Spring 2020

Integrating Synthetic Biology Derived Variables into Ecological Risk Assessment Using the Bayesian Network – Relative Risk Model: Gene Drives to Control Nonindigenous M. musculus on Southeast Farallon Island

Ethan A. Brown
Western Washington University, browne40@wwu.edu

Spring 2020

Integrating Synthetic Biology Derived Variables into Ecological Risk Assessment Using the Bayesian Network – Relative Risk Model: Gene Drives to Control Nonindigenous M. musculus on Southeast Farallon Island

Ethan A. Brown
Western Washington University, browne40@wwu.edu

Integrating Synthetic Biology Derived Variables into Ecological Risk Assessment Using the Bayesian Network – Relative Risk Model: Gene Drives to Control Nonindigenous *M. musculus* on Southeast Farallon Island

By

Ethan A. Brown

Accepted in Partial Completion

Of the Requirements for the Degree

Master of Science

ADVISORY COMMITTEE

Chair, Dr. Wayne G. Landis

Dr. Jenise M. Bauman

Dr. John D. Stark

GRADUATE SCHOOL

David L. Patrick, Interim Dean

MASTER'S THESIS

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Western Washington University, I grant to Western Washington University the non-exclusive royalty-free right to archive, reproduce, distribute, and display the thesis in any and all forms, including electronic format, via any digital library mechanisms maintained by WWU.

I represent and warrant this is my original work, and does not infringe or violate any rights of others. I warrant that I have obtained written permissions from the owner of any third party copyrighted material included in these files.

I acknowledge that I retain ownership rights to the copyright of this work, including but not limited to the right to use all or part of this work in future works, such as articles or books.

Library users are granted permission for individual, research and non-commercial reproduction of this work for educational purposes only. Any further digital posting of this document requires specific permission from the author.

Any copying or publication of this thesis for commercial purposes, or for financial gain, is not allowed without my written permission.

Ethan A. Brown

21 May 2020

Integrating Synthetic Biology Derived Variables into Ecological Risk Assessment Using the Bayesian Network – Relative Risk Model: Gene Drives to Control Nonindigenous *M. musculus* on Southeast Farallon Island

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By

Ethan A. Brown

21 May 2020

Abstract

Ecological risk assessment has not been conducted for the proposed environmental applications of synthetic biology. To develop a quantitative framework for risk assessment of synthetic biology, I selected Southeast Farallon Island as a case study for modeling the deployment of gene drive modified house mice to reduce impacts to threatened species. Southeast Farallon Island is part of the Farallon Islands National Wildlife Refuge. The island is populated by invasive house mice that impact indigenous species. Gene drive technology has been proposed as a method to suppress invasive rodent populations through CRISPR-mediated genome editing. I applied the Bayesian Network – Relative Risk Model to predict the outcomes of a gene drive mouse eradication on Southeast Farallon Island. The Bayesian Network – Relative Risk Model is able to probabilistically evaluate multiple causal pathways, incorporating the influence of multiple stressors on multiple endpoints. I used a modified version of the R-based model MGDrivE to simulate population genetics and population dynamics of gene drive mouse eradication strategies. I conducted simulations for three unique gene drive mouse release schemes across two assumed gene drive homing rates and two levels of supplemental rodenticide use; for a total of twelve simulated scenarios. I compared the relative eradication probabilities of these scenarios within a Bayesian network. Sensitivity analyses were conducted to compare the relative influence of rodenticide use and homing rate on the probability of successful mouse eradication. I found that increasing the assumed homing rate of the gene drive had a higher influence on mouse eradication than the addition of supplemental rodenticide. For most scenarios, simulations showed successful mouse eradications as early as seven years after gene drive deployment, with high probabilities of eradication within ten years.

KEY WORDS: Bayesian network, Relative Risk Model, synthetic biology, gene drive, risk assessment

Acknowledgements

I would like to acknowledge Jared Bennett and Héctor Manuel Sánchez Castellanos (UC Berkeley – John Marshall Lab) for their assistance with “MGDrivE” source code interpretation and modification. I would also like to acknowledge Andy Bunn (WWU – IES) for technical assistance with package modification in R. Additionally, I would like to acknowledge Colter Lemons (WWU – IETC) for assistance with GIS and April Markiewicz (WWU – IETC) for technical assistance through the Institute of Environmental Toxicology and Chemistry. Finally, I would like to acknowledge my committee: Wayne Landis, Jenise Bauman, and John Stark for their support and guidance throughout this project.

Notes

This work builds upon the framework for synthetic biology ecological risk assessment, as outlined by chapter six of *Gene Drives on the Horizon* (NASEM 2016). The Bayesian network files are available electronically or upon request. Download the free version of Netica to view the models without a license (https://norsys.com/netica.html).

The content and format of this paper are intended for submission to *Risk Analysis*.

TABLE OF CONTENTS

List of Figures

List of Tables

List of Acronyms and Abbreviations

BN	Bayesian Network
BN-RRM	Bayesian Network – Relative Risk Model
Cas	CRISPR-Associated
CRISPR	Clustered regularly interspaced short palindromic repeats
GD1/GD2/GD3	Gene drive release schemes 1, 2, & 3
GDM	Gene Drive Modified
IPM	Integrated Pest Management
NASEM	National Academies of Sciences, Engineering, and Medicine
RRM	Relative Risk Model
SEFI	Southeast Farallon Island

1. INTRODUCTION

Proposed environmental applications of synthetic biology bring with them a host of novel management and regulatory considerations. Chapter 6 of *Gene Drives on the Horizon* (NASEM 2016) and Landis et al. (2020a) describe frameworks for risk assessment of synthetic biology using the Bayesian Network – Relative Risk Model (BN-RRM), with gene drives as an example of a synthetic biology-derived stressor. Using the BN-RRM framework, I constructed a probabilistic risk assessment model for the theoretical introduction of gene drive modified (GDM) house mice (*Mus musculus*) to suppress the invasive mouse population on Southeast Farallon Island.

1.1 Introduction to Synthetic Biology

The National Academies of Sciences, Engineering, and Medicine (NASEM) define synthetic biology as the generation of novel traits or organisms via the implementation of chemical or computational processes that do not occur naturally. One branch of synthetic biology is genome editing, defined as the introduction of new alleles or genes into an organism (NASEM 2016). Clustered regularly interspaced short palindromic repeats (CRISPR) Cas (CRISPR-Associated) systems are a common tool in genome editing. For example, CRISPR-Cas9 systems can modify specific DNA sequences using a complementary guide-RNA (gRNA) complexed with a DNA-targeting endonuclease (Cas9) that creates a double strand break at the target site in an organism's genome. The new sequence is then inserted and replicated throughout the genome, making the organism homozygous for the desired allele (Webber et al. 2015; Knott and Doudna 2018). The level of gene editing precision offered by CRISPR-Cas systems has the potential to alter the germ lines of populations. Genome editing at the population scale is the conceptual foundation behind gene drive technology (NASEM 2016).

1.2 Gene Drives and their Environmental Applications

Gene drives, also termed "selfish genetic elements", are naturally occurring or engineered genes that

transfer to offspring at proportions greater than a Mendelian expectation (i.e. above 50%) (NASEM 2016). CRISPR-Cas9-based gene drives have been synthesized (Hammond et al. 2016) to "drive" engineered traits through target populations. Intended applications of gene drives fall into two broad categories: population replacement and population suppression. The goal of population replacement is to substitute a target gene with an engineered gene, avoiding a fitness reduction in the target organisms. The goal of population suppression is to substitute the target gene with a gene that causes reduced organismal fitness, leading to a steep reduction or elimination of the target population (Marshall and Akbari 2016). Gene drives have been proposed for controlling vector-borne diseases, suppressing invasive species, and inducing pesticide tolerance or sensitivity in agricultural settings (NASEM 2016).

1.2.1 Controversies and Uncertainties Around Gene Drive Use

Proposed gene drive applications have come under scrutiny, with researchers questioning the ability of these constructs to successfully suppress or replace populations, in addition to their potential for adverse environmental impacts. For example, Webber et al. (2015) notes the possibility of dispersal of GDM organisms into non-target populations, potentially leading to unintended extinctions and alterations to community dynamics. Roberts et al. (2017) suggests that hybridization of GDM organisms with similar species in a region could spread gene drives to non-target species, leading to unintended population suppression or replacement.

The development of organismal resistance to the homing properties of gene drives has also been investigated. Champer et al. (2017) modified *Drosophila melanogaster* with two types of CRISPR-Cas9 gene drives in a laboratory study, monitoring inheritance patterns and mechanisms. The work of Champer et al. revealed two types of mutated resistance that resulted in the inability of the CRISPR gRNA to recognize the resulting DNA sequence. The first type of mutation, termed in-frame resistance,

resulted in zero fitness reduction to organisms, essentially creating a new genotype with no fitness reduction. The second type of mutation, termed out-of-frame resistance, led to a high organismal fitness cost.

To overcome potential gene drive resistance, studies have proposed using a multiplexing approach to create gene drive constructs that target multiple DNA sequences in a gene using multiple gRNA sequences. This approach is expected to minimize the probability of resistance development by increasing the number of mutations required to negate the homing capabilities of a gene drive (Marshall et al. 2017; Noble et al. 2017; Noble et al. 2019). However, multiplexing has not undergone laboratory testing. Additionally, numerical models used to predict the resulting population dynamics and population genetics of proposed multiplexed gene drives have been largely deterministic (Noble et al. 2017; Noble et al. 2019), failing to provide probabilistic estimates of successful population suppression or replacement. Risk assessment is a well-suited tool for evaluating the influence of resistance development on the probabilities of achieving management goals using gene drive technology.

