

Ecosystem Vulnerability Analysis and Population Dynamics
Modelling of Gene Drive Releases for the case of
Drosophila suzukii

by

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Declaration of Authorship

I, **Carina Roberta Lalyer**, hereby declare that I am the sole author of this work; no assistance other than that permitted has been used and all quotes and concepts taken from unpublished sources, published literature or the internet have been identified with precise source citations.

Date

Signature

Abstract

Gene drive techniques are being developed in order to suppress populations or alter the organisms' properties. They are able to bypass the natural Mendelian law of heredity by increasing the chances of an allele to be inherited. Gene drives are supposed to be applied on wild populations and therefore represent a new stage in the release of genetically modified organisms.

To prospectively explore the impact of releasing gene drive organisms in wild populations, this study aims to identify the most important consequences at the ecosystem and species level. To assess this impact, the state of the science is reviewed and collated to develop a framework for an ecosystem vulnerability analysis. This analysis contains three main criteria: exposure, sensitivity and adaptive capacity. To prepare the basis for an event-based analysis of vulnerability, a hazard impact map for a suppression gene drive in *Drosophila suzukii*, an invasive fruit fly native to Southeast Asia, was created to visualize the spectrum of potential initial effects in an ecosystem. Finally, to further explore the case of *D. suzukii*, a stable population was modelled depending on temperature data of a native habitat of the fly in Japan. Moreover, a *Medea* gene drive was simulated in order to explore the invasiveness of the gene drive in the *D. suzukii*-population.

The work represents an early stage ecosystem vulnerability analysis for gene drives. It is concluded that there is a trend for high exposure potential at the different analyzed levels. Many knowledge gaps were already identified concerning the biology, ecology, and interactions of species but also uncertainties at the ecosystem level have been recognized that should be addressed in further investigations. According to the hazard impact map there is evidence for many potential cascading effects that have to be explored more in detail. According to the model results, the gene drive is efficient at spreading and the wild-type genotype is suppressed.

Key words: gene drives, ecosystem vulnerability analysis, hazards, population dynamics model, *Drosophila suzukii*

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Chapter 1 Introduction

Recent discoveries in gene drive techniques have put forward new ways to potentially suppress or modify natural populations of sexually reproducing organisms. This technology is currently debated in science and in the regulatory sphere concerning the methods and the ethics of harnessing it (Oye *et al.*, 2014, Webber *et al.*, 2015, Min *et al.*, 2017).

Deploying gene drive carrying organisms into the environment might have effects at different organizational levels, from species, communities to landscapes. Understanding the complex interactions between the numerous biotic and abiotic elements present in an ecosystem is highly important in order to be able to perform an assessment of the potential effects of releasing gene drive organisms into wild populations (David *et al.*, 2013, Hayes *et al.*, 2018).

Developing an ecosystem vulnerability analysis framework is one way to investigate and estimate the potential weaknesses of an ecosystem. This framework identifies and explores different characteristics at the species and ecosystem level that concern the exposure potential, sensitivity and adaptive capacity of an ecosystem facing a hazard. Creating hazard impact maps one can illustrate the interactions between the different characteristics and explore the potential hazards that can arise. Further on, developing system dynamics models, the interaction between certain elements can be better understood and analyzed.

Proper actions need to be taken for those ecosystems that prove to be vulnerable (Weißhuhn *et al.*, 2018). However, the complexity of the natural world and the lack of knowledge in certain areas, make it difficult to perform a thorough investigation at the ecosystem level.

Thus, the use of gene drives in controlling natural populations raises uncertainties when it comes to the potential negative effects that they might have at the ecosystem and species level. The current knowledge regarding ecosystems, species and gene drive technologies has many gaps that first need to be identified and filled. As a consequence, the precautionary principle (European Commission, 2000, Renn, 2008) concerning safe gene drive deployment is recommended to be invoked.

The following chapters give an overview on the state of the science on gene drives, ecosystem ecology and identifies several knowledge gaps while implementing an ecosystem vulnerability analysis on a case study. *Drosophila suzukii* (the spotted wing fruit fly) is used as an example. This insect is an emerging pest that originates from Southeast Asia and has been rapidly

expanding to the rest of the world (Asplen *et al.*, 2015). Females are able to damage ripening fruits of many berries and stone fruits causing crop damages (Cini *et al.*, 2012).

1.1. Problem statement

The recent developments of gene editing techniques may enable new methods for the control of populations. These tools could be used in the benefit of human health, agriculture and conservation efforts. However this raises questions about their implementation, ethics and the possible unwanted harmful effects on the environment.

1.2. Research question

Following a potential release of gene drive (GD) modified individuals of *Drosophila suzukii* that carry a suppression gene in Europe or North America, the possible ecological consequences are explored. Thus the main research question is:

- What are the potential consequences at the species and ecosystem level?

1.3. Research objectives

- The first objective of this thesis was to collate into one framework the available research done on ecosystem vulnerability analysis. The framework consists of three steps: exposure, sensitivity and adaptive capacity. The main characteristics of an ecosystem and of a species were identified for each step and then applied to the case study of *Drosophila suzukii*.
- The second objective was to identify the potential hazards that might arise from an initial release of a gene drive with a suppression drive. By creating a hazard impact map, the direct links between different levels and characteristics are shown after an initial stressor. Further on, this hazard impact map was applied to the case study.
- The third objective of the present work was to use a population dynamics model in order to evaluate the seasonal influences on a *Drosophila suzukii* population and the invasiveness of a *Medea* gene drive after the release of gene drive carrying males.

Chapter 2 Gene drive technology

2.1 Introduction

Gene drive technologies developed at a fast pace in the previous years (Min *et al.*, 2017). This led the scientific communities and regulators to have high profile conversations about if and how to harness the technology (Burt, 2003, Webber *et al.*, 2015, Esvelt and Gemmell, 2017).

Gene drives are molecular genome editing tools able to modify, insert and/or delete genes (Burt, 2003). The drive refers to its ability to bypass the normal Mendelian law of inheritance (Fig. 1) by increasing the chances of an allele to be inherited by the offspring with more than 50% thus, driving it into a population (Hammond *et al.*, 2016, Champer *et al.*, 2017) (Fig. 2).

The use of this technology could have the potential to benefit human health, agriculture and conservation efforts (Gantz *et al.*, 2015, Champer *et al.*, 2016, Baltzegar *et al.*, 2018). However, deploying such organisms into wild populations must ensure avoidance of unwanted harmful effects on the environment (Esvelt and Gemmell, 2017).

In this chapter some of the available gene drive techniques will be reviewed, their mechanism and their potential applications will be explained.

2.2.Natural drives

Natural selfish genetic elements have been discovered in both prokaryotes and eukaryotes (Werren, 2011). For example, transposable elements are now known to be the most abundant element in the eukaryotic genome, constituting approximately 50% of the human genome, more than 70% of the genome of some grass species (Wessler, 2006) and 15% of the *Drosophila melanogaster* genome (Biemont and Cizeron, 1999 cited in Hurst and Werren, 2001). These selfish genetic elements can be found in unique sites that are conserved in the genome or in multiple locations throughout the genome (Hurst and Werren, 2001).

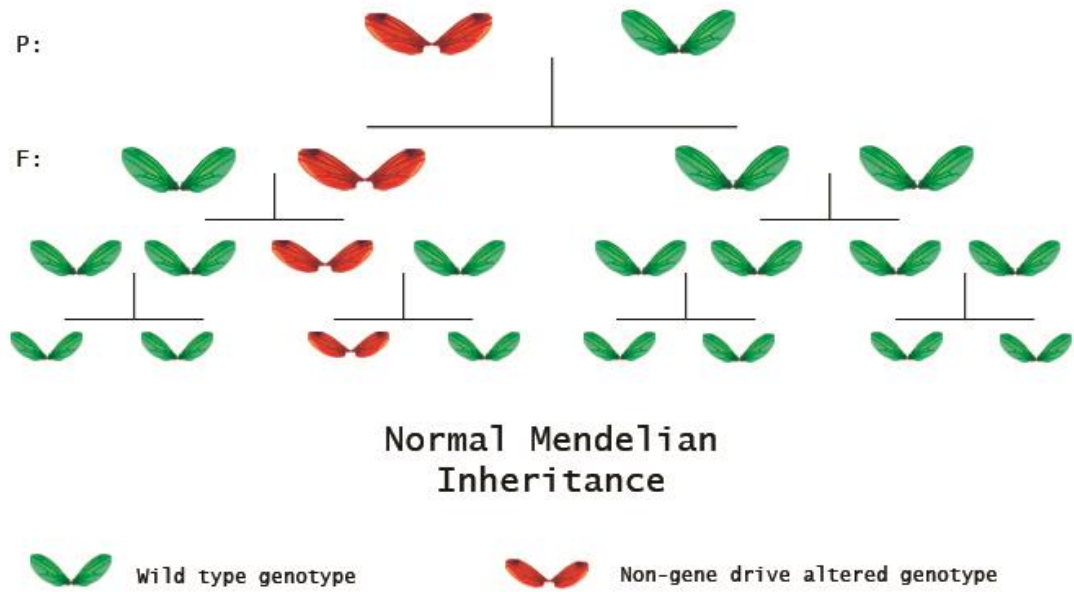


Figure 1. Illustration of a Mendelian inheritance, the offspring (F) have a 50% chance to inherit a copy of the gene from the parents (P)

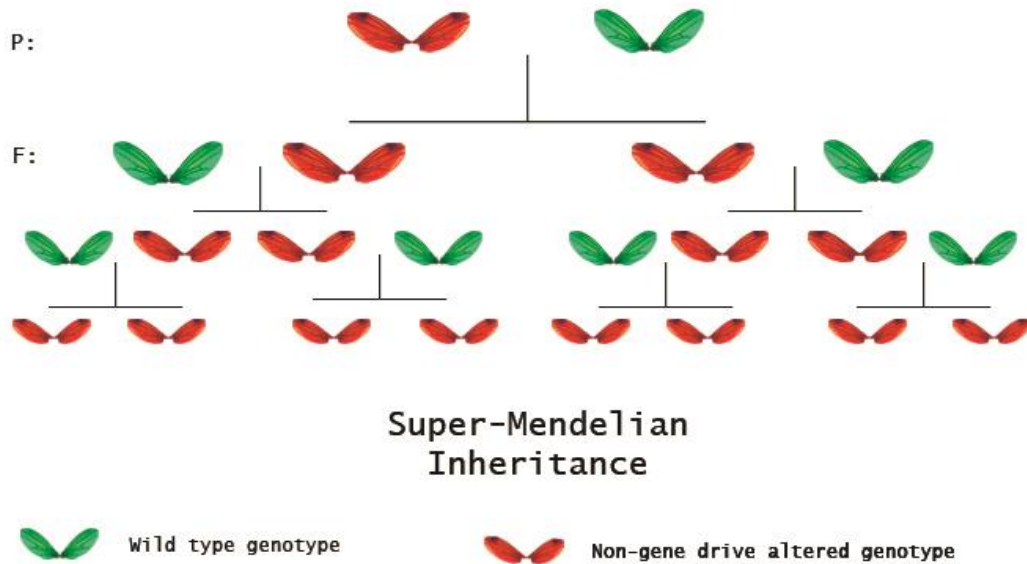


Figure 2: Illustration of super-Mendelian inheritance where the offspring (F) have a 100% chance to inherit a copy of the gene from the parents (P)

Following Hurst and Werren's (2001) classification of natural selfish elements, the next examples are organized by the respective mechanism with which they spread through the population

a. Autonomous selfish elements

These elements include plasmids, endoviruses and transposable elements (Werren, 2011). For example, the latter can spread through a population even if they cause harmful mutations in the genome in most of the cases (Hickey 1982 cited in Hurst and Werren, 2001). This is due to the ability to copy and move themselves in the genome, giving their ability to accumulate (Doolittle and Sapienza, 1980, Orgel and Crick, 1980 cited in Werren, 2011). Subsequently, transposable elements are able to spread through a population from initially low frequencies (even if they impose a fitness cost) because they increase their probability to be inherited (Marshall and Akbari, 2016). These elements are capable of lateral transfers between species (Bartolomé *et al.*, 2009 cited in Werren, 2011) or even taxons (Werren, 2011). The most known example of a transposable element crossing species, is the P element in *Drosophila* where almost all of the global population of *D. melanogaster* inherited the P element from *D. willistoni* within a few decades (Preston and Engels, 1989 cited in Marshall and Akbari, 2016). When wild type males of *D. melanogaster* that contain the P element are mated with a laboratory strain of females *D. melanogaster* that lack the P element, the offspring can display different types of harmful phenotypical traits such as sterility, chromosomal aberrations and high frequencies of mutations. (Griffiths *et al.*, 1999).

b. Biased gene converters

This category includes homing endonucleases which preferentially insert themselves onto the homologous site in the genome (Werren, 2011). They use the DNA's natural repair mechanism called homologous recombination in order to insert themselves onto the homologous chromosome at the specific site where the endonuclease cuts the DNA double-strand (Hurst and Werren, 2001). This type of selfish element will often spread to fixation in a population, as it is inherited in almost all meiotic products (gametes) (*ibid.*). Homing endonucleases have the ability to avoid host resistance by inserting themselves

into important loci of the genome responsible for the survival of the embryo (Werren, 2011).

c. Meiotic drivers

This class of drivers contains certain alleles that increase their frequency during meiosis to be transmitted to functional gametes. These types of selfish elements include the t-locus in mice or B chromosomes in animals and plants. (Hurst and Werren, 2001, Werren, 2011) B chromosomes are non-essential to the function of organisms and are the first natural selfish genes to be called “genomic parasites” by Östergren (1945 cited in Werren, 2011). The t-complex in mice (*Mus* genus) was discovered almost a century ago, in 1927 (Dobrovolskaya-Zavadskaya and Kobozieff, 1927 cited in Safronova and Chubykin, 2013). Only heterozygous males exhibit non-Mendelian inheritance (up to 90% in laboratory grown mice) (Safronova and Chubykin, 2013), thought to be a result of the flagellar dysfunction of the wild-type sperms that results from heterozygous males (Silver, 1985 cited in Carroll *et al.*, 2004). The offspring show different effects such as sterility, sex biasing in favour of males, and male aggressive tendencies (Carroll *et al.*, 2004, Safronova and Chubykin, 2013).

d. Post-segregation distorters

It refers to those selfish elements that act after embryogenesis. They reduce the survival/fitness of the embryo/offspring who do not carry the drive element (Hurst and Werren, 2001, Werren, 2011). This kind of system uses a “modification-rescue”/ toxin-antidote mechanism: the parent has a modification that has to be rescued in the progeny, if the progeny is missing the selfish element, then the rescue cannot occur and the offspring will be negatively affected (Werren, 2011). One example is the *Medea* locus discovered in the flour beetle (*Tribolium castaneum*) which is described as being a maternal effect allele that kills the offspring that do not inherit the allele (Beeman *et al.*, 1992 cited in Hurst and Werren, 2001).

e. Heritable organelle and Microbes

This class contains nuclear genes, cytoplasmic organelle or even prokaryotes or viruses (Hurst and Werren, 2001, Werren, 2011). According to the endosymbiosis theory, mitochondria and plastids were prokaryotes that were engulfed in the eukaryotic cells and became symbionts (Alberts *et al.*, 2002). Cytoplasmic elements are transmitted through the egg cell's cytoplasm, thus they are transmitted maternally (with some exceptions) (Werren, 2011). Nuclear elements can be transmitted by both sexes (*ibid.*). Heritable microorganisms and organelles are selfish elements that have “genetic interests” (Werren, 2011) to be allocated to one sex or the other (Hurst and Werren, 2001, Werren, 2011). They can be either beneficial to their host or harmful because they increase their probability of being transmitted by manipulating the host's reproduction (Werren, 2011). This manipulation entitles conversion of males to functional females, induction of parthenogenesis, male-killing or cytoplasmic incompatibility (Werren, 2011). For example, cytoplasmic incompatibility (CI) is induced by the *Wolbachia sp.* bacteria in many insects (20-75%) (Hurst and Werren, 2001). *Wolbachia sp.* Are present in up to 70% of the natural populations of arthropods (Kremer *et al.*, 2009 cited in Werren, 2011) and even in *Drosophila suzukii* (Tochen *et al.*, 2014, Cattell *et al.*, 2016). CI involves a “killer-rescue mechanism” where the offspring that do not inherit the element will perish (Hurst and Werren, 2001) The *Wolbachia* infected males have their sperm modified so it produces sperm-egg incompatibility if the egg cell does not contain the same strain of *Wolbachia*. This incompatibility results in the death of the offspring. (Hurst and Werren, 2001, Werren, 2011)

As shown in the short review of natural selfish genes, they can often be found in natural populations. The selfish elements and their hosts have co-evolved to have either mutualistic or parasitic interactions and as a response, the hosts evolved defense mechanisms against them (DNA methylation, different regulatory pathways etc.) (Werren, 2011). Their discoveries have inspired scientists to create synthetic gene drives in an attempt to use them to control natural populations.

2.3.Synthetic gene drives

Synthetically engineered gene drives are based on natural selfish genetic elements, as shown in the previous paragraphs. They have the ability to be inherited over generational time at increased frequencies despite lowering the organisms' fitness (Champer *et al.*, 2016). Starting with the 1940s, scientists suggested to control agricultural insect pests or insect disease vectors with the use of translocations and transposable elements (Serebrovskii, 1940, Curtis, 1968, Kidwell and Ribeiro, 1992 cited in Marshall and Akbari, 2016). Currently, with the advances of molecular biology and synthetic biology, engineering gene drives has become a reality. So far, the scientific efforts have created gene drives that are capable of spreading into wild populations for a few species of yeasts (*Saccharomyces cerevisiae*), fruit flies (*Drosophila melanogaster* and *Drosophila suzukii*) and species of mosquitoes from the *Anopheles* and *Aedes* genus (Champer *et al.*, 2016, Li *et al.*, 2017, Buchman *et al.*, 2018).

Gene drives are classified into two main categories depending on the effects that they have on the organisms and populations as a whole: modification drives that have the purpose to transmit a desired gene throughout a population and suppression drives that have the goal to suppress or eradicate a population. The two main mechanisms of GDs are either *homing* which, reliant on homology directed repair, is copying themselves onto the homologous chromosome creating homozygotes or lowering the survival or fitness of the offspring that do not inherit the gene drive complex. (Champer *et al.*, 2016) Thresholds are also an important characteristic of the gene drive. They determine the minimum ratio of organisms carrying a gene drive in the population which is necessary for the propagation of the drive and whether they would reach fixation or not (Champer *et al.*, 2016, Min *et al.*, 2018). GDs that spread fast and require low initial releases are homing-based drives (threshold-independent) while the ones that require a high threshold are those that are based on the underdominance or toxin-antidote systems (threshold-dependent) (Akbari *et al.*, 2013, Champer *et al.*, 2016, Min *et al.*, 2018).

The following section is meant as a short overview of some of the different gene drive mechanisms divided by their mechanism of action and according to the descriptions and characterizations of Champer *et al.* (2016) and Marshall and Akbari (2016).

a. *Homing-based drives*

This class of synthetic gene drives was first proposed to be used in natural populations, in 2003 by Burt (Burt, 2003). Homing endonuclease genes (HEGs) that are located on

one allele have the ability to convert their homologous allele by using the endonuclease to cut the other chromosome at a specific site inducing a double-strand break (Deredec *et al.*, 2008). Once this break is created, one of several repairing mechanisms can be activated, one of which called homology direct repair (HDR) (Champer *et al.*, 2016). Through HDR, the chromosome with the HEG allele is used as a template to copy the missing sequence, thus the HEG is copied into the allele (Stoddard, 2011 cited in Champer *et al.*, 2016). This complex of endonuclease can be accompanied by an engineered payload gene, desired to be spread in the population (Esvelt *et al.*, 2014, Champer *et al.*, 2016). If the HDR occurs in the germline of the organism, then its offspring have a super-Mendelian probability (higher than 50%) to inherit the desired gene (Champer *et al.*, 2016). HEGs can be used to either modify or suppress a population by inducing sterility, sex ratio biasing, reducing lifespan, decreasing fitness etc. In the case of suppression, the offspring that are homozygous by inheriting one allele from each parent would be the ones that are affected due a disruption that leads to recessive lethality or sterility (Burt, 2003, Deredec *et al.*, 2008 cited in Champer *et al.*, 2016). The most promising *homing-based drive* relies on CRISPR/Cas9, discovered in the adaptive immune process of bacteria in the CRISPR (clustered, regularly, interspaced short palindromic repeats) locus (Doudna and Charpentier, 2014). It works by having the Cas9, an endonuclease that is guided by gRNAs to cut at a specific sequence (Doudna and Charpentier, 2014, Marshall and Akbari, 2016, Min *et al.*, 2017).

b. Sex linked meiotic drives

They were proposed starting in the 1960s for distorting the sex ration of *Aedes aegypti* (Hickey and Craig, 1960 cited in Champer *et al.*, 2016). These distortions are made by preventing one sex or the other to mature by preventing the maturation of the gametes that lack the meiotic drive (ibid.). For example the *X-shredder* technique consists of an endonuclease that targets and cuts the X chromosome during spermatogenesis. In this way, only the Y chromosomes are passed on and it ensures that only males are being born. Over time, without females to mate with, the population collapses. This technique has been developed already in the *Anopholes gambiae* mosquito by Windbichler et al. (Windbichler *et al.*, 2008, 2011).

c. *Medea* (maternal effect dominant embryonic arrest)

Medea is a toxin-antidote-engineered mechanism similar to the one that was discovered to naturally occur in the flour beetle (Beeman *et al.*, 1992, Champer *et al.*, 2016). The synthetic *Medea* consists of a microRNA which acts as the toxin (Champer *et al.*, 2016). This is expressed during oogenesis in the *Medea* bearing females and it prevents all of the embryos to survive no matter if the embryos inherited the wild-type or *Medea*-allele (ibid.). This is because the microRNA is inherited in all of the gametes produced, as it exists in the cytoplasm (ibid.), generating a pre-toxic state (Akbari *et al.*, 2014). The second part of the *Medea* system is a tightly linked antidote (a transgene) which is a version of the toxin targeted gene but which is immune to the toxin (ibid.), thus it acts as the zygotic rescue (Akbari *et al.*, 2014). The offspring that do not inherit the *Medea*-allele from the *Medea*-bearing mother will die (Akbari *et al.*, 2014). In this way, there will be an increase in frequency of the *Medea* drive (ibid.). The embryos that do not inherit the antidote (50% from a heterozygote female crossed with a wild-type male and 25% from a heterozygote female crossed with a heterozygous male, shown in red in Fig. 3) will fail to develop (ibid.). The *Medea* synthetic drive has so far been developed for *Drosophila melanogaster* (Chen *et al.*, 2007, Akbari *et al.*, 2014 cited in Champer *et al.*, 2016) and *Drosophila suzukii* (Buchman *et al.*, 2018). Payload genes can be inserted in the system thus being rapidly driven into a wild population (Champer *et al.*, 2016).

Parent		Male					
Female	Genotypes	+/+		M/+		M/M	
	+/+	+/+	+/+	+/M	+/+	+/M	+/M
		+/+	+/+	+/M	+/+	+/M	+/M
	M/+	M/+	M/+	M/M	M/+	M/M	M/M
		+/+	+/+	+/M	+/+	+/M	+/M
	M/M	M/+	M/+	M/M	M/+	M/M	M/M
		M/+	M/+	M/M	M/+	M/M	M/M

Figure 3. *Medea* inheritance.

+/+ Wild type; M/+ heterozygous *Medea* carriers; M/M homozygous *Medea* carriers. The genotypes highlighted in red do not survive due to the lack of inheritance of the *Medea* drive

d. Underdominance gene drives

These rely on the fact that heterozygotes and their progeny have a lower fitness than parental homozygotes (Champer *et al.*, 2016). Balanced reciprocal chromosomal translocations is an example for underdominance because half of the offspring of translocation-bearing heterozygotes die due to unbalanced gene sets (*ibid.*). So far it has been developed in *Drosophila melanogaster* (Akbari *et al.*, 2013, Reeves *et al.*, 2014 cited in Champer *et al.*, 2016) and can be used for population suppression and modification (Champer *et al.*, 2016). Champer *et al.* (2016) argue that this system can be linked to an RNA guided endonuclease to make the translocations happen at specific sites in the genome.

2.4.Design criteria

All synthetic gene drives are designed or desired to have certain characteristics that would ensure their efficacy and contain unwanted ecological effects (Champer *et al.*, 2016, Marshall and Akbari, 2016). Their efficacy depends on a number of traits like the ability to compensate for the loss of fitness in the host organism (*ibid.*). Due to the fact that the drives carry large genes and associated regulatory elements, they impose an intrinsic fitness cost on the organism (Braig and Yan, 2001 cited in Marshall and Akbari, 2016). Despite of this cost, they have to be able to spread throughout the population in a relatively short time that is meaningful for controlling/managing the organism's population (Braig and Yan, 2001 cited in Marshall and Akbari, 2016). Another characteristic is that the design has to take into account evolutionary stability (Akbari *et al.*, 2013, Esvelt *et al.*, 2014, Champer *et al.*, 2016, Braig and Yan, 2001 cited in Marshall and Akbari, 2016). Concerning HEG-drives, natural populations would develop drive resistant alleles due to 1) non-homologous end joining (NHEJ), a DNA repairing mechanism that can be triggered when there is a cut in the chromosomes, 2) polymorphism in the genome sequence or 3) naturally evolved resistance to inhibit the drive (Esvelt *et al.*, 2014). In a recent study developing a *Medea* drive in *Drosophila suzukii*, Buchman *et al.* (2018) discovered a 78% frequency in resistant allele in the initial population. For example, one way to avoid the rise of resistance in RNA-guided GDs is to target multiple sites in the genome (Esvelt *et al.*, 2014) or to target highly conserved sequences in the organism (Champer *et al.*, 2016).

