\frac{1}{\sqrt{15}}}(P - 2) \quad \Rightarrow \quad S = P$$

When  $SE_p = SE_s$ , the risk axis will pass through the origin  $(0, 0)$ . Because the risk axis in this case passes through  $(0, 0)$ , it can be represented simply by any multiple of  $[1 \ 1]^T$ . Projection of the 2-dimensional Gaussian onto the risk axis gives:

$$proj_r(\mathbf{X}) = \frac{1}{2} \begin{pmatrix} 1 & 1 \\ 1 & 1 \end{pmatrix} \mathbf{X}$$

The distance from  $\mu$  after projection onto the risk axis is

$$D(\mathbf{x}_k) = \frac{[1 \ 1]^T \cdot [P_k - \hat{\mu}_p \ S_k - \hat{\mu}_s]^T}{\sqrt{1^2 + 1^2}} = \frac{P_k + S_k - 4}{\sqrt{2}}$$

The resulting standard error is calculated as

$$\hat{SE}_r = \frac{\sqrt{2}(\frac{1}{\sqrt{15}})^2}{\sqrt{(\frac{1}{\sqrt{15}})^2 + (\frac{1}{\sqrt{15}})^2}} = \frac{1}{\sqrt{15}}$$

One can observe that given equal standard error on each coordinate axis, the projection onto the risk axis results in equivalent standard error. To divide the newly linear data into equally probable thirds, we simply multiply this standard error by  $\pm 0.431$ .

$$T_1 = \frac{-0.431}{\sqrt{15}} \quad T_2 = \frac{0.431}{\sqrt{15}}$$

We define risk categories as

| Low                                                                              | Medium                                                                                                                         | High                                                                            |
|----------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| $D(\mathbf{x}_k) \leq \frac{-0.431}{\sqrt{15}}$                                  | $\frac{-0.431}{\sqrt{15}} < D(\mathbf{x}_k) < \frac{0.431}{\sqrt{15}}$                                                         | $\frac{0.431}{\sqrt{15}} \leq D(\mathbf{x}_k)$                                  |
| $\frac{P_k+S_k}{\sqrt{15}} \leq \frac{-0.431\sqrt{2}}{15} + \frac{4}{\sqrt{15}}$ | $\frac{-0.431\sqrt{2}}{15} + \frac{4}{\sqrt{15}} < \frac{P_k+S_k}{\sqrt{15}} < \frac{0.431\sqrt{2}}{15} + \frac{4}{\sqrt{15}}$ | $\frac{0.431\sqrt{2}}{15} + \frac{4}{\sqrt{15}} \leq \frac{P_k+S_k}{\sqrt{15}}$ |

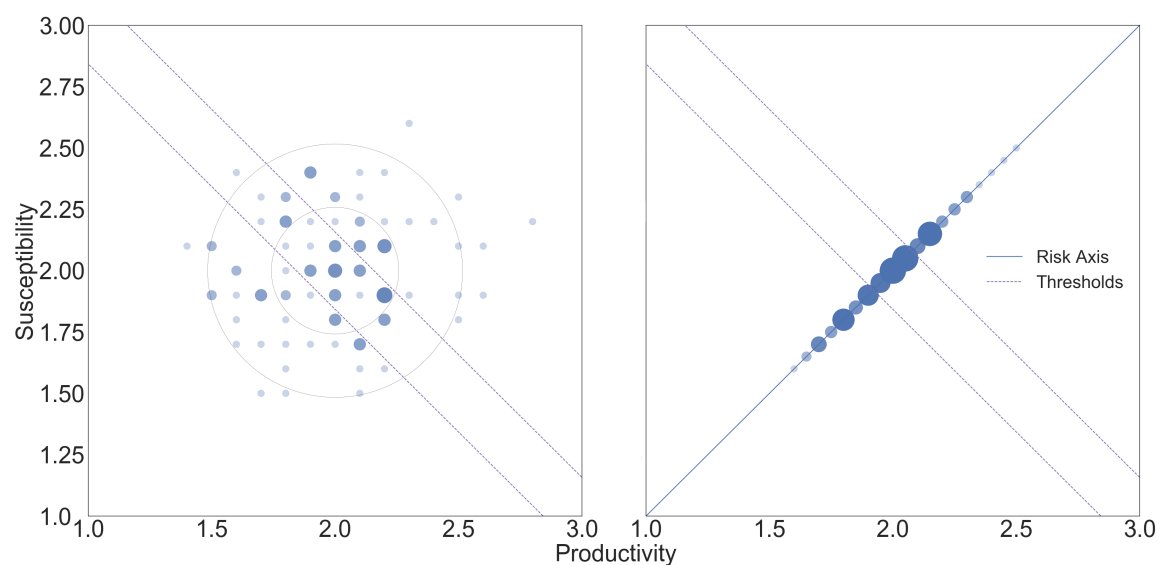


Figure A.8: 100 simulated species plotted on a PSA plot (left).  $P$  and  $S$  are arithmetic means of 10 attribute values each. Circles represent 1 and 2 z-scores from the bivariate mean. A risk axis is defined and the species are orthogonally projected onto this line (right). Those further to right along the line are higher risk. Thresholds are drawn to divide the distribution of species in thirds by probability.

**Example 2: a complex case**

In this second example, productivity is composed of 10 weighted attributes and susceptibility is composed of 12 weighted attributes. Productivity is additive, while susceptibility is multiplicative. Here I show how to handle this more complex case.

Let's set the productivity weights as [1, 2, 2, 2, 4, 2, 3, 2, 3, 2] and the susceptibility weights as [2, 2, 2, 3, 4, 4, 1, 2, 1, 2, 2, 3]. As discussed above, the mean for productivity axis is arithmetic, while the mean for the susceptibility axis is geometric.

$$\hat{\mu}_p = 2 \qquad \hat{\mu}_s = \frac{\log(6)}{3}$$

The standard error of the weighted mean is given as

$$\begin{aligned} \hat{SE}_p &= \left( \frac{2}{3} \sum_{i=1}^{10} w_i^2 \right)^{1/2} = \left( \frac{2(1^2 + 6 * 2^2 + 2 * 3^2 + 4^2)}{3 * 23^2} \right)^{1/2} = 0.273 \\ \hat{SE}_s &= (0.2058 \sum_{j=1}^{12} w_j^2)^{1/2} = 0.2058 \left( \frac{2 * 1^2 + 6 * 2^2 + 2 * 3^2 + 2 * 4^2}{28^2} \right)^{1/2} = 0.141 \end{aligned}$$

$$P_k = \sum_{i=1}^N w_i p_{i,k} \qquad S_k = \sum_{j=1}^{N'} w_j \log(s_{j,k})$$

$$\Sigma = \begin{pmatrix} 0.273^2 & 0 \\ 0 & 0.141^2 \end{pmatrix} = \begin{pmatrix} 0.074 & 0 \\ 0 & 0.020 \end{pmatrix}$$

The risk axis can be defined by the equation:

$$S - \frac{\log 6}{3} = \frac{0.273}{0.141}(P - 2)$$

When this line does not pass through  $(0, 0)$ , projection on the 2-dimensional Gaussian onto the line requires an extra transformation after multiplication with the projection matrix to recenter the mean. Projection of the 2-dimensional Gaussian onto the risk axis gives:

$$proj_r(\mathbf{X}) = \begin{pmatrix} 0.211 & 0.408 \\ 0.408 & 0.788 \end{pmatrix} (\mathbf{X} - \boldsymbol{\mu}) + \boldsymbol{\mu}$$

The distance from  $\boldsymbol{\mu}$  after projection onto the risk axis is

$$D(\mathbf{x}_k) = \frac{[0.141 \ 0.273]^T \cdot [P_k - \hat{\mu}_p \ S_k - \hat{\mu}_s]^T}{\sqrt{0.141^2 + 0.273^2}} = \frac{0.141P_k + 0.273S_k - 0.445}{0.307}$$

The resulting standard error is calculated as

$$\hat{SE}_r = \frac{\sqrt{2}(0.273)(0.141)}{\sqrt{(0.273)^2 + (0.141)^2}} = 0.177$$

To divide the newly linear data into equally probable thirds, we simply multiply this standard error by  $\pm 0.431$ .

$$T_1 = -0.431 * 0.177 = -0.076 \qquad T_2 = 0.431 * 0.177 = 0.076$$

We define risk categories as

| Low                              | Medium                                | High                             |
|----------------------------------|---------------------------------------|----------------------------------|
| $D(\mathbf{x}_k) \leq -0.076$    | $-0.076 < D(\mathbf{x}_k) < 0.076$    | $0.076 \leq D(\mathbf{x}_k)$     |
| $0.141P_k + 0.273S_k \leq 0.422$ | $0.422 < 0.141P_k + 0.273S_k < 0.469$ | $0.469 \leq 0.141P_k + 0.273S_k$ |

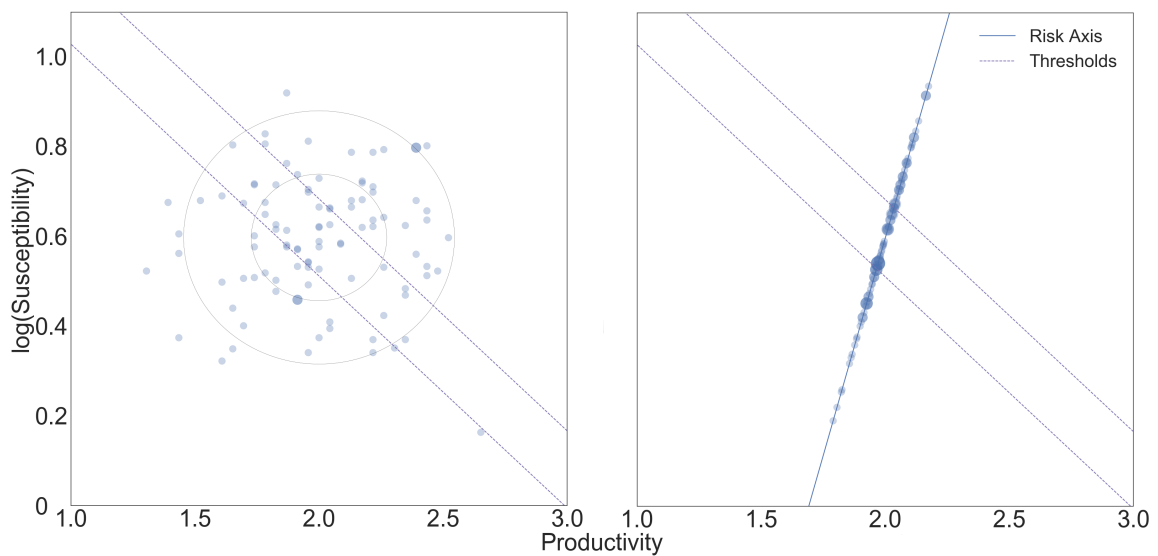


Figure A.9: 100 simulated species plotted on a semilog PSA plot (left).  $P$  is the weighted arithmetic mean of 10 attribute values, and  $S$  is the weighted geometric mean of 12 attribute values. Ellipses represent 1 and 2 z-scores from the bivariate mean. A risk axis is defined and the species are orthogonally projected onto this line (right). Those further to right along the line are higher risk. Thresholds are drawn to divide the distribution of species in thirds by probability. Note that it is the log transformation of the susceptibility axis that produces what appears to be a non-orthogonal projection and skewed thresholds in the right panel.