1.3 The Need for Adaptive Management of Synthetic Biology

Given the lack of empirical data concerning environmental applications of gene drives, *adaptive management* has been suggested as a strategy to investigate proposed applications of gene drives in the environment (NASEM 2016; Landis et al. 2020a). Adaptive management is an iterative approach to decision making that provides a specific set of goals for governance and regulation of ecological systems. Adaptive management involves altering management strategies with the acquisition of new knowledge. This “iterative” process occurs throughout the course of working towards a management goal and follows an explicit set of guidelines to orient thinking and evaluation around an evolving management scheme. An intrinsic property of adaptive management is that decisions are treated as experiments that

inform future management actions (Holling 1978). Walters (1986) outlines three specific goals of adaptive management:

1. Management concerns should be framed as explicit questions to be evaluated in regards to the system being studied.
2. The ecological system should be clearly defined so that all relevant variables can be evaluated in terms of available information and the inherent assumptions and limitations of available data.
3. The history of the study region and its variance through time should be accounted for in regards to modeling techniques and proposed management strategies (Walters 1986).

Landis et al. (2017) suggests the BN-RRM as an empirical risk assessment model to inform adaptive management processes. Because there is very little empirical information on environmental applications of synthetic biology, I reiterate that an adaptive management approach in the form of the BN-RRM would be a powerful tool for informing management decisions around gene drive technology.

1.4 Why Risk Assessment?

Risk assessment is a causal and empirical process that is well-suited for calculating probabilistic estimates of synthetic biology-related impacts to ecological systems. Risk assessment is defined as the estimation of probabilities of impacts to one or more endpoints due to the effects of one or more stressors (NASEM 2016). Landis and Wiegers (2005) outline the Relative Risk Model (RRM) as a framework for ranking the relative impacts of multiple stressors on multiple endpoints, where an *endpoint* is defined as some entity of management or regulatory concern. The RRM involves constructing a conceptual framework of the risk region, formulating a causal diagram of all variables that lead to impacts within the region (Landis and Wiegers 2005; Colnar and Landis 2007). Creating these conceptual frameworks involves listing *sources* that release *stressors* that occur in *habitats*, potentially causing *effects* and eventually *impacts* to management-defined endpoints (Figure 1).

The RRM is currently applied within the BN-RRM where these sources-to-impacts conceptual frameworks are used to construct Bayesian Networks (BNs). BNs are quantitative models that relate variables through conditional probability (Woodberry et al. 2004; Marcot et al. 2006; Pollino et al. 2006). BNs can be used to calculate risk to endpoints in the form of probability distributions representing the severity of impacts (Johns et al. 2017). Because of the BN-RRM's causal structure, it would be an ideal framework for a systems-based evaluation of structures and processes involved in modeling impacts of synthetic biology in the environment. Additionally, the probabilistic nature of risk assessment is well-suited for accounting for the high mathematical uncertainty that arises from the limited body of empirical information concerning synthetic biology and gene drives.

1.4.1 Bayesian Networks

BNs are acyclic (i.e. no feedback loops), probabilistic models that relate a network of variables through conditional probability. Nodes are separated into two primary classifications: *parent nodes* and *child nodes* (Marcot et al. 2006). In the BN-RRM, arrows in a BN represent causal linkages between variables, with parent nodes influencing child nodes (Landis et al. 2020a; Landis et al. 2020b). Child nodes contain *conditional probability tables* (CPTs) that hold the probabilistic relationship between a child node and its parent nodes. Nodes within a BN contain multiple variable states and CPTs contain the probabilities of each child node's variable states given all possible combinations of parent node states (Marcot et al. 2006; McDonald et al. 2015).

1.5 Model Selection Criteria

Risk assessment using the BN-RRM requires the selection of numerical models to calculate the probabilistic relationship between variables in the BN framework. These models should conform to the criteria put forward by chapter 6 of *Gene Drives on the Horizon* (NASEM 2016) and Landis et al. (2020a). These criteria include:

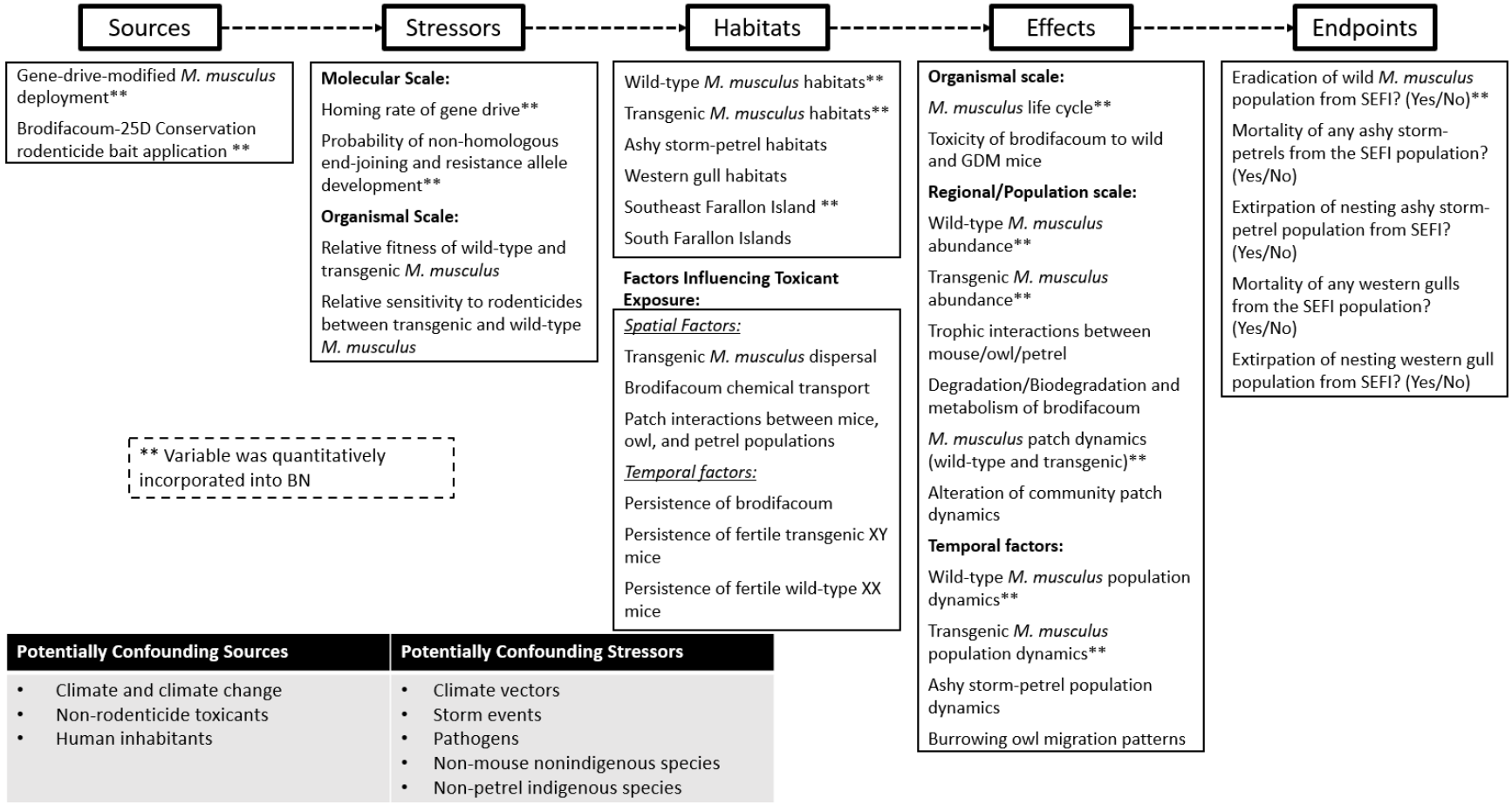

Figure 1. Conceptual model for the SEFI invasive *M. musculus* case study, constructed using the framework of the Relative Risk Model, with *sources* releasing *stressors* that persist in *habitats*, causing *effects*, and eventually impacts to management *endpoints*. (**) indicates variables that that were quantitatively incorporated into parameterizing the BN model in figure 3.

- Models should quantitatively trace causal pathways outlined in the conceptual framework for the risk assessment scenario. These models should reflect the state-of-the-knowledge on causal interactions between variables.
- Models should be able to calculate risk to endpoints, incorporating the concerns of management, stakeholders, and the public.
- Models should be able to elucidate sources of uncertainty and weigh the relative influence of variables or parameters on risk.
- Models should allow for comparison between multiple management strategies. Costs and benefits should be comparable in the context of multiple endpoints.

Meeting these requirements is requisite for the use of risk assessment as a tool for informing research and adaptive management.

1.6 Case Study: Southeast Farallon Island

The South Farallon Islands are part of the Farallon Islands National Wildlife Refuge and are located about 30 miles west of San Francisco. Southeast Farallon Island (SEFI) is the largest island, with an area of approximately 40 hectares (Figure 2). The South Farallones are home to a variety of seabirds, pinnipeds, crickets, and salamanders. Additionally, SEFI is populated by invasive house mice (*M. musculus)* which prey upon endemic species such as the camel cricket (*Farallonophilus cavernicolus*), the maritime goldfield (*Lasthenia maritima*), and the juveniles and eggs of the Farallon arboreal salamander (*Aneides lugubris farallonensis*) (USFWS 2019a).

The invasive mice serve to attract the migratory burrowing owl (*Athene cunicularia*). Burrowing owls are thought to feed primarily on *M. musculus* until the SEFI mouse population reaches a low abundance in late fall. Once the mouse population reaches its low point, ashy storm petrels (*Oceanodroma homochroa*) become the primary food source for owls. Ashy storm petrels are an endangered species (IUCN 2018) of which half the nesting population resides in the South Farallones. The high abundance of

invasive house mice increases the number of overwintering owls on SEFI. This leads to more owl-petrel predation in winter and spring months (USFWS 2019a).