To avoid unwanted effects of the GD on the ecosystem, the design must ensure containment to the targeted species or even targeted population (Champer et al., 2016). This can be done by using “precision drives” that target unique sequences that are found only in the targeted population (Min et al., 2018) or by using a version of homing endonuclease drives in the form of daisy-chains with multiple but interdependent drive elements that will get lost over time with every new generation and thus limiting the spread of the drive (Noble et al., 2016). Reversibility is another key design characteristic which can be called for in the case of unwanted effects on the ecosystem or due to public concerns or protest (Esvelt et al., 2014, Champer et al., 2016). Champer et al. (2016) propose that this could be done by either removing the GD entirely from the population or modifying it to a neutral configuration, although, very important to note is that the wild type genotype could never be restored. . Reversibility is thus a rather misleading term because it suggests that the impact of a gene drive release could be reversed.

2.5. Gene drive applications

Marshall and Akbari (2016, p. 194) stated: “As a technology capable of engineering or eliminating entire species, the development of gene drive systems carries with it both great promise and great responsibility”. As stated in the beginning of this chapter, GD can be used for combating vector-borne diseases, invasive species, agricultural pests or be used for conservation purposes by introducing beneficial genes into threatened populations. The next section will give an overview of the potential applications of gene drives and the current state-of-the-art.

a. Public health

Diseases such as malaria, dengue, yellow fever or Zika could be mitigated by developing gene drives that target the vector species that spread them (e.g. mosquitoes) by either immunizing the vectors against the parasite, blocking its transmission or directly by suppressing or eradicating the vectors (Min *et al.*, 2017). Hammond et al. (2016, 2017) designed a CRISPR/Cas9 system that causes sterility in *Anopheles gambiae* mosquitoes in caged experiments. The transmission rate of the gene drive to the progeny was 91.4% to

99.6%, reaching a frequency of 72-77% after G₄ (Hammond *et al.*, 2016, 2017). The starting population was 600 individuals that were replicated twice, and the sex ratio was 1:1 (*ibid.*). Even if the GD spread rapidly in the population, resistant alleles were formed in such a way that by G₂₅ the frequency of the GD was less than 20% (Hammond *et al.*, 2017). In a previous study, Gantz *et al.* (2015) successfully established a CRISPR/Cas9 gene drive in the Asian mosquito *Anopheles stephensi* in a caged population that started with 680 individuals. The drive targeted eye-color phenotypes that were tightly linked to an anti-pathogen effector. In G₃, it had a frequency of up to 99.5% but the organisms developed resistances which, according to the authors might have been due to NHEJ (Gantz *et al.*, 2015).

b. Agriculture

Arthropod pests were estimated to cause a \$470 billion damage to global agriculture (Culliney, 2014 cited in Scott *et al.*, 2018). Pest management in agriculture mainly relies on the use of pesticides, herbicides, tilling etc., (Min *et al.*, 2017) which can cause many harmful effects (Hallmann *et al.*, 2017, Tsvetkov *et al.*, 2017), while the targeted organisms are capable of developing pesticide-resistance in time (Scott *et al.*, 2017). Gene drives can be designed to sensitize a certain pest and make them vulnerable to certain compounds that are otherwise harmless to the environment or humans (Min *et al.*, 2017). Another way would be to alter the organisms so they no longer consume crops or directly remove the entire population from the location (*ibid.*). In 2018, Buchman *et al.* demonstrated a *Medea* system in *Drosophila suzukii*. Using the system, they proposed to drive a conditionally lethal cargo gene into the population with *Medea* that can only be activated by an environmental cue like a gene expressed under a diapause-promoter (Akbari *et al.*, 2014) or the use of certain chemicals like tetracycline with which was experimented in sterile insect techniques (Schetelig *et al.*, 2016).

c. Conservation

Invasive species are thought to be an important factor in the extinction of many species (Scalera, 2010). Doherty *et al.* (2016) through their meta-analysis study concluded that

58% of the contemporary mammal, bird and reptile species extinctions are due to invasive predators. These extinctions are due to predation (Doherty et al., 2015 cited in Doherty et al., 2016), competition (Harris and Macdonald, 2007 cited in Doherty et al., 2016, Bonesi and Palazon, 2007), disease transmission (Wyatt et al., 2008 cited in Doherty et al., 2016) or facilitation with other invasive species (Simberloff, 2011 cited in Doherty et al., 2016) but also due to synergetic effects (Brook et al., 2008).. In a 2010 study, Scalera, indicated that in the last 15 years prior to 2009, the European Commission has spent €132 million for the management of invasive alien species. Thus, it is undeniable that the invasion of alien species is one of the most pressing problems in conservation.

Esvelt et al. (2014) proposed to use gene drives for the management of invasive species in the scope for conservation efforts but, in a 2017 publication, Esvelt and Gemmell (2017) retract the use of GDs for conservation purposes reasoning that GDs can be highly invasive and can lead to a global spread, potentially eradicating an entire species.

Further on, Piaggio et al. (2017) suggest that due to the increasing loss of biodiversity, it is time to explore other options to conserve biodiversity, such as synthetic biology and gene drives. New Zealand in 2016 declared their plan to eliminate all species of rats, possums and stoats that were brought to the islands by human migration and threaten the native bird and reptile species (Predator Free 2050 Limited, no date, Esvelt and Gemmell, 2017). In their plan, they are also taking into consideration the use of gene drives to achieve their goals (ibid.) but Dearden et al. (2018) warn that more research needs to be undertaken and public acceptance achieved before using gene drive technologies to control pests in New Zealand. Leitschuh et al. (2017) propose the use of GDs to combat invasive rodents on islands, they argue it would have the potential to be species-specific, to be more humane than poisons and also safer for humans.

d. Research

As stated in the beginning of this chapter, GDs have the possibility to insert or delete genes in the genome. With the discovery of gene drives, especially CRISPR, molecular research and synthetic biology can explore new frontiers. For example, in a study published in *Nature Communications*, an antidote for the highly venomous Box jellyfish (*Chironex fleckeri*) was discovered using CRISPR (Lau et al., 2019). In the

study, the research group used CRISPR to determine which components in the cell are affected by the venom, by functionally isolating them through genome-scale loss of function screening (ibid.). In another study, the same technique (genome-wide CRISPR screen) was used to identify HIV host dependency factors (Park *et al.*, 2017). CRISPR/Cas can be used for novel discoveries at the molecular level due to its ability to precisely cut the DNA at specific locations and to induce gene knockouts/knockins.

2.6. Gene drive limitations and difficulties

Synthetic gene drives are meant to spread within a population at a fast pace. Therefore, they can only be designed for organisms that exhibit certain features.

a. Sexual reproduction

Gene drives bias inheritance and require to be transmitted through sexual reproduction (Moro *et al.*, 2018). Organisms that mainly reproduce asexually, self-fertilize or inbreed at high frequencies are anticipated to quickly develop resistance to GDs. In this category viruses and such organisms like bacteria, yeasts, nematodes and many species of plants are included (Min *et al.*, 2018). The reproductive traits such as short generation time, high fecundity, large number of offspring, minimal mate selection are important for the rapid spread of the GD construct (Moro *et al.*, 2018). As a consequence, insects and rodents are animal models for developing GDs. Developing for and releasing GDs in slow reproducing organisms would likely not tackle the relevant issues in an appropriate time frame.

b. Evolutionary stability

As previously discussed, organisms can and will develop resistance against the GD construct. Bull (2015) suggests that evolutionary resistance would rise accordingly to the GD mechanism, thus it depends on the respective GD mechanism. One way to overcome resistance-alleles in *homing* based gene drives is to target the genome at multiple sites (Min *et al.*, 2017).

c. Genome sequencing, technology development and organism rearing

In order to realize a GD, firstly it is important to sequence the target organisms' genome for the specific gene sequences (Moro *et al.*, 2018). Moro *et al.* (2018) exemplify that genes

responsible for female embryo development or male sex determination are highly important in order to be able to develop a GD that would bias the sex ratio. Deploying a GD requires a high number of individuals that have to be genetically modified and released into natural populations. Thus, breeding these organisms and maintaining the colonies can prove difficult (Moro *et al.*, 2018). Although, there are facilities that breed the Mediterranean fruit fly for the sterile insect technique (Hendrichs and Robinson, 2009), breeding rodents at such a scale can prove difficult.

d. Containment

There are two types of containment for gene drives; first in the laboratory to make sure that the organisms cannot escape their confinement and secondly, once they are deployed, to ensure that the GD would stay in the targeted species and population (Min *et al.*, 2018). Marshall and Hay (2011) developed stochastic and deterministic models for a comparative analysis of the confinements of different gene drives. Akbari *et al.* (2015) and others (Mary Ann Liebert Inc., 2008, Heitman *et al.*, 2016, van der Vlugt *et al.*, 2018) proposed several ways to confine the gene drive in the laboratory. The following indications for gene drive confinement are given in Akbari *et al.* (2015) and Min *et al.* (2017):

- through physical barriers which the organism cannot pass,
- molecular confinement where the gene drive is separated in its components,
- ecological confinement such that in the case of an escape, the organisms cannot survive or find suitable mates in nature is confronted with conditions that prevent survival
- reproductive confinement when the organisms cannot reproduce with a wild-type.

e. Possible ecological consequences

There can be numerous ecological effects as a consequence to releasing gene drive organisms into the wild. In the next chapters (4 to 6) this issue will be tackled and further explored with a case study in Chapter 7.

Chapter 3 Ecosystem ecology

3.1. Introduction

Ecosystems are formed by multiplexed interactions between numerous biotic and abiotic elements also termed as pools (Chapin et al. 2011, p. 4) and they determine and regulate the biogeochemical processes of the planet (Loreau et al. 2001). These processes, also known as fluxes or flows between different pools, are influenced by many environmental qualities such as temperature or by population dynamics and community interactions (Chapin et al. 2011, pp. 4-5).

Ecosystem ecology studies these links between organisms and their physical environment as an integrated system at a planetary level. Understanding the complex interactions of the system's physical and biological processes improves our knowledge as to how and why certain effects and responses exist and how they regulate the environment, leading to a better sustainable management and use of the resources. (Chapin et al. 2011, p. 3)

Ecosystems' dynamics are influenced by external and internal factors. Stability is maintained through internal feedback mechanisms which denotes the system's resistance to perturbations (Oliver et al., 2015). Mitchell et al., (2000) argue that stability is maintained by the ecosystem resilience and resistance (Webster et al., 1975 and Leps et al., 1982 in Mitchell et al., 2000). The steady state of an ecosystems is defined by a balance between the input and output of the systems that have no change over time (Bormann and Likens, 1979 in Chapin et al., 2011). However, the steady state theory accepts that there are natural temporal and spatial variations within the ecosystem's dynamics (Chapin et al., 2011). Although it has been studied intensively in the past decades and debated (Ives and Carpenter, 2017, Mitchell et al., 2000), stability of ecosystems is still not yet fully understood (Grman et al, 2010). But, Mitchell et al., (2000) propose that stability depends on the scale and human spatial and temporal scales influence what is perceived to be stable.

3.2. Alternative stable states

Alternative stable states have been proposed for the first time in the late 1960s by (Lewontin 1969 cited in Beisner *et al.*, 2003) in reference to communities of organisms (Beisner *et al.*, 2003). It has been proposed that there is not one stable equilibrium in which the ecosystem

can be (Chapin et al., 2011, p.7) but rather that systems may have alternative stable states caused by abrupt shifts (Oliver et al., 2018) determined by large disturbances (Beisner et al., 2003).

According to Beisner et al. (2003), the concept of alternative stable states is being used in ecology in two ways: first, it refers to stability in population ecology (Lewontin 1969 and Sutherland 1974 in Beisner et al. 2003). In population ecology, the environment is in a fixed state where the biotic community has “different stable configurations”. Secondly, the ecosystem perspective focuses on the effects of environmental change (May, 1977 in Beisner et al. 2003). The variables and characteristics of the communities or ecosystems will persist in different possible arrangements, contributing to an alternate stable state (Beisner et al. 2003). The scientific community still debates when a different state can be considered alternative but it is agreed that identification of critical variables and how they are affected require a thorough understanding of species interactions and feedbacks between the biotic and abiotic elements of the ecosystem (Beisner et al. 2003).

3.3. Disturbance

Disturbances are a natural occurrence in nature and historically, ecosystems have always gone through environmental changes. However, human disturbances increasingly alter ecosystems’ dynamic interactions at an unprecedented rate and intensity (Oliver et al., 2015, Ives and Carpenter, 2017, Peterson et al. 1998). Disturbance is defined by White and Pickett (1985, in Chapin et al. 2011) as an event in time and space that modifies the structure of ecosystems, communities, populations and causes transformations in the physical environment or to resources (Chapin et al., 2011). The impact of a disturbance is determined by its severity, type and how sensitive the ecosystem is.

Ecosystem sensitivity depends on its properties and the time of the event occurrence. Species’ characteristics that ensure survival of a severe event are the ones that will dictate the new trajectory of the ecosystem.

However, when the disturbance is too large and the biotic and environmental conditions surpass the ecosystem’s resilience, then it is likely to shift the system into an alternative state. This threshold between alternative states is exceeded once the adaptive range is surpassed due to the alteration or loss of the different factors that maintain resilience: diversity, plasticity and

buffering capacity of ecosystems (Chapin et al., 2011). The change can lead to new dynamics and communities that may develop a new resilience. This change can occur even though there were efforts made for the initial stressor to be removed (Williams and Jackson, 2007, Chapin et al., 2011).

According to Ellis and Ramankutty (2008, in Chapin et al. 2011, p. 321), humans have altered approximately 75% of the ice-free surface of Earth through deforestation, land use change, species introductions or extinctions, ecosystem management etc. (Foley et al., 2005, Chapin et al., 2011). It is still unclear to what extent anthropogenic disturbances threaten stability (Grman et al., 2010).

Recent advances in gene drive systems opened new possibilities for the scientific community, governments and interested parties to address problems in the agricultural, conservation and public health sector, albeit it is at a highly debatable phase. Gene drives can be used to transform, suppress or even eliminate specific species (Meghani and Kuzma, 2017) that a) act as disease vectors, b) reduce biodiversity or c) have become agricultural pests. Current methods to control organisms require continuous applications and frequently lead to a short term suppression of the population (Moro *et al.*, 2018). Currently, one of the biggest threats to biodiversity is the establishment of invasive species (Scalera, 2010). However, using gene drives in wild populations requires important considerations because the impact of the use of this new technology is uncertain, can lead to GD invasiveness and/or disturbances in the ecosystem (David *et al.*, 2013, Esvelt and Gemmell, 2017, Moro *et al.*, 2018).

According to (Chapin *et al.*, 2011) ecosystems' behaviour is influenced by current and past environmental fluctuations and disturbances. The history of ecosystems is important because their properties change in response to changes already imposed on them, deeming ecosystems to be adaptive systems. Bengtsson *et al.*, (2003) propose that in order for a system to remain stable, the network of species, their interactions with the environment and the interspecific ones, as well as the structural reorganization of the system after a disturbance constitute the "ecological memory" of the system which is an important aspect of resilience (Thompson et al. cited in Bengtsson *et al.*, 2003). The ecological memory of the system as conceptualized by Bengtsson *et al.* (2003) is linked to the concept of "biodiversity as insurance" further explained in the section "Biodiversity and stability". (Chapin *et al.*, 2011) propose that "the most urgent need in ecosystem ecology is to understand resilience and change in ecological systems".

3.4. Resilience

Through resilience, ecosystems maintain relatively stable functionality over long periods of time despite fluctuations in the environment. Holling (1973, p.17) introduced resilience in ecosystem theory as the capacity to “absorb changes of state variables, driving variables, and parameters, and still persist.” (Holling 1973, 17). Thereby, resilience determines the persistence of systems – or their extinction. According to Thrush et al. (2009), resilience is the potential for recovery from disturbance (Pimm, 1991 cited in Thrush et al. 2009). This definition of resilience is also known as engineering resilience. An indicator for engineering resilience is the “duration of the recovery phase” (Weißhuhn *et al.*, 2018). Mitchell et al., (2000) state that when its resilience is high an ecosystem returns faster to equilibrium after a perturbation. The second definition is that of the ecological resilience and means a variable of the ecosystem that can move “within and between stability domains” (Ludwig *et al.*, 1997, Gunderson, 2000 cited in Thrush *et al.*, 2009) or according to De Lange et al., (2010) ecological resilience is a “measure of resistance to disturbances and the speed with which the system returns to the equilibrated stable state”. According to Thrush et al. (2009), engineering resilience can be used to measure resilience empirically, while ecological resilience requires measurement over a long time period. Besides the differentiation of engineering and ecological resilience, a transition in the notion of resilience occurred in that it was once focused on the conservation of structure integrity and is now also considering reorganization of the affected system (Oliver *et al.*, 2015).

Particularly, ecosystems are resilient to regimes of natural variations like daily, seasonal or annual cycles and to extreme events that occurred already throughout their history. In this way organisms have adapted by their evolutionary history. Positive and negative feedbacks are what maintain the internal dynamics of an ecosystem (Hanski et al. 2001, Chapin et al., 2011). Negative feedbacks stabilize the system and confer resilience (Chapin et al. 2011). High genetic or species diversity and high plasticity enable resilience because the functions of the ecosystem are supported through the organisms (Chapin *et al.*, 2011, p. 342).

Another concept related to ecosystem stability, namely “resistance” is defined by the ability of the system to avoid a shift altogether following a disturbance (Leps et al. 1982 in Mitchell et al., 2000) but Holling (1973 cited in Mitchell *et al.*, 2000) refers to this ability as resilience.

3.5. Biodiversity and stability

The dynamics of the ecosystems depends on the traits of organisms; their evolutionary histories and interactions in the community (Chapin *et al.*, 2011). Therefore it is important to understand the role of organisms in their community. Recently, the role of biodiversity in ecosystem functioning is gaining popularity and appreciation (Diaz *et al.*, 2006 in Chapin *et al.* 2011, p. 3).

The “ubiquitous occurrence of species interactions” that strongly affect ecosystem processes is a feature of ecosystem functioning (Chapin *et al.*, 2000 in Chapin *et al.* 2011). Biodiversity is considered to be the “biological diversity in a system, taking into account the genetic, species diversity and their functional roles but also ecosystem diversity in a landscape” (Chapin *et al.* 2011). Functional traits represent characteristics that allow a species to survive and reproduce and they impact their fitness. The loss or gain of species within a system can alter ecosystem processes due to the change in the species’ functional traits that have large impacts on the system, namely effects on supplies or limiting resources, microclimate, intraspecific or interspecific interactions and effect on disturbance regimes (*ibid.*).

In 1996, Johnson *et al.* (cited in Mitchell *et al.*, 2000) collected four theories regarding the role of species in ecosystem stability. First, the diversity-stability hypothesis suggests that species diversity is directly proportional to ecosystem productivity and resilience (MacArthur, 1955). Then, in 1981 (Ehrlich and Ehrlich, 1981 in Mitchell *et al.* 2000) proposed the rivet hypothesis which puts forward the idea that an ecosystem functions normally as long as the loss of species is not too severe and that there is no loss of critical species, also known as keystone species (Paine, 1969 cited in Bond 1994). The redundancy hypothesis suggests that resilience is maintained by the ability of the species to compensate through their functional role in case species are lost (Walker 1992 cited in Mitchell *et al.*, 2000). Peterson *et al.* (1998) added to this hypothesis suggesting that resilience depends on the functional groups, thus the ecological role of species. Lastly, Lawton (1994 cited in (Mitchell *et al.*, 2000) postulates that the ecosystem is too complex and it is unpredictable to determine the magnitude and direction of the change, even if there is evidence that its function changes with diversity (termed as the idiosyncratic hypothesis).

It is thought that the more species there are in a system, the wider will be the range of conditions under which ecosystem processes can be maintained at their characteristic state (Chapin *et al.*, 2011). It denotes diverse responses that allow ecosystem resilience to variation and change (Bengtsson *et al.*, 2003). This is due to the theory of “diversity as insurance” (Chapin *et al.*,

2011). Diversity ensures functionality under extreme or novel conditions because different species do not respond in the same way in a potential perturbation due to their evolution and life history. In other words, species diversity stabilizes ecosystem processes when annual variations happen or extreme events occur because it is unlikely that all species that perform a functional role go extinct (Walker et al., 1995 in (Chapin *et al.*, 2011), p. 333).

Following a disturbance, there are changes in ecological processes, including niche clearance and the possibility of niche filling by new individuals (Chapin et al. 2011). Alien or exotic species have the potential to alter the physical and biotic environment by changing the abundance of the native species or even eliminating them altogether (Chapin et al. 2011). For example, in the past 200 years, humans have introduced to New Zealand all of its terrestrial mammals and half of the plants (Kelly and Sullivan, 2010 in Chapin et al. 2011). Rats, mice, stoats and other rodents caused the extinction of 25% of New Zealand's native birds (Tennyson, 2010 in Chapin et al. 2011). As with ecosystem disturbances, species extinctions and migrations are natural processes but the intervention of humans have almost doubled in frequency these events thus it is changing biodiversity. Because of this rapid increase, it is highly important to understand the species' ecological function and how or if a change in species would lead to large ecosystem consequences (Chapin et al. 2011, p.322).

Therefore, there is a need to bridge these knowledge gaps and identify the most important ecological consequences on the environment. De Lange et al. (2010) suggest that when a specific risk is desired, in this case the release of GD organisms with the intention to suppress a population, it is appropriate to perform a vulnerability analysis. In the light of the potential power of current GD systems and the fact that a gradual release approach as for common GMOs is impossible, an *a priori* analysis is necessary to determine how vulnerable an ecosystem is, by identifying its weaknesses and its capacity to recover following an initial hazard (Weißhuhn *et al.*, 2018).

Chapter 4 Ecosystem analysis approaches

4.1. Introduction

In order to explore the vulnerability of an ecosystem two approaches were developed. The first approach attempts to perform an ecosystem vulnerability analysis (eVA). A framework (Fig. 4) has been elaborated using collated ecosystem and species characteristics from published literature. These criteria for the analysis of vulnerability will be described in the following sections. Further on the concept of regime shifts will be discussed. The second approach consists of a complementary hazard impact map (Fig. 5) that was accomplished in order to understand the potential interactions between some of the characteristics found at different levels in the ecosystem vulnerability analysis.

Ecosystem vulnerability assessment

Turner et al. (2003) describes vulnerability as the “degree to which a system, subsystem or system component is likely to experience harm due to exposure to a hazard, either a perturbation or stress”. “Vulnerability” is being used in both social and natural science disciplines, where authors define it in different ways, without a consensus of its’ conceptualization (Füssel, 2007). Newell et al. (2005, cited in Füssel, 2007) even suggested that the term vulnerability is a “conceptual cluster” in interdisciplinary research.

For ecosystem vulnerability assessment it is important to detect potential weaknesses and adaptive capabilities of an ecosystem under threat (Weißhuhn *et al.*, 2018). By such an analysis (eVA), it may be possible to estimate “the inability of an ecosystem to tolerate stressors over time and space” (Williams and Kapustka, 2000).

According to Liverman (1990, cited in Füssel, 2007) vulnerability is related to concepts of “resilience, marginality, susceptibility, adaptability, fragility and risk” where Füssel (2007) added the concepts of “exposure, sensitivity, coping capacity... and robustness”. When describing vulnerability, it has been stated that for it to be relevant, it is better to specify the system and its vulnerability to specified hazards as well as to mention the time frame (Brooks 2003 cited in Füssel, 2007). Recent work aimed to create a more interdisciplinary framework and defined vulnerability as a function of exposure, sensitivity and adaptive capacity (Füssel,

2007, Frazier *et al.*, 2014, Weißhuhn *et al.*, 2018). This recent definition of vulnerability is the framework that will be used in the present study.

The fundamentals of a vulnerability analysis were set by two “reduced-form models” (Turner *et al.*, 2003) developed in the realm of environmental and climate assessments (White 1974 and Cutter 2001 cited in Turner *et al.*, 2003). First, the risk-hazard models were put into place in the 1970s and 1980s and they defined the impact of a hazard as a function of exposure to the hazard and the “dose-response” (sensitivity) of the system exposed (Burton *et al.* 1978, Kates 1985 cited in (Turner *et al.*, 2003). Due to the shortcoming of these models, like the failure to take into account the system’s abilities to amplify or reduce the impacts (Kasperson *et al.* 1988, Palm 1990 cited in (Turner *et al.*, 2003, Weißhuhn *et al.*, 2018) or the fact that the systems is comprised of different sub-elements that react differently to the hazard (Cutter 1996, Cutter *et al.* 2000 cited in (Turner *et al.*, 2003, Frazier *et al.*, 2014), the “pressure-and-release” models were developed. In these type of models, risk is defined as a function of stress and the explicit vulnerability of the exposed system (Blaikie *et al.* 1994 cited in (Turner *et al.*, 2003). Although, mainly these models address social vulnerabilities in the face of natural hazards, they did put forward the basis of a vulnerability analysis (Turner *et al.*, 2003). The ecosystem vulnerability analysis follows a biocentric view where the environment is the one that is being exposed to a disturbance as opposed to it being the source of hazards (Birkmann and Wisner, 2006, Weißhuhn *et al.*, 2018).

As the definition of an ecosystem is the interactions between its biotic and abiotic elements (Weißhuhn *et al.*, 2018), there is a need for ecosystem characterization regarding its biological systems (De Lange *et al.*, 2010). The analysis of a vulnerable ecosystem requires an investigation at different levels, from a species characterization to the organism’s interactions with the environment and the environment’s abiotic attributes (De Lange *et al.*, 2010). Those ecosystems that turn out to be vulnerable need proper management (Weißhuhn *et al.*, 2018) or a different strategy.