### A.11.3 Case study details

Data from 5 empirical case studies used in the analysis and results were collected. In the Patrick et al. NOAA assessment, 10 Productivity and 12 Susceptibility attributes are used to derive Productivity and Susceptibility scores. This data, including labeled attributes is available at <https://github.com/grewelle/rPSA>. We have also listed the attributes below:

| <b>Productivity</b>                        | <b>Susceptibility</b>        |
|--------------------------------------------|------------------------------|
| Growth Rate ( $r$ )                        | Management Strategy          |
| Maximum Age                                | Areal Overlap                |
| Maximum Size                               | Geographic Concentration     |
| vob Bertalanffy Growth Coefficient ( $k$ ) | Vertical Overlap             |
| Estimated Natural Mortality                | Fishing rate relative to $M$ |
| Measured Fecundity                         | Biomass of Spawners          |
| Breeding Strategy                          | Seasonal Migrations          |
| Recruitment Pattern                        | Schooling-Aggregation        |
| Age at Maturity                            | Morphology Affecting Capture |
| Mean Trophic Level                         | Post Release Mortality       |
|                                            | Value of the Fishery         |
|                                            | Fishery Impact to EFH        |

Table A.1: Attributes included in the NOAA PSA.

We have performed a comparison analysis in Fig. A.10 across the four possible treatments of each axis.

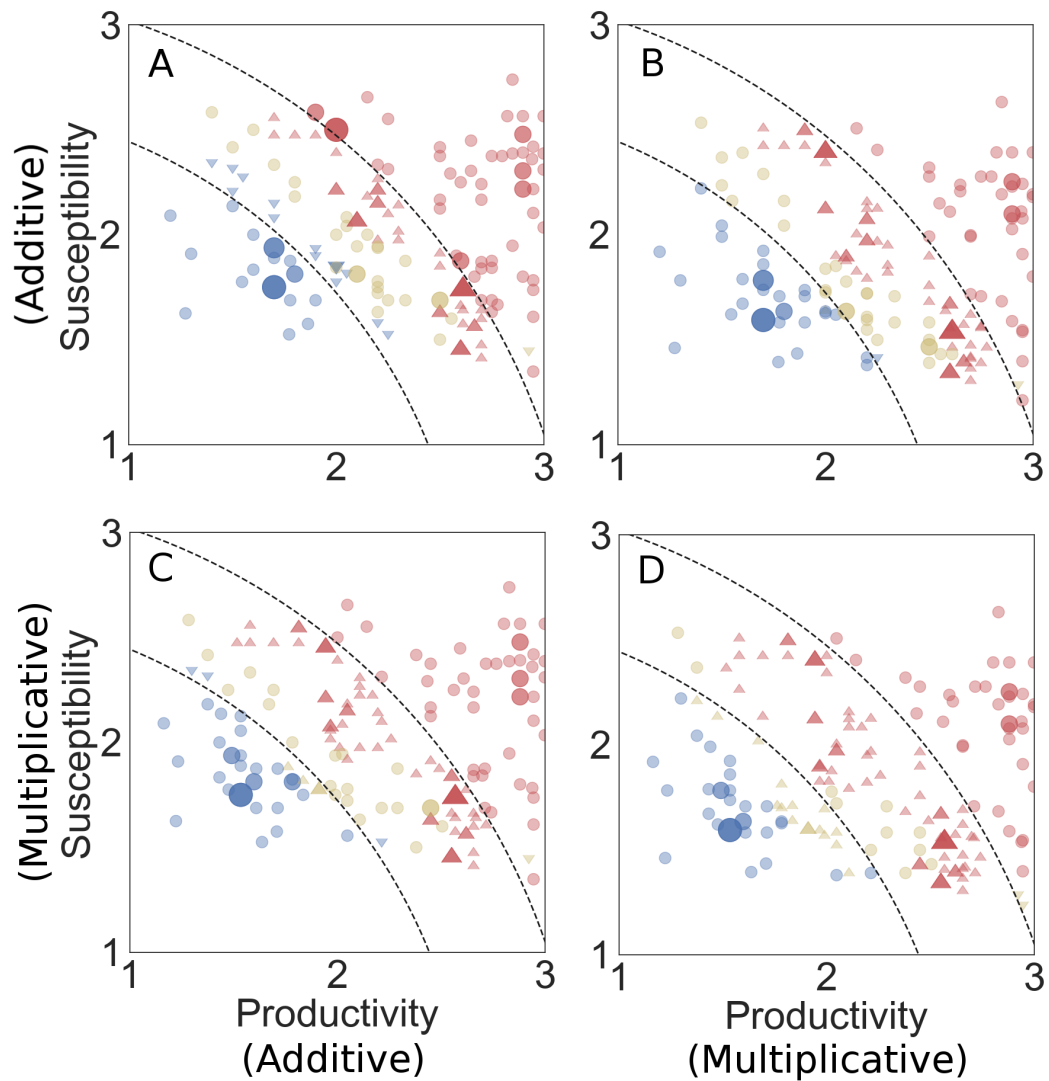


Figure A.10: Patrick et al. comparison of the sPSA and rPSA across the four permutations of model choices (additive and multiplicative) for the axes. Down triangles represent lower risk categorization with the rPSA, circles represent the same categorization, and up triangles represent higher risk categorization compare to the sPSA. Colors give the categorization per the rPSA (blue = low, yellow = medium, red = high). Dotted lines show the sPSA thresholds. (A) Both susceptibility and productivity are additive – 16 species categorized lower, 42 higher, 108 same with rPSA; (B) susceptibility is additive, productivity is multiplicative – 2 lower, 61 higher, 103 same; (C) susceptibility is multiplicative, productivity is additive – 4 lower, 63 higher, 99 same; (D) both are multiplicative – 2 lower, 85 higher, 79 same.



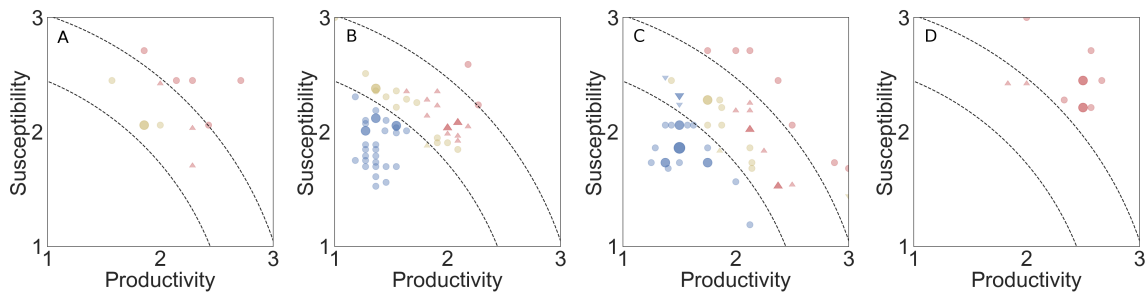


Figure A.11: 4 additional case studies evaluated in the main text. (A) Cotter et al., Sri Lanka; (B) Dee et al., aquaria; (C) Lin et al., Taiwan; (D) Ponton-Cevallos et al., Galapagos. Each study varies in species composition and scoring procedure, producing different layouts of points on the plot, which influences the changes observed when applying the rPSA. Point shapes, sizes, and colors are represented according to the system specified in the caption of figure A.10.

Figure A.6 in the main text reports the changes observed when analyzing all 5 empirical case studies with the sPSA and rPSA. These studies varied in geographical location, species studied and attributes used. Below are the species included in each study.

| <b>Cotter et al.</b> |
|----------------------|
| Swordfish            |
| Barracuda            |
| Flying Fishes        |
| Kingfish             |
| Sailfish             |
| Sea Barramundi       |
| Travelly/ Jackfish   |

|                                 |
|---------------------------------|
| Bream                           |
| Flying fish                     |
| Skipjack Tuna                   |
| Yellow fin tuna                 |
| Dolphin fish                    |
| <b>Dee et al.</b>               |
| Angelfish                       |
| Azure demoiselle                |
| Banggai cardinalfish, Indonesia |
| Bicolor dottyback               |
| Blacktail humbug                |
| Blue devil damselfish           |
| Blueband goby                   |
| Brown tang                      |
| Butterflyfish                   |
| Caerulean damselfish            |
| Canary wrasse                   |
| Citron goby                     |
| Clarkii clownfish               |
| Cleaner wrasse                  |

|                        |
|------------------------|
| Dogface puffer         |
| Dragon wrasse          |
| Elegant firefish       |
| Engineer goby          |
| Fairy wrasses          |
| Fire clownfish         |
| Firefish goby          |
| Flame damselfish       |
| Goldtail demoiselle    |
| Green chromis          |
| Harlequin sweetlips    |
| Koran angelfish        |
| Lagoon triggerfish     |
| Lawnmower blenny       |
| Longhorn cowfish       |
| Lyretail anthias       |
| Mandarinfish           |
| Maroon clownfish       |
| Moon wrasse            |
| Needlespine coral goby |

|                      |
|----------------------|
| Neon damselfish      |
| Panther grouper      |
| Pennant coralfish    |
| Porcupine puffer     |
| Powder brown tang    |
| Red lionfish         |
| Redtooth triggerfish |
| Royal dottyback      |
| Saddleback clownfish |
| Sailfin tang         |
| Scissortail goby     |
| Sergeant major       |
| Six line wrasse      |
| Snowflake moray eel  |
| Spotted prawn goby   |
| Steinitz' prawn goby |
| Strawberry dottyback |
| Threespot dascyllus  |
| Tomato clownfish     |
| Tomini tang          |

|                               |
|-------------------------------|
| Valentinni's sharpnose puffer |
| Velvet damselfish             |
| Yellow angelfish              |
| Yellow clown goby             |
| Yellow prawn goby             |
| Zebra goby                    |
| Zebra lionfish                |
| <b>Lin et al.</b>             |
| Silky shark                   |
| Blue shark                    |
| Pacific bluefin tuna          |
| Shortfin mako                 |
| Narrow barred mackerel        |
| Japanese Spanish mackerel     |
| Bigeye tuna                   |
| Black marlin                  |
| Indo-Pacific sailfish         |
| Red seabream                  |
| Yellowfin tuna                |
| Yellowback sea bream          |

Striped marlin

Albacore

Mangrove red snapper

Red Bigeye

Blackhead seabream

Large yellow croaker

Blue marlin

Blackmouth croaker

Japanese jack mackerel

Japanese scad

Escolar

Swordfish

Silver pomfret

Japanese butterfish

Striped bonito

Greater amberjack

Wahoo

Common dolphinfish

Mi-iuy croaker

Dogtooth tuna

Moonfish

Blue mackerel

Chub mackerel

Yellow croaker

Threadfin porgy

Shrimp scad

Crimson snapper

Cobia

Largehead hairtail

Skipjack tuna

Oilfish

Redtail scad

Torpedo scad

Silver-stripe round herring

Bullet tuna

Round herring

Frigate tuna

Butterfish

Flathead grey mullet

Spotted catfish

| Patrick et al.            |
|---------------------------|
| Shortfin mako             |
| Blue shark                |
| Common thresher           |
| Porbeagle                 |
| Oceanic whitetip          |
| Bigeye thresher           |
| Longfin mako              |
| Sixgill shark             |
| Sharpnose sevengill shark |
| Sandbar shark             |
| Blacktip shark            |
| Spinner shark             |
| Silky shark               |
| Bull shark                |
| Tiger shark               |
| Nurse shark               |
| Lemon shark               |
| Scalloped hammerhead      |



|                           |
|---------------------------|
| Great hammerhead          |
| Smooth hammerhead         |
| Dusky shark               |
| Caribbean reef shark      |
| Night shark               |
| Bignose shark             |
| Galapagos shark           |
| Sandtiger shark           |
| Bigeye sandtiger shark    |
| White shark               |
| Basking shark             |
| Whale shark               |
| Atlantic sharpnose shark  |
| Bonnethead shark          |
| Blacknose shark           |
| Finetooth shark           |
| Angel shark               |
| Smalltail shark           |
| Caribbean sharpnose shark |
| Alaska skate              |

|                           |
|---------------------------|
| Aleutian skate            |
| Commander skate           |
| Whiteblotched skate       |
| Whitebrow skate           |
| Roughtail skate           |
| Bering skate              |
| Mud skate                 |
| Roughshoulder skate       |
| Big skate                 |
| Longnose skate            |
| Butterfly skate           |
| Deepsea skate             |
| California sheephead      |
| Cabazon                   |
| Kelp greenling            |
| Rock greenling            |
| California scorpionfish   |
| Monkyface prickelback     |
| Black rockfish            |
| Black-and-yellow rockfish |

|                    |
|--------------------|
| Blue rockfish      |
| Brown rockfish     |
| Calico rockfish    |
| China rockfish     |
| Copper rockfish    |
| Gopher rockfish    |
| Grass rockfish     |
| Kelp rockfish      |
| Olive rockfish     |
| Quillback rockfish |
| Treefish rockfish  |
| Pacific sardine    |
| Northern Anchovy   |
| Pacific mackerel   |
| Jack mackerel      |
| Market squid       |
| Pacific herring    |
| Pacific bonito     |
| Pacific saury      |
| Albacore           |