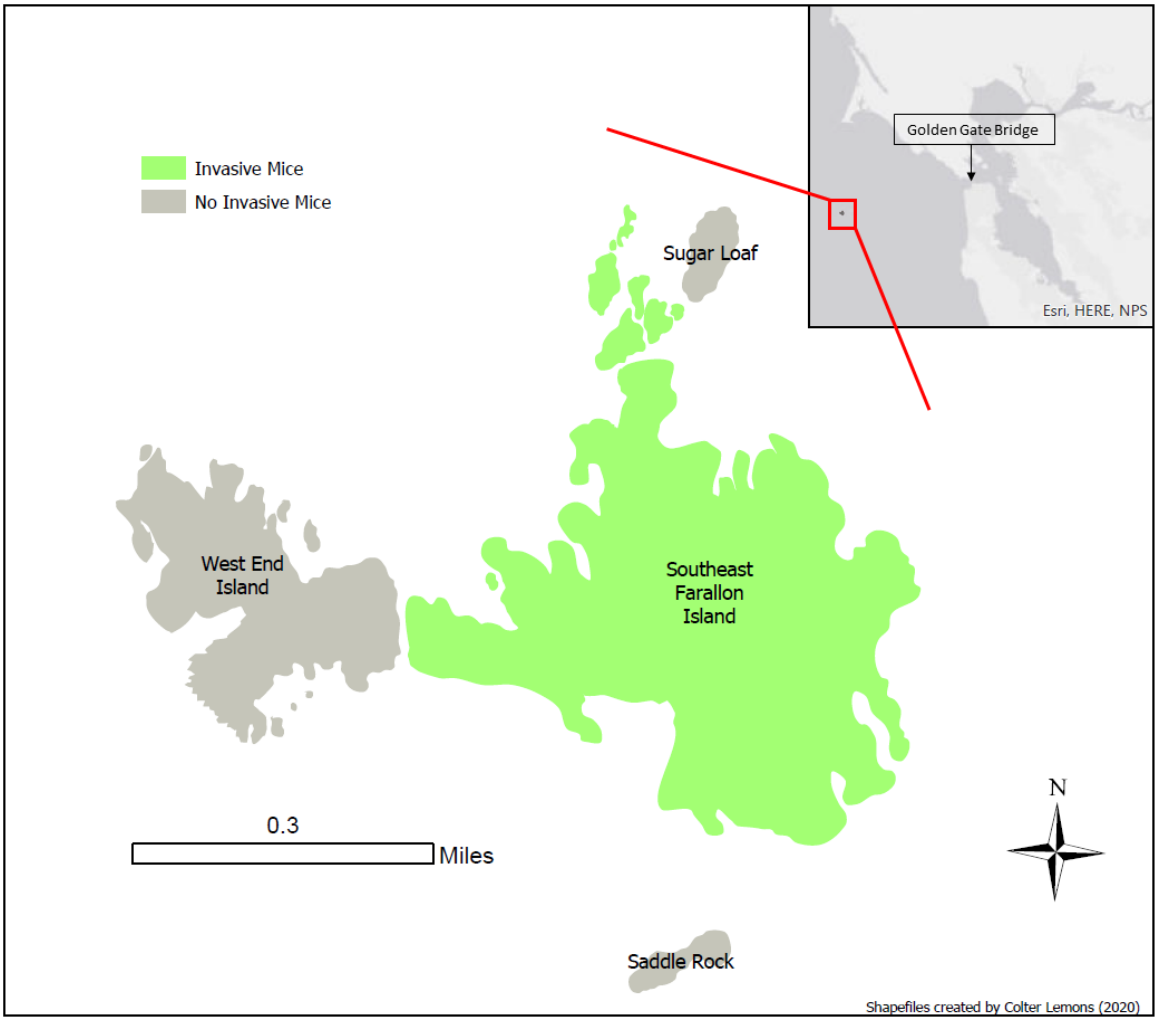

Figure 2. Map of the South Farallon Islands. SEFI is populated with invasive *M. musculus*. A locator map is included to highlight SEFI's location relative to San Francisco, CA.

A 2019 Environmental Impact Statement (EIS) by the United States Fish and Wildlife Service (USFWS) proposed deployment of the chemical rodenticide, brodifacoum, as the preferred management strategy for eradicating invasive mice on SEFI. Brodifacoum is an anticoagulant that functions through vitamin K antagonism. It is deployed in the form of 25 ppmw pellets that can be spread via aerial bombardment or

hand baiting (USFWS 2019a). This eradication plan was originally scheduled to occur November 2019 (USFWS 2019a), but has since been withdrawn by USFWS due to concerns voiced by the California Coastal Commission (Newberry 2019).

Using SEFI as a case study, I employed the BN-RRM to evaluate the risk of mouse eradication given a number of scenarios, simulating three hypothetical population suppression gene drive approaches, with and without supplemental brodifacoum deployment.

1.7 *Sox9* CRISPR-Cas9 Gene Drive

A CRISPR-Cas9 gene drive has been proposed to spread the *Sox9* male development gene to mouse offspring at a proportion greater than 95%, causing XX offspring to develop as males, resulting in sterility (Prowse et al. 2017). This estimated homing rate estimate was based on laboratory testing of a CRISPR-Cas9 gene drives for *Anopheles gambiae* (Hammond et al. 2016). At the time of this work, laboratory testing of CRISPR-Cas9 gene drives on mice has not been conducted. To incorporate dynamics of this gene drive into a SEFI mouse eradication, I used a simulation approach to predict outcomes of twelve unique GDM mouse deployment scenarios (Table 1). I used the R-based model MGDrivE (Sánchez et al. 2020b) for the simulations. MGDrivE is able to simulate population genetics, population dynamics, patch dynamics, and user-specified gene drive parameters into a Monte Carlo simulation framework. MGDrivE was originally intended for simulation of mosquito populations (Sánchez et al. 2020a). I modified MGDrivE to simulate mouse populations, altering functions affecting mating behavior and changing density-dependent and density-independent mortality equations. I also added functions to incorporate stochastic toxicological effects into MGDrivE simulations. See sections 2.2.3 and 2.2.3.1 for more details on MGDrivE and the modifications.

1.8 Rodenticide Use

Because the focus of this work was to probabilistically evaluate gene drives in pest management, rodenticide use, as specified in the SEFI EIS (USFWS 2019), was not considered in isolation when simulating mouse eradication scenarios. However, gene drive simulations were run with and without supplemental rodenticide use in conjunction with GDM mouse deployment. Brodifacoum is also toxic to birds (Eason and Spurr 1995a), with reported LD_{50} values as low as 0.5 mg/kg. Because the Farallon Islands are home to a variety of seabird species, IPM is necessary for considering potential pathways of non-target chemical impacts. However, while the BN includes variables representing non-target pesticide impacts (Figure 3), these variables are unparameterized. In other words, no numerical models have yet been implemented to calculate the probabilities of impacts to non-target species. The calculations focused solely on mice, calculating the influence of rodenticide on mouse abundance and eradication risk.

1.9 Findings

I used the modified version of MGDrivE to simulate three unique GDM *M. musculus* deployment schemes. Each release scheme was simulated across two hypothetical rates of resistance allele generation by the *Sox9* CRISPR-Cas9 gene drive. Additionally, each gene drive scenario was simulated with and without the inclusion of rodenticide-induced mortality, for a total of twelve simulations each run for 250 Monte Carlo repetitions.

Altering the assumed resistance allele generation rate of the gene drive had more influence on the probability of a successful SEFI mouse eradication than the addition of a rodenticide. Resistance rate also had a relatively high influence on the time to reach successful mouse eradication. For one of the gene drive release schemes, eradication was not achieved in any of the Monte Carlo iterations unless a high gene drive homing rate was assumed. The addition of rodenticide increased the probability of

mouse eradication in all tested scenarios, but to a lesser extent than increasing homing rate. These results elucidate the importance of empirically determining homing rates in proposed gene drive constructs.

2. METHODS

First, I framed the Farallon Islands case study within the BN-RRM, delineating specific endpoints based on management goals for SEFI. To accomplish this, I delineated the pest management strategies to be incorporated into the risk assessment, constructing a conceptual model of sources, stressors, habitats, and effects that could alter risk to endpoints (Figure 1). Next, I outlined the step-by-step methodology I used to create a BN-RRM framework for the SEFI pest management scenario. Finally, I described the specific quantitative methods I used to parameterize the BN (Figure 3), focusing on the R-based model (MGDrivE) that I modified and used to simulate GDM *M. musculus* population dynamics and population genetics on SEFI.

2.1 Framing the Farallones Case Study with the BN-RRM

It is important to orient the formulation of a BN-RRM around specific management or regulatory goals for which the probability of impacts to these endpoints can be calculated. A BN-RRM framework is a region-specific probabilistic model that takes a systems-oriented and causal approach to incorporating variables relevant to calculating risk. To organize the SEFI case study within this context, I first outline the specific endpoints to be included in the BN-RRM. Then, I outline the specific management strategies that were incorporated into the model. These twelve simulated release strategies are summarized in Table 1. They include three GDM mouse release schemes simulated across two levels of gene drive resistance generation and two levels of supplemental pesticide. The ability to compare many management strategies in the context of multiple stressors and multiple endpoints is a distinct advantage of the BN-RRM.