An ecosystem can be considered vulnerable when it has a high degree of exposure and sensitivity and low adaptive capacity (Mumby *et al.*, 2014). Here, adaptive capacity has been introduced, which theoretically links the concepts of resilience and vulnerability and collates them into the form of vulnerability assessments (Weißhuhn *et al.*, 2018). According to Weißhuhn and de Lange *et al.* *vulnerability* is a function of *exposure*, *sensitivity* also known as potential impact and *adaptive capacity* (AC) (De Lange *et al.*, 2005, Weißhuhn *et al.*, 2018).

In a review paper, Morris (2003) reduced the theory of ecology to fundamental principles that “all organisms do”: require space for living, consume resources, live in dynamic environments, interact with organisms from the same or a different species and copy their genes. The range of environmental characteristics in which a species lives in is called the species’ ecological niche (Hutchinson, 1957 cited in Chase, 2011). The aforementioned niche results from evolutionary processes while species interact with their environment and other organisms (Chase, 2011). According to Chase and Leibold (2003 cited in Chase, 2011) the niche defines a species’ spatial existence, biogeography, interspecies interactions, abundance and ecological role. Chase (2011) in his review on niche theory, describes that there are two components in which the definition of a niche can be divided into: the range of biotic and abiotic characteristics that enable a species to persist in a space (Grinnell, 1917; Hutchinson, 1957 cited in Chase, 2011), also named the requirement component (Chase, 2011) and secondly, the impact the species has on its given environment (Elton, 1927 cited in Chase, 2011), known as the impact component (Chase, 2011).

Exposure

Exposure describes the likelihood of the ecosystem to come into contact with a stressor (De Lange *et al.*, 2010). To assess exposure, Frazier *et al.* (2014) recommend to examine the probability of a disturbance or its spatial proximity, whereas, Dong *et al.* (2015) suggest to determine the threatened area. As it is shown in Figure 1, the ecosystem’s exposure in this study is divided into qualities of the ecosystem and qualities of the targeted species. In order to assess the exposure potential of the ecosystem, as suggested by de Lange *et al.* (De Lange *et al.*, 2010), a measure of ecosystem exposure is the spatial scale of exposure.

As stated before, De Lange *et al.* (2010) in their framework for assessing ecosystem vulnerability, proposed that exposure can be analyzed at different levels, starting from the ecological traits of the species or the ecosystem. In order to determine the spatial scale, the current study has compiled the following characteristics:

1. Ecosystem characteristics

- a. Distribution of adequate habitat conditions
- b. Biogeographical barriers

Across distant locations, species tend to differ from each other due to evolutionary processes typical for the location in question that shaped a certain species' genotype and phenotype. According to (Cox *et al.*, 2016, pp. 91–92), the distribution of species is limited by geographical barriers that can be of different types: physical barriers that prevent organisms to disperse such as mountains or rivers; climatic barriers that impede certain organisms to thrive due to their own physiological characteristics; biological barriers in the form of predation, parasitism, interspecies competition; historical and geological barriers that have shaped the surface of Earth; microclimates can also be barriers to a species' dispersal, especially to specialized species in microhabitats, e.g. insects in a rotting log.

In spite of these natural barriers that have confined species to certain locations, organisms have been able to establish themselves in places far away from their native ranges (Capinha *et al.*, 2015). This “breakdown” of biogeographical barriers arose from human assisted dispersal through travel and trade (Capinha *et al.*, 2015) and causes an intermixing of biota that puts the native species under additional pressure (Capinha *et al.*, 2015, Montgomery *et al.*, 2015). It is being predicted that the locations with intensified trading relations and those that are closely located will suffer the greatest homogenization of biota (Capinha *et al.*, 2015). The new biota community would be formed by competitive generalists that will be composed of few but widespread species (McKinney and Lockwood, 1999).

c. Density of species' population

Habitat selection differs from species to species, within time and space (Rosenzweig, 1991). Density-dependent habitat selection portrays the mechanisms behind habitat selection in relation to population size. According to Svardson (1949 cited in Rosenzweig, 1991), intraspecific competition “can cause a greater variety in habitats”, meaning that as the population density grows, the population expands into suboptimal habitats, making the individuals less selective (Mayr, 1926, Svardson 1949, Morisita, 1950 cited in Rosenzweig, 1991). Svardson (1949 cited in Rosenzweig, 1991) also suggested that when there is interspecific competition, such as when a second species competes for the same resources, there is a tendency for the species in question to narrow its habitat and become selective again.

The size of a population in a certain habitat is bound to density-dependent processes, due to the fact that a population can grow in size as long as the carrying capacity of the habitat allows it (Morris, 2003). Fretwell and Lucas (1970, cited in Rosenzweig, 1991) suggested that these processes are based on the optimal foraging and intraspecific competition principles.

The concept of carrying capacity is used in different disciplines, ranging from population ecology to resource management and human society (del Monte-Luna *et al.*, 2004). According to a review by del Monte-Luna *et al.* (2004), the concept dates from 1798 when Malthus (cited in del Monte-Luna *et al.*, 2004) proposed a model to assess the exponential increase in the human population while there is an increase in available food. From here, Verhulst in 1838 (cited in del Monte-Luna *et al.*, 2004) modified this model and included a “saturation level” where an environment can support a maximum population given finite resources. Del Monte-Luna *et al.* (2004) propose a general definition of the carrying capacity which is: “the limit of growth or development of each and all hierarchical levels of biological integration, beginning with the population, and shaped by processes and interdependent relationships between finite resources and the consumers of those resources”. Thus, in populations, although this model considers only the maximum population size, the carrying capacity concept takes into account the individuals and the factors (e.g. food availability) that control their growth (Menczer, 1998 cited in del Monte-Luna *et al.*, 2004). The finite number of limiting resources is not constant over time, it varies according to the stochasticity of the environment, but when abstracted in models, it may be expressed as a fixed parameter (del Monte-Luna *et al.*, 2004).

The following equation denotes a discrete logistic equation that expresses the growth in size of a population over time:

$$\text{Equation 0: } \frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right); \text{ adapted from (Morris, 2003)}$$

where: $\frac{dN}{dt}$ = change in population size over time; N= population size; r= growth rate; K= carrying capacity

The distribution of densities among habitats comes in its simplest form under the name of the free ideal distribution where the organisms have achieved fitness equilibrium by proportionally distributing themselves among the habitats (Rosenzweig, 1991). However, in reality, individuals may not be free and ideal (Jonzén *et al.*, 2004). This theory can also be seen that it incorporates the idea that the densities of consumers correlate perfectly with the densities of resources in the habitats (*ibid.*), but the same author admits that this may not always be the case, and that empirical testing should be performed. As more species, variables and spatial or temporal fluctuations are taken into account, the more difficult it is to predict the dynamics of a population (Rosenzweig, 1991, Morris, 2003, Jonzén *et al.*, 2004).

d. Distribution of food sources

Another variable in habitat selection is the quality of resources and where can these be found. In the natural world, habitats undergo fluctuations regarding their quality over time and space (Jonzén *et al.*, 2004). The variation is a consequence of the habitat itself or of the number of organisms using it, in relation to their density (Jonzén *et al.*, 2004).

2. Species characteristics

To continue to assess the degree of exposure a gene drive can have on a certain ecosystem, certain characteristics of the species in question are required to be known, especially because the gene drive is hypothetically inserted into a natural population.

a. Habitat choice

Morris (2003) defined habitat selection as the process through which individuals of a certain population preferentially choose to occupy or use a certain habitat based on particular variables. The selection of habitat is related to population density regulation, community interactions and the origin and maintenance of biodiversity (Morris, 2003).

“Habitat” according to Whittaker *et al.* (1973 cited in Chase, 2011) portrays the “environmental features” where a species can live. Whereas in Morris (2003, p. 2), the given definition for habitat is “*a spatially bounded area, with a subset of physical and biotic conditions, within which the density of interacting individuals, and at least one of the parameters of population growth, is different than in adjacent subsets*”. Huey (1991) argues the importance of the environmental physical conditions (temperature, humidity, salinity etc.) and of the organisms’ physiology to perform in a given habitat in order to choose one specific habitat. For example, ectotherms in particular, are sensitive to the temperature of a certain habitat (Porter and Gates, 1969 cited in Huey, 1991) and it influences their habitat selection.

Environmental temperature is hard to predict from standard meteorological measurements due to the fact that body temperature depends on both local factors such as wind or radiation and particular factors such as heat-transfer properties that are modified by the colour and shape of the individual (Huey, 1991). Important to note is that behaviour in the laboratory may differ from that in the field (Huey 1982 cited in Huey, 1991). For example, the organisms’ behaviour,

resource distribution and interaction with other organisms impacts an individual's performance and how it perceives the environmental temperature (Huey 1991). Therefore, most adult organisms can move if the microhabitat is unsuitable for their physiology and search for a microhabitat that is more favourable.

- b. Biology and ecology of the target species
- c. Seasonal influences on the population

Seasonality produces environmental variability in terms of temperature, humidity, resource availability etc. which influences the life-history traits of organisms (Turchin 2003 cited in Taylor *et al.*, 2013).

- d. Gene flow in the target population (adapted from Moro *et al.*, 2018)

Moro *et al.* (2018) argues that the spread of a gene throughout an ideal population is determined by random mating and whether the gene flow is high or not.

- e. Rapidness of the GD to spread in the target population

In gene drive systems that depend on thresholds, the spread of the GD will be determined by the release of the gene drive organism (GDO) into the wild population above a certain frequency (Marshall and Akbari, 2016).

- f. Ability of dispersal

This trait would determine how far can the organism travel from the source population (adapted from Moro *et al.*, 2018). Also, it would determine the gene flow between populations (Mitton, 2013, Onstad and Gassmann, 2014).

- g. Potential of the GD to affect non-target populations (adapted from Moro *et al.*, 2018)

Gene flow facilitated by dispersal could spread the GD to non-target populations. However, Oye *et al.* (2014) warn that scientists have little experience with manipulating natural systems for evolutionary robustness. Thus, they argue that precision drives could prevent the drive from spreading into non-intended populations, but its reliability requires further research (Oye *et al.*, 2014). Other ways to prevent the spread to other populations than the intended one is by molecular confinement, threshold drives that will not fixate into the population at low frequencies, targeting very specific DNA sequences that are population specific or gene drives

that transform the population to be sensible to a specific chemical (Marshall and Hay, 2011, Esvelt *et al.*, 2014, Marshall and Akbari, 2016).

h. Potential of the GDO to hybridize (adapted from Moro *et al.*, 2018)

David *et al.* (2013) suggest that the presence of a driver gene in a wild-type population can raise concern of interspecific gene flow. Gene flow between species could happen through hybridization, introgression (David *et al.*, 2013) or horizontal gene transfer (Werren, 2011). There are confirmed instances where two species of the same genus hybridize naturally in so called hybridization zones. One example is the hybrid zone in Romania, where the fire-bellied toads *Bombina bombina* and *B. variegata* naturally interbreed (Vines *et al.*, 2003).

Sensitivity

Sensitivity of the ecosystem is the susceptibility to disturbances (Weißhuhn *et al.*, 2018). It expresses the degree to which the system can be affected by a certain disturbance or stress and it depends on the intensity of the disturbance and may change depending on the length of the exposure due to the development of increased tolerance (Weißhuhn *et al.*, 2018). De Lange *et al.* (De Lange *et al.*, 2010) emphasise the need to know the sensitivity of the species, functions within the ecosystem and the trophic relationships amongst other aspects.

1. Ecosystem characteristics

The following characteristics for the ecosystem sensitivity have been collated:

- a. Structural biodiversity (adapted from De Lange *et al.*, 2010) represented by species composition, population structure and number of individuals
- b. Key functional traits of the species in the ecosystem: functional role of the species (adapted from De Lange *et al.*, 2010)

The dynamics of the ecosystems depends on the traits of organisms; their evolutionary histories and interactions in the community (Chapin *et al.*, 2011). Therefore it is important to understand the role of organisms in their community.

Functional traits represent characteristics that allow a species to survive and reproduce and they impact their fitness. The loss or gain of species within a system can alter ecosystem processes due to the change in the species' functional traits that have large impacts on the system, namely

effects on supplies or limiting resources, microclimate, intraspecific or interspecific interactions and effect on disturbance regimes (Chapin *et al.*, 2011). Especially important if the targeted species is or affects a key stone species.

- c. Species redundancy within functional groups (Difference in sensitivity of functionally similar species) (adapted from De Lange *et al.*, 2010)

The redundancy hypothesis suggests that resilience is maintained by the ability of the species to compensate through their functional role in case species are lost (Walker 1992 cited in Mitchell *et al.*, 2000; Fonseca and Ganade, 2001)).

It is thought that the more species there are in a system, the wider will be the range of conditions under which ecosystem processes can be maintained at their characteristic state (Chapin *et al.*, 2011). It denotes diverse responses that allow ecosystem resilience to variation and change (Bengtsson *et al.*, 2003). This is due to the theory of “diversity as insurance” (Chapin *et al.*, 2011). Diversity ensures functionality under extreme or novel conditions because different species do not respond in the same way in an eventual perturbation due to their evolution and life history. In other words, species diversity stabilizes ecosystem processes when annual variations happen or extreme events occur because it is unlikely that all species that perform a functional role go extinct (Walker *et al.*, 1995 in Chapin *et al.*, 2011, p. 333).

- d. Trophic relationships within the community (adapted from De Lange *et al.*, 2010)

Energy and nutrient flow in an ecosystem is regulated through food webs (Chapin *et al.*, 2011, p. 300). The trophic relationships that determine food webs are complex but can be narrowed down to bottom-up (e.g. productivity of plants regulate herbivore numbers) and top-down controls (e.g. predators that regulate prey population)(ibid.).

- e. Emergent properties (adapted from De Lange *et al.*, 2010)

According to (Reuter *et al.*, 2005), emergent properties are new qualities that form at higher integration levels and constitute more than the sum of the low-level components. The emergence concept is based in a hierarchical structure of nature, such as the different organizational levels ranging from an individual, to community, ecosystem and landscape (Reuter *et al.*, 2005). For example, the ecological interactions between different individuals produce processes and dynamics within the ecosystem (ibid.).

- f. Seasonal climatic influence (adapted from De Lange *et al.*, 2010)
- g. Impact of climate change over time

Impact of climate change could lead to additive effects. An additive effect is when the combined effects of multiple drivers are equal to the sum of the individual effects (Crain *et al.*, 2008). Synergistic cumulative effect is when the combined effect is greater than the sum of the individual effects (*ibid.*). Antagonistic cumulative effect is when the combined effects is less than the sum of the individual effects (*ibid.*).

2. Species characteristics

At the species level, the following qualities and impacts were found to be relevant:

- a. Genetic diversity of the species
- b. Human pressures on the species

Stressors such as pressures produced by humans (habitat destruction, hunting, use of pesticides) often interact and produce combined effects on biodiversity or ecosystem services (Crain *et al.*, 2008), termed additive effects.

- c. Influence of climatic changes (adapted from De Lange *et al.*, 2010)

Adaptive Capacity

The third step in the vulnerability assessment is to investigate its adaptive capacity (AC). According to Weißhuhn, adaptive capacity describes the system's ability to compensate the impacts of disturbances (Weißhuhn *et al.*, 2018). AC is scarcely properly described for natural systems (Weißhuhn *et al.*, 2018), however according to Folke *et al.* (2002) it is related to genetic diversity, biological diversity and landscape heterogeneity (Peterson *et al.*, 1998, Carpenter *et al.*, 2001, Bengtsson *et al.*, 2003) cited in Folke 2002).

Weißhuhn *et al.* (Weißhuhn *et al.*, 2018) suggest that AC can be measured through:

1. Genetic variability (direct relationship)
2. Species ability to reproduce (Díaz *et al.*, 2013 cited in Weißhuhn *et al.*, 2018)
3. Species ability to disperse in/invade into disturbed environments (Díaz *et al.*, 2013 cited in Weißhuhn *et al.*, 2018)

4. Response diversity within functional groups

Elmqvist *et al.* (2003, p. 488) define response diversity as “the diversity of responses to environmental change among species that contribute to the same ecosystem function.” In order to maintain desirable states of an ecosystem, after a disturbance, it is important that the diverse functional groups are available to reorganize (Lundberg and Moberg, 2003 cited in Elmqvist *et al.*, 2003).

Mumby *et al.* (Mumby *et al.*, 2014) highlight the general relevance of Biodiversity for the capacity of an ecosystem to adapt. Biodiversity is considered to be the “biological diversity in a system, taking into account the genetic, species diversity and their functional roles but also ecosystem diversity in a landscape” (Chapin *et al.* 2011). The debate about the role of biodiversity in ecosystem resilience is ongoing.

Although different authors define AC as either “potential of recovery” or “resilience” (Weißhuhn *et al.*, 2018), both of the concepts are being characterized by the ecosystem’s biotic elements (Thrush *et al.*, 2009, Oliver *et al.*, 2015, Weißhuhn *et al.*, 2018). But apart from a mixture with the capacity to adapt, the full potential of resilience can only be tapped when both terms are applied separately. However, although this listing may be tempting to derive resilience from a mere description of the system under study, Thrush *et al.* (2009) argues that empirical studies are not sufficient to measure resilience. Instead, there is a need to develop models and identify the positive feedbacks that would drive systems to change.

4.1.1. Susceptibility to regime shifts

According to Thrush *et al.* (2009) changes in the ecosystem can be predicted up to a certain point when the drivers of change are strong enough to force an ecological system into an alternative state. However, the same authors argue that it is impossible to predict ecosystem shifts but the implications can be discerned. Regime shifts are extreme broad-scale changes in species composition and function. They can be detected by assessing the loss of specific species or groups that have important functions in the ecosystem.

However the ecological definition of resilience states that a variable of the ecosystem can move “within and between stability domains” (Ludwig *et al.*, 1997, Gunderson, 2000 cited in Thrush *et al.*, 2009). Systems may have alternative stable states (Chapin *et al.*, 2011, p. 7) caused by

abrupt shifts (Oliver et al., 2018) that are determined by large disturbances (Beisner et al., 2003). Alternative stable states have been proposed for the first time in the late 1960s by (Lewontin 1969 cited in Beisner et al., 2003) in reference to communities of organisms (Beisner et al., 2003). According to Beisner et al. (2003), the concept of alternative stable states is being used in ecology in two ways: first, it refers to stability in population ecology (Lewontin 1969 and Sutherland 1974 in Beisner et al. 2003). In population ecology, the environment is in a fixed state where the biotic community has “different stable configurations” and secondly, the ecosystem perspective focuses on the effects of environmental change (May, 1977 in Beisner et al. 2003). The variables and characteristics of the communities or ecosystems will persist in different possible arrangements, contributing to an alternate stable state (Beisner et al. 2003).

Therefore, if an ecosystem is resilient, it may enter into an alternative stable state, but if resilience is reduced by for example limiting species redundancy, reducing response diversity or other human made pressures, the ecosystem may abruptly shift to a less desirable state (Folke *et al.*, 2004). The scientific community still debates when a different state can be considered alternate but it is agreed that identification of critical variables and how are they affected require a thorough understanding of species interactions and feedbacks between the biotic and abiotic elements of the ecosystem (Beisner et al. 2003). Thrush *et al.* (2009) suggest the following indicators for implications of disturbances or when there is a risk of regime shift:

- Communities homogenise
- The complexities of food webs decrease
- Diversity within functional groups decreases
- Habitat structure produced by organisms decreases
- Size of organisms decrease
- Decrease in abundance in key species or key functional groups
- Changes in productivity
- Changes in recruitment and juvenile mortality
- Changes in the timing of events which leads to a decoupling of processes

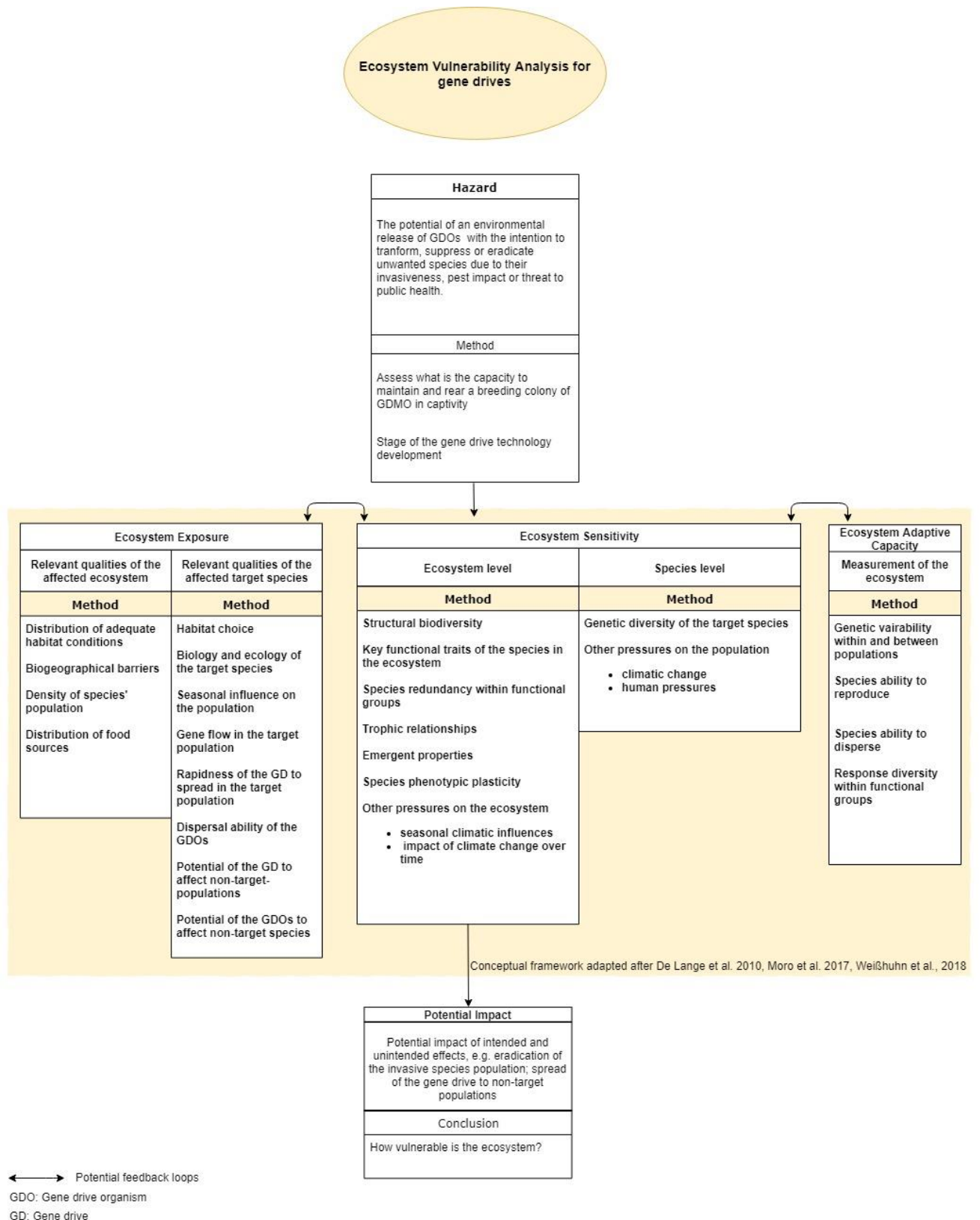


Figure 4. Ecosystem vulnerability analysis Framework

Hazard impact identification and hazard mapping

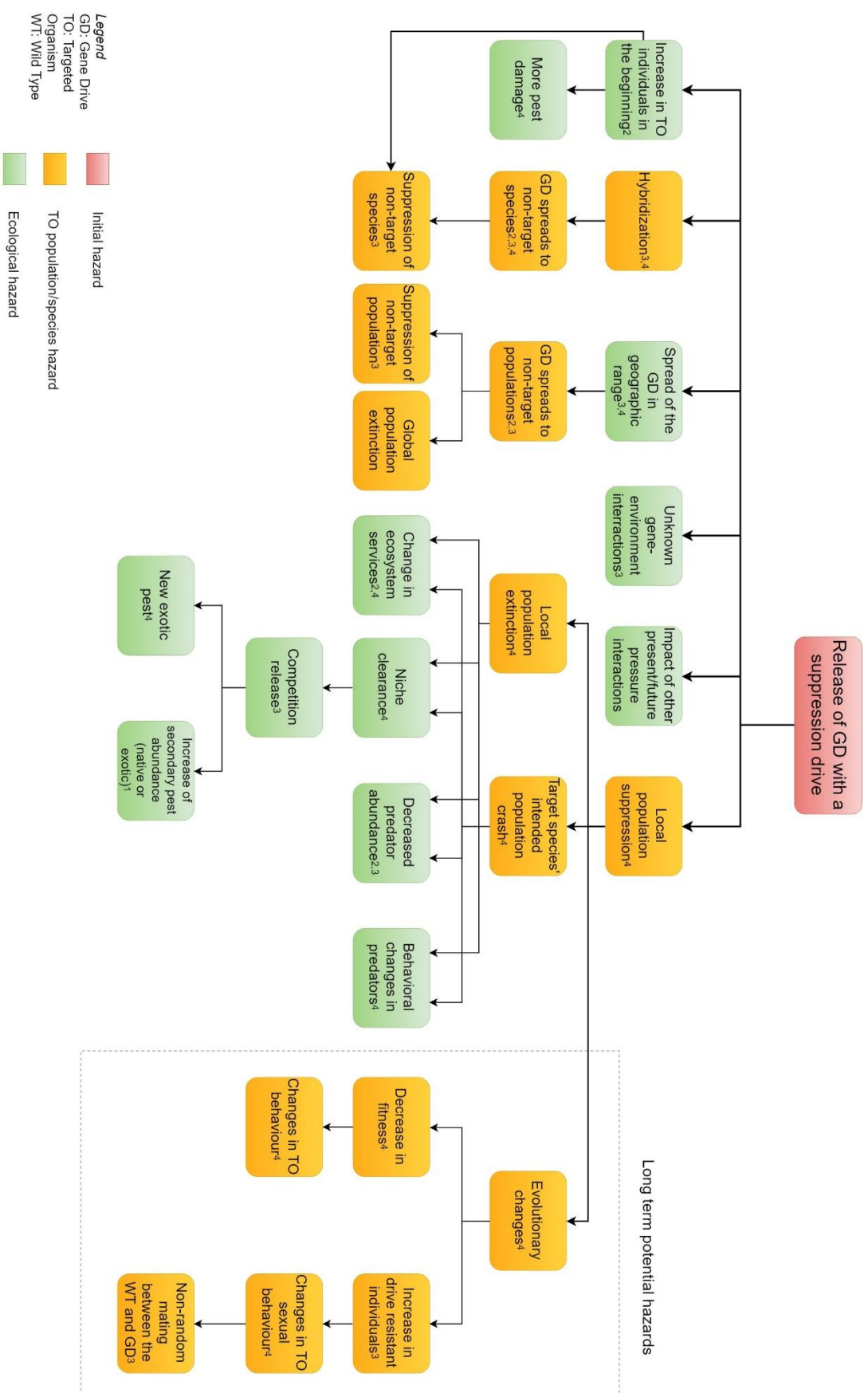
Structured hazard analysis aims to illustrate the potential effects that are caused at different scales by an initial hazard (Hayes *et al.*, 2018). Effects can be seen at the molecular level of the gene drive carrying organism and spanning from the individual level to the ecosystem level (De Lange *et al.*, 2010, Hayes *et al.*, 2018, Moro *et al.*, 2018). The concept of “hazard” is defined here in accordance with the United Nation’s internationally agreed glossary of basic terms as: “A threatening event, or the probability of occurrence of a potentially damaging phenomenon within a given time period and area” (United Nations, no date cited in Hayes *et al.*, 2018)

The color code in Fig 5 represents hazard that affect at the species/population level (yellow), ecosystem level (green), initial hazard (red).