Bigeye Tuna

Black Marlin

Bullet Tuna

Pacific Pomfret

Blue Shark

Bigeye thresher shark

Blue Marlin

Dolphin Fish

Brilliant Pomfret

Kawakawa

Spotted Moonfish

Longfin Mako Shark

Salmon Shark

Striped Marlin

Oilfish

Northern Bluefin Tuna

Roudi Escolar

Pelagic Thresher Shark

Sailfish

Skipjack Tuna

Shortfinned Mako Shark

Short Bill Spearfish

Broadbill Swordfish

Flatheat Pomfret

Dagger Pomfret

Sickle Pomfret

Wahoo

Yellowfin Tuna

Oceanic Whitetip Shark

Silky Shark

Common Thresher Shark

Escolar

Albacore

Bigeye Tuna

Black Marlin

Bullet Tuna

Pacific Pomfret

Blue Shark

Bigeye thresher shark

Blue Marlin

|                        |
|------------------------|
| Dolphin Fish           |
| Brilliant Pomfret      |
| Kawakawa               |
| Spotted Moonfish       |
| Longfin Mako Shark     |
| Salmon Shark           |
| Striped Marlin         |
| Oilfish                |
| Northern Bluefin Tuna  |
| Roudi Escolar          |
| Pelagic Thresher Shark |
| Sailfish               |
| Skipjack Tuna          |
| Shortfinned Mako Shark |
| Short Bill Spearfish   |
| Broadbill Swordfish    |
| Flatheat Pomfret       |
| Dagger Pomfret         |
| Sickle Pomfret         |
| Wahoo                  |

|                           |
|---------------------------|
| Yellowfin Tuna            |
| Oceanic Whitetip Shark    |
| Silky Shark               |
| Common Thresher Shark     |
| Escolar                   |
| GM Cod                    |
| GB Cod                    |
| GM Haddock                |
| GB Haddock                |
| Redfish                   |
| Pollock                   |
| CC-GM Yellowtail Flounder |
| GB Yellowtail Flounder    |
| SNE Yellowtail Flounder   |
| American Plaice           |
| Witch Flounder            |
| GM Winter Flounder        |
| GB Winter Flounder        |
| SNE-MidA winter Flouder   |
| GM-GB Windowpane          |

|                                                                                                                                                                                                                                                                                  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>SNE-MA Windowpane</p> <p>Ocean Pout</p> <p>White Hake</p> <p>Halibut</p> <p>Sand Tilefish</p> <p>Bar Jack</p> <p>Rock Sea Bass</p> <p>Margate</p>                                                                                                                             |
| <b>Ponton-Cevallos et al.</b>                                                                                                                                                                                                                                                    |
| <p>Sailfin grouper</p> <p>Pacific mutton hamlet</p> <p>Pacific graysby</p> <p>Leather bass</p> <p>Olive grouper</p> <p>Starry grouper</p> <p>Misty grouper</p> <p>Pacific creole-fish</p> <p>Graery threadfin seabass</p> <p>Grape-eye seabass</p> <p>White-spotted sandbass</p> |



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Table A.2: Species included in each PSA case study.

#### A.11.4 Dependency among attributes and between axes

Among the set of attributes chosen for a given analysis, some or all of those attributes may be correlated. If weakly correlated, the method presented in the main text can apply without correction. However, given a subset of attributes that are significantly correlated, the degree of clustering of points will not be as dramatic, and the number of attributes used does not inform completely the degree to which points will cluster. There are two paths to follow: remove hard-to-measure attributes that are highly correlated with other attributes or estimate the number of effectively independent attributes. If attributes are not removed from analysis due to correlation, a principal component analysis can be used to describe the effective number of independent attributes. All attribute values should be normalized by dividing by 3 and subtracting 0.5.

$$\bar{P}_{i,k} = \frac{P_{i,k}}{3} - \frac{1}{2} \quad \bar{S}_{i,k} = \frac{S_{i,k}}{3} - \frac{1}{2} \quad (\text{A.31})$$

Two covariance matrices can be produced: one for productivity attributes ( $\Sigma_P$ ) and another for susceptibility attributes ( $\Sigma_S$ ).  $\Sigma_P$  will be an  $N \times N$  matrix, and  $\Sigma_S$  will be an  $M \times M$

matrix. Let  $\lambda_P$  be the set of  $N$  eigenvalues of  $\Sigma_P$  and  $\lambda_S$  be the set of  $N'$  eigenvalues of  $\Sigma_S$ . Then let

$$\bar{\lambda}_P = \frac{N\lambda_P}{\sum_{i=1}^N \lambda_{P_i}} \quad \bar{\lambda}_S = \frac{M\lambda_S}{\sum_{j=1}^M \lambda_{S_j}} \quad (\text{A.32})$$

The number of effectively independent attributes for productivity and susceptibility are given respectively as

$$N_P = \alpha_P + N - \sum_{i=1}^{\alpha_P} \lambda_{P_i} \quad N_S = \alpha_S + M - \sum_{i=1}^{\alpha_S} \lambda_{S_i} \quad (\text{A.33})$$

where  $\alpha_P$  and  $\alpha_S$  represent the number of values greater than 1 in the vectors  $\lambda_P$  and  $\lambda_S$ , respectively. The analysis can be performed as described preceding this subsection by replacing  $N$  and  $M$  with  $N_P$  and  $N_S$ , respectively, in all equations. We recommend in future work that standardized covariance matrices be created to describe the relationships among attributes to be used across studies. In their absence, covariance matrices can be created for each study, particularly for studies including more than 20 species.

Equation A.14 in the main text gives the transformation of the standard error of the bivariate gaussian distribution  $\mathbf{X}$  to the risk axis. This equation assumes independent contributions of the component standard errors  $\hat{S}E_p$  and  $\hat{S}E_s$ . However, this equation can be generalized to cases in which productivity and susceptibility are not independent of each other. Consider the covariance matrix ( $\Sigma$ ) expressed in equation A.11 (main text). Equation

A.14 can be expressed in terms of this covariance matrix:

$$\hat{S}E_r = \sqrt{\frac{2\det(\Sigma)}{\hat{S}E_p^2 + \hat{S}E_s^2}} \quad (\text{A.34})$$

Accounting for expected covariance between productivity and susceptibility allows for calculation of  $V_p$  via equations A.16 and A.22 (main text).  $V$  is a reliable metric regardless of the covariance relationship between P and S, and therefore needs no recalculation when P and S are dependent because covariance is symmetrical.

### A.11.5 Example tables

Presented below are example tables to rapidly assign a risk category to each species analyzed. For each species, find the cell in the table corresponding to number of productivity attributes used and the number of susceptibility attributes used. There are four numbers in that cell. Multiply the top (first) number ( $n_1$ ) in the cell by the productivity score ( $P$ ) and the second number ( $n_2$ ) by the susceptibility score ( $S$ ). Add these products together to yield a standardized risk score (vulnerability). If the vulnerability value is lower than the third value in the cell, the species is low risk. If the vulnerability value is between the third and fourth value in the cell, the species is medium risk. High risk species will have vulnerability scores above this fourth value. The vulnerability value is standardized and can be used to rank species according to risk, even if differing numbers of attributes are used for each species.

$$V = n_1S + n_2P \quad (\text{A.35})$$

The first table presented assumes attributes are equally weighted for productivity and susceptibility and that productivity and susceptibility scores are arithmetic means of these attribute values. The second table presented assumes attributes are equally weighted for productivity and susceptibility and that the productivity score is the arithmetic mean of productivity attribute values. The susceptibility score, however, is the geometric mean of the susceptibility attribute values. Similar tables can be created to represent any variant of assumptions using equation A.19 in the main text. Otherwise, computation can be handled

using the software associated with this paper.