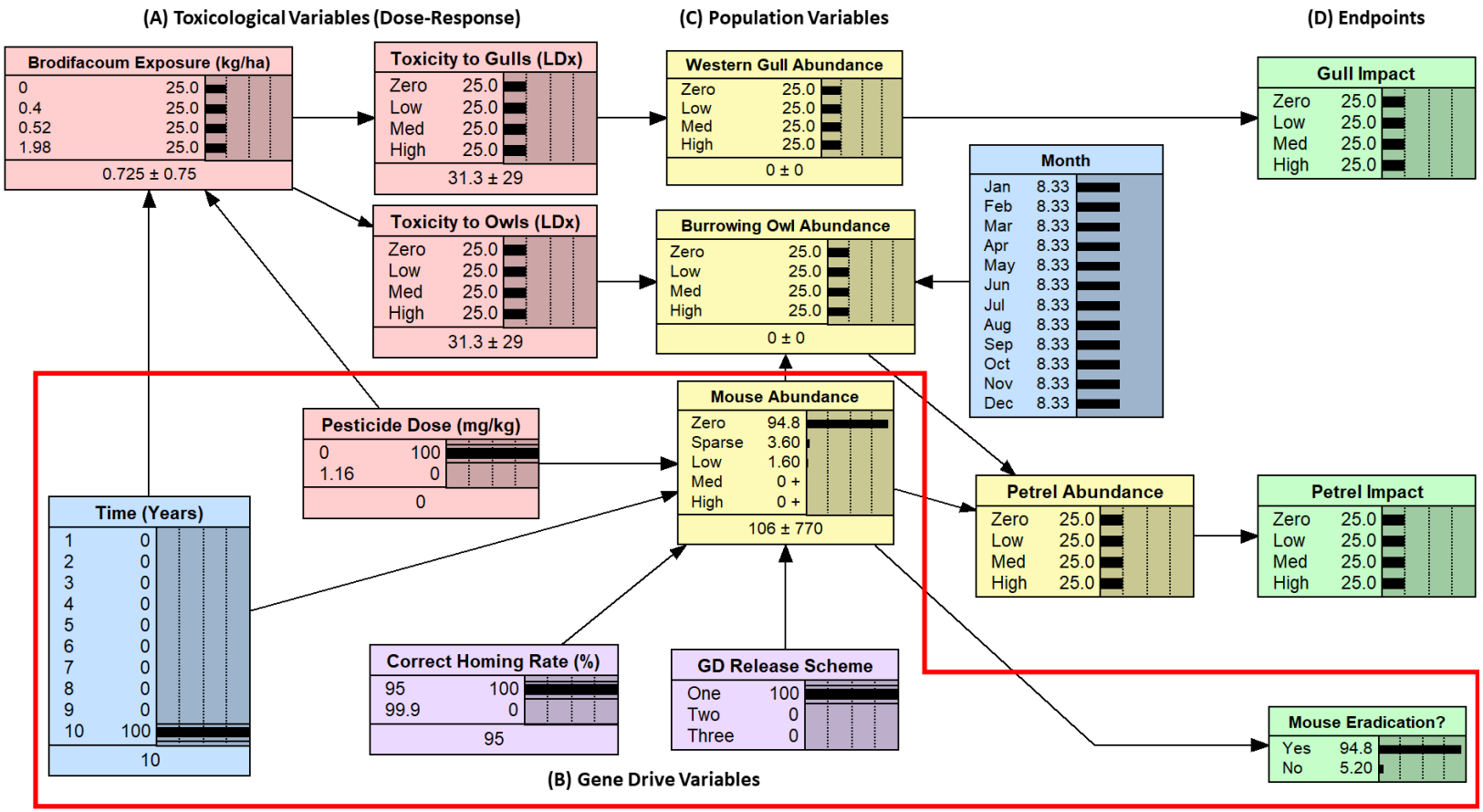

Figure 3. Bayesian network for the SEFI mouse eradication scenario. The highlighted nodes (surrounded by a red border) were parameterized using MGDrivE simulations. The sensitivity analyses described in section 3.3 and Figure 5 were performed using only the bordered nodes. Non-bordered nodes follow a uniform distribution between node states, as their CPTs have not been defined using numerical modeling techniques. Red nodes (A) represent toxicological variables; yellow nodes (C) represent population variables; purple nodes (B) represent gene drive variables; and green nodes (D) represent endpoints. The model as depicted here is showing the results of scenario 1 simulations after 10 years, as defined in table 1. The full model is available on WWU CEDAR.

2.1.1 Management Goals

Integrated pest management (IPM) is a strategy employed in pest eradications that focuses on minimizing the use of chemical pesticides to prevent effects to non-target species. The purpose of IPM is to alter the risk of non-target impacts by decreasing the amount of pesticide used, or by employing other methods to reduce the probability of non-target species toxicity from pesticides. In the context of the SEFI mouse eradication, the EIS (USFWS 2019a) suggested a number of IPM strategies to reduce non-target impacts of brodifacoum deployment. These strategies included gull hazing, using various visual and aural methods to keep western gulls away from SEFI, hypothetically reducing their exposure to brodifacoum; timing brodifacoum deployment to correspond with the annual low point in the *M. musculus* population that occurs in late fall or early winter; and timing the pesticide deployment to co-occur with the low point in annual ashy storm petrel mating cycle (USFWS 2019a). In the context of this work, gene drive deployment corresponds with IPM, potentially reducing or eliminating the need for pesticides.

Table 1. List of gene drive scenarios simulated using the modified version of the R-based model MGDrivE. Each of the following scenarios was simulated for 250 Monte Carlo repetitions used to calculate the mouse abundance and eradication probabilities input into the BN depicted in figure 2.

Scenario Number	Release Scheme	Rodenticide?	Correct Homing Rate (%)
1	GD1	No	95
2	GD1	No	99.9
3	GD1	Yes	95
4	GD1	Yes	99.9
5	GD2	No	95
6	GD2	No	99.9
7	GD2	Yes	95
8	GD2	Yes	99.9
9	GD3	No	95
10	GD3	No	99.9
11	GD3	Yes	95
12	GD3	Yes	99.9

2.2 Analysis Tools

To calculate the influence of GDM *M. musculus* and rodenticide on the risk of SEFI mouse eradication, three numerical models were used. First, I applied exposure-response analysis using the "drc" package in R (Ritz and Streibig 2020) to model the probability of *M. musculus* mortality given a specified dosage of brodifacoum. Next, I constructed a BN in accordance with the BN-RRM to causally relate variables in the SEFI mouse eradication scenario through conditional probability. Finally, I modified and used the R-based model "MGDrivE" (Sánchez et al. 2020b) to simulate GDM mouse population dynamics and population genetics given various release strategies.

2.2.1 Pesticide Exposure-Response Analysis using R-`drc`

I used the "drc" package in R (Ritz and Streibig 2020) to generate a dose-response equation relating dosage of brodifacoum with *M. musculus* mortality (Figure 4; Eq. 1). A two-parameter log-logistic regression model was used to generate the dose-response curve and equation. 95% prediction intervals and 95% confidence intervals were calculated to show the degree of confidence in point estimates (LDx values) on the curve. Prediction intervals were assumed to represent where outcomes (data points) will occur with a specified degree of confidence and a specific number of future predictions, while confidence intervals were assumed to represent where the fitted model (regression curve) will fall with a specified degree of confidence.

$$7d\ Lethality = \frac{1}{1+\exp\left(-3.30(\log(dose)-\log 1.76)\right)} \qquad \text{(Eq. 1)}$$

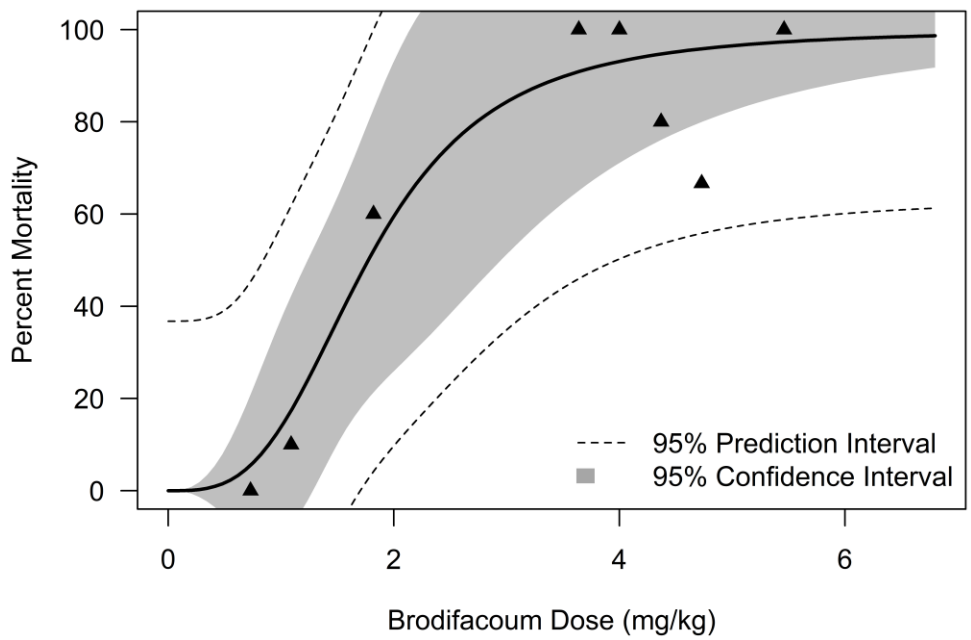

Figure 4. Two-parameter log-logistic dose-mortality curve for 7-day brodifacoum toxicity to *M. musculus* with plotted 95% confidence and prediction intervals. Data from Wheeler et al. (2019).

2.2.2 Bayesian Network – Relative Risk Model

Using the conceptual model of the SEFI mouse eradication scenario, I created a BN framework incorporating the nodes to be used for the evaluation of risk to a variety of pest management and conservation-based endpoints (Figure 3). While the model includes nodes for mice and other non-target species, the work focused only on the mouse eradication endpoint in terms of the application of numerical models and analysis, leaving other endpoints present, yet unparameterized.

2.2.2.1 Endpoints

Endpoints should be framed as specific questions with quantifiable measures of success, including explicit experimental units and methods for data acquisition. In the BN-RRM, endpoints must consist of an *entity* and an *attribute*. An entity is a specific ecological feature such as a species, group of species, or ecological service. An attribute is a measurable property of an entity such as population abundance or extinction. Endpoints should correspond to site-specific management goals. I included four endpoints

within the BN for the SEFI case study. The first endpoint was the probability of mouse eradication, where eradication is defined as having zero mice remaining on SEFI. The other endpoints included the probability of impacts to ashy storm petrel and western gull abundance on SEFI. I did not parameterize these endpoints as part of this work, but the unparameterized nodes were still included in the BN to show causality. Unparameterized nodes are nodes that have not been informed by empirical information or numerical models. For the most part, unparameterized nodes are assumed to follow a uniform distribution until specific analyses have been performed to generate a more informative parameterization. An advantage of this approach is that knowledge gaps are clearly depicted by unparameterized nodes. Experts with relevant knowledge can then assist in developing or applying techniques to parameterize these nodes. This work focused on parameterizing the mouse eradication endpoint using gene drive simulations.

2.2.2.2 Bayesian Network Model Structure

In the BN for the SEFI scenario (Figure 3), I included nodes based on the conceptual model in Figure 1, with groupings for rodenticide variables (group A), gene drive variables (group B), population variables (group C), and endpoints (group D). I also included temporal variables to show how mouse eradication risk changes through time. Gene drive variables included the specific release scheme of GDM mice, with options for three unique release patterns for GDM mice (hereafter termed GD1, GD2, GD3 - see section 2.2.3 for descriptions). I used Netica™ (Norsys Software Corp.) for all Bayesian network construction and analysis.