Most of these analysis tools were developed for industrial or aerospace systems (Kumamoto and Henley, 1996 cited in Hayes *et al.*, 2018) and examples are Fault Tree Analysis (FTA), Event Tree Analysis, Qualitative Mathematical Modelling etc. (Hayes *et al.*, 2018). These tools were used in eco-toxicological assessments (Ankley *et al.*, 2009 cited in Hayes *et al.*, 2018) but using them in ecological assessments is rather a new concept (Hayes *et al.*, 2018). One difficulty using these tools, is that there is a need for a thorough understanding of the system in question (ibid.). In particular to biological or ecological systems, there are many knowledge gaps leading to the possible incompleteness of the hazard impact analysis (ibid.). Regardless, there are examples of such tools being used in ecological assessments. (Murphy *et al.*, 2010) performed a FTA to identify the possible ecological and social hazards and their related consequences to releasing Wolbachia infected *Aedes aegypti* mosquitoes in Australia. After an expert meeting they found 50 possible hazards that were divided in the two categories (Murphy *et al.*, 2010).

Identifying the potential hazards that could arise is important to help researchers and regulators to discover how they can occur but also how to help mitigate them.

Hazard mapping refers in the current study to creating an easy to understand graphical illustration of the identified hazards in the form of an initial hazard and its potential cascading hazards and potential effects. The graphical illustration and the hazard impact analysis can be seen in *Chapter 8 Applied hazard impact identification and hazard mapping*.



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Figure 5 Hazard Impact Map

Chapter 5 Modelling approach and System Dynamics

5.1. Introduction

Models help to understand real-world complex processes by attempting to recreate them in a simplified way and based on various assumptions (Hannon, 2014). The same author stresses the fact that models should be kept as simple as possible because the role of a model is not to represent all parts of the real system but to understand the desired cause and effect relationships (ibid.).

A system dynamics model aims to represent a system whose variables and rates change over time (Hannon, 2014). Thus, such a model is needed in order to examine a population's behaviour that is dependent on variables that change over time.

The present work attempts to model a *Drosophila suzukii* (D.s.) population using a stock-flow model. As D.s. individuals' biology and the species population's behavior are temperature dependent, the input variables that influence the modelled population dynamics are also temperature and time dependent.

The model attempts to explore the ecosystem vulnerability analysis and more exactly two characteristics that influence exposure at the species level:

- What is the invasiveness of the GD in the target population?
- How do seasonal temperature fluctuations influence the gene drive organisms?

Two models have been elaborated in order to answer the questions mentioned above. The first model aims to depict a stable wild-type population of D.s. whose population dynamics are temperature-dependent. This model explores how the seasonal temperature fluctuations influence the population. In the second model a *Medea* gene drive is added to the population through the release of *Medea* homozygous males (M/M). It is important to note that the *Medea* gene drive does not carry a cargo gene, hence it does not confer additional effects to the population. In this model, two more distinct population were created with the same properties as the wild-type population. These populations represent the two distinct genotypic individuals that are produced after the release of the gene drive carrying individuals: heterozygotes (M/+) and homozygotes (M/M). The second model attempts to explore both questions mentioned above.

The phrase “suppress the wild-type population” used in this chapter and chapter 9 refers to the suppression of the wild-type genotype and not to be confused with an actual suppression of population numbers

5.2. Software

STELLA Professional[®] (iseesystems.com) is a graphical dynamic modelling program (Hannon, 2014). It is a discrete, differential and difference equation model (Ogden *et al.*, 2005) that allows the user to build the model with icons. Aside of using STELLA for modelling Excel 2013 (Microsoft Corporation) was used to calculate the seasons and the mortality/fertility rates for the model.

5.3. Model building

The model consists of several elements that are connected through feedback loops. The stock-flow model consists of four components (Fig. 6).

- Stocks represent state variables can be viewed as an indicator for the current state of the model (Hannon, 2014). Conserved variables represent stocks, an accumulation of individual insects (in this case) and non-conserved variables which can also be stocks that represent temperature (in this case).
- Flows are elements in the model that “update” the state variables at each time step of the model, they can represent either the input or output of the stocks (Hannon, 2014), an example for this is the “Oviposition” in the current model.
- Convertors are parameters that describe the relationships of the elements of the model (Hannon, 2014), one example is the “Fertility rate” in this model. They control how the flows and stocks behave.
- Connectors are elements that allow the different components to interact with each other. They do not have a numerical value and can be considered as “information arrows” (Hannon, 2014, p. 12,14).

The model runs over a period of 2191 days (six years). Each day is modelled by setting the DT (delta time) to 1 while the integration method used is Euler (Griffiths and Higham, 2010).

A complete list of all the elements of the model with their corresponding formulas and the equations of the models can be found in the Appendix.

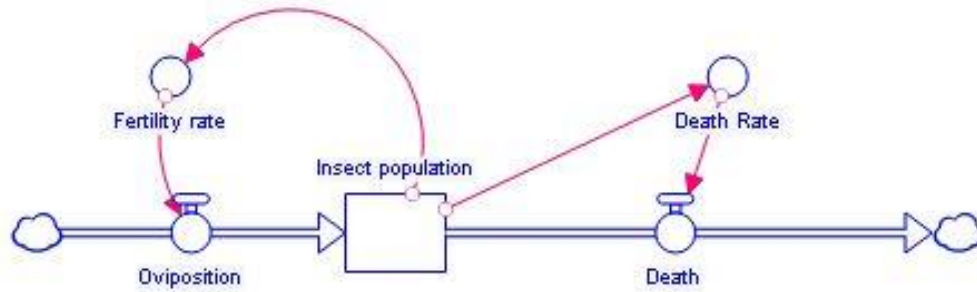


Figure 6. Example of elements in model building: Flow (Oviposition, Death), Stock (Insect population), Convertor (Fertility rate, Death Rate), Connectors (pink arrows)

5.4. Data used

The input data was selected from published research on the developmental, reproductive, population growth rates and gene drive development of D.s. (Tochen *et al.*, 2014, Ryan *et al.*, 2016, Zerulla *et al.*, 2017, Buchman *et al.*, 2018).

The following data and its calculations were used to build both of the models.

The initial numbers of the females and males are 2000 individuals each. These numbers were arbitrarily set so that the model has a starting population. The model run starts in the first day which belongs in the winter season. Because conditions are unfavorable, the individuals gradually die and then they reappear when the temperature allows them again to develop and reproduce. This population can represent the overwintered population re-emerging from diapause.

Temperature

Temperature data was taken from the Weather website wunderground.com from the weather station “suzu04” (Weather Station ID: IKAWASAK99), situated in the city of Kawasaki, Japan at 35.626° N, 139.525° W, at an altitude of 10ft (~3m). The data retrieved is a daily average

temperature from the years 2013-2017. The data was transformed from °F to °C using the following equation:

Equation 1: $(^{\circ}\text{F} - 32) \times \frac{5}{9}$

To filter out daily extreme fluctuations, a filter was applied in the Stella model. Daily extreme temperatures represent temperature data points that differ too much from the ones in the previous and next days. For example, on June 15th, 2014 the recorded temperature was 0°F. This recording might be an error of the station or an extreme temperature due to climatic phenomena. Giving the fact that the population is sensitive to any temperature changes, these extreme fluctuations do not reflect the real behavior of a population. This is because in a natural environment, individuals have the ability to migrate to different microclimatic zones in order to find adequate conditions. Thus, to accommodate for the individuals' ability to tolerate daily extreme fluctuations, the filter (Fig. 7) was applied. The equation for the filter and the filtered temperature can be seen in the Appendix under "Model Equations" and "Filtered Temperature" respectively.

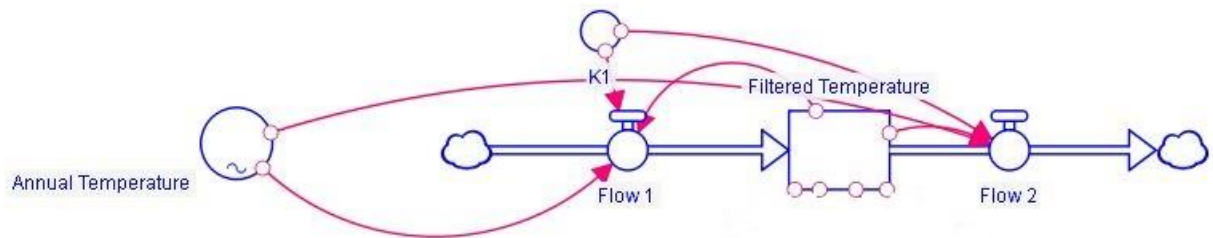


Figure 7. Temperature filter model building

The components of the filters are explained as follows:

- Annual Temperature: the daily average temperatures taken from the weather station were input into the model as a graphical function in a convertor.
- Filtered Temperature represents the output of the filtered Annual Temperature. This represents the data that was used for all the variable of the model.
- Flow 1 (Eq. 2) and Flow 2 (Eq. 2) are used through their equations to filter the input data and output the new values of the temperature data points.

Equation 2: $K1 \times (\text{Annual}_{\text{Temperature}} - \text{Filtered}_{\text{Temperature}})$

Equation 3: $K1 \times (\text{Filtered}_{\text{Temperature}} - \text{Annual}_{\text{Temperature}})$

- K1 represents a convertor that was used to adjust the degree of filtration. This was done through the sensitivity analysis mode of the modelling process. The chosen value of 0.38 proved to be satisfactory and to account for daily extreme fluctuations without changing the original data in a damaging way.

Fertility rate

The fertility rate is expressed in number of eggs produced per female per day and it was taken from the study of Ryan *et al.* (2016) on the thermal tolerances of *Drosophila suzukii*. The rates are temperature dependent. They were implemented in the model as a graphical input according to the data points from Ryan *et al.* (2016). The data is shown in Table 1 in the appendix.

Calibration was used due to the fact that in the study of Ryan *et al.* (2016) the maximum number of eggs deposited by one female in a day was 2. In other studies, it was shown that females can deposit more than two eggs per day (Tochen *et al.*, 2014). The convertor “E” with a value of 2 was added to the “Egg lay” flow.

Mating

The number of matings is represented by the minimum number between the females and males. It makes sure that the individuals mate once per day every day.

Mortality rates

Developmental stages

The mortality rates used for the developmental stages are from the study of Ryan *et al.* (2016). In the study, temperature-dependent development was investigated at 5°C to 35°C. This can be seen in Table 2 in the appendix. The calculations for the daily mortality rates used in the model can be seen in Table 3 in the appendix. Equation 4 was used in order to transform the rates into daily ones:

Equation 4:
$$D \sqrt{\frac{m}{100}};$$

In equation 4, D represents the averages number of days in a season and m represents the survival rate. The final daily mortality rate is shown in Equation 5:

$$\text{Equation 5: } 1 - \sqrt[D]{\frac{m}{100}},$$

In equation 5, 1 represents the maximum mortality rate.

The average number of days in the summer was calculated by calculating the mean value of all days within a season throughout all year.

- mean duration of winter season = 97 days
- spring days mean=47
- summer days mean=133
- high summer days mean=28
- fall days mean=53

Calibration methods were used to adjust the winter developing population. According to published studies, D.s. overwinters only as adults (Zerulla *et al.*, 2015) during the winter and it does not survive cold temperatures in the form of eggs, larva and pupa (Ryan *et al.*, 2016). With this reason a convertor “F” was added to the winter mortality rates for the developing stages. It was calibrated to a value of 10, through a sensitivity analysis until the population reached close to 0 individuals.

Adults

Mortality rate data was taken from the experiments of Zerulla *et al.* (2017) and transformed as follows, the input adult mortality rates can be seen in Table 4. In their study individuals of D.s. were adapted for 6 days at temperatures of 10°C, 20°C and 30°C. After the adaptation period the individuals were released into 3 different temperature gradient cages (10-25°C; 20-35°C; 25-40°C). The experiments were concluded after 25 minutes for each treatment. During this period, the following mortality rates were observed:

- Flies that were adapted to 10°C and were released into a 10°C - 25°C gradient had a mortality rate of 0.7%,
- Flies that were adapted to 20°C and were released into all of the experimental gradients had an average mortality rate of 1.5%,

- Flies that were adapted to 30°C and were released into the 25°C -40°C gradient had a mortality rate of 0.7%,
- Flies that were adapted to 30°C and were released into the 10°C -25°C and 20°C - 35°C gradients had an average mortality rate of 0.35.

For the winter season, the adult overwintering survival rates were taken from the study of Ryan *et al.* (2016). An average of 0.89 mortality rate was recorded for temperatures between -5°C and 5°C included. The study was performed over a period of 6 weeks.

In order to calculate the daily mortality rate for the winter season, the equations 6 and 7 were used:

Equation 6: $\sqrt[97]{\frac{1}{11}}=0.975$ and

In the above equation, 97 is the average number of winter days.

Equation 7: $100 - 89 = 11$ and

11 % represents the survival rate for the 6 week period.

The mortality rate for the winter season used in the model is calculated in equation 8:

Equation 8: $1 - 0.975 = 0.025$

Calibration was used for the winter mortality rate, which was multiplied with 5 to reduce the adult population during the winter. The value 5 was chosen arbitrarily with the reason that the winter population was still at a high number and it did not reflect actual data according to insect trapping studies (Harris *et al.*, 2014, Kinjo *et al.*, 2014). Trapping methods do not reflect population abundance, but rather only presence of individuals. However, the input values follow data retrieved from published research. The value was input in the “X” convertor in the model and it can be changed any time when more accurate data is available. Moreover, another calibration convertor “G” was added to the overall Adult mortality rate to account for the extrinsic mortality rate which can be perceived as predation on the D.s. individuals. The chosen value was arbitrarily set to 7.3 and can be changed.

Table 4 Data that was used for the daily adult mortality rates in the current model

<i>Mortality rate used</i>	<i>Season</i>
0.007	Spring
0.015	Summer
0.007	High Summer
0.0035	Fall
0.025	Winter

Lifespan

The lifespan for the developmental stages (egg to adult) is represented in days to emergence and it is temperature-dependent. It was put into the model as a graphical function with the corresponding data points. The data used is from the studies of Tochen *et al.* (2014) and Ryan *et al.* (2016), depicted in Tables 5.1 and 5.2 in the appendix.

The female and male lifespan represents the number of days until the individuals' death and it is temperature-dependent. It was introduced into the model as a graphical function with the corresponding data points. The data is taken from the study of Tochen *et al.* (2014) and it represents organisms that originally hatched from cherry fruits (data can be viewed in Table 6 in the Appendix).

Seasons

Five seasons were calculated using Microsoft Excel 2013. Based on the daily temperature data, the season and its corresponding temperature range are shown in Fig. 8. The integration of the real temperature data set was used in order to distinguish between the five seasons. In order to determine when a data point belongs to a certain season, a sliding window of 10 data points was used to always compare between the temperature data points and the mean average of a season. The following section describes the calculations used in order to determine the seasons.

Season	Temperature (°C)
Winter	<10
Spring	10-20
Summer	20-30
High Summer	>30
Fall	20-10

Figure 8. Seasons and their corresponding temperature range

Step 1

First, it was determined when the seasons of Winter, Spring, Summer and High Summer end. This was based on the temperature gradients used in Zerulla *et al.* (2017). From these temperatures, the median was calculated between the neighboring seasons as shown in Table 7. The medians also calculate the average between two numbers and reflects the average temperatures in a season. In this way it can be calculated when a particular season is taking place.

Step 2

In order to integrate the temperature, the difference between the first temperature point in the Filtered Temperature and the median values from step 1 is calculated as shown in Table 8. This represents the calculation starting point.

Equation 9: $C2 = B2 - M1;$

Equation 10: $D2 = B2 - N1;$

Equation 11: $E2 = B2 - O1;$

Equation 12: $F2 = B2 - P1;$

$C2$ = winter data point; $D2$ = spring data point; $E2$ = summer data point; $F2$ = high summer data point, $B2$ = first data point of the Filtered Temperature, $M1$, $N1$, $O1$, $P1$ represent the averages of the seasons (winter, spring, summer and high summer respectively).

Table 7. Defined seasonal temperature (median) calculation

	<i>M1</i>	<i>N1</i>	<i>O1</i>	<i>P1</i>
<i>Season</i>	Winter	Spring	Summer	High Summer
<i>°C</i>	0	10	20	30
<i>Median</i>	5	15	25	32.5

Table 8. First integration of the Filtered temperature data point

<i>A2</i>	<i>B2</i>	<i>C2</i>	<i>D2</i>	<i>E2</i>	<i>F2</i>
<i>Time</i>	Filtered Temperature	Winter	Spring	Summer	High Summer
<i>1</i>	1	-4	-14	-24	-31.5

To integrate the rest of the data set, to each seasonal temperature data point, the difference between the next Filtered Temperature point and the median of the respective season is added, as shown in Table 9.

Table 9. Example of calculations for daily seasonal integrated points

A	B	C
TIME	Filtered Temperature	Winter
2	1	-4
3	2.482422222	-6.517577778
4	3.559221551	-7.958356227

For example, in order to calculate C4, the defined temperature for the particular season is subtracted from the Filtered temperature data point. The value is approximately 0 (Equation 13). This value is then added to the previous calculated data point (Equation 14) which represents C4 data point. In order to determine each season, the same is done for all of the Filtered Temperature data points with the corresponding defined seasonal temperature which is given by the median.

Equation 13: $B4 - M1 = -1.44;$

Equation 14: $C3 + (-1.44) = -7.95;$

Where B4= Filtered Temperature for day 4, C3= integrated value for third day of winter, C4= integrated value for the fourth day of winter

Step 3

For the accumulated data points of the four seasons, the standard deviation (std) was calculated. When the standard deviation is smaller (down to 0), the values are closer to each other. A window or an array of 10 values were selected to calculate the std. A window of 10 values was selected in order to smooth the data and correct for outliers (extreme daily fluctuations) present in the temperature data.

Step 4

In order to determine the start and end of the seasons, the minimum value of the std was calculated between the integrated values of the four season within a day. The minimum std, means that the temperature difference between the Filtered Temperature was closer to the average temperature of a season and thus that particular day can be identified as belonging to a particular season.

Using an IF clause function (shown in Appendix), the seasons were able to be coded: 1=Winter, 2=Spring, 3=Summer, 4=High Summer. Because the defined temperatures for spring and fall coincide, every Spring that follows after a Summer was considered Fall, and manually recoded to 5.

In addition to the previous data that were used for both models, the data presented in the following sections were used only in the second model.

Fitness

Fitness is usually measured from the life-history traits of an organism based on their longevity, reproductive success, developmental rate etc. (Bergeron *et al.*, 2011).

The fitness values are provided from Buchman *et al.* (2018) who engineered, studied and modelled a *Medea Drosophila suzukii* population. They estimated that the gene drive has a high fitness cost on the carriers: 65% for the *Medea* homozygous form and 28% for the heterozygous form. In the model the fitness was integrated in the Developmental Death outflow by adding the sum of 1 and Fitness variable. The Developmental Death outflow represents the individuals of the developing stages that die in the model.

Matings

In order to establish the probability of a genotype (+/+, M/+ or M/M) that could arise from the matings of the three genotypically differentiated populations. The equations can be seen in the Equation 15 of the Appendix and the mating interactions between the individuals can be seen in Fig. 19 in the Appendix.

The MIN function takes the minimum number between the two input variables,

5.5. Model assumptions

- a. No immigration in the population from external individuals,
- b. Fitness for WT individuals is 1,
- c. Overlapping generations,
- d. No extrinsic predation rates on the developing stages,
- e. Developmental stages include eggs, larvae, pupae,
- f. Adult capacity limit is 10.000.000 individuals,
- g. Developmental stages capacity limit is 100.000.000 individuals,
- h. Organisms reproduce (mate and oviposit) once a day

- i. Males and females have the same fertility throughout their lives,
- j. The three genotypes are influenced by the same, mortality and lifespan rates,
- k. Sex ratio is 1:1,
- l. It does not take into account density dependence,
- m. It does not take into account the fruit flies' ability to disperse and migrate to better microclimatic conditions in case the temperatures become unfavourable.
- n. Panmyxia (random mating)

III. Case study

Chapter 6 *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae)

Drosophila suzukii, also known as the spotted wing *Drosophila* is a fruit fly native to Southeast Asia that has been rapidly expanding to the rest of the world (Asplen *et al.*, 2015). It was first described by Matsumura in 1931 (Hauser, 2011) but the first account of it in Japan dates back to 1916 (Kanzawa, 1936 cited in Hauser, 2011). D.s. is part of the *melanogaster* group of the *Sophophora* subgenus of Drosophilidae and it is further classified in the *suzukii* subgroup (Hauser, 2011). In the *suzukii* subgroup there are at least 6 other species, including *D. pulchrella* (Lewis *et al.*, 2005). More recently *D. subpulchrella* was added (Takamori *et al.*, 2006). The current state of knowledge is that D.s. does not fulfil key ecosystem services (M.T. Kimura, personal communication).

6.1. Argument for species choice

The D.s. species was chosen as a case study due to its emerging importance as an invasive pest, contributing to significant economic losses for berries and stone fruit growers. Atallah *et al.*, (2014) classify the species as one of the more severe current biological invasions of the Western Hemisphere. Therefore, a gene drive was recently developed for this particular species

(Buchman *et al.*, 2018). These circumstances make it a possible candidate for further research and potential deployment in wild populations if gene drives will be approved.

6.1.1. *The species as a pest*

D.s. is viewed as a highly damaging pest due to the female's ability to oviposit in ripening fruits, as opposed to rotten fruits that most Drosophilidae species use as oviposition sites (Hauser, 2011, Atallah *et al.*, 2014, Hamby *et al.*, 2016). This ability is due to a sclerotized serrated ovipositor that is used to pierce through the fruits' skin (Atallah *et al.*, 2014, Asplen *et al.*, 2015), a characteristics only seen in two other closely related species: *D. pulchrella*, *D. subpulchrella* (Atallah *et al.*, 2014, Karageorgi *et al.*, 2017) and the African fig fly- *Zaprionus indianus* (Bernardi *et al.*, 2017). The fruits are damaged due to the larval feeding of the flesh and because it provides a gateway for other species to oviposit in the fruit (Bernardi *et al.*, 2017) or for yeasts (Hamby *et al.*, 2012) and bacteria to enter the fruit (Walsh *et al.*, 2011). In addition to the ovipositor, females have a preference for ripening fruit, thought to be associated with the evolution of their olfactory sensation (Keesey *et al.*, 2015, Karageorgi *et al.*, 2017). Furthermore, its high fecundity allows it to have between 7 to 15 generations in a year with females capable of laying up to 600 eggs in their lifetime (Cini *et al.*, 2012). Other reasons as to why it is considered an important pest are because it has a wide range of crop and non-crop plant hosts and a high dispersal potential. (Hauser, 2011, Lee, Bruck, Curry, *et al.*, 2011, Asplen *et al.*, 2015, Lee *et al.*, 2015).

6.1.2. *Economic damage and current insect pest management*

A D.s. infestation could lead to a high economic loss for the growers. Reports of yield losses estimate up to 80% due to *Drosophila suzukii* damage (Walsh *et al.*, 2011). For three states in the USA it was estimated that a 20% yield loss in cherries, strawberries, raspberries, blueberries and blackberries would amount to a total of 511\$ million annual loss (Bolda *et al.*, 2010). In Northern Italy, in the year 2011 an estimated 3€ million loss occurred due to D.s. infestation (Ioriatti *et al.*, 2011 cited in Cini *et al.*, 2012).

In Japan, D.s. has been reported to be a cherry fruit pest (Kanzawa, 1939 cited in Asplen *et al.*, 2015). Previous to the intensification of blueberry cultivated areas, there are no records of severe blueberry pests in Japan (Kinjo *et al.*, 2014), but D.s. is now reported as a damaging pest for this crop production (Kawase *et al.* 2007 and Schimizu 2006 cited in Kinjo *et al.*, 2014).

In addition to crop damage, economic losses are also caused by the cost of pesticides and different pest management strategies (Matsuura *et al.*, 2018).