# Number of Productivity Attributes

## Number of Susceptibility Attributes

|    | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    | 14    | 15    |
|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 3  | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.471 | 0.471 | 0.471 | 0.471 | 0.471 | 0.471 | 0.471 | 0.471 | 0.471 | 0.471 | 0.471 | 0.471 | 0.471 |
|    | 1.75  | 1.642 | 1.568 | 1.514 | 1.471 | 1.437 | 1.409 | 1.385 | 1.364 | 1.346 | 1.331 | 1.317 | 1.304 |
|    | 2.021 | 1.877 | 1.778 | 1.705 | 1.649 | 1.603 | 1.565 | 1.533 | 1.506 | 1.482 | 1.461 | 1.442 | 1.425 |
| 4  | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.408 | 0.408 | 0.408 | 0.408 | 0.408 | 0.408 | 0.408 | 0.408 | 0.408 | 0.408 | 0.408 | 0.408 | 0.408 |
|    | 1.642 | 1.531 | 1.456 | 1.4   | 1.357 | 1.322 | 1.293 | 1.269 | 1.248 | 1.229 | 1.213 | 1.199 | 1.186 |
|    | 1.877 | 1.735 | 1.638 | 1.566 | 1.511 | 1.466 | 1.429 | 1.397 | 1.37  | 1.347 | 1.326 | 1.307 | 1.291 |
| 5  | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.365 | 0.365 | 0.365 | 0.365 | 0.365 | 0.365 | 0.365 | 0.365 | 0.365 | 0.365 | 0.365 | 0.365 | 0.365 |
|    | 1.568 | 1.456 | 1.379 | 1.323 | 1.279 | 1.243 | 1.214 | 1.189 | 1.168 | 1.149 | 1.133 | 1.118 | 1.105 |
|    | 1.778 | 1.638 | 1.542 | 1.471 | 1.416 | 1.372 | 1.335 | 1.304 | 1.277 | 1.254 | 1.234 | 1.215 | 1.199 |
| 6  | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.333 | 0.333 | 0.333 | 0.333 | 0.333 | 0.333 | 0.333 | 0.333 | 0.333 | 0.333 | 0.333 | 0.333 | 0.333 |
|    | 1.514 | 1.4   | 1.323 | 1.266 | 1.221 | 1.185 | 1.156 | 1.131 | 1.109 | 1.09  | 1.074 | 1.059 | 1.045 |
|    | 1.705 | 1.566 | 1.471 | 1.401 | 1.347 | 1.303 | 1.266 | 1.236 | 1.209 | 1.186 | 1.166 | 1.147 | 1.131 |
| 7  | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.309 | 0.309 | 0.309 | 0.309 | 0.309 | 0.309 | 0.309 | 0.309 | 0.309 | 0.309 | 0.309 | 0.309 | 0.309 |
|    | 1.471 | 1.357 | 1.279 | 1.221 | 1.176 | 1.14  | 1.11  | 1.085 | 1.063 | 1.044 | 1.028 | 1.013 | 0.999 |
|    | 1.649 | 1.511 | 1.416 | 1.347 | 1.292 | 1.249 | 1.213 | 1.182 | 1.156 | 1.133 | 1.113 | 1.095 | 1.079 |
| 8  | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.289 | 0.289 | 0.289 | 0.289 | 0.289 | 0.289 | 0.289 | 0.289 | 0.289 | 0.289 | 0.289 | 0.289 | 0.289 |
|    | 1.437 | 1.322 | 1.243 | 1.185 | 1.14  | 1.104 | 1.074 | 1.048 | 1.026 | 1.007 | 0.99  | 0.975 | 0.962 |
|    | 1.603 | 1.466 | 1.372 | 1.303 | 1.249 | 1.205 | 1.17  | 1.139 | 1.113 | 1.09  | 1.07  | 1.052 | 1.036 |
| 9  | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.272 | 0.272 | 0.272 | 0.272 | 0.272 | 0.272 | 0.272 | 0.272 | 0.272 | 0.272 | 0.272 | 0.272 | 0.272 |
|    | 1.409 | 1.293 | 1.214 | 1.156 | 1.11  | 1.074 | 1.044 | 1.018 | 0.996 | 0.977 | 0.96  | 0.945 | 0.931 |
|    | 1.565 | 1.429 | 1.335 | 1.266 | 1.213 | 1.17  | 1.134 | 1.104 | 1.078 | 1.055 | 1.035 | 1.017 | 1.001 |
| 10 | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.258 | 0.258 | 0.258 | 0.258 | 0.258 | 0.258 | 0.258 | 0.258 | 0.258 | 0.258 | 0.258 | 0.258 | 0.258 |
|    | 1.385 | 1.269 | 1.189 | 1.131 | 1.085 | 1.048 | 1.018 | 0.992 | 0.97  | 0.951 | 0.934 | 0.918 | 0.905 |
|    | 1.533 | 1.397 | 1.304 | 1.236 | 1.182 | 1.139 | 1.104 | 1.073 | 1.048 | 1.025 | 1.005 | 0.987 | 0.971 |
| 11 | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.246 | 0.246 | 0.246 | 0.246 | 0.246 | 0.246 | 0.246 | 0.246 | 0.246 | 0.246 | 0.246 | 0.246 | 0.246 |
|    | 1.364 | 1.248 | 1.168 | 1.109 | 1.063 | 1.026 | 0.996 | 0.97  | 0.948 | 0.928 | 0.911 | 0.896 | 0.882 |
|    | 1.506 | 1.37  | 1.277 | 1.209 | 1.156 | 1.113 | 1.078 | 1.048 | 1.022 | 0.999 | 0.979 | 0.962 | 0.946 |
| 12 | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.236 | 0.236 | 0.236 | 0.236 | 0.236 | 0.236 | 0.236 | 0.236 | 0.236 | 0.236 | 0.236 | 0.236 | 0.236 |
|    | 1.346 | 1.229 | 1.149 | 1.09  | 1.044 | 1.007 | 0.977 | 0.951 | 0.928 | 0.909 | 0.892 | 0.876 | 0.863 |
|    | 1.482 | 1.347 | 1.254 | 1.186 | 1.133 | 1.09  | 1.055 | 1.025 | 0.999 | 0.977 | 0.957 | 0.939 | 0.923 |
| 13 | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.226 | 0.226 | 0.226 | 0.226 | 0.226 | 0.226 | 0.226 | 0.226 | 0.226 | 0.226 | 0.226 | 0.226 | 0.226 |
|    | 1.331 | 1.213 | 1.133 | 1.074 | 1.028 | 0.99  | 0.96  | 0.934 | 0.911 | 0.892 | 0.875 | 0.859 | 0.845 |
|    | 1.461 | 1.326 | 1.234 | 1.166 | 1.113 | 1.07  | 1.035 | 1.005 | 0.979 | 0.957 | 0.937 | 0.919 | 0.904 |
| 14 | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.218 | 0.218 | 0.218 | 0.218 | 0.218 | 0.218 | 0.218 | 0.218 | 0.218 | 0.218 | 0.218 | 0.218 | 0.218 |
|    | 1.317 | 1.199 | 1.118 | 1.059 | 1.013 | 0.975 | 0.945 | 0.918 | 0.896 | 0.876 | 0.859 | 0.844 | 0.83  |
|    | 1.442 | 1.307 | 1.215 | 1.147 | 1.095 | 1.052 | 1.017 | 0.987 | 0.962 | 0.939 | 0.919 | 0.902 | 0.886 |
| 15 | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.211 | 0.211 | 0.211 | 0.211 | 0.211 | 0.211 | 0.211 | 0.211 | 0.211 | 0.211 | 0.211 | 0.211 | 0.211 |
|    | 1.304 | 1.186 | 1.105 | 1.045 | 0.999 | 0.962 | 0.931 | 0.905 | 0.882 | 0.863 | 0.845 | 0.83  | 0.816 |
|    | 1.425 | 1.291 | 1.199 | 1.131 | 1.079 | 1.036 | 1.001 | 0.971 | 0.946 | 0.923 | 0.904 | 0.886 | 0.87  |

# Number of Productivity Attributes

Number of Susceptibility Attributes

|    | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    | 14    | 15    |
|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 3  | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.262 | 0.262 | 0.262 | 0.262 | 0.262 | 0.262 | 0.262 | 0.262 | 0.262 | 0.262 | 0.262 | 0.262 | 0.262 |
|    | 0.73  | 0.702 | 0.684 | 0.67  | 0.659 | 0.65  | 0.643 | 0.637 | 0.632 | 0.627 | 0.623 | 0.619 | 0.616 |
|    | 0.881 | 0.833 | 0.8   | 0.776 | 0.757 | 0.742 | 0.73  | 0.719 | 0.71  | 0.702 | 0.695 | 0.689 | 0.683 |
| 4  | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.227 | 0.227 | 0.227 | 0.227 | 0.227 | 0.227 | 0.227 | 0.227 | 0.227 | 0.227 | 0.227 | 0.227 | 0.227 |
|    | 0.67  | 0.641 | 0.621 | 0.607 | 0.595 | 0.586 | 0.579 | 0.572 | 0.567 | 0.562 | 0.558 | 0.554 | 0.55  |
|    | 0.8   | 0.754 | 0.722 | 0.699 | 0.681 | 0.666 | 0.654 | 0.644 | 0.635 | 0.627 | 0.62  | 0.614 | 0.609 |
| 5  | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.203 | 0.203 | 0.203 | 0.203 | 0.203 | 0.203 | 0.203 | 0.203 | 0.203 | 0.203 | 0.203 | 0.203 | 0.203 |
|    | 0.629 | 0.599 | 0.579 | 0.564 | 0.552 | 0.542 | 0.535 | 0.528 | 0.522 | 0.517 | 0.513 | 0.509 | 0.506 |
|    | 0.746 | 0.7   | 0.669 | 0.646 | 0.628 | 0.614 | 0.602 | 0.592 | 0.583 | 0.576 | 0.569 | 0.563 | 0.558 |
| 6  | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.185 | 0.185 | 0.185 | 0.185 | 0.185 | 0.185 | 0.185 | 0.185 | 0.185 | 0.185 | 0.185 | 0.185 | 0.185 |
|    | 0.599 | 0.568 | 0.547 | 0.532 | 0.52  | 0.51  | 0.502 | 0.495 | 0.49  | 0.485 | 0.48  | 0.476 | 0.472 |
|    | 0.705 | 0.66  | 0.63  | 0.607 | 0.59  | 0.575 | 0.564 | 0.554 | 0.545 | 0.538 | 0.531 | 0.525 | 0.52  |
| 7  | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.171 | 0.171 | 0.171 | 0.171 | 0.171 | 0.171 | 0.171 | 0.171 | 0.171 | 0.171 | 0.171 | 0.171 | 0.171 |
|    | 0.575 | 0.544 | 0.523 | 0.507 | 0.495 | 0.485 | 0.477 | 0.47  | 0.464 | 0.459 | 0.454 | 0.45  | 0.447 |
|    | 0.674 | 0.629 | 0.599 | 0.577 | 0.559 | 0.545 | 0.534 | 0.524 | 0.516 | 0.508 | 0.502 | 0.496 | 0.491 |
| 8  | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.16  | 0.16  | 0.16  | 0.16  | 0.16  | 0.16  | 0.16  | 0.16  | 0.16  | 0.16  | 0.16  | 0.16  | 0.16  |
|    | 0.556 | 0.525 | 0.503 | 0.487 | 0.475 | 0.465 | 0.457 | 0.45  | 0.444 | 0.438 | 0.434 | 0.43  | 0.426 |
|    | 0.648 | 0.604 | 0.575 | 0.552 | 0.535 | 0.521 | 0.51  | 0.5   | 0.492 | 0.485 | 0.478 | 0.472 | 0.467 |
| 9  | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.151 | 0.151 | 0.151 | 0.151 | 0.151 | 0.151 | 0.151 | 0.151 | 0.151 | 0.151 | 0.151 | 0.151 | 0.151 |
|    | 0.541 | 0.509 | 0.487 | 0.471 | 0.458 | 0.448 | 0.44  | 0.433 | 0.427 | 0.421 | 0.417 | 0.413 | 0.409 |
|    | 0.627 | 0.584 | 0.554 | 0.532 | 0.515 | 0.501 | 0.49  | 0.48  | 0.472 | 0.465 | 0.459 | 0.453 | 0.448 |
| 10 | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.143 | 0.143 | 0.143 | 0.143 | 0.143 | 0.143 | 0.143 | 0.143 | 0.143 | 0.143 | 0.143 | 0.143 | 0.143 |
|    | 0.527 | 0.495 | 0.473 | 0.457 | 0.444 | 0.434 | 0.426 | 0.419 | 0.412 | 0.407 | 0.402 | 0.398 | 0.394 |
|    | 0.61  | 0.566 | 0.537 | 0.515 | 0.498 | 0.485 | 0.473 | 0.464 | 0.455 | 0.448 | 0.442 | 0.436 | 0.431 |
| 11 | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.137 | 0.137 | 0.137 | 0.137 | 0.137 | 0.137 | 0.137 | 0.137 | 0.137 | 0.137 | 0.137 | 0.137 | 0.137 |
|    | 0.516 | 0.483 | 0.461 | 0.445 | 0.432 | 0.422 | 0.413 | 0.406 | 0.4   | 0.395 | 0.39  | 0.386 | 0.382 |
|    | 0.594 | 0.551 | 0.522 | 0.5   | 0.484 | 0.47  | 0.459 | 0.449 | 0.441 | 0.434 | 0.428 | 0.422 | 0.417 |
| 12 | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.131 | 0.131 | 0.131 | 0.131 | 0.131 | 0.131 | 0.131 | 0.131 | 0.131 | 0.131 | 0.131 | 0.131 | 0.131 |
|    | 0.506 | 0.473 | 0.451 | 0.434 | 0.422 | 0.411 | 0.403 | 0.395 | 0.389 | 0.384 | 0.379 | 0.375 | 0.371 |
|    | 0.581 | 0.538 | 0.509 | 0.488 | 0.471 | 0.457 | 0.446 | 0.437 | 0.429 | 0.421 | 0.415 | 0.41  | 0.405 |
| 13 | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 |
|    | 0.497 | 0.464 | 0.442 | 0.425 | 0.412 | 0.402 | 0.393 | 0.386 | 0.38  | 0.374 | 0.369 | 0.365 | 0.361 |
|    | 0.569 | 0.527 | 0.498 | 0.476 | 0.46  | 0.446 | 0.435 | 0.426 | 0.418 | 0.41  | 0.404 | 0.399 | 0.394 |
| 14 | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.121 | 0.121 | 0.121 | 0.121 | 0.121 | 0.121 | 0.121 | 0.121 | 0.121 | 0.121 | 0.121 | 0.121 | 0.121 |
|    | 0.489 | 0.456 | 0.434 | 0.417 | 0.404 | 0.394 | 0.385 | 0.378 | 0.371 | 0.366 | 0.361 | 0.357 | 0.353 |
|    | 0.559 | 0.516 | 0.488 | 0.466 | 0.45  | 0.436 | 0.425 | 0.416 | 0.408 | 0.401 | 0.394 | 0.389 | 0.384 |
| 15 | 0.471 | 0.408 | 0.365 | 0.333 | 0.309 | 0.289 | 0.272 | 0.258 | 0.246 | 0.236 | 0.226 | 0.218 | 0.211 |
|    | 0.117 | 0.117 | 0.117 | 0.117 | 0.117 | 0.117 | 0.117 | 0.117 | 0.117 | 0.117 | 0.117 | 0.117 | 0.117 |
|    | 0.482 | 0.449 | 0.426 | 0.41  | 0.397 | 0.386 | 0.377 | 0.37  | 0.364 | 0.358 | 0.353 | 0.349 | 0.345 |
|    | 0.549 | 0.507 | 0.478 | 0.457 | 0.441 | 0.427 | 0.416 | 0.407 | 0.399 | 0.392 | 0.386 | 0.38  | 0.375 |