Rodenticide nodes included the assumed average dosage of brodifacoum to adult mice on SEFI. I included rodenticide-induced mortality as part of MGDrivE simulations (see section 2.2.3). Other rodenticide variables that I did not parameterize as part of this work included brodifacoum toxicity to western gulls and burrowing owls, in addition to a node for “Brodifacoum Exposure” to represent the persistence of brodifacoum outside of the specified rodenticide deployment period. Because

brodifacoum is highly lipophilic and has a long biological half-life (Godfrey 1985) it is possible for toxicity to occur as a result of predation upon organisms that have ingested brodifacoum baits. Such instances have been documented (Eason and Spurr 1995a; 1995b; Godfrey 1985). There is no toxicity node for ashy storm petrels because they are considered nocturnal marine feeders and therefore have no apparent route of exposure to deployed rodenticide (USFWS 2019a).

Population nodes included abundance variables for house mice, burrowing owls, ashy storm petrels, and western gulls. Mouse abundance is depicted as a parent node to owl abundance, as mice are thought to be the primary attractive force for burrowing owl migration to the South Farallones. Owl abundance is shown as a parent node to petrel abundance, as owls are thought to be one of the main predators of ashy storm petrels after the house mouse population reaches its annual low point in late fall. Population node states were divided into categories for "Zero," "Low," "Medium," and "High" abundance representing ranges of organism counts for each species, with an additional node state, "Sparse," for mice representing when the population was nearing eradication, but not quite at "Zero."

Temporal nodes included a variable for "Time" with node states for years 1-10 following a GDM mouse deployment. I included another temporal node called "Month" which is a parent to the owl abundance node and is currently unparameterized. However, this node could be used to represent migratory patterns for owls roosting on the South Farallones.

2.2.3 Gene Drive Simulation

Sánchez et al. (2020a) proposed the R-based model "MGDrivE" (Sánchez et al. 2020b) to simulate population dynamics of GDM mosquitoes. MGDrivE incorporates population genetics, age-class population dynamics, and functionalities of a variety of gene drives. Additionally, MGDrivE provides options for altering biological parameters of mosquitoes to accommodate a range of species, as well as, allowing for the adjustment of the number of ecological patches and the probabilities of migration

between patches. MGDrivE can be run as a deterministic or stochastic model, with the ability to specify a number of Monte Carlo simulations to be performed for stochastic runs (Sánchez et al. 2020a).

Because I simulated mice and not mosquitoes for this risk assessment, I made a number of modifications to MGDrivE to accommodate mouse population dynamics and the *Sox9* gene drive. Specific modifications are described in section 2.2.3.1.

For each of the two gene drive release scenarios I simulated for this risk assessment framework, I assumed that the SEFI mouse population had a carrying capacity of 50,000 mice, based on spatial house mouse density estimates from the SEFI EIS (USFWS 2019a). I also assumed that the SEFI mouse population was divided into two patches containing 70% and 30% of the total population, with a 20% migration rate between patches. Patches were based on a topographic elevation map of SEFI from the EIS appendices (USFWS 2019b).

I assumed that the *Sox9* gene drive had a *cutting rate* of 99.9%, a *correct homing rate* of either 95% or 99.9%, and an *in-frame resistance allele generation rate* of 33.3%. The cutting rate represented the rate of CRISPR-Cas9 successfully causing strand breakage for the target wild-type (W) allele. If cutting was unsuccessful, it was assumed that the organism would continue to generate W alleles. The correct homing rates represented the probability of successful homology-directed repair by the CRISPR-Cas9 system, substituting gene drive (H) alleles in place of the cut W alleles. If cutting was successful and homing was unsuccessful, out-of-frame resistant (B) alleles were generated via non-homologous end joining, leading to zero fertility in homozygous (BB) offspring and a 90% fertility reduction in mice heterozygous for B alleles. The in-frame resistance generation rate represented the proportion of incorrect homing that would lead to development of in-frame resistant (R) alleles. R alleles were assumed to cause no reduction in fertility. I chose to compare homing rates of 95% and 99.9% percent because 95% is a homing rate that has been demonstrated in laboratory testing of gene drives

(Hammond et al. 2016), and 99.9% is a hypothesized homing rate for multiplexed gene drive constructs (Marshall et al. 2017).

The three release schemes for GDM mouse introduction, GD1, GD2, and GD3 were parameterized as described by Table 2. All release schemes were run across two levels of resistance allele generation, with and without toxicity, for a total of twelve runs of MGDrivE with n=250 Monte Carlo simulations for each run. Table 1 contains a comprehensive list of all simulations and their parameterizations.

Table 2. Release schemes (GD1, GD2, GD3) as parameterized for MGDrivE simulations.

Release Scheme	Number of Releases	Number of Mice Released (per patch)	Interval (days between releases)	Which Patch?	Total GDM Mice Needed
GD1	40	1200	60	Both	96,000
GD2	35	2500	60	Larger*	87,500
GD3	50	750	30	Both	75,000

* The ecological patch containing 70% of the mouse population was considered the "Larger" patch.

2.2.3.1 Modifications to MGDrive

To alter the functionality of MGDrivE for compatibility with modeling population dynamics of *M. musculus*, I made changes to functions affecting mating behavior, density dependent mortality, and recruitment of early life stage mice. I also added functions for modeling the dynamics of the *Sox9* CRISPR-Cas9 gene drive, including rodenticide-induced mortality in simulations, and extracting the numerical output of simulations into a form that could be used to parameterize the BN. I modified the source code of MGDrivE version 1.5.0 found at https://github.com/MarshallLab/MGDrivE. This altered version of MGDrivE can be found at https://github.com/eabrown2378/MGDrivE.

In the original version of MGDrivE by Sánchez et al. (2020b) (hereafter termed "MosquitoGD"), all female mosquitoes are assumed to find a mate and oviposit eggs once each day, assuming panmixia unless genotype-specific mating preferences are specified by the user. In the altered version of MGDrivE

(hereafter termed "MouseGD"), not all female mice mate and conceive offspring every day. Instead, each female has a probability of mating and conceiving new offspring calculated with Eq. 2:

$$P(mating\ and\ conception) = \frac{annual\ number\ of\ litters}{365\ days} \quad \text{(Eq. 2)}$$

Where the annual number of litters is an assumed average for female *M. musculus*. I assumed an average of 7.5 litters in the runs of MouseGD. Because this value was used to calculate a probability, it was not necessary to use an integer for the average number of litters. When running MouseGD as a stochastic model, the probability calculated from Eq. 2 is used in a binomial distribution:

$$Mating\ Females = Binomial(Ad_f, P(mating\ and\ conception)) \quad \text{(Eq. 3)}$$

Where Ad_f is the total female population. MouseGD still assumes panmixia, unless otherwise specified, assigning each mating female to a mate amongst the adult male population.

A Poisson distribution was used to calculate the number of newly gestating offspring resulting from conception, following Eq. 4

$$Newly\ Gestating\ Mice = Poisson(\lambda = average\ number\ of\ pups\ per\ litter) \quad \text{(Eq. 4)}$$

I assumed an average of 6 mouse pups per litter based on parameters used by Prowse et al. (2017), using this value as the rate parameter in draws from the Poisson distributions, with one random draw for each mating female.

In MosquitoGD age-class population dynamics, four mosquito life stages were assumed: egg, pupa, larvae, and adult, where density-independent mortality occurred at all four life stages and density-dependent mortality occurred at the larval stage (Sánchez et al. 2020a). In MouseGD, I also assumed four life stages: gestating, nursing, adolescent, and adult, maintaining density-independent mortality processes at all four stages, but removing density-dependence from the larval (nursing) stage and

adding density-dependent mortality to the adult stage in the form of a carrying capacity modeled by Eq. 5:

$$D = \left(\frac{k * 1/2}{k + Ad}\right)^{\frac{1}{\theta}} \qquad \text{(Eq. 5)}$$

Where D is the probability of surviving the density-dependent process; *k* is the carrying capacity for the adult mice and was set to 50,000 to represent the SEFI population; *Ad* represents the current adult mouse population; and Θ represents a shape parameter used to limit the mouse population to carrying capacity (*k*). A Θ value of 22.4 was used in conjunction with the assumed life cycle parameters for *M. musculus* used in these simulations. I assumed life stage times of 19 days for gestation, 23 days for nursing, 37 days for adolescence, and 690 days for adulthood. I based *M. musculus* life cycle parameters on estimates from Brust et al. (2015). At the time of writing this paper, no adolescent mortality is included in MouseGD. However, abundances of each adolescent life stage are calculated as part of daily mouse population dynamics and mortality processes could be added to the model with relative ease.

In MosquitoGD, properties of gene drives are stored as 3-dimensional arrays called "Inheritance Cubes" which contain the expected offspring frequencies for each genotype for every male-female mating pair, as well as, optional parameters that can specify mechanisms such as the viability of specific mating pairs, genotype-specific fitness reduction, and genotype-specific sex emergence. In MouseGD, I added a novel inheritance cube function for the *Sox9* CRISPR-Cas9 gene drive. Because the *Sox9* CRISPR-Cas9 gene drive is supposed to function through sex-specific organism fitness, this inheritance cube function separated genotypes into sex-specific genotypes for modeling purposes, altering genotype-specific sex-ratio emergence for male and female genotypes respectively. Four alleles (W/H/B/R) were included in the inheritance cube. See sections 1.2.1 and 2.2.3 for contextual descriptions of these alleles. W alleles were assumed to cause no reduction in mating fitness, with homozygotes producing a normal amount of offspring as calculated from the Poisson function in Eq. 4. H alleles were assumed to carry zero fitness

reduction for males; however, they were assumed to cause 100% fitness reduction in both homozygous and heterozygous females. Mice homozygous for B alleles had zero mating fitness, however mice heterozygous for B alleles experienced a fractional reduction of 90%. Similar to W alleles, R alleles caused no reduction in mating fitness, however, R alleles were not subject to the enhanced inheritance rate of the *Sox9* homing element.