Currently, fruit growers use the broad-spectrum pesticides available on the market (Beers *et al.*, 2011) but Haye *et al.* (2016) warn that pesticide-resistance might arise in the following years (Poyet *et al.*, 2017). Although pesticides are the main use for population pest control of D.s., there is emergent research on the possibility of biocontrol. It was found that European larval parasitoids like *Leptopilina heterotoma* are not able to develop on *D. suzukii* due to the host's strong immune response against the parasite (Poyet *et al.*, 2013). However, it has been discovered recently that there are a few specialized parasitoid wasp species of D.s.: *suzukii*-specialised *Ganaspis xanthopoda* and *Asobara sp.*, TK1 that was currently found only in Tokyo (Girod *et al.*, 2018). Another option for pest control would be using predatory insects that feed on the developmental stages of D.s. (Cuthbertson *et al.*, 2014, Haye *et al.*, 2016, Renkema and Cuthbertson, 2018).

Haye *et al.* (2016) argue that it would become increasingly more difficult to combat this pest due to its rapid dispersal ability and wide range of wild host plants. Moreover, landscape spatial distribution influence the flies' behaviour and activity in the sense that forest edges or wild host plants adjacent to crops serve as refugia against pest management, increased temperatures during the summer or as alternative food resources (Lee *et al.*, 2015, Klick *et al.*, 2016, Santoiemma, Mori, *et al.*, 2018).

6.1.3. Gene drive technology development

In 2018 Buchman *et al.* (2018) developed a *toxin-antidote Medea* gene drive system capable to bias normal Mendelian inheritance with up to 100%. The “toxin” in the *Medea* drive is a synthetic miRNA that targets the highly conserved *myd88* gene responsible for dorsal-ventral patterning in embryogenesis. This miRNA was designed to act against the 5'UTR *myd88* sequence and it always resides in the cytoplasm of the gamete. The *Medea* “antidote” consisted of a *myd88* coding sequence that was engineered to be immune to the miRNA because it lacked the targeted 5'UTR sequence. Thus, the individuals that would inherit the “antidote” would survive because the miRNA could not target the gene crucial for embryonic development. As previously discussed in Chapter 2, *Medea* is a threshold drive which means that the gene drive can spread to fixation in the population only at high release proportions. Buchman *et al.* (2018) concluded that the drive was able to spread into the population at release proportion above 90%.

6.2. Species phenotypic description

D. suzukii adults are 2-3mm long drosophilids flies that have a pale brown or yellowish brown thorax, black stripes on the abdomen and red eyes (Cini *et al.*, 2012). Males can be easily distinguished by a dark spot on their top edge of each wing (Cini *et al.*, 2012) that become visible after 2 days from emergence or it could be that some specimens do not show the spots at all (Hauser, 2011). Another feature that males can be identified by is the two black combs on their tarsus (Hauser, 2011). Male *D. subpulchrella* also display similar dark spots as *D.s.* (Takamori *et al.*, 2006). Females are characterised by an enlarged ovipositor with many sclerotized teeth (Hauser, 2011), as shown in Fig. 9.



Figure 9. Serrated ovipositor of female *D. suzukii*

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Adults have two seasonal morphologies:

- Summer-morph

The fruits flies were observed to be most active around 20°C and their activity becomes reduced at temperatures above 30°C (Walsh *et al.*, 2011). Tochen *et al.* (2014) concluded that summer-

morphs cannot develop at temperatures below 7.5°C and they die within a few seconds in confined spaces at temperatures of 40°C (Zerulla *et al.*, 2017).

- Winter-morph

The winter-morph is found to be triggered when temperatures start to be between 10°C -15 °C (Stockton *et al.*, 2018). This cold-hardening enables adults to withstand lower temperatures (1°C) during the winter through different physiological adaptations that include an increased body size, a longer wing length and darker pigmentations (Shearer *et al.*, 2016, Stockton *et al.*, 2018). This phenotypic plasticity induces a winter diapause in *D. suzukii* by down-regulating oogenesis and DNA replication while they adjust different physiological functions in their bodies like up-regulation of the carbohydrate metabolism (Shearer *et al.*, 2016).

The sex ratio can naturally vary during the year. During trapping in Northern Italy, it was observed that the ratio was female based in the beginning of the year until week 25-35 as opposed to the rest of the year where there was a male sex bias (Thistlewood *et al.*, 2018). However, the authors high light that this biasing might be caused by the trapping method (one sex or the other can be more attracted to a trap) or by competitiveness (Thistlewood *et al.*, 2018).

6.3. Interactions with other species

6.3.1. Plant hosts

An extensive list of crop and non-crop host species for D.s. has been collated by Berry (2012). The main crop hosts are *Prunus sp.*, *Rubus sp.*, *Fragaria ananassa*, *Vaccinium sp.*, *Ficus carica*, *Ribes sp.* etc (Lee, Bruck, Curry, *et al.*, 2011, Walsh *et al.*, 2011, Berry, 2012). Although D.s. is a fruit specialist with more than 80 species of crop and non-crop hosts identified in Europe alone (Kenis *et al.*, 2016), in Japan it has been discovered that a few individuals emerged from the flowers of *Styrax japonicus* (Mitsui *et al.*, 2010).

6.3.2. Animal interactions and parasitism

In Japan, *D. suzukii* and *D. subpulchrella* have been observed to have similar seasonal cycle and resource use although *D. subpulchrella* was observed less frequently (Mitsui *et al.*, 2010), for unknown reasons (Kimura and Anfora, 2012). In addition, an examination of the ovipositors

from both species determined that *D. pulchrella* also has an enlarged serrated ovipositor and like *D. suzukii* has the ability to lay eggs in soft skinned fruits with intact skin (Atallah *et al.*, 2014). According to personal communication with M.T. Kimura (2018) *D. suzukii*, *D. pulchrella* and *D. subpulchrella* might be in competition but further research has to be taken.

6.3.3. Parasitoids and parasites

In Japan it seems that D.s. populations are successfully parasitized by wasps that oviposit in the larval stages of D.s.: the "suzukii-specialised" type of *Ganaspis xanthopoda*, also known as *Ganaspis cf. brasiliensis* (Kasuya *et al.*, 2013), *Asobara japonica* and *Asobara sp.* TK1 (Mitsui and Kimura, 2010, Girod *et al.*, 2018). D.s. seems to have a strong immunity resistance to the palearctic figitid wasp *Leptopilina heterotoma* which could be one of the reasons of its invasion success: release of pressure from the European and North American larval parasitoid wasps (Poyet *et al.*, 2013). However, Gabarra *et al.* (2015) discovered that the *Pachycrepoideus vindemmiae* parasitoid wasps are able to successfully develop from D.s. wild pupa, while *Trichopria cf. drosophilae* was able to parasitize the pupae under laboratory conditions.

Like many other arthropods (Weinert *et al.*, 2015), wild populations of *D. suzukii* are infected with the endosymbiotic bacterium *Wolbachia* (wSuz strain). Cattel *et al.* (2016) determined in Europe that an average of 46% of individuals from 23 populations of D.s. are infected with this bacterium.

Moreover, D.s. has been shown to contain a diversity of yeasts and bacteria, some of them are in symbiotic interactions with the fly (Hamby *et al.*, 2012, Hamby and Becher, 2016). Research is currently being developed to exploit these interactions for pest control (Hamby and Becher, 2016) because it is possible that the symbionts provide commensal or parasitic effects on their partners that can be used to develop pest management controls (Klepzig *et al.*, 2009).

6.3.4. Predators

Several arthropods were identified in association with D.s., which denotes that they predate on the developing stages of D.s.: *Orius laevigatus*, *O. insidiosus* and then ants, staphylinids, carabids and spiders. They might have a regulatory role on larvae and pupae (Walsh *et al.*, 2011, Gabarra *et al.*, 2015).

6.4. Distribution and dispersal

The spotted wing *Drosophila* is native to Southeast Asia where it was found in Japan, China, South Korea (Asplen *et al.*, 2015), India and Thailand (Hauser *et al.*, 2009 cited in Hauser, 2011). Using genomic analysis, Ometto *et al.* (2013) were able to confirm the Asian origin of D.s. and its original evolution in montane temperate forest habitats in the Tibetan plateau. But due to the intensification and globalization of trading routes, D.s. has a facilitated migration through fruit shipments (Bolda *et al.*, 2010, Cini *et al.*, 2012, 2014). Berry (2012) reported that there were no D.s. records in New Zealand but it is considered a hazard due to the possibility of entering the country through fruit shipments. Now, the species can be found in most of Europe, North America, Brazil and Hawaii (Cini *et al.*, 2012, Asplen *et al.*, 2015) where it became highly invasive and a major agricultural pest (Hauser, 2011, Cini *et al.*, 2014).

From the point of view of altitude it can be shown that D.s. can be found at various elevations from 40m above sea level in Tokyo (Matsuura *et al.*, 2018) to 2000m above sea level in mountainous regions (Mitsui *et al.*, 2010). According to Mitsui *et al.* (2010) D.s. is capable of altitudinal migration throughout the year to exploit different resources. In the summer it migrates to altitudes of above 1600 to find better or more resources and not to escape high temperatures as it is a heat-tolerant species (Mitsui *et al.*, 2010).

6.5. Reproduction, development and lifespan

The species has three developmental stages: eggs that possess two breathing filaments, three larval instars and a pupa stage. Development is temperature dependent thus the time from egg to adult emergence varies based on environmental conditions: 8-10 days at 25°C and 21-25 days at 15°C (Lee, Bruck, Dreves, *et al.*, 2011) with an optimum at 28.2°C (Ryan *et al.*, 2016)

Females are able to oviposit up to 600 eggs during their life time, with an average of 5.7 eggs per day (Emiljanowicz *et al.*, 2014). Females oviposit at temperatures between 10°C and 32°C (Lee, Bruck, Dreves, *et al.*, 2011) with an optimum at 22.9°C (Ryan *et al.*, 2016). Usually they deposit 1 egg per fruit (Lee, Bruck, Dreves, *et al.*, 2011) but it can be between 2-3 eggs per fruit (Cini *et al.*, 2012). However, in the laboratory no pupal emergence was observed above 31°C and no adult emergence has been observed at temperatures above 30°C (Ryan *et al.*, 2016) while similar results were achieved by Tochen *et al.* (2014). In their laboratory grown

D.s. population, Emiljanowicz *et al.* (2014) concluded that the population is comprised of 25% eggs, 51% larvae, 16% pupae and 8% adults. In their study, the mean total lifespan of an individual was 86 days with a maximum of 154 days (Emiljanowicz *et al.*, 2014). Due to their high fertility rates, there can be between 7 and 15 generations in a year (Cini *et al.*, 2012).

In conclusion, *Drosophila suzukii* is a species that was able to occupy a new ecological niche among the Drosophilides, that of the ripening berry and stone fruits, due to the females' ovipositor modification. Its fast dispersal in the Western hemisphere, polyphagy and high fecundity makes it an important pest for the agricultural sector. More research about the species has to be undertaken as there seem to be knowledge gaps in its functional role in the ecosystem and what the interspecific interactions are between other insects that occupy the same niche and its predators and parasitoids.

Chapter 7 Applied Ecosystem Vulnerability Analysis (eVA)

7. Introduction

As shown in Chapter 4, in order to determine an ecosystem's vulnerability, characteristics that belong to exposure, sensitivity and adaptive capacity have to be analysed. To perform such an investigation, elements from the ecosystem and the species level have to be evaluated in accordance to the framework presented in Chapter 4.

In the following chapter, such an approach is undertaken in order to determine how vulnerable the *Drosophila suzukii* native populations and their Asian native ecosystems are.

7.1.Exposure

1. Ecosystem characteristics

- a. Distribution of adequate habitat conditions: it is important to know what are the conditions required for the target species' survival (De Lange *et al.*, 2010)

Based on the two related definitions for the concept of “habitat” discussed in chapter 4, in the present study I focus first on the importance of abiotic features which allow a certain species to thrive in a given habitat. The environmental conditions and physiological limitations may have considerable influence on the small ectotherms, such as insects and on their population (Stevenson, 1985 cited in Huey, 1991). Habitats are thermally heterogeneous and the ectotherm’s body performance depends on the organisms’ morphology, physiology, behavior (Huey, 1991) but also on its ability for acclimatization (Levins 1968 and Prosser 1986 cite in Huey 1991) and evolutionary change (Levins, 1968; Heinrich 1981; Huey and Kingslover, 1989 cited in Huey, 1991).

In the case of D.s. its phenotypic plasticity allowed it to establish in different regions of Asia. Current observations were made in China, Japan, South Korea, India and Thailand (Hauser, 2011, Asplen *et al.*, 2015).

From the point of view of adequate environmental conditions, in the event of introduced gene drive engineered specimens of D.s. on the Asian continent, the individuals would be able to survive and thrive.

- b. Biogeographical barriers: if it is possible for the GDO to disperse because of or despite physical, physiological or environmental barriers in the landscape (Capinha *et al.*, 2015)

With the increase of global traffic, biogeographical barriers are broken due to the human assisted migration of organisms. Gene drive carrying organisms would be able to cross from Europe or North America to countries like Japan for example. This dispersal can be facilitated unwillingly through fruit shipments or illegally done by various groups that would like to salvage their fruit crops from the damage of the pest. Moreover, the species has been recorded to have the ability to disperse along different altitudes, making it a very mobile insect (Mitsui *et al.*, 2010).

- c. Density of species’ population

Wiman *et al.*, (2016) suggest that early female reproduction is limited by the availability of early host species. Females usually deposit 1 egg in a fruit (Lee, Bruck, Dreves, *et al.*, 2011)

but occasionally they can lay 2-3 eggs per fruit (Cini *et al.*, 2012). In one study conducted in North-East Italy, 17,000 female adult flies were captured in a 200km² area with orchards and forest cover. Out of 4480 cherry fruit there were 675 eggs counted (Santoiemma, Mori, *et al.*, 2018). Although, the flies can be captured through various methods, they still do not reflect the entire population, thus it is difficult to estimate the entire density of an insect population.

- d. Distribution of food sources: determines where the organism might migrate (De Lange *et al.*, 2010);

D.s. is a polyphagous insect that is able to vary its resource use during the year according to what resources are available (Mitsui *et al.*, 2010). Its migration between different habitat types and elevations has also the purpose to search for resources (Mitsui *et al.*, 2010, Klick *et al.*, 2016, Pelton *et al.*, 2016, Santoiemma, Mori, *et al.*, 2018, Santoiemma, Trivellato, *et al.*, 2018). Additionally, crops that are located near forest edges or semi-natural habitat have an increased chance of damage due migration from overwintering individuals from the forest (Klick *et al.*, 2016, Santoiemma, Mori, *et al.*, 2018).

2. Species characteristics

Qualities of the target species are also highly important to know in order to assess the exposure potential of the hazard:

- a. Habitat choice: different habitats hold different living conditions (De Lange *et al.*, 2010)

D.s. shows a wide variety of habitat use, by migrating across forest, forest edges and adjacent crops in search for adequate conditions (Klick *et al.*, 2016, Pelton *et al.*, 2016, Santoiemma, Trivellato, *et al.*, 2018). Thus, the species is not limited to one habitat and has flight capacity in order to move.

- b. Biology and ecology of the target species

D.s. has a wide trophic niche and abilities to withstand winter conditions due to its phenotypic plasticity and its cold-hardening abilities. A more in detail characterization of the insect was done in Chapter six.

c. Seasonal influences on the population

D.s. population dynamics is strongly influenced by temperature and resource availability. In Japan, it has been reported that the population numbers vary in accordance to season and altitude. Thus at elevations of 500-680m, the highest numbers of individuals were recorded in June/July and October/November. However at an altitude of 2000m, the highest numbers were recorded in July/August. (Mitsui *et al.*, 2010)

d. Gene flow in the target population

Buchman *et al.* (2018) engineered the *Medea* gene drive for D.s. using 8 strains of flies from geographically distinct populations: 7 from across the U.S.A. and 1 from Japan. The authors concluded that the drive would be able to spread to fixation in spite of the pre-existing resistant alleles found in the genetically different wild populations (Buchman *et al.*, 2018). Moreover, by analysing the gene flow between populations, the distribution and range expansion can be identified (Goergen *et al.*, 2011 cited in Tait *et al.*, 2017).

e. Rapidness of the GD to spread in the target population

This question is being explored in chapter 9. Through population dynamics modelling it was shown that the number of the initial releases and their timing has an effect on how fast the GD spreads in the population. The earliest the release (during environmental conditions that allow development and reproduction) the fastest would the GD spread.

f. Ability of dispersal: how far can the organism travel from the source population

In a study to test the flight capabilities of D.s. flies, it was concluded that the median flight duration was 2.7 minutes on a distance of 27.16 m. However, the maximum distance was 1.75 km during a 2.35 hour time interval. In the study, it was demonstrated that the flight performance of individuals is affected negatively if the individuals are starved. The authors concluded that the dispersal ability remains within a limited area while other individuals can fly over long distances. (Wong *et al.*, 2018)

Additionally, Tait *et al.* (2018) discovered that individuals can disperse 9 km in a mountainous region in North Italy.

g. Potential of the GD to affect non-target populations

The species has a natural dispersal ability (Tait *et al.*, 2018, Wong *et al.*, 2018) and an increased global migration (Cini *et al.*, 2014) with a velocity of dispersal of 1000 km per year (Poyet *et*

al., 2015). Based on these findings, one could speculate that based on the insect's biology and ecology, the GD can spread to non-target populations, even if it is trans-continental. In addition, this potential spread depends on the type of GD used (high/low threshold, Daisy chain) (Esvelt and Gemmell, 2017, Min *et al.*, 2017).

h. Potential of the GDO to hybridize

Although not recorded so far for D.s. in particular, there is a natural instance where two Drosophilid species hybridized (Sawamura *et al.*, 2016). *D. suzukii* and *D. subpulchrella* are the closest species relatives (M.T. Kimura, personal communication). Thus, further research would have to be done in order to establish if there are hybridization zones (Vines *et al.*, 2003) or possibility for natural gene flow between these species.

7.2. Sensitivity

The analysis of the whole ecosystem's sensitivity is beyond the scope of the current work and thus will not be attempted.

2. Species characteristics

At the species level, the following qualities and impacts were found to be relevant:

a. Genetic diversity of the species

A study performed in Italy, analyzed the genetic variability of nine populations spread across the country. They have concluded that there is high genetic variability defined by a high diversity of alleles and heterozygosity. They also stipulated that there are high migration rates between the populations which are possibly facilitated by fruit transports within the country. Even more, it is suggested that within this population individuals could have also immigrated from South America, northern Europe, Spain and the U.S.A. due to fruit transports. (Tait *et al.*, 2017)

b. Human pressures on the species

Today, fruit growers use different techniques to combat the pest. In general, broad-spectrum pesticides are being used (Beers *et al.*, 2011) but also other management methods are used such as nets to cover the crops, insect traps, bio-pesticides or biocontrol (Woltz *et al.*, 2015, Haye *et al.*, 2016). As there are already other measures to suppress the populations of D.s. further

investigation is recommended to explore if there is a possible interaction between using pesticides for example in combination with GD. It is known that insects develop pesticide-resistance, through modifying their genotype. This could lead to an interaction between the pesticide-resistant forms and the GD carrying organisms.

- c. Influence of climatic changes (adapted from De Lange *et al.*, 2010)

For understanding of the influences of climatic changes refer to the previous sections and Chapter 6.

7.3. Adaptive Capacity

- a. Genetic variability (direct relationship) (adapted from Weißhuhn *et al.*, 2018)

Indicated by the Italian study (Tait *et al.*, 2017), the genetic variability within and among different populations of D.s. is very high. Thus, the occurrence of natural gene drive-resistant alleles might also be high. However, according to the study of Buchman *et al.* (2018) even if the initial resistant allele frequency was at 78%, the gene drive was still able to spread to fixation.

- b. Species ability to reproduce (Díaz *et al.*, 2013 cited in Weißhuhn *et al.*, 2018)

The flies have a high ability to reproduce, being able to oviposit up to 600 eggs in a female lifespan (Emiljanowicz *et al.*, 2014) and have between 7 to 15 generations per year (Cini *et al.*, 2012).

- c. Species ability to disperse in/invoke into disturbed environments (Díaz *et al.*, 2013 cited in Weißhuhn *et al.*, 2018)

The species has a very high ability to disperse as previously shown. This characteristic, coupled with their high ability to reproduce and high genetic variability should confer the species a high adaptive capacity due to the fact that there is a large possibility of pre-existing resistant allele against the GD or developing them in time.

- d. Response diversity within functional groups

The potential ability of *D. pulchrella* and *D. subpulchrella* to fill the vacant ecological niche in the case of a D.s. population suppression confers the ecosystem an ability to reorganize and

have an adaptive capacity. However, there are at least two parasitoid species that have been discovered to be specialized on D.s.: the "suzukii-specialised" type of *Ganaspis xanthopoda*, also known as *Ganaspis cf. brasiliensis* and *Asobara sp*, TK1 (Mitsui *et al.*, 2010, Girod *et al.*, 2018). If indeed they can only deposit eggs on the larva of D.s. then it could mean that their population would also be suppressed.

Chapter 8 Applied hazard impact identification and hazard mapping

A graphical structured hazard impact map was created to identify the possible ecological consequences that could emerge from the introduction of a GD with a suppression capability in a natural population. In the diagram entitled "Hazard identification and Mapping" I have investigated what the release of a suppression drive into a wild population would mean to the affected population and ecosystem as a whole. The hazards in Fig. 5 are colour coded to distinguish between the initial hazard (red), hazards at the ecological, community level (green) and at the individual/species level (yellow).

According to Scott (2017) to manage pests using gene editing technology would be the easiest to achieve with a population suppression drive, by either targeting genes necessary for female development, fecundity or survival. Conversely, it is highly desired that a GD is contained in the targeted population or targeted species and does not spread to the native habitat, where it is a species that has co-evolved with the native flora and fauna and could lead to adverse effects. In the following chapter these adverse effects are further explored.

The following analysis deals exclusively with the case study *Drosophila suzukii*.

Initial hazard: Release of GD with a suppression drive

In the targeted invaded ecosystems, such as the agro-ecosystems where D. s. causes damage to crops, the wild type population of D. s. is intended to come into contact with the suppression drive delivered through GD modified insects, and thereby come into contact with the ecosystem themselves. In 2018, Buchman *et al.* announced the development of a synthetic gene drive system to bias Mendelian inheritance with an efficiency close to 100%, however there is

evidence of drive resistance and fitness costs (Buchman *et al.*, 2018), as discussed in Chapter 6, section “Gene drive technology development”. The method would imply to release modified males into the wild which would reproduce with wild-type females that ultimately would carry the “toxin” coupled with the linked embryonic “antidote”. By releasing males, the active spread of the gene drive is delayed by a generation until it spreads into the population (also shown in Chapter 9). The reason for releasing males in this case, is to avoid an increased pest damage due to an increased abundance in the population. In a CRISPR/Cas9 gene drive system, a 1:10 ratio of GD insects and wild types respectively, could suppress the population within 10-20 generations (Scott *et al.*, 2017).

8.1. Increase in target organism individuals in the beginning

Due to the release of the modified insects, there will be an increase in the local population abundance of the target species. Depending on the gene drive (threshold dependent or independent), the release number of engineered individuals can vary. In the case of the *Medea* gene drive, there is a threshold dependency thus (Champer *et al.*, 2016), the initial release of GD organisms would be high. Buchman *et al.*, (2018) concluded that the gene drive does not fixate into the population at releasing frequencies lower than 50% but fixates at least for the duration of 19 generations (the length of the experiment) at frequencies higher than 90%.

a) More pest damage

David *et al.*, (2013) suggest that a transitory increase in the targeted population can lead to an increase in its damaging effects. Depending of the target species and the sex of the released organisms a transient increase of harmful effects or crop damage is therefore likely. The initial release of the male *D. suzukii* would not cause more damage due to the fact that female *D. suzukii* are the ones that deposit their eggs in the soft skinned fruits (Cini *et al.*, 2012). Thus, in this particular case, the hazard “More pest damage” does can be easily circumvented.

b) Suppression of non-target species

Organisms form different kinds of interspecific relationships. If the targeted organism is in competition for resources with another species, then an increase of its abundance might intensify the competition and thus suppress the other species by out-competition

(David *et al.*, 2013). Even more, it is suggested that *D. subpulchrella* has a similar seasonal occurrence and resource use as *D. suzukii* (Mitsui *et al.*, 2010). *D. suzukii*, *D. pulchrella* and *D. subpulchrella* could be competitors (M. T. Kimura, personal communication, Cini *et al.*, 2012) for oviposition sites due to their ability to oviposit in soft skinned fruits (Cini *et al.*, 2012).

Although the fruit flies *Rhagoletis sp* are found in Europe or North America, it is important to note that they lays eggs only in cherry and sour cherry fruits. Because of this, in the invaded territories of D.s.,the *Rhagoletis sp* flies could become suppressed.

8.2.Hybridization

According to Bock (1984), there are 1,500 species of *Drosophila* that have been described. Currently, there are six other species included in the *suzukii* subgroup with *D. pulchrella* and *D. subpulchrella* being the closest relatives of *D. suzukii* (Lewis *et al.*, 2005, Takamori *et al.*, 2006, M.T. Kimura personal communication). To date, it has been discovered that *D. suzukii* is in complete reproductive isolation from *D. pulchrella* (Takamori *et al.*, 2006) since there is no evidence for *D. suzukii* hybridizing with *D. subpulchrella* (M.T. Kimura, personal communication).