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## **Appendix B**

# **Data-poor Ecological Risk Assessment of Multiple Stressors**

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## B.1 Abstract

1. Ecological Risk Assessment is a formal process widely applied to terrestrial, marine, and freshwater ecosystems to evaluate the likelihood of adverse ecological effects occurring as a result of exposure to natural or anthropogenic stressors. For many species, data is sparse and semi-quantitative methodologies provide valuable insight for ecosystem management. Recent statistical developments have improved the quality of these analyses, yet a rigorous theoretical framework to assess the cumulative impact of multiple stressors is lacking despite threats to species of conservation and commercial value being numerous (e.g. harvesting, pollution, disease, climate change).
2. We present EcoRAMS (Ecological Risk Assessment of Multiple Stressors), a web application and open-source software module that provides easy-to-use, statistically-robust ecological risk assessments of multiple stressors in data-poor contexts. This method extends the Productivity-Susceptibility Analysis, which is applied to a single stressor. The software receives attribute scores for two variables (e.g. exposure-sensitivity, productivity-susceptibility, severity-likelihood) via CSV templates and outputs results according to a probabilistic metric of risk.
3. We demonstrate comparative results across a range of assumptions, using simulated and empirical datasets including up to five stressors. Accounting for multiple stressors even when data is limited provides a more detailed analysis of risk that may otherwise be understated in single stressor analyses.

4. This application will allow quantification of risk across data-poor contexts for which statistical results have been previously unavailable. The web app format of EcoRAMS.net lowers the barrier of use for practitioners and scientists at any level of statistical training.

**Keywords**

*Cumulative Impacts, Ecological Risk Assessment, Ecosystem Based Management, Productivity Susceptibility Analysis, Vulnerability*



## B.2 Introduction

The development of management policies for species of conservation and commercial interests requires clear identification of potential threats to target species, evaluation of associated risk, and establishment of priorities for intervention. In data-poor systems, such as many small scale fisheries that together support the livelihoods of over 117 million people and provide essential nutrition for many more, conducting these steps is challenging (FAO, 2020). Therefore, a wide variety of qualitative and semi-quantitative approaches is deployed to measure risk of species to stressors, which are often human-mediated. These stressors can be unique to each ecosystem or even to species within an ecosystem. Common stressors of wildlife result from harvesting, mining, recreational, or construction activities and those chosen for analysis are relevant to the species affected and stakeholder interests (Hope, 2006). In some contexts, the effects of a stressor can be precisely quantified under controlled conditions, for instance when a physiological or behavioral response is measured for increasing concentration of a contaminant (Norton et al., 1992). However, in many contexts, the quantitative relationship between stressor and response is unknown or under-studied. Semi-quantitative methodologies have been developed to assess risk based on limited information of a stressor and the life history traits of species of conservation interest. With incomplete data, these approaches often rely on expert input and standardized scoring procedures (Pilling et al., 2009b). This is particularly common when it is important to assess vulnerability to harvesting or risk of bycatch for a variety of species

in small-scale fisheries because data collection is sparse. The Productivity-Susceptibility Analysis (PSA) has become the most widely used approach to conduct ecological risk assessments for data-poor fisheries and has been applied widely to other ecological contexts, including terrestrial systems. The PSA calculates Vulnerability, which is a metric of risk that incorporates qualitative scores of life history characteristics of species (Productivity) and fishing activity that overlaps with each species (Susceptibility). It has been incorporated within the broader Ecological Risk Assessment for the Effects of Fishing (ERAEF) framework which uses risk estimates from the PSA to prioritize evaluation by managers, scientists, and stakeholders (Hobday et al., 2011c; Hobday et al., 2007b; I. Stobutzki et al., 2001b). Management efforts can account for all species, guilds, and communities with this approach and is the goal of ecosystem based management (Fletcher, 2005; Townsend et al., 2019b). For this reason, data-poor methodologies have been widely adopted (Battista et al., 2017b; Marine Stewardship Council, 2019b; Pontón-Cevallos et al., 2020b). However, data-poor ecological risk assessments, like the PSA and others employing Exposure and Sensitivity or Effect as risk determinants, have historically lacked a statistical basis.

In our previous work, we presented a novel, statistically-robust approach to derive Vulnerability from Productivity and Susceptibility scores that addresses several problems with the original PSA (Grewelle et al., 2021). Productivity and Susceptibility are each scored as the mean of their respective set of several attributes, each given values between 1 (low) and 3 (high). This procedure produces a non-uniform distribution of species on the PSA plot,

therefore the Euclidean distance of each species from the origin is not a suitable metric for Vulnerability. The statistical distribution of plotted species changes with common variations of the analysis, including choice of multiplicative or additive scoring model and the number of attributes used to score Productivity and Susceptibility. Our revised PSA (rPSA) framework is able to incorporate these variations to assess Vulnerability by projecting the two-dimensional distribution of species onto a one-dimensional risk axis, along which species' risk to fishing activity increases (Fig. B.1).

Vulnerability in our previous work is presented in two forms: a probability ( $V_p$ ) taking values between 0 (lowest risk) and 1 (highest risk), which are comparable between studies, and a relative value ( $V$ ) that is easily calculated and is useful to categorize and rank species within a study but cannot be compared between studies with different assumptions. These formulations of Vulnerability rely on precise characterization of the distribution of expected scores on both axes of the PSA plot, allowing both  $V_p$  and  $V$  to be statistical quantities.

While PSA-like approaches have been widely used in both marine and terrestrial ecosystems, these methods usually consider a single threat or pressure (e.g. a specific fishery or fishing gear), whereas populations are often subject to multiple stressors at the same time (Halpern et al., 2009b; Van den Brink et al., 2016). Thus it is crucial to have robust methods to assess vulnerability to cumulative impacts. Building on the original PSA framework, Micheli et al. presented an empirical formula to compute an Aggregated Susceptibility (AS) that broadened the definition of Susceptibility to multiple fishing gears or stressors

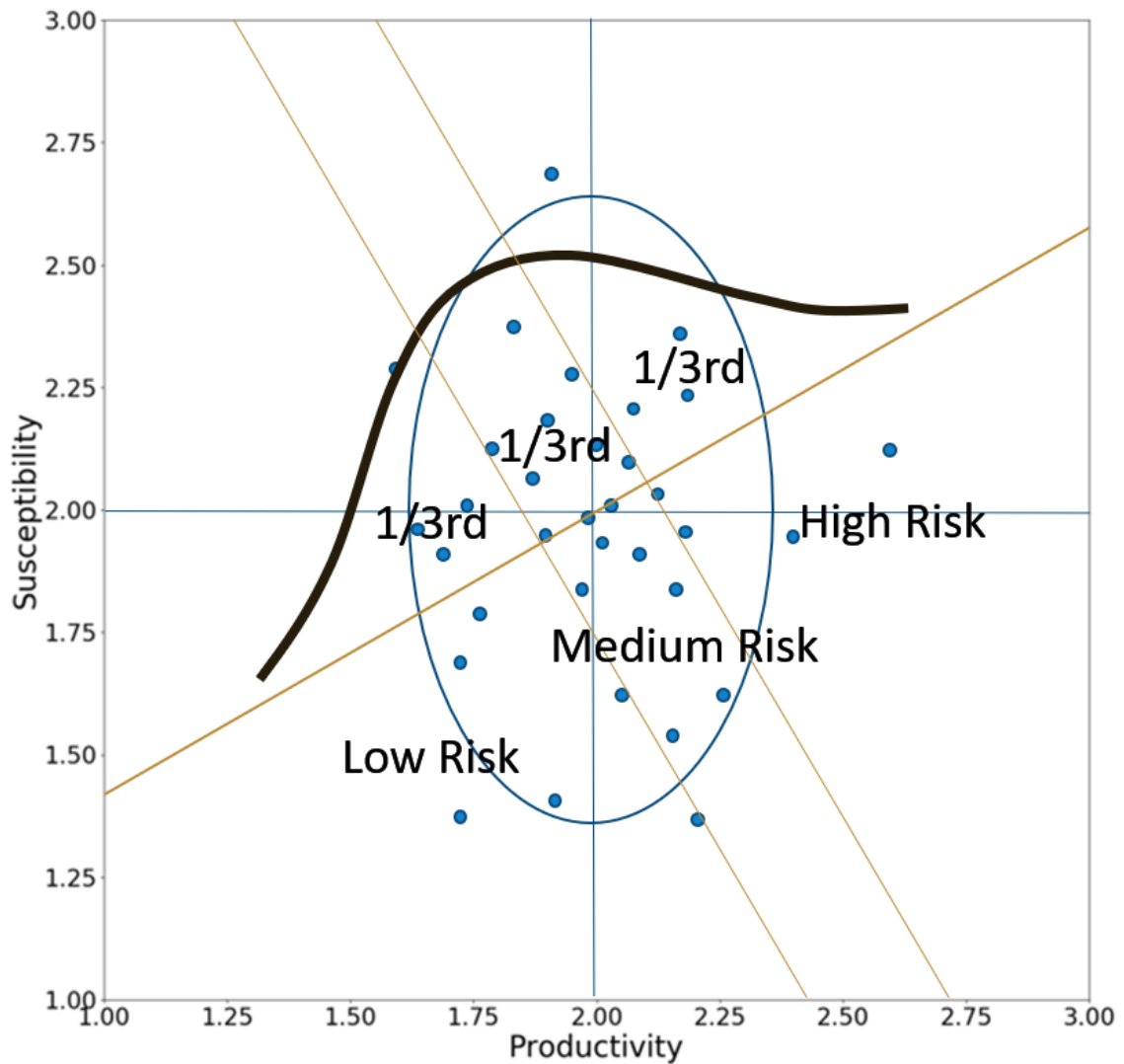


Figure B.1: In the scoring model used by the PSA, a bivariate normal distribution (blue ellipse) is produced along the Productivity and Susceptibility axes. This can be projected along a Risk Axis (bold orange, ascending left to right), which is defined by the likelihood properties of the analysis, to form an ordered set of points along a one dimensional normal distribution (bold black). This new distribution is used to score Vulnerability. Vulnerability scores fall into risk categories delineated by thresholds (light orange, descending left to right) which divide the distribution of points into equal partitions by probability.

(Micheli et al., 2014c). Aggregated Susceptibility is truncated at a maximum value of 3 and takes a minimum value of 1 to remain within the scoring bounds of the PSA.

$$AS = \min\{ 3, 1 + \sqrt{\sum_{i=1}^n (S_i - 1)^2} \} \quad (\text{B.1})$$

When Susceptibility ( $S_i$ ) to a stressor  $i$  is 1, this stressor does not increase AS. When  $S_i > 1$  for two or more stressors, then AS is greater than or equal to Susceptibility from each stressor. Vulnerability in the original PSA was calculated as the Euclidean distance from the origin.

$$V = \sqrt{P^2 + S^2} \quad (\text{B.2})$$

AS is substituted for S in the calculation of Vulnerability to multiple stressors. Though practical, the empirical formula to calculate AS was not derived on the basis of rigorous statistical principles. The primary aim of this work is to create a statistical interpretation of AS that can be integrated with the rPSA framework which will produce a robust measure of Vulnerability for when one or more stressors contribute to species' Susceptibility. We generalize this framework for data-poor ecological risk assessment to evaluate any two-dimensional scoring procedure, including Sensitivity-Exposure (Rodier & Norton, 1992), Impact-Probability (Dumbravă & Iacob, 2013), or Severity-Likelihood based analyses (Woodruff, 2005).