Rodenticide-induced mortality was included within the daily population dynamics of MouseGD simulations, applying a daily probability of mortality to each adult mouse using random draws from a binomial distribution following the form:

$$Surviving\ Adults = Binomial(Ad, 1 - P(lethality)) \quad \text{(Eq. 6)}$$

For simulations that included rodenticide-induced mortality, there was a ~2.9% probability of adult mouse lethality for a period of 70 days coinciding with the start of GDM mouse deployment. This probability corresponds to an assumed 1.16 mg/kg average dose of brodifacoum to all adult mice over the 70-day toxicity period. This dosage was calculated from the 2-parameter log-logistic regression model depicted in Eq. 1 and was assumed to correspond to a 7-day LD_{20} for *M. musculus* mortality from brodifacoum. The daily lethality was assumed to be the 7-day lethality calculated from Eq. 1 divided by 7. This dose-response curve (Figure 4) was generated using toxicity data from Wheeler et al. (2019).

Changes to the source code of MGDrivE version 1.5.0 (Sánchez et al. 2020b) was conducted using RTools version 3.5.0.4 (Ripley et al. 2020), RStudio version 1.2.5024 (RStudio Team 2020), and R version 3.6.3 (R Core Team 2020).

3. RESULTS

For each of the twelve MouseGD runs used to parameterize the BN (Table 1), I used the 250 Monte Carlo iterations to calculate the probability of the SEFI mouse population falling into each of five

abundance categories in the mouse abundance node. I defined "High" mouse abundance as greater than 25,000 mice on SEFI; "Medium" mouse abundance as 10,000-25,000 mice; "Low" as 1,000-10,000 mice; "Sparse" as 1-1,000 mice; and "Zero" as zero mice. The probability of the "Yes" state on the "Mouse Eradication" node was equal to the probability of "Zero" in the "Mouse Abundance" node, while the probability of the "No" state was equal to all non-zero states of the "Mouse Abundance" node combined.

Looking at distributions of mouse abundance and the risk of mouse eradication generated by each simulated scenario, I made some general comparisons between the outcomes of each gene drive scenario. Then I looked at specific instances of risk to the mouse eradication endpoint, noting the influence of time, resistance generation, and pesticide usage. Finally, I used Netica's built-in sensitivity analysis function to calculate the relative entropy reduction of pesticide deployment and gene drive resistance rate on risk to the mouse eradication endpoint. Entropy is a measure of statistical uncertainty, so a higher entropy reduction for a variable means that the variable has a higher influence on changes in the node being evaluated (Hosack et al. 2008; Marcot 2012). I conducted sensitivity analyses separately for GD1, GD2, and GD3 at each time point included in the BN.

3.1 Gene Drive Release Scheme Comparisons

GD1 simulations predicted a 94.8% probability of successful mouse eradication within 10 years of GDM mouse deployment when assuming no rodenticide and a 95% correct homing rate of the gene drive, surpassing GD2 and GD3 simulations which showed 10-year eradication probabilities of 49.6% and 0% respectively. GD1 overall had the highest probability of successful mouse eradication, however, eradication tended to occur later than GD2 or GD3. GD1 would involve the highest number of GDM mice being released over the longest time period, with a total of 96,000 mice being released over 6.5 years.

GD2 simulations showed earlier eradication of mice than GD1, with 7-year eradication probabilities ranging from 23.6-100% depending on gene drive resistance rate and the presence or absence of rodenticide. 10-year eradication probabilities ranged from 49.6-100%. GD2 would require less GDM mice that GD1, but more GDM mice than GD3, with 87,500 mice being released over 5.75 years.

GD3 simulations predicted the earliest possible mouse eradications out of the three release schemes, however, the overall probability of achieving eradication was lowest out of all the scenarios. Unless I assumed a 99.9% homing rate for the gene drive, there was a 0% probability of eradication with GD3. Although, 99.9% homing and the addition of rodenticide yielded a 40.8% probability of a "Sparse" mouse population after 5 years which was the earliest of any simulated scenarios to reach a "Sparse" mouse abundance. GD3 would involve the lowest number of released GDM mice, with a total of 75,000 GDM mice being released over a 4-year period.

The BN model is available is a supplementary resource file in the WWU CEDAR repository or upon request. The model can be viewed using the free version of Netica available at http://www.norsys.com/netica.html.

3.2 Risk to Mouse Eradication Endpoint

In addition to looking specifically at the properties of GD1-3, I also looked at the general trends of mouse eradication risk in terms of time, resistance development, and toxicity, as they appeared across all three release schemes.

For the "Time" variable, I looked at the earliest times that each mouse population threshold was reached for "Medium," "Low," and "Sparse," abundance as well as, the earliest time eradication was predicted across all simulations. 52.8% of simulations reached the "Medium" population threshold across all simulated scenarios after 3 years. After 4 years, the first simulations reached the "Low" threshold at a proportion of 12.1%. The first simulations reached a "Sparse" population at year 5 at a

proportion of 3.4%. One simulation reached eradication at year 6: This was a simulation for GD3 with rodenticide and a 99.9% correct homing rate. Knowing the probabilities of these population thresholds in addition to the expected times to reach these reduced abundances is a useful management tool in conjunction with knowing the overall probability of eradication, especially when considering that a drastically reduced mouse population could minimize the presence of burrowing owls, even if total mouse eradication has not yet been achieved. Therefore, these probabilistic results for mouse abundance thresholds could be useful in anticipating risk of impacts to ashy storm petrels.

The assumed correct homing rate increased the probability of achieving eradication within 10 years for all tested scenarios, with 82.4% of simulations across all scenarios reaching eradication if a 99.9% homing rate was assumed, and only 54.9% of simulations reaching eradication with an assumed homing rate of 95% assumed. For GD1 and GD2, 100% of simulations showed eradication within 10 years if 99.9% homing was assumed. For GD3, no simulations reached eradication with 95% homing, but 47.2% reached eradication within 10 years for a 99.9% homing rate. These results suggest that empirical testing of an engineered gene drive's resistance generation is essential to evaluating the viability of management decisions.

Addition of rodenticide to simulations caused an increase in eradication probability across 10/12 release scenarios. The exceptions were the two GD3 simulations at 95% homing rate where eradication was not achieved in any simulations. Averaged across all tested scenarios, 64.5% of simulations reached eradication within 10 years when no rodenticide was present and 72.8% of simulations reached eradication when rodenticide was included. Currently the BN nodes for toxicity to burrowing owls and laughing gulls are unparameterized, however, I expect that an increase in the amount of deployed brodifacoum would lead to increased risk of toxicity-related impacts to non-target organisms on SEFI.

3.3 Uncertainty and Sensitivity Analysis

Sensitivity analysis can show the relative influence of parent nodes on a child node in a BN via the calculation of entropy reduction. In the sensitivity analyses I primarily focused on the relative entropy reductions of the "Correct Homing Rate" and "Pesticide Dose" nodes of the BN, holding "Time" and "GD Release Scheme" constant. Because unparameterized nodes interfere with sensitivity analysis calculations, I removed all unparameterized nodes from the BN before calculating entropy reduction. Overall, correct homing rate of the gene drive had a higher entropy reduction than the addition of rodenticide meaning that in the simulation I ran, the resistance generation rate had more influence on the risk of mouse eradication than the influence of toxicity. However, the relative entropy reduction between homing rate and rodenticide changed depending on the GD release scheme and the time since GDM mouse deployment.

First, I ran sensitivity analyses on GD1-3 at year 7 (Figure 5), since this is when simulations started to show mouse eradication. For GD1, "Pesticide Dose" and "Correct Homing Rate" had entropy reductions of 0.16% and 2.36% respectively suggesting that neither of these variables had much influence of GD1's eradication probability. For GD2, pesticide and homing rate had entropy reductions of 7.2% and 38.3% respectively meaning these variables explain a high proportion of the variance in eradication probability for GD2, with homing rate exerting the most influence. For GD3, pesticide and homing rate had entropy reductions of 8.47% and 16.7% respectively, meaning that these variables had high influence on eradication probability. However, as opposed to GD2, the influence of rodenticide in GD3 was more comparable to the influence of homing rate.

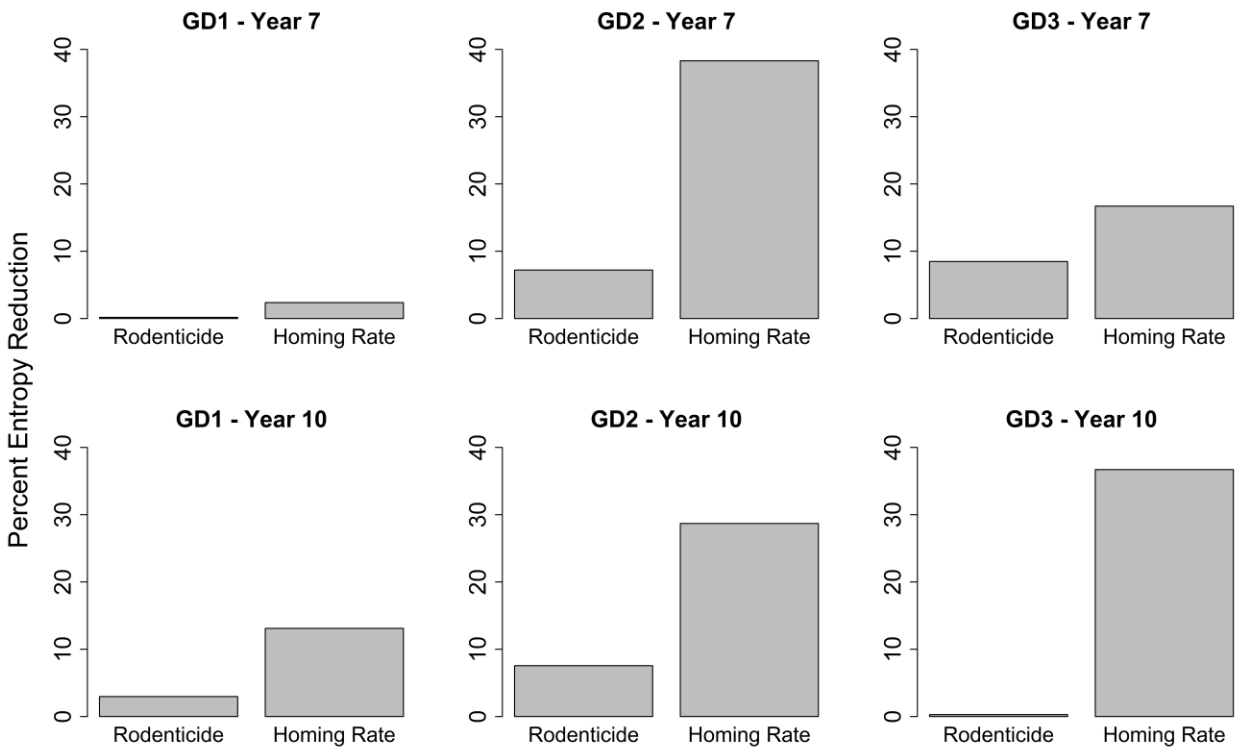

Figure 5. Results of sensitivity analyses for GD1-3 at years 7 and 10. Percent entropy reduction represents the influence of each variable on the risk of mouse eradication. I compared the influence of supplementary rodenticide and gene drive homing rate. Homing rate had higher entropy reduction across all scenarios. I performed sensitivity analyses using only parameterized nodes (see figure 3). I used Netica for all calculations.