Although it is rare, there are instances where natural hybridization has occurred between two species. Sawamura et al (2016) have described *D. cf. parapallidosa* to be a cross between *D. ananassae* and *D. parapallidosa* on Penang Island in Malaysia. If natural hybridization has already occurred throughout the *Drosophila* genus, further investigation should be warranted to see if *D. suzukii* can hybridize naturally.

a) GD spreads to non-target species

David *et al.* (2013) suggest that the presence of a driver gene in a wild-type population can raise concern of interspecific gene flow. Gene flow between species could happen through hybridization, introgression (David *et al.*, 2013) or horizontal gene transfer (Werren, 2011). Although horizontal gene transfer does not occur from hybridization, it is still important to mention it under the possible hazard of the GD spreading to non-target species. It has been established that a third of the transposable elements found in the *Drosophila* genomes originate from interspecies lateral transfers (Bartolomé *et al.*, 2009 cited in Werren, 2011). There are known instances where interspecific flow of an

engineered gene has occurred between a genetically engineered crop and wild plants (Zapiola and Mallory-Smith, 2012 cited in David *et al.*, 2013). There are few to none examples of transgenic animals that had a gene flow with the wild-type counterpart.

b) Suppression of non-target species

If the GD spreads to other species, then the modified gene would cross to another species, with the possibility to cause population suppression, in a non-target species (David *et al.*, 2013). According to Besansky *et al.* (1997 cited in David *et al.*, 2013) there is evidence of interspecific gene flow between *Anopheles gambiae* and *Anopheles arabiensi*.

8.3. Unknown gene-environment interactions

Tabachnick (2003) argues that in wild populations it is hard to predict what the effects of the environment on the the genes are. Single genes can produce different phenotypes in different environmental conditions, a property called the gene's "array of reaction" (ibid.). For example, the number of eye facets present in *D. melanogaster* depends on the individual's genetic background and temperature (ibid.). Saeed *et al.* (2018) studied the combined effects of temperature and *Wolbachia* infection on D.s. They concluded that at 29°C, *Wolbachia* infected females had a shorter survival period than the wild type, but at the same temperature *Wolbachia* had a positive effect on egg hatch rates and viability. The natural environmental conditions might influence the fitness of the gene drive organisms or have other effects that cannot be predicted.

8.4. Impact of other present/future pressure interactions

The changing conditions of the environment or human pressures could lead to an unpredictable change in the organism's abundance, dispersal, behaviour etc. Using GDs for population control is not regarded a "silver bullet" (Webber *et al.*, 2015) but more as an additional management tool. Thus, there is a concern about the interacting effects of chemicals on the gene drive genotypes.

8.5. Spread of the GD in geographic range

Drosophila suzukii is a highly invasive species that has been reported in Europe, North America, Hawaii islands, Brazil and its native ranges of South-east Asia (Hauser, 2011, Cini *et al.*, 2012, Asplen *et al.*, 2015). Its expansion has been characterized to be due to the species' wide climatic niche, absence of environmental challenges upon colonization and increased human mediated migration (Fraimout and Monnet, 2018). Moreover, the occurrence of *D.s.* outside of its climatic niche is due to microhabitats created by humans where they can thrive (ibid.).

Murphy *et al* (2010). have identified a possible hazard for the *Aedes aegypti* mosquito to increase its geographic range beyond prediction or at a faster pace. This hazard can apply to *D. suzukii* which would increase its geographic range. According to recent trends, *D. suzukii* is classified as a major invasive insect pest (Asplen *et al.*, 2015) which is spreading rapidly and is of major concern (Cini *et al.*, 2012).

Scott *et al* (2017). and Oye *et al.* (2014) emphasize the need to ensure that the gene drive is contained to only the desired targeted populations and that the eradication takes place only in its non-native habitat.

a) GD spreads to non-target population

Due to the fact that the release of GD *D. suzukii* would be attempted to be done on a continent, the spread of the insects to other population than the targeted one is likely. In Europe, USA and other non-Asian countries, *D. suzukii* is an invasive species. Therefore, the spread to the invasive populations is a desired effect. Noble *et al.* (2017) argue through mathematical models that gene drives can be invasive and can spread worldwide even after a small release of modified organisms in the beginning.

However, the hazard analysis focuses on the potential effects of *D. suzukii* gene drive carrying individuals that reach their native habitat. Due to the increased social and economic global migration (Webber *et al.*, 2015), there is a high probability that such organisms would reach the native populations of South-east Asia. Nevertheless, if a high threshold or a daisy-chain gene drive is applied, then it could be that even if some individuals would reach non-targeted populations, the drive would not be able to spread to fixation (Min *et al.*, 2017). But, a low-threshold gene drive would be able to become highly invasive and spread globally (Esvelt and Gemmell, 2017).

b) Suppression of non-target population

Due to *D. sukii*'s remarkable dispersal capabilities, there is a possibility of intraspecific gene flow between populations. Admixture might have a multitude of effects, including a reduction of fitness (Facon et al. 2011). It is known that the laboratory strains differ from the ones that are found in nature. Therefore, if a laboratory strain or individuals from a geographically distant population are used to be modified for a gene drive and released in a natural population, admixture effects can happen. Moreover, there have been records of an engineered gene being able to cross from genetically engineered crops (Bt corn) to non-engineered crops (landraces) (Mercer and Wainwright, 2008 cited in David *et al.*, 2013).

c) World population extinction

The dispersal of GD *D. sukii* to its native populations, might induce the suppression of the species in its natural ecosystem where it might play a role in the function of the system and to ecosystem services (Esvelt and Gemmell, 2017). This could lead to ecological consequences with negative impacts (David et al, 2013).

8.6. Local population suppression

a) Local population extinction

Murphy *et al.* (2010) have identified a possible hazard for the release of *Wolbachia* infected *Aedes aegypti* mosquitoes: local population extinction, which again can apply to *D. sukii* as a final outcome of population suppression by a *Medea*-drive as well. Several cascading effects can result from this local extinction presented below.

b) Target species' intended population crash

If the population crash were to happen in the native habitat of *D. sukii*, thought to be in Japan (Cini et al, 2012), then the ecological consequences can be negative. Further research has to be undertaken in order to establish these impacts.

i. Change in ecosystem services

One of the negative consequences is the possibility of changes in ecosystem services. So far, according to personal information from M.T. Kimura, *D. suzukii* has no role as a pollinator in its native habitat in Japan (personal communication with M.T. Kimura, 2018). Further research has to be performed in order to find evidence if *D. suzukii* is of relevance for any ecosystem services in its native ecosystem.

Scott *et al* (2017) suggest that because *D.s.* is a new invader in Europe and USA, the ecological impacts of *D. suzukii* population suppression are not thought to be significant. This insect was first documented in Europe in 2008 (Cini *et al.*, 2012) and in Hawaii in 1980 and continental US in 2008 (Hauser, 2011). In Australia *Ae. Aegypti* is considered to be an exotic species thus, Murphy *et al.* (2010) found that no other species depend on it because it has not co-evolved with the native ecosystem (Murphy *et al.*, 2010)

ii. Niche clearance

Due to local population extinction or in the worst case a population crash of the native target species, ecological niches will be made available for other organisms.

- Competition release

May be evoked if the affected targeted population competes with other organisms and would be removed. This could lead to:

- New exotic pest

Murphy *et al.* (2010) suggest in their hazard analysis that once *Ae. aegypti* goes extinct, a new exotic mosquito species can emerge. Among the Drosophilides, *D. suzukii* distinguishes itself by being able to deposit its eggs in healthy soft skinned fruits due to its serrated ovipositor (Cini *et al.*, 2012). There is a possibility that *D. subpulchrella* is also able to do so (Cini *et al.*, 2012, Atallah *et al.*, 2014). Although the two species are found ranging in the same geographical area (Takamori *et al.*, 2006, Girod *et al.*, 2018), there could be that *D. subpulchrella* can expand its niche where it was previously not found due to a suppression release. Further investigations for this suggested hazard have to be undertaken.

- Increase in secondary pest abundance (native or exotic)

Single species pest management is most appropriate where there is one species that causes the majority of the crop damage (Baltzegar *et al.*, 2018). If there is a complex of pests damaging the crops, then eliminating one species would cause the increase of the second pest abundance due to the release of competition (ibid.) Kimura and Anfora (2012) mention that *D. suzukii* is more abundant than *D. subpulchrella* in Japan, but the reasons are unknown. Even more, it is suggested that *D. subpulchrella* has a similar seasonal occurrence and resource use as *D. suzukii* (Mitsui *et al.*, 2010). *D. suzukii*, *D. pulchrella* and *D. subpulchrella* could be competitors for the same resources (M. T. Kimura, personal communication, 2018) due to their ability to oviposit in soft skinned fruits. One hypothesis for *subpulchrella* being less abundant, could be that it is being suppressed by *D. suzukii*. In the hypothetical scenario that *D. suzukii* is suppressed in its native habitat, and if indeed it keeps under control the other species, it would be likely that there will be a rise in *D. subpulchrella* population abundance. Of course, further research has to be undertaken to test this hypothesis and see if *D.s.* keeps a pressure on *D. subpulchrella*

iii. Decreased predator/parasitoid abundance

Further investigation on *D. suzukii*'s predators is to be performed in order to determine if there are predators that in the short time the species has been present in Europe or U.S have become dependent on it as prey. Also, predators in the native habitat. Moreover, there is a possibility that due to its small average biomass, it is not an important food source.

Another important issue that might arise is the decreased abundance of parasitoids. In the native habitat in Southeast Asia, there are some parasitoid species of wasps that attack the larvae of *D. suzukii*, such as some strains from the *Asobara* genus, most importantly *Asobara* sp. TK1, so far only found in Tokyo and thought to be specific to *D. suzukii* (Girod *et al.*, 2018). Furthermore, it has been discovered that there is a specialized insect that attacks *D. suzukii* and possibly the two related species, *D. pulchrella* and *D. subpulchrella* (ibid.). *Ganaspis cf. brasiliensis*, a figitid wasp, also

identified previously as the ‘suzukii-specialised’ type of *Ganaspis xanthopoda* (Kasuya *et al.*, 2013) was the most frequent parasitoid that emerged from *D. suzukii* in studies made in China and Japan (Girod *et al.*, 2018).

iv. Behavioral changes in predators/ parasites

Due to a decrease in prey species abundance (*D. suzukii*) the predators have to change their behaviour and prey on other species.

c) Evolutionary changes

i. Increase in drive resistant individuals

Scott *et al.* (2017) suggest that if a population is suppressed over years, without it being eradicated, it can undergo rapid resistance evolution. Resistance can develop naturally in the target organisms in response to the drive (Champer *et al.*, 2016). The emergence of resistance alleles can occur through mutations, errors in the DNA’s repair mechanism, replication or pre-existence in the wild type population and will prevent the gene drive system from spreading (ibid.). In *Drosophila suzukii*, Buchman *et al.* (2018) discovered a 78% frequency in initial resistant allele in the population.

ii. Decrease in fitness

In *Mus musculus* there are fitness disadvantages for using the T-complex as a gene drive as males present a reduced ability to hold territories (Carroll *et al.*, 2004). Buchman *et al.* (2018) showed that the *Medea* drive imposes a high fitness cost on the individuals, an estimated 28% in heterozygotes and 65% in homozygotes.

- Changes in TO behaviour

Possible changes in TO behaviour could arise from the insertion of the gene drive in the population and its interactions within the genome and with the environment. More research in this area has to be done. On the other hand, if the GD is meant as a modification drive, for example weakening the female’s ovipositor, then it might be that the females would have to change their behaviour and oviposit into decaying fruits.

- Changes in TO sexual behaviour

Gemmell and Tompkins (2017) argue that females of rodents preferably choose heterozygous males to reproduce with. Heterozygosity is usually associated to a better fitness, disease resistance, developmental rate and general condition (Brown, 1997). In *Drosophila*, it has been discovered that males with a higher frequency of heterokaryotypes performed mating rituals, like in the case of *Drosophila pavani* (Brncic and Koref-Santibanez, 1963 cited in Brown, 1997) or mated more rapidly such as *Drosophila pseudoobscura* (Spiess and Langer, 1964 cited in Brown, 1997). Mating choices in the favour of heterozygosity can reduce negative effect of deleterious alleles in the offspring. Thus, *D. suzukii* females could develop sexual selection against the gene drive carrying males.

- Non-random mating between WT and GD

The success of the gene drive would depend on random mating (Scott *et al.*, 2017) so that it ensures an equilibrated genetic distribution. But if mating partners that carry gene drives would not be selected for reproduction, then random mating will be lost.

Chapter 9 Applied Modelling Approach

9.1. Results and Discussion

In the following section, the modelling outputs are presented. Model 1 depicts a stable wild type population of *Drosophila suzukii* that is temperature-dependent. Model 2 consists of adding a *Medea* gene drive with no cargo gene to the wild type population. The modified populations are represented by individuals that are either heterozygous or homozygous for the drive. Using this model, the spread of the GD through the wild type population exposed to

seasonal fluctuations is explored. A list of all the variables, elements and their values or equations can be viewed in the Appendix.

Model 1

The wild-type population of *D. suzukii* is depicted in Fig. 11. Performing a visual analysis, it can be observed that the male and female populations which are represented by the “WT Males” and “WT Females” stocks in the model builder, are the highest during the summer, beginning of the high summer and fall seasons. During the high summer season there is a visible depression in the population, with a daily population that can even reach 0 if the temperature is 33°C. The high population at the beginning of the high summer could be because the adult individuals take time to die as they are influenced by their mortality rates and lifespan. A strong bottleneck is present during the winter months. The population starts reproducing during the spring season, as seen in Fig. 10. This depicts that fertility rates of the population are the highest in the summer season. However, during the high summer season or after a few days of the highest temperatures, the fertility rate declines significantly to 0. The time delay between the start of the fertility rates per adult and the start of the “Developmental Stages” population is due to their lifespan which is temperature dependent.

Validation

The results can be roughly validated by trap captures of individuals performed in Tokyo on a blueberry plantation by Kinjo et al. (2014). The most adults were caught in the months of June/July and October/November. During the summer months with high temperatures (~30°C), there were no adults trapped. This study was used as a comparison for the current models because it was performed in Tokyo. From this location the temperature data set was used to build the current models.

Model 2

In the previous model it was shown a stable insect population whose variables are temperature dependent. In order to answer the two questions proposed and to explore the exposure potential at the species level of a *Medea* drive, the following analysis was performed.

In this model, 6 scenarios were created with the following characteristics:

- A release ratio of ~50% of the initial wild-type population. The releases were done for three consequent times at an interval of ten days.
- A release ratio of ~80% of the initial wild population. This was a single release.

The scenarios were chosen in order to assess when is the best time to deploy the GD and suppress the “WT Population”, how long would it take for the “WT Population” to be suppressed and to see which genotypes would dominate.

1. One release of M/M Males in day 435 when the first developmental stages appear. The initial release constitutes of 15000 individuals which represent ~80% of the Adult population present on that day.
2. One release of M/M Males in day 495, in the end of the first spring. The initial release constitutes of 2.020.000 individuals which represent ~80% of the Adult population present on day 495.
3. One release of M/M Males in day 549, in the middle of the summer season. The initial release constitutes of 8.000.000 individuals which represent ~80% of the Adult population present on day 190.
4. Three releases of M/M Males starting with day 435 when the first developmental stages appear. Each release constitutes of 10.000 individuals which represent half of the Adult population present on that day. The three releases are each 10 days apart from each other (in days 435, 445, 455).
5. Three releases of M/M Males in days 495, 505 and 515, at the end of the first spring season. Each release constitutes of 1.260.000 individuals which represent half of the Adult population present day on that day.
6. Three releases of M/M Males starting on day 549, in the middle of the summer season. Each release constitutes of 5.000.000 individuals which represent half of the Adult population present day 549. The three releases are each 10 days apart from each other.

According to the graphical illustrations (Fig. 12-17), in Scenarios 3 and 6, the wild type population starts to be suppressed immediately after the release of the GD. These two scenarios coincide with the release of the GD in the middle of the summer when the fertility rates are highest (Table 10).

In Scenarios 2 and 5, the wild-type populations starts to be suppressed but it is still in high numbers. On the contrast, Scenarios 1 and 4 seem to have the least impact in the year of release. The release dates for these scenarios coincide with the beginning of the spring season, when the fertility rate is the lowest. The suppression of the wild-type population starts in the second year after the release.

From these preliminary results, it can be supposed that the spread of the GD is more efficient when the fertility rate is high, thus there are more eggs that are being laid and more individuals that hatch and develop. As the results from the scenarios seem to be coupled based on the release day and not the number of the released individuals, it seems that the timing of the release is most important. Within these coupled scenarios, it can be illustrated that a multiple release is more effective than a single one.

Table 10. *Fertility rates expressed in number of eggs/female/day at the initial releases of the GD*

<i>Day</i>	Filtered Temperature	Fertility Rate per adults
435	17.9459601	1.06
495	19.3837966	1.4
549	24.9673326	2.08

In Table 11, an example of all the 6 scenarios and the population number is shown at a specific time. By a visual analysis, the wild type population was the strongest suppressed in Scenario 6. Based on this result, it can be concluded that the best practice to deploy the GD is at the middle of the summer when the fertility rate is the highest.

Important to note is that in all of the scenarios the M/+ genotype is the dominant one. This might be due to the fact that M/M is the genotype that carries the strongest fitness cost on the individuals. However, there is a possibility that the gene drive carrying populations have not yet become stable during the six years while the model runs. Thus, it may be that the M/M genotype will become the dominant one.

In conclusion, from the preliminary data exploration and analysis, it can be illustrated that the spread of the GD depends first on the timing of its deployment and secondly on the number of individuals initially released. As the population dynamics of the fruit fly is temperature

dependent, the invasiveness of the GD and its efficacy are also dependent on this particular abiotic factor, as it seems that it is more efficient when the fertility rate is high.

Table 11. Example of the population numbers on a specific day under the 6 scenarios

Day 2114

	Scenario 1	Scenario 1	Scenario 1
	M/+ Population	M/M Population	WT Population
<i>Individuals</i>	4943491.127	3997142.868	987372.9045
	Scenario 4	Scenario 4	Scenario 4
	M/+ Population	M/M Population	WT Population
<i>Individuals</i>	4935545.007	4013807.83	978652.734
	Scenario 2	Scenario 2	Scenario 2
	M/+ Population	M/M Population	WT Population
<i>Individuals</i>	4930326.742	4024791.153	972886.2364
	Scenario 5	Scenario 5	Scenario 5
	M/+ Population	M/M Population	WT Population
<i>Individuals</i>	4900708.11	4088250.75	939025.552
	Scenario 3	Scenario 3	Scenario 3
	M/+ Population	M/M Population	WT Population
<i>Individuals</i>	4839775.057	4227684.711	860392.416
	Scenario 6	Scenario 6	Scenario 6
	M/+ Population	M/M Population	WT Population
<i>Individuals</i>	4756273.48	4421091.45	750315.795

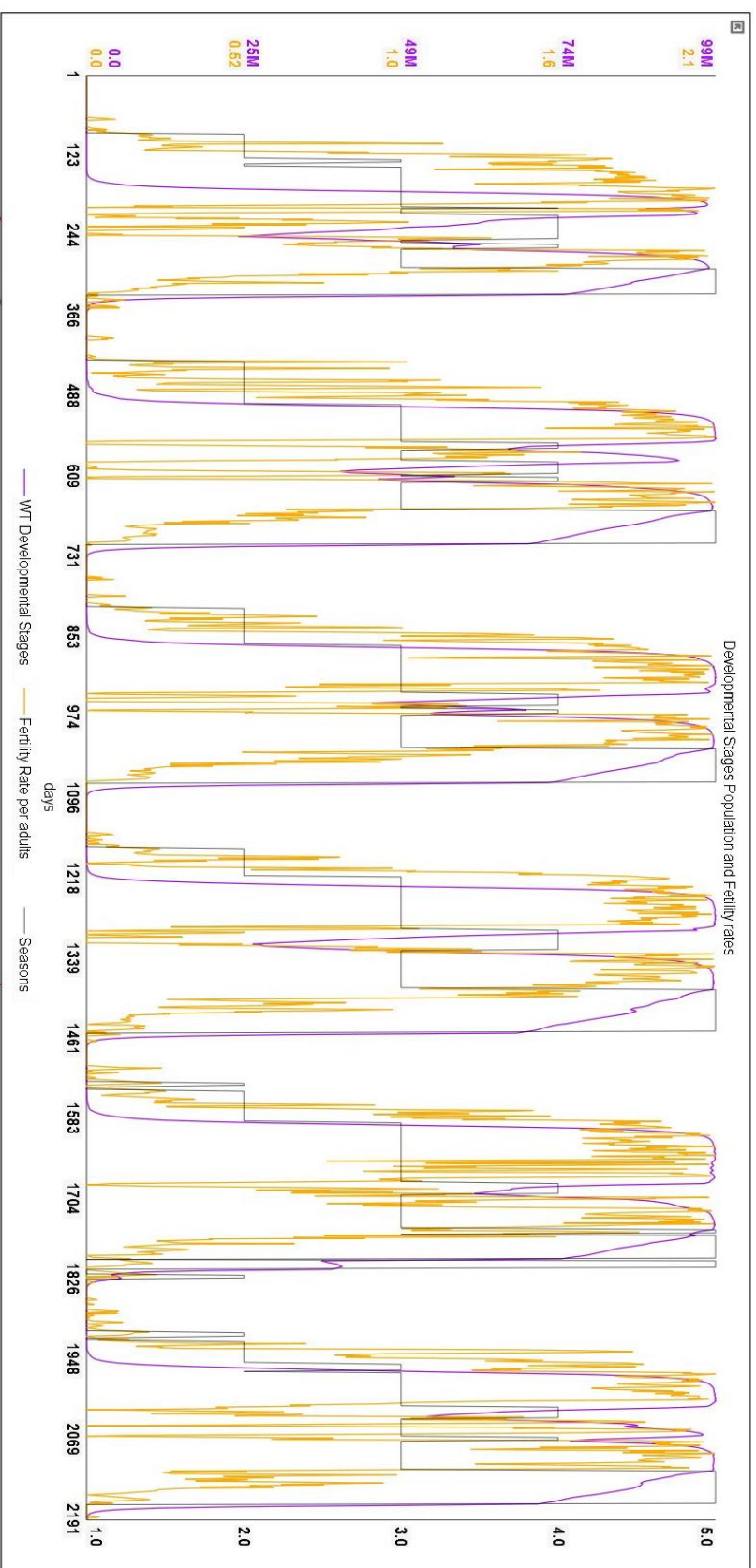


Figure 10. Temperature-dependent population dynamics of the developmental stages and Fertility rate.

Note: Different scales on the Y axis

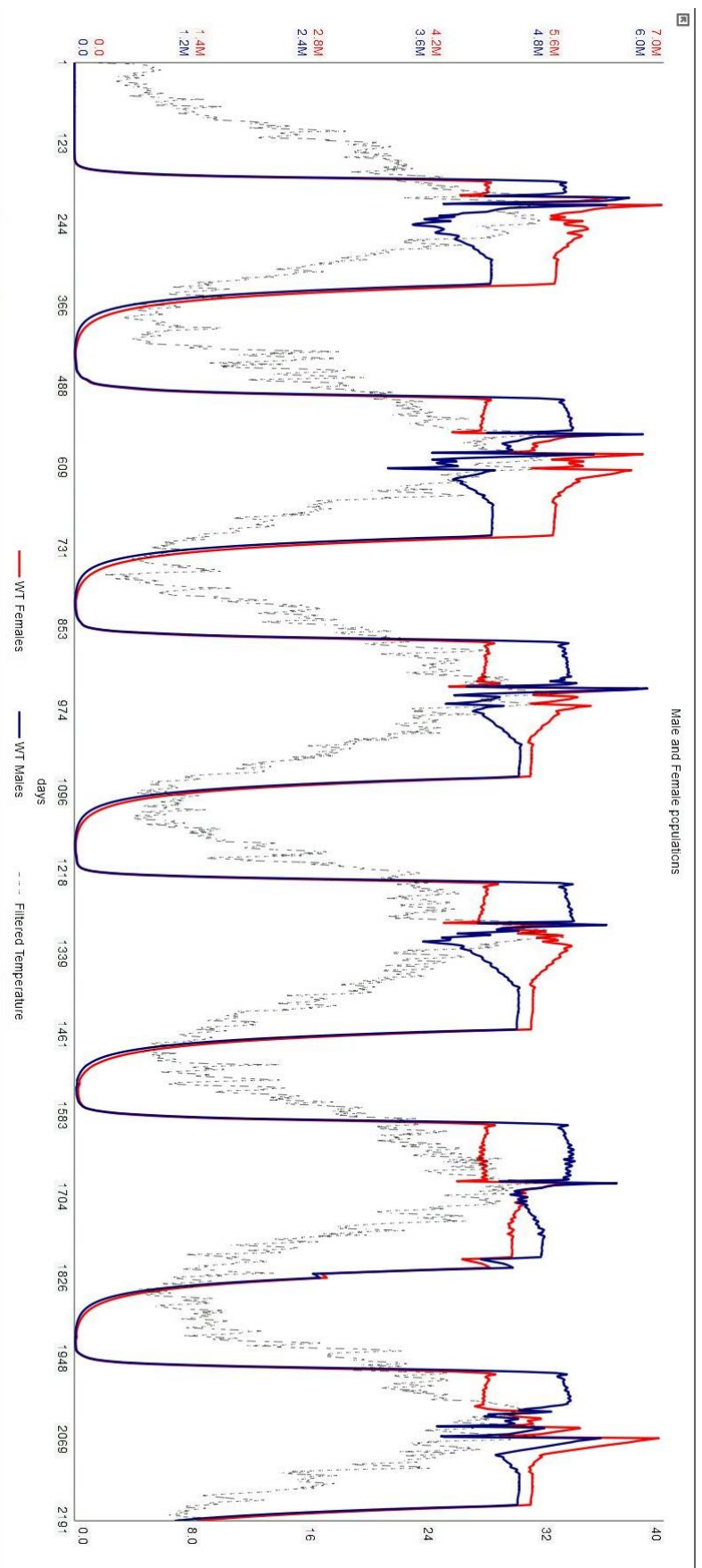


Figure 11. WT Female and Male populations depending on temperature.