### B.3 Methods

In our previous work we show that Productivity and Susceptibility variables, which are scored by calculating the mean of several attributes, are normally distributed with a standard error that depends on conditions of the analysis, such as the number of attributes used (Grewelle et al., 2021). Other variations of data-poor ecological risk assessments exist that evaluate risk using different variables (e.g. Sensitivity-Exposure) but with equivalent calculations (Samhoury et al., 2019b). Therefore, the statistics we develop apply equally across these analyses. To generalize the statistics for multiple stressors developed here, we denote  $\alpha$  as any response variable (Productivity, Sensitivity, Effect, Severity, Impact etc.) determined by characteristics of the species or endpoints studied and  $\beta$  as any stressor variable (Susceptibility, Exposure, Likelihood, Probability etc.) determined by the probability of contact with stressors. It is assumed that  $\beta$  is a composite variable with basis  $\{\beta_1, \beta_2, \dots, \beta_n\}$ , while  $\alpha$  is a single variable. Though, this method can be extended in future work to treat  $\alpha$  as a composite variable. Each  $\beta_i$  represents a separate source of stress to species in the analysis. We modify the equation for AS to a statistical form to calculate  $\beta$ .  $\beta$  must be Gaussian; however, the formula for AS does not form a statistical distribution. Each stressor variable,  $\beta_i$  forming the basis of  $\beta$  is Gaussian because they are scored by calculating the mean of several attributes. With modification we can standardize the expression within the square root of AS to form a chi distribution:

$$Y = \sqrt{\sum_{i=1}^n \left( \frac{y_i - \mu_i}{\bar{\sigma}_i} \right)^2} \quad (\text{B.3})$$

Each species or endpoint is evaluated with  $n$  stressor variables.  $y_i$  is the score of each stressor variable distributed according to  $\beta_i \sim N(\mu_i, \bar{\sigma}_i)$ .  $\bar{\sigma}_i$  is the standard error of the mean expected score determined by scoring criteria.  $Y$  is a statistic that is chi distributed

$$Y \sim \chi(n) = f(y; n) \quad (\text{B.4})$$

with cumulative distribution function  $F_+(y; n)$ . This function gives  $Pr(Y \leq y)$  where  $Y > 0$ . Each term in the summation of  $Y$  is a squared Z-score, and the sign of each Z-score is not preserved. We form a piecewise function  $F$  which translates the sign and magnitude into a continuous cumulative distribution function on  $y \in (-\infty, \infty)$ . Let  $\zeta = \sum_{i=1}^n \text{sgn}(Z_i) Z_i^2$ , where  $Z_i$  is the Z-score of each stressor variable.

$$F(y; n) = \begin{cases} 0.5 + \frac{F_+(y; n)}{2} & \zeta > 0 \\ 0.5 - \frac{F_+(y; n)}{2} & \zeta < 0 \\ 0.5 & \zeta = 0 \end{cases}$$

This result follows from the additive property of the chi distribution:

$$F_+\left(\sqrt{\sum_{i=1}^n Z_i^2}; n\right) = \bigcup_{i=1}^n F_+(Z_i; n) \quad (\text{B.5})$$

$f(y; n)$  is a symmetric probability density function centered at  $y = 0$ . To integrate this distribution with the rPSA, we transform the modified chi distribution to a normal distribution centered at  $y = 2$  with a chosen variance. By the probability integral transform theorem, we can map the chi-distributed points  $\{p\}$  to a normal distribution with mapping  $m(\{p\})$ :

$$m(\{p\}) = F_N^{-1}(F(\{p\}; n)) \quad (\text{B.6})$$

$$\beta = \min\{3, m(\{p\})\} \quad (\text{B.7})$$

$F_N(y; \hat{\sigma})$  is a normal cumulative distribution function whose standard error,  $\hat{\sigma}$ , can be chosen arbitrarily because the mapped points and this standard error are used to generate Vulnerability statistics when incorporated in the rPSA framework, which standardizes values by any chosen standard error. For visualization we choose to standardize the calculation of  $\hat{\sigma}$  to be similar in magnitude to the standard errors of each contributing stressor variable. For those variables log-transformed to produce a normal distribution, corresponding standard errors can be adjusted for subsequent calculation using the lognormal relation:

$$\text{adjusted } \bar{\sigma}_i = \sqrt{(e^{\bar{\sigma}_i^2} - 1)e^{4+\bar{\sigma}_i}} \quad (\text{B.8})$$

Additive stressor variables require no transformation, and therefore their associated



adjusted standard errors are equivalent to their standard errors without adjustment. We propagate error for the mean of the stressor variables, assuming variables are independent of one another:

$$\mu = \frac{1}{n} \sum_{i=1}^n \mu_i \quad (\text{B.9})$$

$$\bar{\sigma}_\beta = \sqrt{\sum_{i=1}^n \left(\frac{\partial \mu_i}{\partial \mu}\right)^2 (\text{adjusted } \bar{\sigma}_i)^2} \quad (\text{B.10})$$

$$= \sqrt{\frac{1}{n} \sum_{i=1}^n (\text{adjusted } \bar{\sigma}_i)^2} \quad (\text{B.11})$$

The probability density function for the normal mapping is  $f_N = N(2, \bar{\sigma}_\beta)$ . Recalling from (Grewelle et al., 2021) any vector  $\mathbf{x}_k = [\alpha_k - \hat{\mu}_\alpha \quad \beta_k - \hat{\mu}_\beta]^T$  can be projected along the risk vector,  $\mathbf{r}$ , to map  $\mathbf{X}$  to one dimension.  $k$  is the species index. After mapping all points to the risk vector, the distance of each point  $x_k = (\alpha_k, \beta_k)$  from the mean is the magnitude of the projection:

$$D(\mathbf{x}_k) = \frac{\mathbf{r} \cdot \mathbf{x}_k}{|\mathbf{r}|} \quad (\text{B.12})$$

The projection results in a linear transformation of  $\mathbf{X}$  with standard error

$$\bar{\sigma}_r = \frac{\sqrt{2}\bar{\sigma}_\alpha\bar{\sigma}_\beta}{\sqrt{\bar{\sigma}_\alpha^2 + \bar{\sigma}_\beta^2}} \quad (\text{B.13})$$

The cumulative distribution function that yields the probabilistic metric of Vulnerability is:

$$V_p = \frac{1}{2} + \frac{1}{\sqrt{\pi}} \int_0^{\frac{D(\mathbf{x}_k)}{\bar{\sigma}_r\sqrt{2}}} e^{-t^2} dt \quad (\text{B.14})$$

This metric can be compared across all varieties of input conditions, including variable number and type of stressors and attributes. However, comparisons across studies are most explicable when conditions of the analyses are similar.

### B.3.1 Compounding Stressors

The above framework implemented in EcoRAMS ([data-poor] Ecological Risk Assessment of Multiple Stressors) measures Vulnerability from multiple stressor variables, standardized by the distance of each stressor score from each mean set by the attribute scoring procedure and the chosen model (additive or multiplicative). Vulnerability values calculated in this way will fall between Vulnerability values calculated for each stressor independently, and therefore, this metric provides maximum resolution to distribute points across the low, medium, high risk range. This interpretation of Vulnerability assumes that stressors hold values close to their mean when not included in analysis (i.e. the null model is the mean for

each stressor). Nevertheless, it may be useful to assume that stressors hold values close to their minimum when otherwise not included in analysis (i.e. the null model is the minimum for each stressor). In these instances, Vulnerability should increase as stressors are added to the analysis. In fact, this is the impetus for the concept of Aggregated Susceptibility. EcoRAMS incorporates compounding stressors by calculating the standardized distance of each score from a minimum to form the inner expression of  $Y$ .

$$Y_{compound} = \sqrt{\sum_{i=1}^{n-c} \left( \frac{y_i - \mu_i}{\bar{\sigma}_i} \right)^2 + \sum_{j=1}^c \left( \frac{y_j - min_j}{\bar{\sigma}_j} \right)^2} \quad (\text{B.15})$$

$c$  is the number of stressor variables to be compounded.  $1 \leq n - c$  because at least one variable must be standardized at the mean, not the minimum. For an additive model, the default minimum is set to 1 as attribute scores take values between 1 and 3. In the multiplicative model, the default minimum is  $\log(1) = 0$ .

### B.3.2 Scoring Practices

The EcoRAMS method requires several steps prior to submitting template CSV files to receive results (Fig. B.2). After the analysis conditions are setup, the  $\alpha$  and  $\beta$  attributes must be chosen. Attributes should be approximately independent from each other. Redundant attributes should be removed, or otherwise, the effective number of attributes can be estimated using the method outlined in the supplementary information of (Grewelle et al., 2021). Percentiles are then chosen for scoring each attribute, adhering to relevant expected

## EcoRAMS Workflow

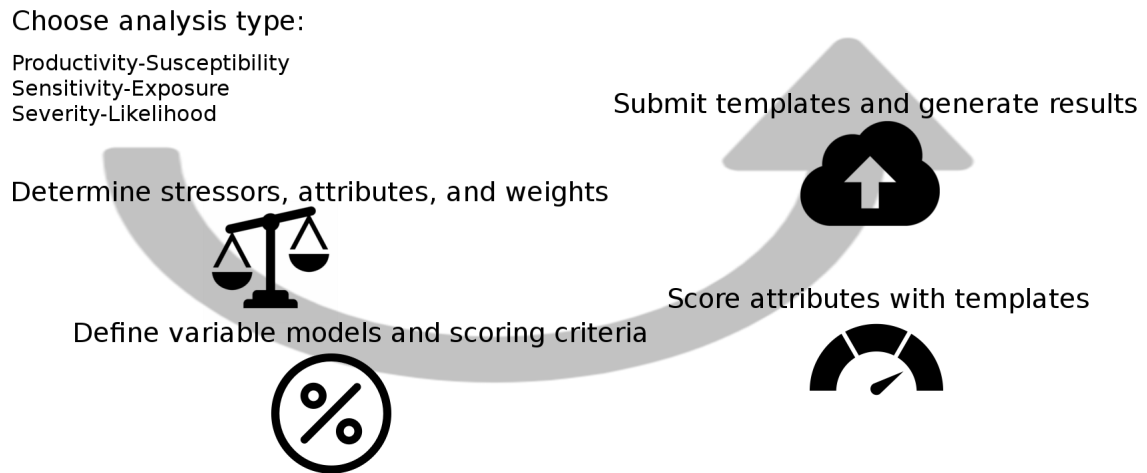


Figure B.2: Workflow diagram for EcoRAMS analysis. The analysis type chosen determines which of the pairs of variables is used in the risk assessment, which will constrain the types of attributes used for scoring. Included stressors, attribute weights, and their associated models and scoring criteria will define the scope and structure of the analysis. After attributes are scored using provided templates, users submit templates simultaneously on the EcoRAMS web app at EcoRAMS.net and results are automatically generated.

ranges. For ease, we recommend equal bin sizes: 1 = 0-33%, 2 = 33-67%, 3 = 67-100%.

When attributes are irrelevant or lack expert input or data for scoring, the attributes should not be scored. Leaving the corresponding cells blank in the CSV templates provided will adjust the number of attributes used for the species and broaden standard error estimates for calculating Vulnerability and scored attributes will be appropriately weighted. The  $\alpha$  and  $\beta$  values are calculated as the weighted means of their respective attribute scores. Weights can be applied on the basis of importance or data quality, and efforts to justify weights via supporting mechanism or data should be made. The mean calculated depends on the model assumed for the attributes (geometric mean = multiplicative, arithmetic mean = additive).