I also ran sensitivity analysis on GD1-3 at year 10 (Figure 5) to show which variables had the highest overall influence on the probability of a successful SEFI mouse eradication. For GD1, pesticide and homing rate had entropy reductions of 2.96% and 13.1% respectively; for GD2, entropy reductions were 7.55% and 28.7% respectively; and GD3 entropy reductions were 0.29% and 36.7% respectively.

In conclusion, sensitivity analysis revealed that the correct homing rate of a deployed gene drive has high influence on how early a planned eradication is likely to be achieved, and also on the overall likelihood of eradication using GDM mice.

4. DISCUSSION

This BN-RRM risk assessment framework for gene drives demonstrates that outcomes and uncertainties pertaining to environmental applications of gene drives can be probabilistically evaluated with clearly-documented modeling assumptions. I will now discuss this the connection with NASEM criteria for risk assessment of gene drives, how this model fits into the broader context of synthetic biology and ecological risk assessment, conclusions, and next steps.

4.1 Alignment with NASEM Criteria

Chapter 6 of *Gene Drives on the Horizon* (NASEM 2016) outlines some key properties that should be present in an ecological risk assessment of gene drive technology:

1. The risk assessment should be able to provide probabilistic estimates for potential harm and benefits of gene drive deployment using numerical models.

BNs can combine quantitative modeling techniques and empirical information into calculations of probability distributions representing risk. The model demonstrates this property by generating distributions of mouse abundance and mouse eradication from gene drive simulations. BNs can compare relative impacts to multiple endpoints due to the influence of multiple stressors.

2. The model should allow for comparison between multiple management strategies.

The model contains the risk distributions from 12 simulated gene drive management scenarios, showing their efficacy across a 10-year period. The outcomes of these scenarios were calculated to inform specific management goals by calculating risk to endpoints.

3. The model should be able to incorporate the concerns of relevant management, stakeholders, and publics.

The endpoint nodes in this BN reflect specific management concerns around the invasive mouse problem on SEFI, showing the probability of accomplishing these management goals given the implementation of various mouse eradication strategies. Landis et al. (2017) emphasized the ability of the BN-RRM to evaluate a wide range of endpoints. These endpoints can be viewed in terms of their costs and benefits to various stakeholder groups.

4. The model should have utility in identifying sources of uncertainty and forming an adaptive management scheme around gene drives.

Sensitivity analysis was used to determine the relative influence of variables on the probability of mouse eradication on SEFI. These analyses could be used to prioritize research investigating the efficacy of various gene drive constructs. Landis et al. (2017) incorporates risk assessment and uncertainty analysis into an explicit adaptive management framework. Feedback from management decisions and research can be used to update the BN-RRM with new knowledge, informing future management decisions and research directions.

In short, this model meets the recommended criteria for ecological risk assessment of gene drives outlined by *Gene Drives on the Horizon* chapter 6 (NASEM 2016) and is an appropriate framework to start utilizing for directing empirical gene drive research and developing site-specific adaptive management schemes for potential environmental applications of gene drives.

4.2 Risk Assessment for Synthetic Biology

The properties of this risk assessment framework for gene drives are broadly applicable to any ecological use of synthetic biology. The usefulness of probabilistic estimates of environmental outcomes is wide-ranging. Additionally, the flexible and causal nature of the BN-RRM is suitable to a plethora of potential scenarios related to emerging biotechnology. Utilizing the MGDrivE framework to parameterize this BN allowed me to account for population dynamics, population dynamics, patch

dynamics, and specific gene drive parameters when simulating a SEFI mouse eradication. Adapting MGDrivE to risk assessment indicated the flexibility of the BN-RRM to incorporate a wide range of advanced numerical modeling techniques into a causal BN framework.

4.3 Conclusions

I demonstrated an approach to parameterizing a risk assessment model for the environmental deployment of GDM organisms, providing a framework that can compare multiple management strategies and isolate sources of scientific uncertainty. The purpose of this framework is to assist in prioritizing research needs and developing adaptive management strategies, enabling responsible investigations into the usefulness of gene drive technology and synthetic biology as a whole.

In simulating the deployment of GDM mice on SEFI, I found that the risk of successful mouse eradication was greatly influenced by the resistance allele generation rate of the simulated *Sox9* gene drive. These results suggest that thorough laboratory investigations into the homing properties of gene drives would be useful for predicting the likelihood of successful population suppression using a CRISPR homing system.

4.4 Next Steps

The risk assessment model presented in this study serves as a template for developing decision making models and orienting future research around synthetic biology and gene drives. However, before risk assessment frameworks can be used to direct management decisions in the field, they must be tested and validated using supplemental experimentation and monitoring. As I demonstrated with sensitivity analysis of the BN, studies to evaluate the performance of gene drives in terms of successful cutting and homing of the CRISPR-Cas9 system would be useful in calculating the probabilities of various outcomes in a field deployment of GDM organisms.

Another set of variables that were not fully evaluated in the BN were the conservation-based endpoints, or the impacts to non-target species on SEFI due to the deployment of a pesticide or gene drive. For example, processes such as hybridization or horizontal gene transfer could potentially lead to the transfer of gene drives to non-target species (NASEM 2016). Pesticides can also cause non-target toxicity via multiple routes of exposure, including predation of dosed organisms and non-target consumption of bait. Many other potentially confounding variables were excluded from the analyses including seasonal and predatory influence on mouse population dynamics; residual toxicity, fate, and transport of brodifacoum rodenticide; early life stage mortality processes for mice; and potential differences in rodenticide sensitivity for various mouse life stages and for newly released GDM mice.

Chapter 5 of *Gene drives on the Horizon* outlines a phased testing approach for gene drives (Figure 6). This process involves research and deployment phases encapsulating preliminary lab and field testing, field deployment of GDM organisms, and continuous monitoring of post-deployment impacts. This approach is intended to be iterative and cautious, with criteria for revisiting research or deployment phases when new information or uncertainties arise. Risk assessment is intended to inform this phased testing approach at all stages, revealing research needs and guiding adaptive management of gene drives. The BN-RRM is a well-suited framework for informing the NASEM-outlined goal of responsible and thorough methods for evaluating proposed environmental uses of gene drives and synthetic biology.

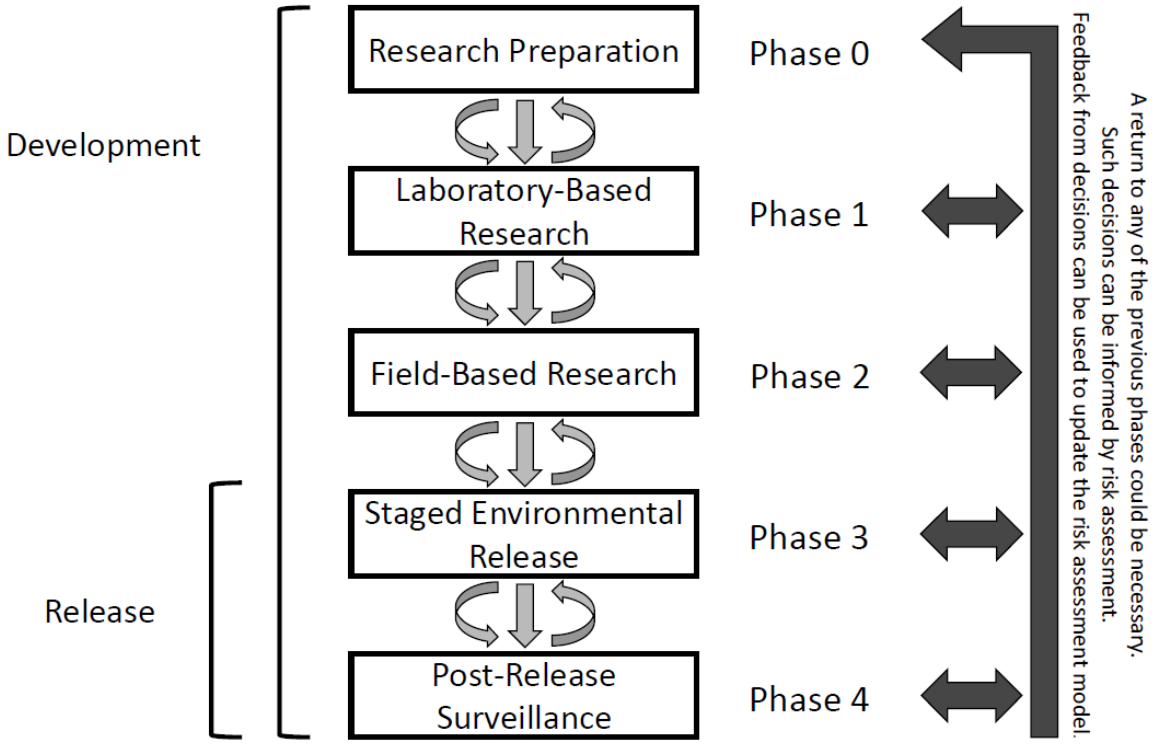

Figure 6. A "phased testing" approach to gene drive research and deployment as outlined by *Gene Drives on the Horizon*, including steps for laboratory testing, field testing, GDM organism release, and post-release monitoring. This is intended to be an iterative process, with criteria for returning to previous stages if novel uncertainties arise. Modified from NASEM (2016).