Note: Different scales on the Y axis

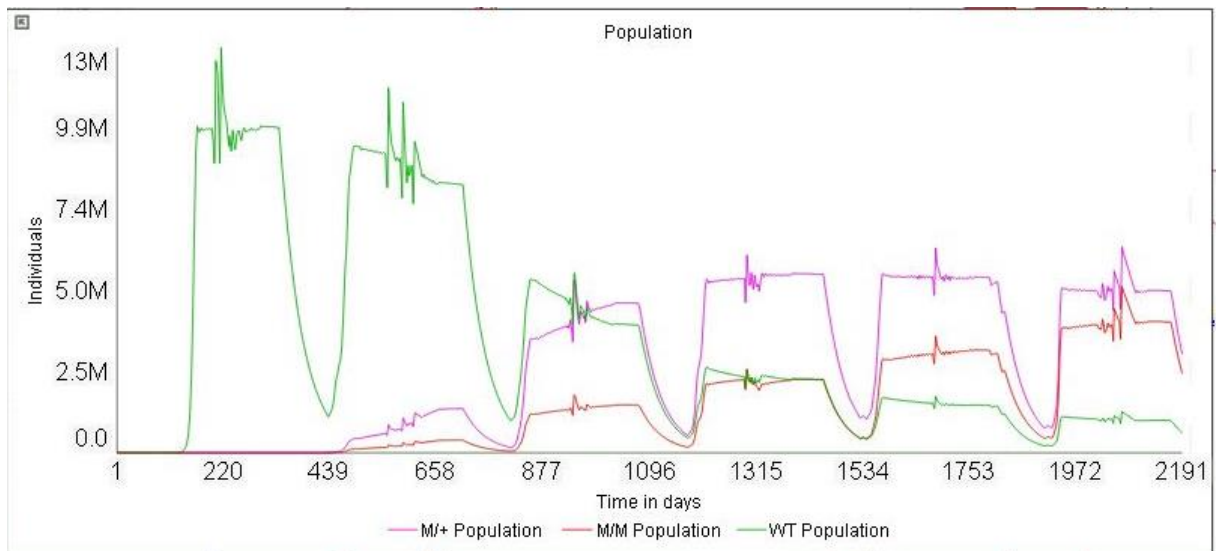


Figure 12. Scenario 1. Graphical output of the three populations

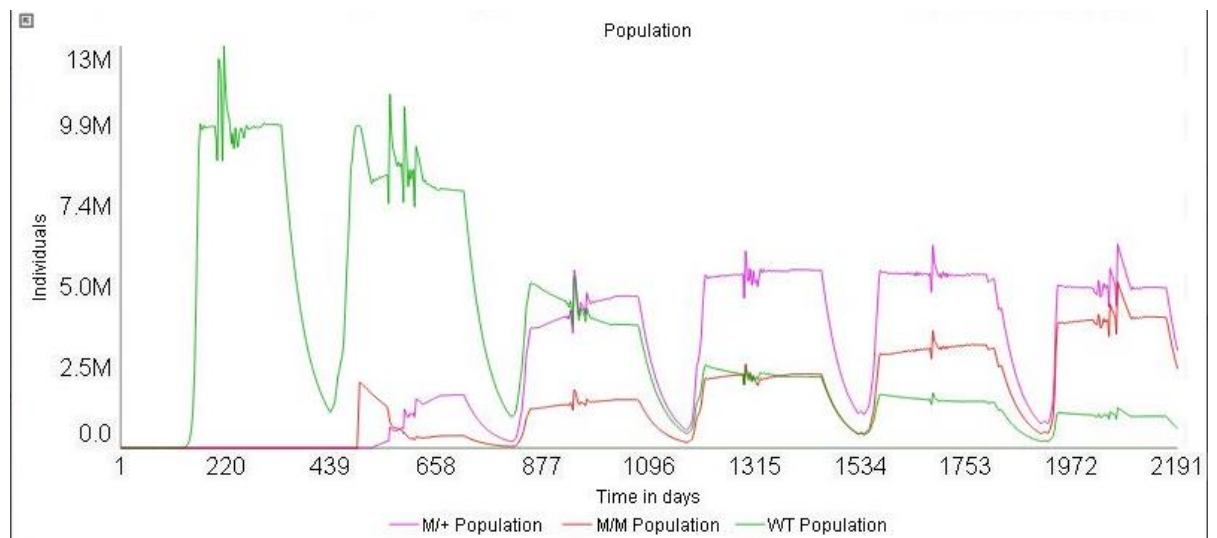


Figure 13. Scenario 2. Graphical output of the three populations

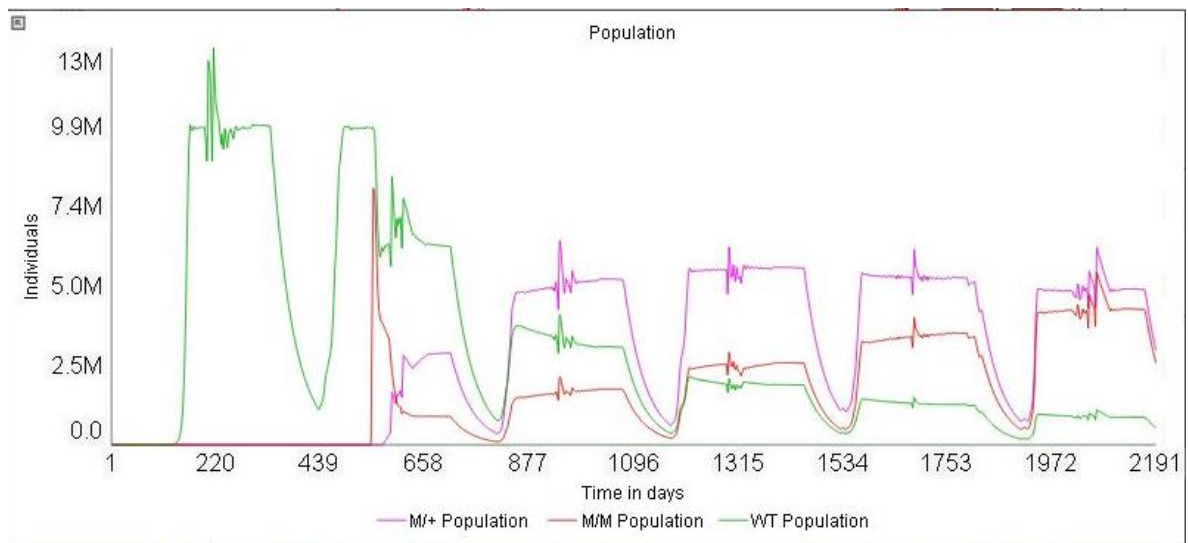


Figure 14. Scenario 3. Graphical output of the three populations

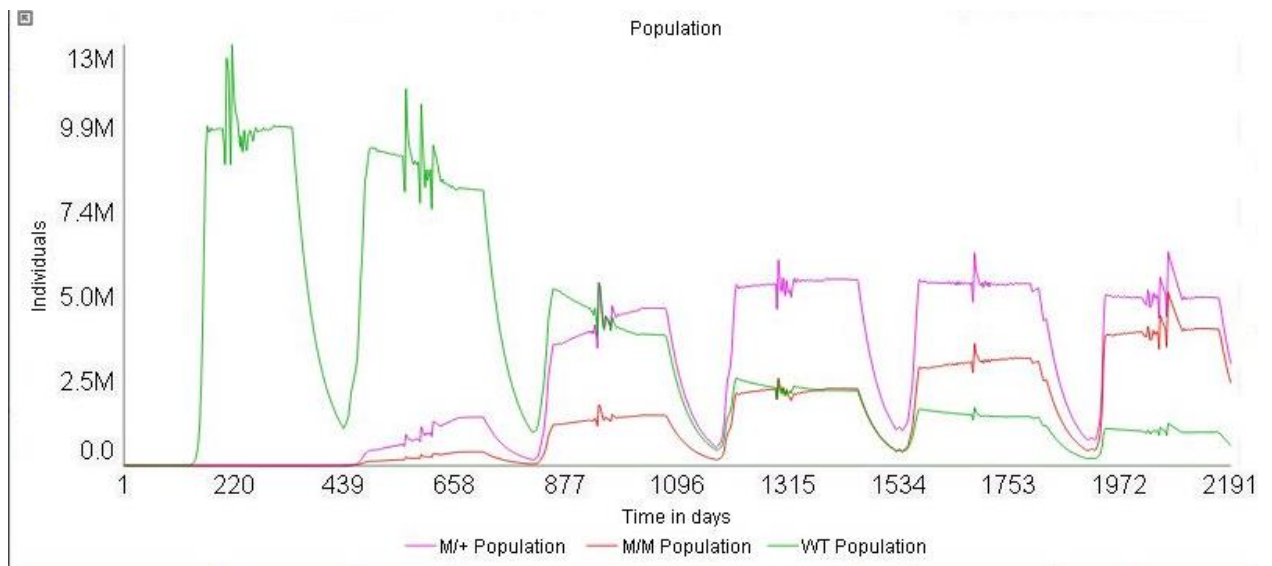


Figure 15. Scenario 4. Graphical output of the three populations

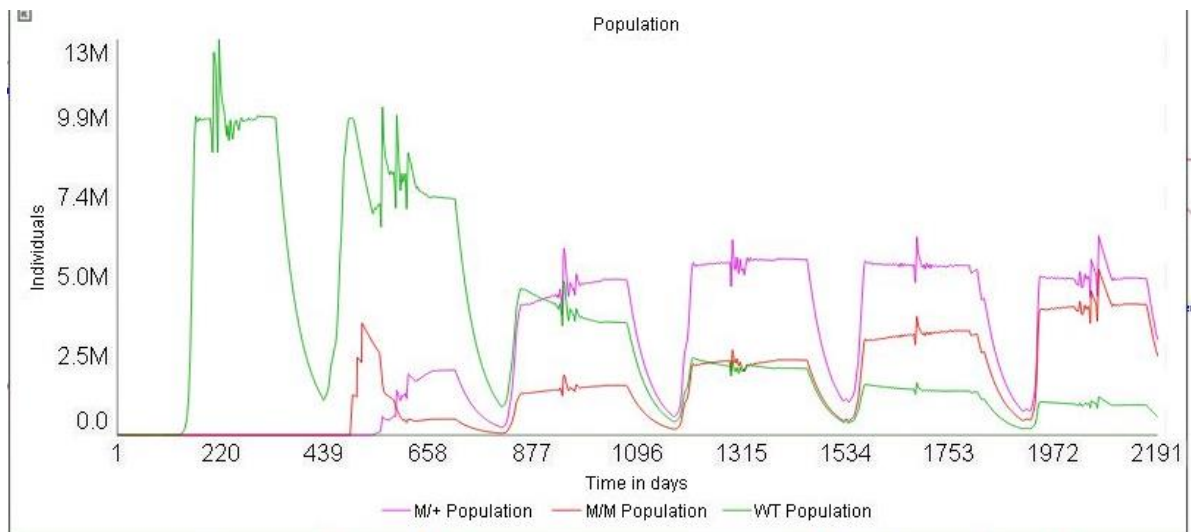


Figure 16. Scenario 5. Graphical output of the three populations

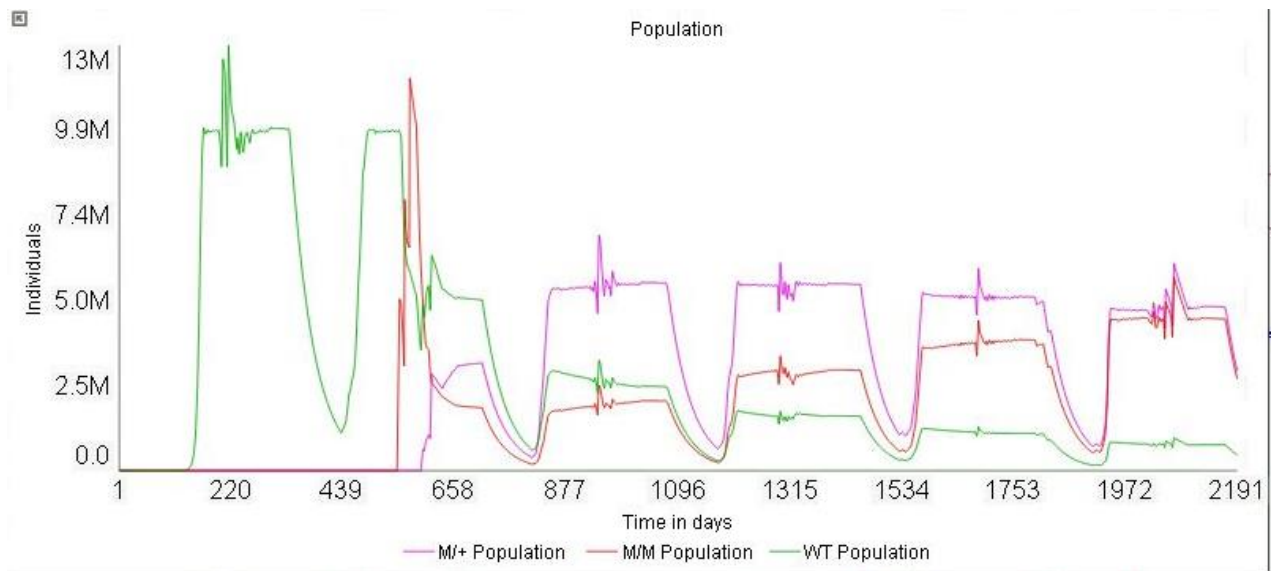


Figure 17. Scenario 6. Graphical output of the three populations

IV Discussion and conclusion

Chapter 10 Conclusions

10.1. Introduction

The current proposal put forward nowadays to control organisms with gene drives requires in depths research and analysis at different organizational levels spanning from genetics to ecosystems and landscapes. The work presented in the previous chapters attempted to gather the information available on gene drives, ecosystem ecology, vulnerability assessment methods and species biology in order to give a broad picture on the difficulty of the issue. While doing so, knowledge gaps have been discovered and many parts of the eVA require much more resources for investigations. As shown in the results section, there is insufficient knowledge to fully investigate the vulnerability of an ecosystem when facing a potential release of gene drive carrying organisms. However, there is a trend for high exposure potential. Therefore, the precautionary approach is invoked to reduce the risk of causing changes to the ecosystem that could lead to regime shifts.

The discovery of natural selfish genetic elements has enabled researchers to create synthetic gene drive systems with the purpose to control agricultural pests or insect vectors that spread diseases. The concept has since extended to the conservation field in order to eradicate invasive species, although this concept is highly debated.

The design of gene drives have to incorporate several characteristics that would allow them to be efficient and targeted. One of the main problems that arose is the issue of containment of the gene drive. More exactly, the immediate containment to the targeted population or the potential transfer to a non-targeted species in time. Implementing gene drives in animals, mobile organisms whose dispersal cannot be controlled, is risky in the face of containing a gene drive to a specific population. With an increase in trading across continents and a lack of quick and safe techniques to determine if a fruit shipment would contain engineered organisms inside, the global spread of the drive is a facing possibility.

So far there is little experience with genetically modified animal organisms released in the wild (see control of Dengue with modified mosquitoes Alphey, 2014). The dynamics of an ecosystem are complex and there are many underlying interactions that can be difficult to account for. Although ecosystems are resilient systems with alternative stable states, the tipping

points or the points of no return to a damaged system are difficult to foresee. To date, disturbance regimes, the role of biodiversity in ecosystem stability and the complex interactions are still being researched and theorized. But one aspect is certain: after the release of engineered animals, even if the drive would not be efficient, thus phenotypically the organisms would be the same, genotypically the organisms will always be altered. Subsequently, the wild-type as it is known now would be lost to a certain point.

Apart from the ecological consequences that might arise, there are numerous social impacts to releasing gene drives that have not been discussed in the current work as they were beyond the scope. One of these issues would be the agreement between countries (neighboring or worldwide) when one of the countries would want to deploy GDs. This issue is due to the fact that once released in one country there is a strong probability that it is going to spread (unintentionally or maliciously intentional) to the neighboring countries. To date there are still many unknown factors of how could gene drives act in natural populations as they were used only in laboratory or cage experiments.

The case of *Drosophila suzukii* was chosen because this species has a double status and thus more possible ecological implications. On the one hand, it is considered invasive due to its high ability to disperse and establish in different climatic regions of the world. On the other hand, the status of a pest is earned due to the ability of the females to oviposit in ripening fruits, the high number of generations per year and multiple crop host plants

10.2. Discussion

The present work developed an eVA framework based on the current literature available. Using this framework, it was attempted to analyze the vulnerabilities of an ecosystem. Different levels of the ecosystem as a whole and their interactions have to be taken into account which can be proven difficult due to many uncertainties revolving the interactions in the ecosystem and between biota. In order to account for these cascading uncertainties, a breakdown of the system in question was realized. These parts can then be analyzed and categorized in order to investigate the vulnerability of an ecosystem to a hazard. Many parts of the eVA require much more resources for investigations. Some of the results are discussed here:

- The current state of knowledge is that D.s. does not fulfil key ecosystem services, further investigations should be done in order to determine what exactly the functional role of the species is.

- *D. suzukii* uses a wide range of non-crop plants. This interaction raises the question if the species might have an effect on the natural plant species composition. For example, if it acts as a control for the wild host species as it damages the fruits and consequently the seeds. In order to test this theory, further investigations could be taken in this sense of direction.
- There is a concern about the interacting effects of chemicals and/ or Wolbachia on the gene drive carrying insects.
- *D. suzukii* and *D. subpulchrella* seem to be the closest species. Further research could be done to investigate their hybridization potential.

Following the analysis of the impacts of the introduction of gene drive carrying organisms in the native habitats of Southeast Asia, multiple hazards have been identified. The most relevant impacts or knowledge gaps were found to be:

- *D. pulchrella* and *D. subpulchrella* seem to share parts or the same ecological niche in Japan. The competition between these species might have some suppressing effects on each other. Following a *D. suzukii* population crash, the other two species might be released from competition and fill in the space in the niche. These two species might have the ability to become pests as well. Of course, further investigation has to be undertaken in the future to confirm or refute this hypothesis.
- Several parasitic wasps seem to be able to oviposit only in the larva of *D. suzukii*. The suppression of the fly in its native territories might have cascading negative effects on other species that seem to depend on it for their reproduction. Further investigations are recommended to see what the potential cascading effects are, of suppressing the two specialized species of parasitic wasps. More over further research should be done to investigate what the functional traits and trophic relations of the parasitic wasps are.

Additionally, on the European or North American continent *D.s.* might suppress a local species, *Rhagoletis sp.*, but a knowledge gap was found in regards to the interactions of this species and others that use fruits for oviposition. For examples, further research could be taken to see the competition effects between *D. suzukii* and *Rhagoletis sp.* This latter species lays eggs only in cherry and sour cherry fruits which means that has a smaller host range than *D. suzukii*.

Additionally to the development of an ecosystem vulnerability framework, and the hazard impact mapping, a system dynamics modelling approach is a complementary tool to simplify and understand the interactions between certain elements. In chapter 9 it is shown that a model for a stable D.s. population was successfully set up over a course of six years. The population dynamics is influenced by temperature relevant to the species' native habitat environmental conditions. It is shown that the population behaves accordingly to trapping results available in the literature. For example a strong winter bottleneck effect is shown due to low temperatures or also a summer decrease in abundance due to high temperatures. However, although the data available in literature is helpful to some extent, it does not necessarily reflect the wild population's behavior. This is because organisms are affected by many other abiotic factors such as humidity, wind or light. Moreover, animals are mobile and can find better microclimatic conditions that would allow them to escape high temperatures during the summer or low ones during the winter.

In the second model, a *Medea* gene drive was successfully implemented to the stable wild-type population. The gene drive model includes a specific *Medea* impact on the fitness of flies. Creating different scenarios, it was concluded that the invasiveness of a gene drive mostly depends on the timing of the release. The gene drive was more efficient at spreading when temperatures allowed maximal conditions for reproduction. Moreover, in the model it has been demonstrated that the gene drive will suppress the wild-type genotype and become dominant. A run model over a larger time frame is required in order to stipulate which genotype would become the dominant one.

10.3. Conclusion

In conclusion, understanding the biology and ecology of a species is highly important when attempts are being made to control its population. As every species and ecosystem is different such an approach must be done on a case-by-case basis. With the potential of gene drives to disperse across populations (possibly species) and countries, a thorough investigation has to be done in order to try and account what the consequences might be or what control options are available in order to decide if such deployments are too risky to try. The knowledge gaps that still exist have to be first filled in order to consider such deployments. Moreover, in the case of failure to contain the gene drive, due to the current state of the loss of insect biodiversity, a suppressing gene drive raises concerns in potentially adding to biodiversity loss and taking away even more species from the world's biota.

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Appendix

Table 1. Fertility rates for *D. suzukii*

Adapted from Ryan et al., 2016

Temperature (°C)	eggs/female/day
5	0
6	0
7	0
8	0
9	0
10	0.1
15	0.29
20	1.6
25	2.09
30	0.49
31	0
32	0
33	0
34	0
35	0

Table 2. Temperature dependent mortality rate for *D. suzukii* (egg-adult)

Adapted from Ryan et al., 2016

Temperature (°C)	Mortality rate
5	1
6	1
7	1
8	1
9	0.7
10	0.6
15	0.3
20	0.3
25	0
30	0.4
31	1
32	1

Table 3. Daily mortality rates calculations for the Developmental Stages

Temperature (°C)	Average mortality	Survival rate in %	Daily survival rate	Daily mortality rate	Season
<10	0.94	6	0.971	0.029	winter
10-20	0.4	60	0.989	0.011	spring/fall
20-30	0.2333333333	76.66666667	0.998	0.002	summer
>30	0.8	20	0.944	0.056	high summer

Table 5.1. Female lifespan in days

Adapted from *Tochen et al., 2014*

Temperature(°C)	(females)Time of emergence (d)
10	35
14	27.3
18	18.2
22	10.5
26	12.8
28	10.7
30	2

Table 5.2. Male lifespan in days

Adapted from *Tochen et al., 2014*

Temperature(°C)	(males)Time of emergence (d)
10	31
14	20.7
18	16.8
22	13.2
26	12.8
28	10.1
30	0

Table 6. Developmental stage (egg to pupa) lifespan in days

Temperature(°C)	Time of emergence (d)	Source
9	87	Ryan <i>et al.</i> 2016
10	79.3	Tochen <i>et al.</i> 2014
14	28.8	Tochen <i>et al.</i> 2014
18	18.2	Tochen <i>et al.</i> 2014
22	14	Tochen <i>et al.</i> 2014
26	10.8	Tochen <i>et al.</i> 2014
28	9.9	Tochen <i>et al.</i> 2014
30	12	Tochen <i>et al.</i> 2014

Equation 15: Genotype probabilities as a result of the matings between the three populations in Model 2

Wild type genotype: $1 \times \text{MIN}(\text{WT_Females}, \text{WT_Males}) + 0.5 \times \text{MIN}(\text{WT_Females}, \text{"M/+_Males"}) + 0.5 \times 0 \times \text{MIN}(\text{"M/+_Females"}, \text{WT_Males}) + 0.25 \times 0 \times \text{MIN}(\text{"M/+_Females"}, \text{"M/+_Males"})$

M/+genotype: $((0.5 \times \text{MIN}(\text{"M/+_Males"}, \text{WT_Females})) + (0.5 \times \text{MIN}(\text{"M/+_Females"}, \text{WT_Males}))) + (0.5 \times \text{MIN}(\text{"M/+_Females"}, \text{"M/+_Males"})) + (1 \times \text{MIN}(\text{"M/M_Males"}, \text{WT_Females})) + (1 \times \text{MIN}(\text{WT_Males}, \text{"M/M_Females"})) + (0.5 \times \text{MIN}(\text{"M/+_Females"}, \text{"M/M_Males"})) + (0.5 \times \text{MIN}(\text{"M/M_Males"}, \text{"M/+_Females"}))$

M/M genotype: $(0.25 \times \text{MIN}(\text{"M/+_Females"}, \text{"M/+_Males"})) + 0.5 \times \text{MIN}(\text{"M/+_Females"}, \text{"M/M_Males"}) + 0.5 \times \text{MIN}(\text{"M/M_Females"}, \text{"M/+_Males"}) + 1 \times \text{MIN}(\text{"M/M_Females"}, \text{"M/M_Males"})$

Equation 15. Temperature filter

$\text{Filtered_Temperature}(t) = \text{Filtered_Temperature}(t - dt) + (\text{Flow_1} - \text{Flow_2}) \times dt$

INIT Filtered_Temperature = TIME

INFLOWS:

$\text{Flow_1} = K1 \times (\text{Annual_Temperature} - \text{Filtered_Temperature})$

OUTFLOWS:

$\text{Flow_2} = K2 \times (\text{Filtered_Temperature} - \text{Annual_Temperature})$

*Note that K1=K2

IF clause used for Season coding

=WENN(K14=G14,1,WENN(K14=H14,2,WENN(K14=I14,3,WENN(K14=J14,4,0))))