However, as a default,  $\alpha$  variables should be additive, while  $\beta$  variables should be multiplicative. A multiplicative model should be used when the magnitude of risk associated with a variable depends on interactions of the attributes with each other such that absence of risk for any attribute substantially reduces risk as a whole. Values of variables scored in this way act much like probabilities, and in fact, a geometric mean is an isotone (order-preserving) mapping of probability and is indistinguishable in the results of the EcoRAMS analysis. The model used can be different for each variable  $\beta_1, \beta_2, \dots, \beta_n$  if different attributes are used to score each, though we encourage consistency for ease of interpretation. Not all risk analysis are amenable to the above scoring procedure, and if not, EcoRAMS should not be used. Risk analyses scored differently may be adapted to use with EcoRAMS by defining scoring criteria within the range of 1-3.

## B.4 Case Studies

To demonstrate the use of EcoRAMS, we generated a set of data for 100 species using a standard scoring procedure for the PSA used in the Ecological Risk Assessment for the Effects of Fishing. We adopt the same scoring procedure by which attributes of  $\alpha$  and  $\beta$  variables are scored 1 (low risk), 2 (medium risk), or 3 (high risk). Note that these scores reflect association with risk, not association with the  $\alpha$  or  $\beta$  variables per se. For example, in a PSA high Productivity is associated with low risk (Hobday et al., 2007b), and therefore Productivity attribute scores should be transformed by subtracting from 4 if

originally scored as 1 = low Productivity, 2 = medium Productivity, 3 = high Productivity.

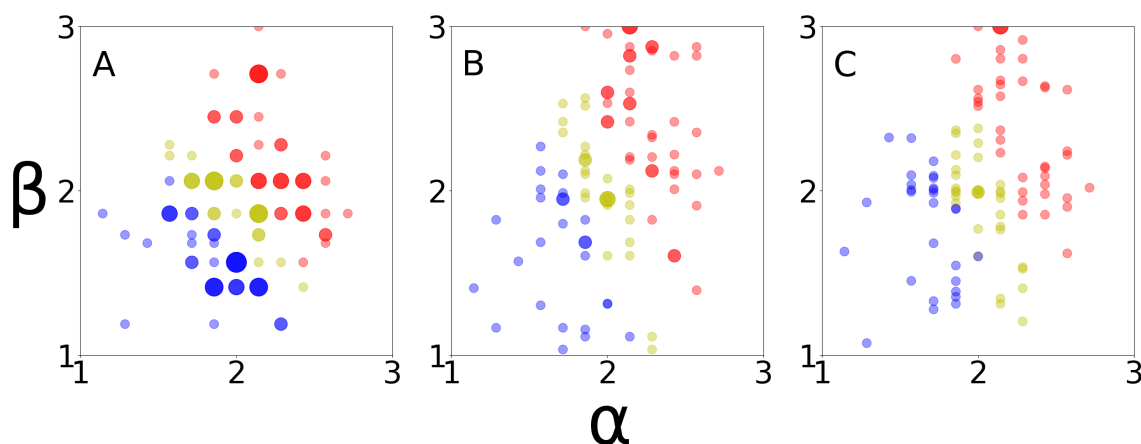


Figure B.3: Risk classification of 100 simulated species by Vulnerability scores in which stressors do not compound. Low risk species are in blue, medium risk in yellow, high risk in red. The size of a dot corresponds to the number of species sharing overlapping positions in the plot. (A) A single stressor ( $\beta_1$ ) is used in an analysis. (B) Two stressors are used in an analysis, the second ( $\beta_2$ ) does not compound on the first shown in panel A. (C) Three stressors ( $\beta_1, \beta_2, \beta_3$ ) are used in an analysis.

In our simulated analysis, 7  $\alpha$  and 4  $\beta$  attributes are scored randomly on a uniform distribution of integers between 1 and 3. The  $\alpha$  score is calculated as the arithmetic mean assuming an additive model. The  $\beta$  score is calculated as the geometric mean assuming a multiplicative model. Attributes are weighted equally. Fig. B.3 presents the results of EcoRAMS when  $\beta$  variables are not compounded for 1-3 stressors, while Fig. B.4 presents results when stressors 2 and 3 compound on stressor 1.

When stressors do not compound and are instead standardized by the expected mean, additional stressors will not affect the distribution of risk categorization. When scoring is uniformly distributed, approximately one-third of species should fall into each category.

This procedure is useful for maximally discriminating risk of species within a study for downstream assessment. However, in some studies when it assumed that stressors not directly measured are negligible, the addition of stressor variables presents an opportunity to assess cumulative impacts of two or more stressors. In these cases, Vulnerability scores are expected to increase with added stressors for which scores exceed the minimum, and risk compounds. Fig. B.4 shows how the distribution of risk rapidly shifts as stressors compound and Vulnerability scores increase.

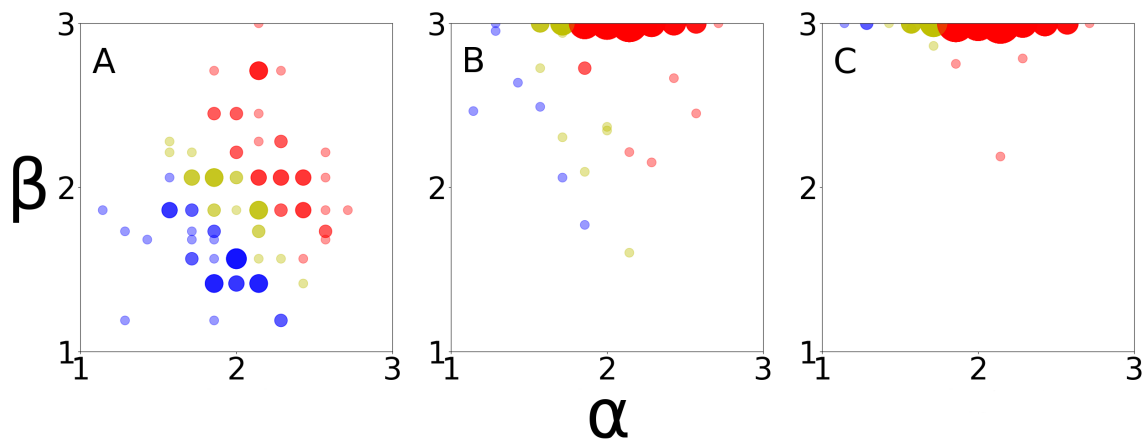


Figure B.4: Risk classification of 100 simulated species by Vulnerability scores in which stressors compound. Low risk species are in blue, medium risk in yellow, high risk in red. The size of a dot corresponds to the number of species sharing overlapping positions in the plot. (A) A single stressor ( $\beta$  variable) is used in an analysis. (B) Two stressors are used in an analysis, the second ( $\beta_2$ ) compounds on the first shown in panel A. (C) Three stressors ( $\beta_1, \beta_2, \beta_3$ ) are used in an analysis, the second and third compound on the first.

When a minimum score is used to standardize additional stressors, fewer species are expected to remain in the low or medium risk categories, especially with three or more stressors.  $\beta$  scores are truncated at 3, which ensures that some species will remain in these

categories when  $\alpha$  scores are sufficiently low. In many scenarios a far greater number of species will remain in lower categories than depicted in Fig. B.4 because scoring of additional stressors will be skewed right, favoring lower scores than the stressor of greatest impact, which may be the initial target of analysis. We see this illustrated in an empirical case study of 81 fished species in Baja California, Mexico. We re-analyzed these species from (Micheli et al., 2014c) using the EcoRAMS framework (Fig. B.5). Each type of fishing activity (e.g. gear type) is treated as a separate stressor.

Despite the majority of species receiving Vulnerability scores placing them in the low risk category for each single stressor (Fig. B.5 A-E), the cumulative impacts of each of the five stressors produces a higher Susceptibility score, and therefore, a higher Vulnerability score when compounded. Compared with the single stressor of highest impact (set gillnets), accounting for multiple stressors results in 26% higher net risk: 23% low, 31% medium, 46% high for multiple stressors compared to 49% low, 10% medium, 41% high for set gillnets. Although this analysis highlights application to multiple sources of fishing pressure, EcoRAMS is fully flexible to analyze any system for which multiple independent sources of stress introduce risk to subjects of the analysis. For example, the PSA has been used to evaluate terrestrial ecosystems, although it was designed for data-poor fisheries. Similar risk-based frameworks that use Exposure and Sensitivity (or Effect) or Severity and Likelihood as variables have been applied to marine and terrestrial systems and have also been extensively developed for data-rich ecological risk assessments (Council et al., 2009;



Samhouri et al., 2019b). These applications extend beyond ecology and include analysis of risk in business (Koller, 2005), human health (Ravindra & Mor, 2019; World Health Organization et al., 2020), engineering (Zio, 2018), and others. We provide a cohesive statistical framework, associated software, and web application to easily and robustly analyze risk in any data-poor context, including when accounting for multiple stressors is important for the assessment. EcoRAMS requires no prior statistical or programming knowledge, as full functionality of this software is deployed as a web app that only requires users to complete template CSV files with their data. We view wide and reliable access to robust statistical methods as imperative to the progress of ecosystem-based management and risk analysis. Because 90+% of marine species are considered data-poor (FAO, 2020; Mora et al., 2011), EcoRAMS has the opportunity to be widely adopted to improve analyses marine sciences, and we anticipate EcoRAMS to have similar value to other ecological risk assessments of terrestrial and freshwater systems when data is sparse. The best use of EcoRAMS is with input from scientists, managers, practitioners, and stakeholders to allocate time and resources to species for which conservation provides mutual benefits to the ecosystem and the people within it (Finkbeiner et al., 2017b; Oestreich et al., 2019b).

## B.5 Considerations

Statistical (probabilistic) methods have been deployed for ecological risk assessments where data is available. We provide a statistical framework to conduct ecological risk assessments in data-poor contexts. Even in data-rich contexts, the use of statistical risk assessments is not universally accepted practice. Challenges like increased complexity, greater data needs, and difficulty in communicating results to stakeholders slow widespread uptake (Hope, 2006). Conventional non-statistical methods like hazard quotients or guidelines set to fixed thresholds without regard to uncertainty in data or mechanistic interpretation of the underlying model are often simple and convenient interpretations of risk and are used by decision-makers (Tannenbaum et al., 2003), but these benefits become less tenable when statistical practices are highly accessible. Changing convention will take time, and ecological risk assessments will benefit from the transition to statistical methods, as they provide more reliable insights into risk management. Adoption of statistical methods for data-poor ecological risk assessments may similarly require time, and the key to improving the state of the field in the shortest time is to make methods accessible. EcoRAMS represents a sophisticated statistical software with easy inputs and easily understood results for any audience. Engagement with stakeholders and downstream efforts to prioritize management is crucial for any risk assessment, and EcoRAMS can facilitate these synergies.