REFERENCES

Brust V, Schindler PM, Lewejohann L. 2015. Lifetime development of behavioural phenotype in the house mouse (*Mus musculus*). *Frontiers in Zoology* 12(suppl 1):517.

Champer J, Reeves R, Oh SY, Liu C, Liu J, Clark AG, Messer PW. 2017. Novel CRISPR/Cas9 gene drive constructs reveal insights into mechanisms of resistance allele formation and drive efficiency in genetically diverse populations. *PLOS Genetic* 13(7):1006796.

Colnar AM, Landis WG. 2007. Conceptual model development for invasive species and a regional risk assessment case study: the European Green Crab, *Carcinus maenas*, at Cherry Point, Washington USA. *Human and Ecological Risk Assessment* 13:120-155.

Eason CT, Spurr EB. 1995a. The toxicity and sub-lethal effects of brodifacoum in birds and bats: a literature review. *Science for Conservation* 6:5-16.

Eason CT, Spurr EB. 1995b. Review of the toxicity and impacts of brodifacoum on non-target wildlife in New Zealand. *New Zealand Journal of Zoology* 22:371-379.

Godfrey MER. 1985. Non-target and secondary poisoning hazards of “second generation” anticoagulants. *Acta Zoologica Fennica* 173:209-212.

Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, Gribble M, Baker D, Marois E, Russel S, Burt A, Windbichler N, Crisanti A, Nolan T. 2016. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. *Nature Biotechnology* 34:78-83.

Hayes EH, Landis WG. 2004. Regional ecological risk assessment of a near shore marine environment: Cherry Point, WA. *Human and Ecological Risk Assessment* 13:299-325.

Holling CS. 1978. Adaptive Environmental Assessment and Management. London (UK): John Wiley & Sons. 377 p.

Hosack GR, Hayes KR, Dambacher JM. 2008. 2008. Assessing Model Structure Uncertainty through an Analysis of System Feedback and Bayesian Networks. *Ecological Applications* 18:1070–1082.

International Union for Conservation of Nature (IUCN). 2018. The IUCN red list of endangered species: Ashy storm-petrel. Available at: https://www.iucnredlist.org/species/22698562/132653646. Accessed 5 May 2019.

Knott GJ, Doudna JA. 2018. CRISPR-Cas guides the future of genetic engineering. *Science* 361:866-869.

Johns AF, Graham SE, Harris MJ, Markiewicz AJ, Stinson JM, Landis WG. 2017. Using the Bayesian Network Relative Risk Model Risk Assessment Process to Evaluate Management Alternatives for the South River and Upper Shenandoah River, Virginia. *Integrated Environmental Assessment and Management* 13:100-114.

Landis WG, Brown EA, Eikenbary S. 2020a. An initial framework for the environmental risk assessment of synthetic biology derived organisms with a focus on gene drives. In B. D. Trump et al. (Eds.) *Synthetic Biology 2020: Frontiers in Risk Analysis*. Springer Nature Switzerland AG pp 257-268.

Landis WG, Chu VR, Graham SE, Harris MJ, Markiewicz AJ, Mitchell CJ, Von Stackelberg KE, Stark JD. 2020b. Integration of chlorpyrifos acetylcholinesterase inhibition, water temperature, and dissolved oxygen concentration into a regional scale multiple stressor risk assessment estimating risk to chinook salmon. *Integrated Environmental Assessment and Management* 16(1):28-42.

Landis WG, Markiewicz AJ, Ayre KK, Johns AF, Harris MJ, Stinson JM, Summers HM. 2017. A general risk-based adaptive management scheme incorporating the Bayesian Network Relative Risk Model with the South River, Virginia, as case study. *Integrated Environmental Assessment and Management* 13(1):115-126.

Landis WG, Wiegers JK. 2005. Chapter 2: Introduction to the regional risk assessment using the relative risk model. In W. G. Landis (Ed.) *Regional Scale Ecological Risk Assessment Using the Relative Risk Model*. CRC Press Boca Raton pp 11-36.

Marcot BG, Steventon JD, Sutherland GD, McCann RK. 2006. Guidelines for developing and updating Bayesian belief networks applied to ecological modeling and conservation. *Canadian Journal of Forest Research* 36:3063-3074.

Marcot BG. 2012. Metrics for evaluating performance and uncertainty of Bayesian network models. *Ecological Modelling* 230:50-62.

Marshall JM, Akbari OS. 2016. Gene drive strategies for population replacement. In *Genetic Control of Malaria and Dengue*. Elsevier Inc pp 169-200.

Marshall JM, Buchman A, Sánchez CHM, Akbari OS. 2017. Overcoming evolved resistance to population-suppressing homing-based gene drives. *Scientific Reports* 7:3776.

McDonald KS, Ryder DS, Tighe M. 2015. Developing best-practice Bayesian Belief Networks in ecological risk assessments for freshwater and estuarine ecosystems: A quantitative review. *Journal of Environmental Management* 154:190-200.

National Academies of Science, Engineering, and Medicine (NASEM). 2016. *Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values*. Washington, DC: The National Academies Press.

Newberry L. 2019. Plan to dump rat poison on Farallon Islands questioned by California Coastal Commission. *LA Times*. Available at: https://www.latimes.com/local/lanow/la-me-farallon-islands-rat-poison-plan-20190710-story.html. Accessed 20 May 2020.

Noble C, Olejarz J, Esvelt KM, Church GM, Nowak MA. 2017. Evolutionary dynamics of CRISPR gene drives. *Science Advances* 3:1601964.

Noble C, Min J, Olejarz J, Buchthal J, Chavez A, Smidler AL, DeBenedictis EA, Church GM, Nowak MA, Esvelt KM. 2019. Daisy-chain gene drives for the alteration of local populations. *PNAS* 116(17):8275-8282.

Norsys Software Corp. Netica™. Vancouver, B.C., Canada. Copyright 2014. Available at: http://www.norsys.com/netica.html. Accessed 15 May 2015.

Pollino CA, Woodberry O, Nicholson A, Korb K, Hart BT. 2006. Parameterisation and evaluation of a Bayesian network for use in an ecological risk assessment. *Environmental Modelling and Software*. 22: 1140-1152.

Prowse TAA, Cassey P, Ross JV, Pfitzner C, Wittmann TA, Thomas P. 2017. Dodging silver bullets: good CRISPR gene-drive design is critical for eradicating exotic vertebrates. *Proceedings of the Royal Society B: Biological Sciences* 284(1860):20170799.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at https://www.R-project.org/. Accessed 24 April 2020.

Ripley B, Murdoch D, Ooms J. 2020. RTools: Building R for Windows. Available at https://cran.r-project.org/bin/windows/Rtools/history.html. Accessed 24 April 2020.

Ritz C, Streibig JC. 2020. Analysis of dose-response curves. Package "drc" version 3.0-1. Available at https://github.com/DoseResponse/drc. Accessed 22 April 2020.

Roberts A, de Andrade PP, Okumu F, Quemada H, Savadogo M, Singh JA, James S. 2017. Results from the workshop "Problem Formulation for the Use of Gene Drive in Mosquitoes." *The American Journal of Tropical Medicine and Hygene* 96(3):530-533.

RStudio Team. 2020. RStudio: Integrated Development for R. RStudio Inc. Boston (MA). Available at http://www.rstudio.com.

Sánchez CHM, Wu SL, Bennett JB, Marshall JM. 2020a. MGDrivE: A modular simulation framework for the spread of gene drives through spatially explicit mosquito populations. *Methods in Ecology and Evolution* 11:229-239.

Sánchez CHM, Bennett JB, Wu SL, Marshall JM. 2020b. MGDrive: Mosquito Gene Drive Explorer. Package "MGDrivE". Available at https://CRAN.R-project.org/package=MGDrivE. Accessed 22 April 2020.

United States Fish and Wildlife Service (USFWS), 2019. South Farallon Islands Invasive House Mouse Eradication Project: Final Environmental Impact Statement. Available at https://www.fws.gov/uploadedFiles/South_Farallon_Island_%20Invasive_House_Mouse_Eradication_Project_Final%20EIS.pdf. Accessed 22 April 2020.

United States Fish and Wildlife Service (USFWS), 2019b. South Farallon Islands Invasive House Mouse Eradication Project – Final Environmental Impact Statement – Appendices.

Walters C. 1986. Adaptive Management of Renewable Resources. Caldwell (NJ): The Blackburn Press. 374 p.

Wheeler R, Priddel D, O'Dwyer T, Carlile N, Portelli D, Wilkinson I. 2019. Evaluating the susceptibility of invasive black rats (Rattus rattus) and house mice (Mus musculus) to brodifacoum as a prelude to rodent eradication on Lord Howe Island. *Biological Invasions* 21:833-845.

Webber BL, Raghu S, Edwards OR. 2015. Opinion: Is CRISPR-based gene drive a biocontrol silver bullet or global conservation threat? *PNAS* 112(34):10565-10567.

Woodberry O, Nicholson AE, Korb KB, Pollino CA. 2004. Parameterising Bayesian networks. In Webb GI, Xinghuo Y. (Eds.) *AI 2004: Advances in Artificial Intelligence: 17th Australian*

Joint Conference on Artificial Intelligence, Cairns, Australia. Springer Berlin Heidelberg pp 1101-1107.

SUPPLEMENTARY MATERIALS

- Netica file containing the SEFI Bayesian network model described in this work
- Source code for the modified version of the R package MGDrivE (MouseGD)
- R code for each of the 12 simulations run and input into the BN
- *.csv file output for each of the 12 MouseGD simulations run and used in BN
- R code used to generate the dose-response model in figure 3 and eq. 1 for *M. musculus* brodifacoum-induced mortality

*these files are available in the WWU CEDAR repository (will have to get the URL later)