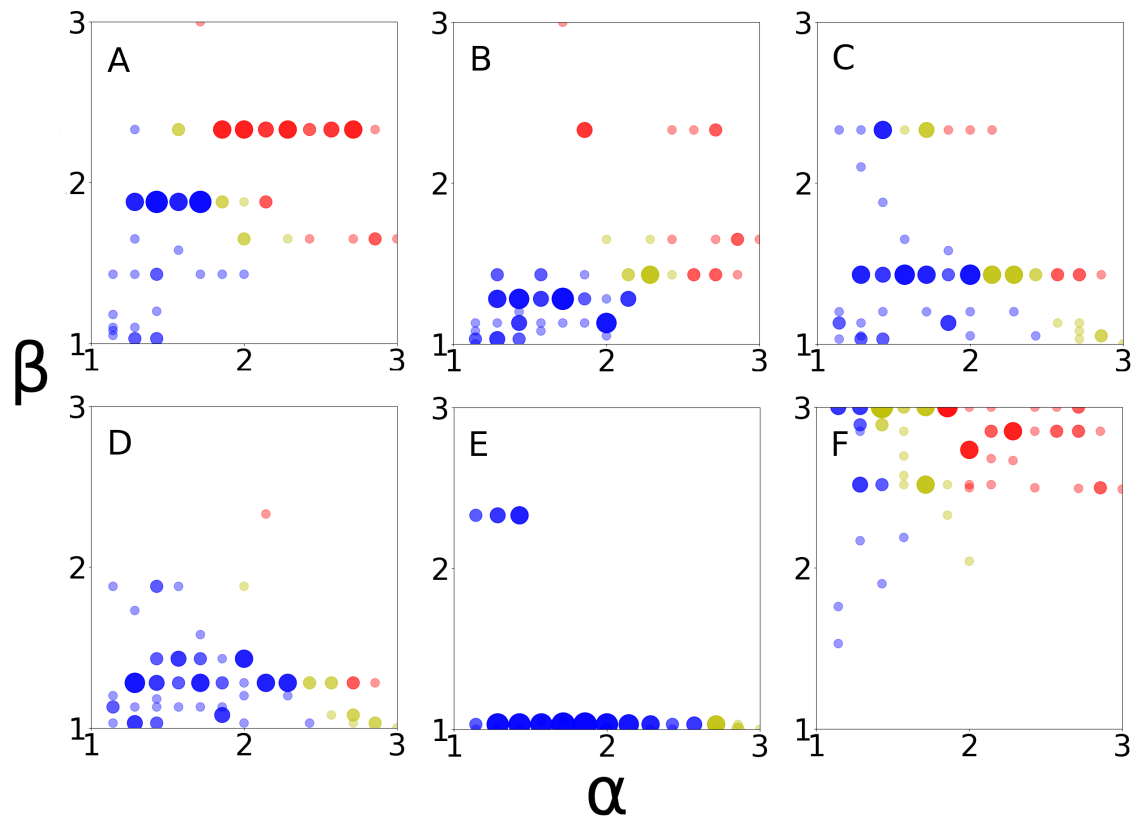


Figure B.5: Risk classification of 81 marine species in Baja California, Mexico analyzed by Micheli et al. Low risk species are in blue, medium risk in yellow, high risk in red. The size of a dot corresponds to the number of species sharing overlapping positions in the plot.  $\alpha$  = Productivity and  $\beta$  = Susceptibility. (A) Analysis of stressor of highest impact, set gillnets, followed by analyses of four other fishing stressors in descending order of impact: (B) drift gillnets, (C) lobster traps, (D) fish traps, (E) dive fishing. (F) These stressors are analyzed together in the EcoRAMS framework assuming stressors compound, revealing higher Vulnerability scores due to increased Susceptibility than for any stressor alone.

## **B.6 Acknowledgements**

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## **B.7 Authors' Contributions**

Conceptualization, REG; Methodology, REG; Software, REG; Analysis, REG & EM; Visualization, REG; Writing – Original Draft, REG; Writing – Review & Editing, REG, EM, FM, GDL; Funding Acquisition, REG, FM, GDL

## **B.8 Conflict of Interest**

The authors declare no conflict of interest.

## **B.9 Data Availability**

Software associated with this study is available for download and use at

<https://github.com/grewelle/EcoRAMS>. Empirical data evaluated is publicly available in referenced works. The web app is deployed at [EcoRAMS.net](https://EcoRAMS.net).

## B.10 Supporting Information

Downloadable templates and instructions for EcoRAMS, also found at

<https://github.com/grewelle/EcoRAMS/blob/main/README.md>

EcoRAMS is software used to perform ecological risk assessments in data-poor contexts. It provides a statistical interpretation of risk for each species or endpoint analyzed. This metric is probabilistic Vulnerability ( $V_p$ ). Inputs are attribute scores and weights for two variables: response (e.g. Productivity, Effect, Sensitivity, Severity) and stressor (e.g. Susceptibility, Exposure, Likelihood). EcoRAMS is designed to incorporate multiple stressors that when aggregated can have compound impacts on risk or not. This software is associated with Grewelle et al. 2021, Data-Poor Ecological Risk Assessment of Multiple Stressors. This paper builds on previous work which constructs a statistical framework for data-poor risk assessments in fisheries with single stressors, Grewelle et al. 2021, Redefining Risk in Data-Poor Fisheries <https://onlinelibrary.wiley.com/doi/abs/10.1111/faf.12561>, <https://github.com/grewelle/rPSA> and earlier work on multiple fishery stressors Micheli et al. 2014, A risk-based framework for assessing the cumulative impact of multiple fisheries <https://web.stanford.edu/group/MicheliLab/pdf/a%20risk%20based%20framework.pdf>. EcoRAMS is free to use, and we welcome feedback to improve the usability of the software. We only ask that you credit our work and cite our papers in any byproducts resulting from use of EcoRAMS.

Below is a guide to using EcoRAMS. After downloading and completing templates,

analysis occurs within a few seconds of upload. If your data is well organized to be input into templates, a full analysis from template download to results can occur within a few minutes. The instructions are divided into three sections: pre-download of templates, template completion, and EcoRAMS analysis.

1. Pre-download of templates

- Determine the types of response and stressor variables used in analysis (e.g. Productivity-Susceptibility, Exposure-Effect/Sensitivity, Severity-Likelihood) - Generate a set of one or more stressor variables that independently contribute to risk - Decide which set of attributes will be used to assess each of the variables. If more than one stressor is included, different attributes may be used for different types of stressors, though care should be taken to interpret results appropriately given the added complexity of the analysis. Chosen attributes should be approximately independent from each other. Highly correlated/redundant attributes should not be included in the analysis. If moderately-highly correlated attributes are used, refer to the supplement of Grewelle et al. 2021, *Redefining Risk in Data-Poor Fisheries* to estimate the effective number of attributes used for each variable. - Classify each variable as additive or multiplicative in nature. When an additive model is used for a variable, it is assumed that each attribute contributes to a fraction of risk proportional to its weight (see following instruction on weighting). Therefore, adding all attribute contributions to risk gives the full risk associated with the variable. When a multiplicative model

is used for a variable, it is assumed that each attribute's contribution is affected by the contributions of other attributes. Simply, if risk from one or more attributes is absent, overall risk associated with the variable would be absent as well even when high risk is associated with other attributes. This model is often used when attributes measured operate in a sequence or are probabilistic (e.g. Likelihood). By default, response variables are additive and stressor variables are multiplicative in the templates. Different models can be used for each stressor, though care should be taken to interpret results appropriately given the added complexity of the analysis.

- Set criteria for low, medium, and high risk for each variable. This consists of two percentile cut-offs, below the first is low, above the second is high, and between them is medium risk. These cut-offs can be chosen as any percentile provided they are symmetric (i.e. low and high categories are of equal range). The template defaults assume equally sized categories.
- Assign weights to each attribute. Weights can take any numerical value and are only important relative to weights of other attributes for a single species or endpoint. Weights can differ within an attribute but across species. The template defaults to equal weighting.
- Score attributes for all species. Attribute scores should take values between 1 and 3. These values should fall within intended categories based on the percentile cut-offs assigned above. Defaults correspond to 1 = low risk, 2 = medium risk, 3 = high risk.

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## 2. Template completion

- Download one alpha template and one beta csv template. - Open the alpha template. The top four rows have six sets of values to input. a) Additive or Multiplicative attributes? Acceptable inputs: Additive, Multiplicative b) Low and high percentile cut-offs for attribute scoring. Acceptable inputs: any two fractions that as cut-offs produce a symmetric distribution of score ranges. Numerators and denominators must include a decimal. Values must remain in fraction form (i.e. do not give the decimal equivalent of the fraction). c) Number of attributes. Acceptable input: a whole number corresponding to the number of attribute columns. d) Low and high thresholds. Acceptable inputs: The second value must be larger than the first. Numerators and denominators must include a decimal. Values must remain in fraction form (i.e. do not give the decimal equivalent of the fraction). These thresholds determine the risk categories following Vulnerability scoring. e) Scoring in reversed risk order? Acceptable inputs: Y, N. In some cases (e.g. Productivity) the attributes of the alpha variable may be scored such that high values represent low risk. If scores input in the template were scored in this way, assign Y to this field. If high values correspond to high risk, assign N to this field. f) Axis label. Acceptable inputs: any x-axis label for the resulting plot, preferably the name of the alpha variable. - Row 5 must be left empty. Row 6 is the dataset header, and values in these cells can be changed without affecting the analysis. - Columns must be organized accordingly: column 1 is for higher level organization of species and will not be output in results. Column 2 is the list of species and will be reported in results alongside Vulnerability scores and risk categories. Input your list of species in



column 2. No input is required for column 1 unless it is helpful for your organization. Blank rows can be included in between species or chunks of species for aesthetics without affecting the analysis provided the blank rows are placed consistently for all templates so that species fall on the same row. - Columns 3+ are for attribute scores and weights. Add or remove columns to rows 6+ to add or subtract attributes. Two empty columns must be kept between attribute scores and attribute weights. Attribute weight columns must be in the same order, left to right, as the attribute columns. For example, for 5 attributes, columns 1 and 2 would report group (optional) and species (or endpoint generally). Columns 3-7 would report attribute scores. Columns 8-9 would be blank. Columns 10-14 would report attribute weights. When an attribute is unscored due to lack of data or irrelevance for the species, both the attribute score and weight cells should be left blank. Default attribute scores are randomly chosen between 1 and 3. Default weights are equal. - Save the alpha template as `alpha_xxx.csv` where `xxx` is any string you choose. The file must be saved in UTF-8 format. Note: Microsoft Office for Mac incorrectly encodes the UTF-8 format, so upload errors may be a result of incorrect encoding. Use LibreOffice, Google Sheets, or Numbers on a Mac. Microsoft Office works correctly on a PC for csv encoding. - Open the beta template. Like the alpha template, the top four rows have six sets of values to input. These sets of values can be entered according to the guidelines for the alpha template above except for (e). Here the entry differs: Compound model? Acceptable inputs: Y, N. This entry refers to whether the stressor is statistically standardized by the expected mean or the

expected minimum. By default, this value should be N for the first beta template to yield a comparable analysis to the rPSA for a single stressor. Subsequent stressors can be treated as compounding (increasing risk with more stressors – Y) or not (N) by completing additional beta templates for each stressor. - The same rules apply for column and row formatting and data entry for both beta and alpha templates. It is recommended to rank stressors in order of impact, with the highest impact stressor entered in the first beta template, and the lowest impact stressor entered in the last beta template. All values can differ between stressors except for (d) low and high thresholds. These thresholds will be the same across all templates, including the alpha template, as the thresholds are applied to Vulnerability scores at the end of the analysis. The software is setup to take the threshold values from the alpha template, so modifying the thresholds in the beta templates will not change results. - Save each beta template as beta1\_xxx.csv, beta2\_xxx.csv, beta3\_xxx.csv, etc. in the same folder as you saved the completed alpha template. Ordering of these completed templates matters in the upload stage, as all files are selected simultaneously. Therefore, in the folder, files must appear in the following order: alpha\_xxx.csv, beta1\_xxx.csv, beta2\_xxx.csv, beta3\_xxx.csv, etc.

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### 3. EcoRAMS analysis

- Navigate to the main page of EcoRAMS.net. - Click on the 'Choose Files' button after which a file browser window will appear. Navigate to the folder hosting your completed

templates. The order the files appear is the order they will be uploaded and should be in the order described above. Use ctrl (or cmd) + select or shift select to select all files to be analyzed. - After opening these files, the homepage will read the number of files selected. Click 'Submit' to analyze your data. - After a few seconds, a display page will appear with results. Results will appear in order of the stressors uploaded with stressor 1 corresponding to beta1\_xxx.csv. These are single stressor results for each stressor standardized by the expected mean. Each plot will precede a list of all species and their associated probabilistic Vulnerability score between 0 (lowest) and 1 (highest) and their associated risk category determined by the thresholds set. The final result is the multiple stressor result. - All figures can be downloaded by saving the image with a right click, and the data tables can be copied and pasted directly into any format like a csv file.

## Appendix B. Bibliography

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