Model 1 connectivity

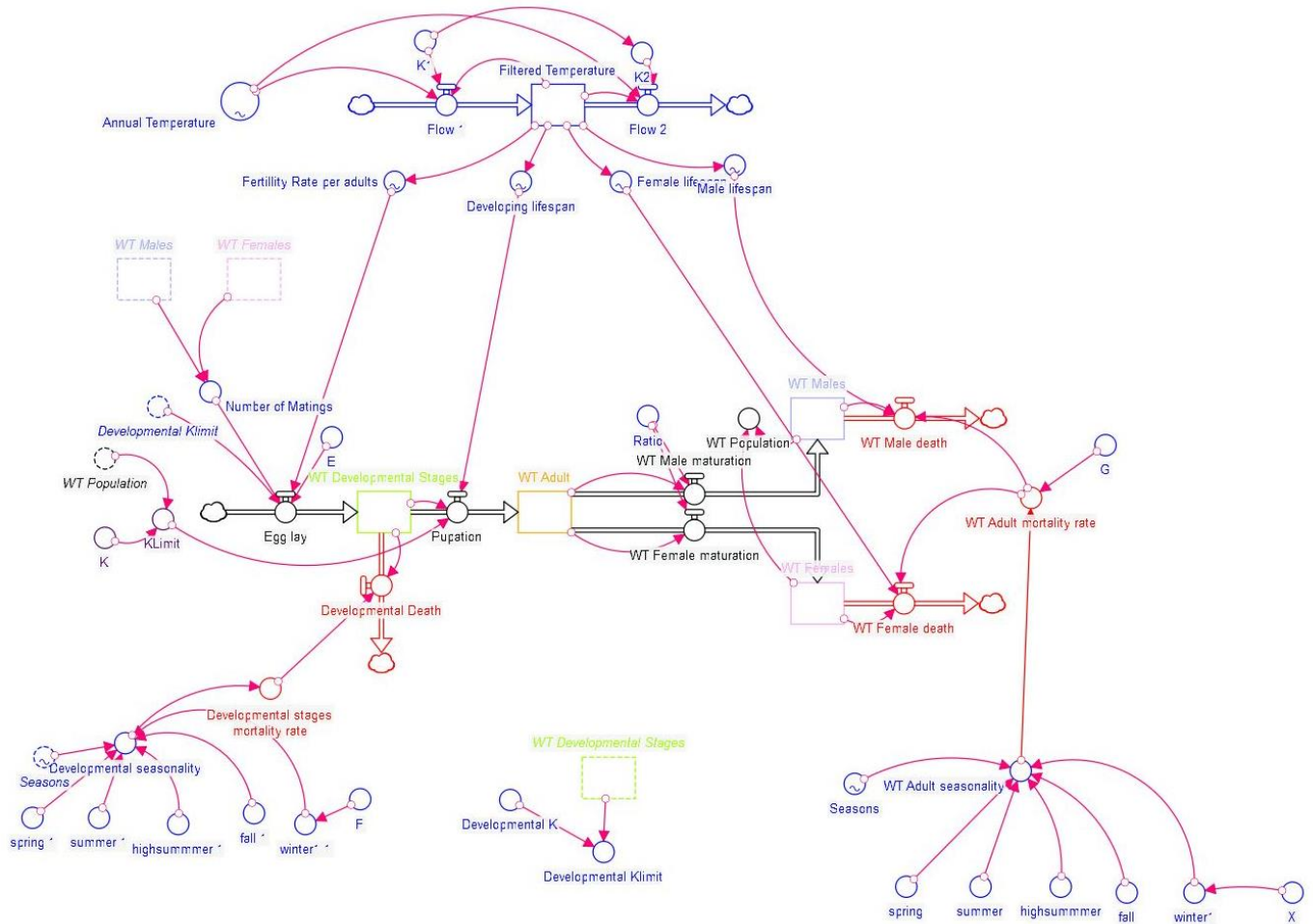


Figure 18. Model 1 development showing the causality between the model's elements

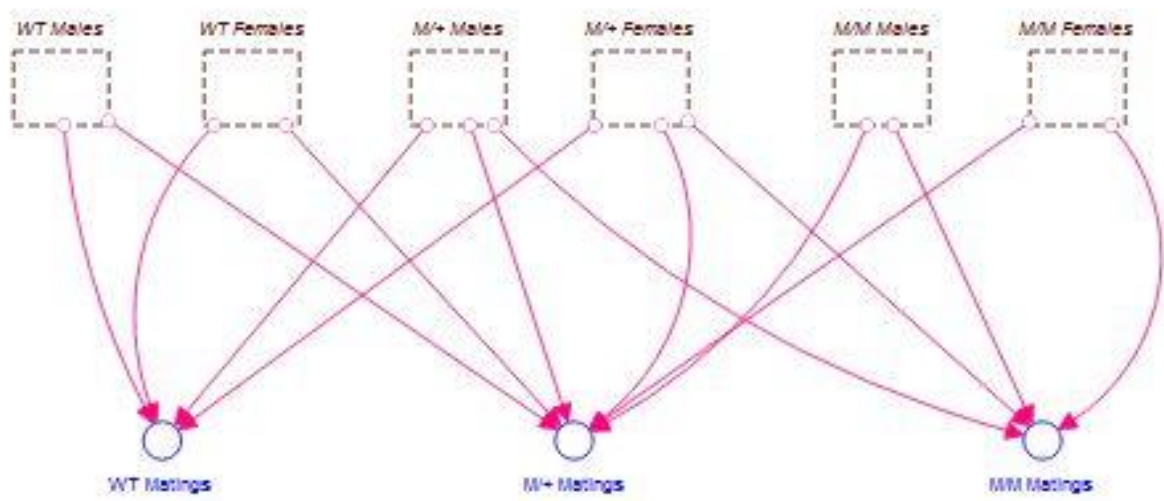


Figure 19. Genotype production probability and mating interactions

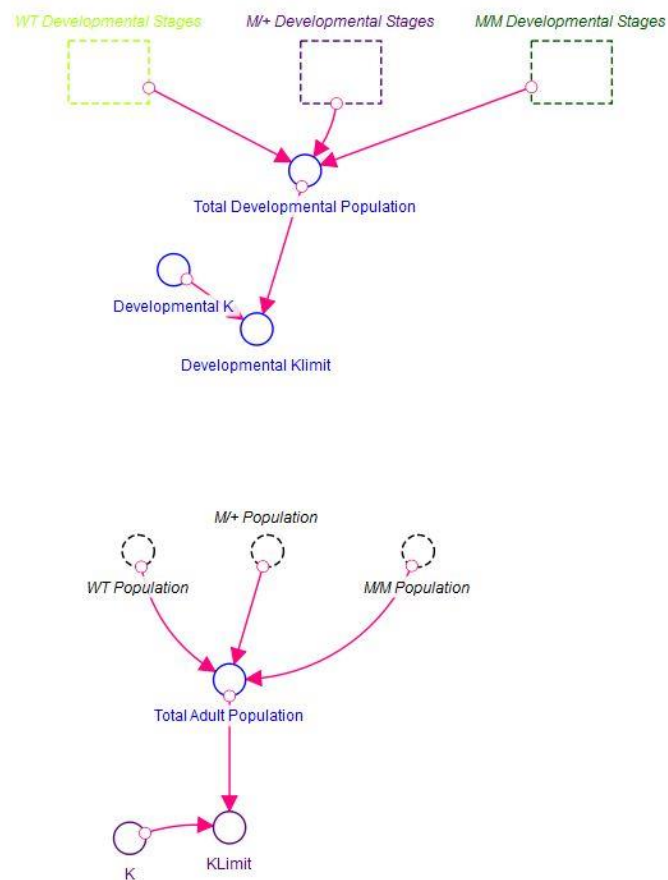


Figure 20. Illustration of the Total Population model building

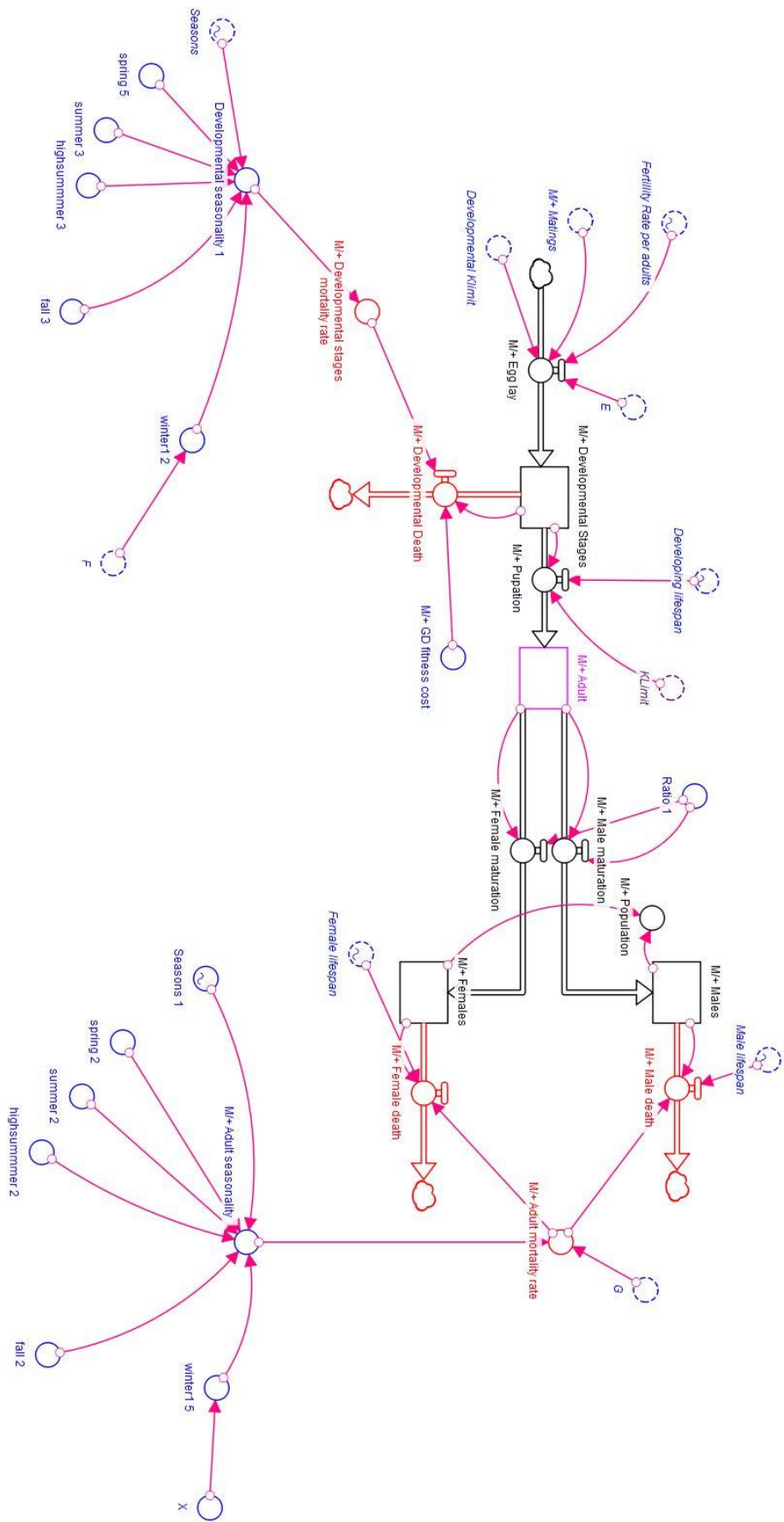


Figure 21. *Heterozygous ($M/+$) population*

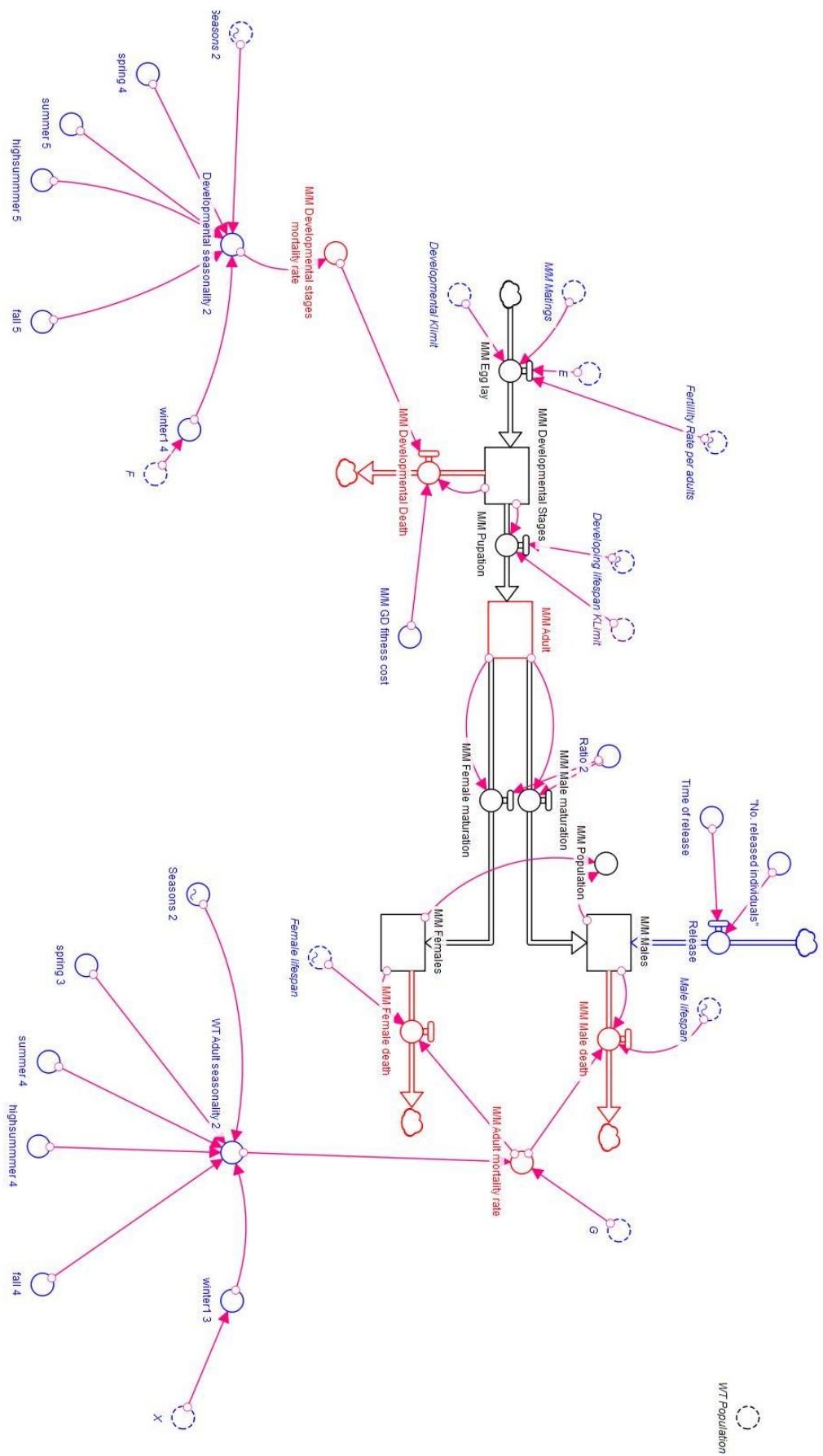


Figure 22. Homozygous (M/M) population

Insect population model building elements

4 stocks:

- Developmental Stages: represent the egg to adult stages of the insect's life cycle (eggs, larvae, pupae)
- Adults: represents the wild type adult population after pupation
- Males: represents the sexually active wild type males
- Females: represents the sexually active wild type females

7 Flows:

Inflows

1. (Develop. stages) Egg lay: "Number of matings"*"Fertility rate per adults"/"K limit"
2. (Adult) Pupation: "Developmental Stages"/"Developing lifespan"
3. (Males): Male maturation: "Adults"/"Male lifespan"
4. (Females): Female maturation: "Adults"/"Female lifespan"

Outflows

1. (Developmental stages) Developmental stage death: "Developmental Stages"*"Developmental stages mortality rate"
2. (Males) Male death: "Males"*"Adult mortality rate"
3. (Females) Female death: "Females"*"Adult mortality rate"

Variables

Egg lay

1. Developmental K (Population capacity limit number) 100.000.000
2. Developmental K limit (formula): $(1 - ("Developmental stages"/"K"))$
3. Matings: MIN(Females, Males) minimum of females or males
4. E: random variable to adjust the fertility rate
5. Fertility rate per adults/day: graphical function

Developmental death

1. Developmental stages mortality rate- depends on the "Dev. seasonality"- constant rates depending on the season
 - 1.1. Developmental seasonality: IF clause function: "(IF(seasons=1) THEN winter1_1 ELSE IF(seasons=2) THEN spring_1 ELSE IF(seasons=3) THEN summer_1 ELSE IF(seasons=4) THEN highsummer_1 ELSE IF (seasons=5) THEN fall_1 ELSE 0)"
 - 1.1.1. Seasons: graphical function depending on the model's TIME function
 - 1.1.2. Spring 1: developmental stages mortality rate in spring
 - 1.1.3. Summer 1: developmental stages mortality rate in summer
 - 1.1.4. Highsummer 1: developmental stages mortality rate in high summer
 - 1.1.5. Fall 1: developmental stages mortality rate in fall
 - 1.1.6. Winter 1: developmental stages mortality rate in winter
 - 1.1.6.1.F: random variable to adjust the winter developmental mortality rate

Pupation

1. Developing lifespan: graphical function based on the “Filtered temperature”
2. Developmental Stages
3. K limit: $1 - (\text{Total_Adult_Population}/K)$
4. K: 10.000.000

Male Maturation

1. Adult/2

WT Female Maturation

2. Adult/2

Male Death and Female death

1. Adult mortality rate: depends on the “WT Adult seasonality” variable which is based on constant rates depending on the season
 - 1.1 Seasons: graphical function depending on the model’s TIME function
 - 1.1.1. Spring : developmental stages mortality rate in spring
 - 1.1.2. Summer : developmental stages mortality rate in summer
 - 1.1.3. Highsummer : developmental stages mortality rate in high summer
 - 1.1.4. Fall : developmental stages mortality rate in fall
 - 1.1.5. Winter : developmental stages mortality rate in winter
 - 1.1.5.1.X: Random variable to adjust the winter adult mortality rate
 - 1.2. G: Random variable to adjust the adult mortality rate
2. Male lifespan: graphical function depending on the “Filtered temperature”
3. Female lifespan: graphical function depending on the “Filtered temperature”

In Model 2 there are additional elements:

Convertors:

1. M/+ GD fitness cost that interacts with the M/+ Developmental Death
2. M/M GD fitness cost that interacts with the M/M Developmental Death
3. Total Adult population: a summation of the three genotypic adult populations
4. Total Developmental Population: a summation of the three genotypic developing populations

Model 1 Equations

Top-Level Model:

```

Filtered_Temperature(t) = Filtered_Temperature(t - dt) + (Flow_1 -
Flow_2) * dt
INIT Filtered_Temperature = TIME
INFLOWS:
    Flow_1 = K1*(Annual_Temperature-Filtered_Temperature)
OUTFLOWS:
    Flow_2 = K2*(Filtered_Temperature-Annual_Temperature)
WT_Adult(t) = WT_Adult(t - dt) + (Pupation - WT_Male_maturation -
WT_Female_maturation) * dt
INIT WT_Adult = 0
INFLOWS:
    Pupation = WT_Developmental_Stages/Developing_lifespan*KLimit
OUTFLOWS:
    WT_Male_maturation = WT_Adult*Ratio
    WT_Female_maturation = WT_Adult*(1-Ratio)
WT_Developmental_Stages(t) = WT_Developmental_Stages(t - dt) + (Egg_lay
- Pupation - Developmental_Death) * dt
INIT WT_Developmental_Stages = 0
INFLOWS:
    Egg_lay =
E*Fertillity_Rate_per_adults*Number_of_Matings*Developmental_Klimit
OUTFLOWS:
    Pupation = WT_Developmental_Stages/Developing_lifespan*KLimit
    Developmental_Death =
WT_Developmental_Stages*Developmental_stages_mortality_rate
WT_Females(t) = WT_Females(t - dt) + (WT_Female_maturation -
WT_Female_death) * dt
INIT WT_Females = 1000
INFLOWS:
    WT_Female_maturation = WT_Adult*(1-Ratio)
OUTFLOWS:
    WT_Female_death =
WT_Females*WT_Adult_mortality_rate/Female_lifespan
WT_Males(t) = WT_Males(t - dt) + (WT_Male_maturation - WT_Male_death) *
dt
INIT WT_Males = 1000
INFLOWS:
    WT_Male_maturation = WT_Adult*Ratio
OUTFLOWS:
    WT_Male_death = WT_Males*WT_Adult_mortality_rate/Male_lifespan
Annual_Temperature = GRAPH(TIME)
(1, 4.444444444), (2, 6.666666667), (3, 5.555555556), (4, 5.555555556),
(5, 4.444444444), (6, 4.444444444), (7, 6.666666667), (8, 4.444444444),
(9, 5.555555556), (10, 5.555555556), (11, 6.666666667), (12,
4.444444444), (13, 5.555555556), (14, 4.444444444), (15, 2.777777778),
(16, 3.333333333), (17, 4.444444444), (18, 4.444444444), (19,
5.555555556), (20, 1.111111111), (21, 2.222222222), (22, 5.555555556),
(23, 5.555555556), (24, 2.222222222), (25, 3.333333333), (26,
3.333333333), (27, 2.777777778), (28, 2.222222222), (29, 4.444444444),
(30, 4.444444444), (31, 3.888888889), (32, 6.666666667), (33,
2.777777778), (34, 2.222222222), (35, 6.111111111), (36, 4.444444444),
(37, 3.333333333), (38, 6.666666667), (39, 5.555555556), (40,
4.444444444), (41, 4.444444444), (42, 5.555555556), (43, 5.555555556),
(44, 4.444444444), (45, 4.444444444), (46, 8.333333333), (47,
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(28.00, 9.9), (30.00, 12.0), (31.00, 5.0)
Developmental_K = 100000000
Developmental_Klimit = 1-(WT_Developmental_Stages/Developmental_K)
Developmental_seasonality = IF(Seasons=1) THEN winter1_1 ELSE
IF(Seasons=2) THEN spring_1 ELSE IF(Seasons=3) THEN summer_1 ELSE
IF(Seasons=4) THEN highsummer_1 ELSE IF (Seasons=5) THEN fall_1 ELSE 0
Developmental_stages_mortality_rate = Developmental_seasonality
E = 2
F = 10
fall = .0035
fall_1 = 0.011
Female_lifespan = GRAPH(Filtered_Temperature)
(10.00, 35.00), (14.00, 27.30), (18.00, 18.20), (22.00, 10.50), (26.00,
12.80), (28.00, 10.70), (30.00, 2.00)
Fertility_Rate_per_adults = GRAPH(Filtered_Temperature)
(9.00, 0.000), (10.00, 0.100), (15.00, 0.290), (20.00, 1.600), (25.00,
2.090), (30.00, 0.490), (31.00, 0.000)
G = 7.3
highsummer = 0.007
highsummer_1 = 0.056
K = 10000000
K1 = 0.38
K2 = K1
Klimit = 1-(WT_Population/K)
Male_lifespan = GRAPH(Filtered_Temperature)
(10.00, 31.0), (14.00, 20.7), (18.00, 16.8), (22.00, 13.2), (26.00,
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Number_of_Matings = MIN(WT_Males, WT_Females )
Ratio = 0.5
Seasons = GRAPH(TIME)

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1.000), (2186, 1.000), (2187, 1.000), (2188, 1.000), (2189, 1.000),
(2190, 1.000), (2191, 1.000)
spring = 0.007
spring_1 = 0.011
summer = 0.015
summer_1 = 0.002
winter1 = .069*X
winter1_1 = 0.03*F
WT_Adult_mortality_rate = WT_Adult_seasonality*G
WT_Adult_seasonality = IF(Seasons=1) THEN winter1 ELSE IF(Seasons=2)
THEN spring ELSE IF(Seasons=3) THEN summer ELSE IF(Seasons=4) THEN
highsummer ELSE IF (Seasons=5) THEN fall ELSE 0
WT_Population = WT_Males+WT_Females
X = 4
{ The model has 47 (47) variables (array expansion in parens).
  In 1 Modules with 0 Sectors.
  Stocks: 5 (5) Flows: 9 (9) Converters: 33 (33)
  Constants: 16 (16) Equations: 26 (26) Graphicals: 6 (6)
}

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Model 2 Equations

Top-Level Model:

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Filtered_Temperature(t) = Filtered_Temperature(t - dt) + (Flow_1 -
Flow_2) * dt
  INIT Filtered_Temperature = TIME
  INFLOWS:
    Flow_1 = K1*(Annual_Temperature-Filtered_Temperature)
  OUTFLOWS:
    Flow_2 = K1*(Filtered_Temperature-Annual_Temperature)
"M/+_Adult_1"(t) = "M/+_Adult_1"(t - dt) + ("M/+_Pupation" -
"M/+_Male_maturation" - "M/+_Female_maturation") * dt
  INIT "M/+_Adult_1" = 0
  INFLOWS:
    "M/+_Pupation" =
"M/+_Developmental_Stages"/Developing_lifespan*KLimit
  OUTFLOWS:
    "M/+_Male_maturation" = "M/+_Adult_1"*Ratio_1
    "M/+_Female_maturation" = "M/+_Adult_1"*(1-Ratio_1)
"M/+_Developmental_Stages"(t) = "M/+_Developmental_Stages"(t - dt) +
("M/+_Egg_lay" - "M/+_Pupation" - "M/+_Developmental_Death") * dt
  INIT "M/+_Developmental_Stages" = 0
  INFLOWS:
    "M/+_Egg_lay" =
E*Fertillity_Rate_per_adults*"M/+_Matings"*Developmental_Klimit
  OUTFLOWS:
    "M/+_Pupation" =
"M/+_Developmental_Stages"/Developing_lifespan*KLimit
    "M/+_Developmental_Death" = "M/+_Developmental_Stages" *
(1+"M/+_GD_fitness_cost") * "M/+_Developmental_stages_mortality_rate"
"M/+_Females"(t) = "M/+_Females"(t - dt) + ("M/+_Female_maturation" -
"M/+_Female_death") * dt
  INIT "M/+_Females" = 0
  INFLOWS:
    "M/+_Female_maturation" = "M/+_Adult_1"*(1-Ratio_1)
  OUTFLOWS:
    "M/+_Female_death" =
"M/+_Females"*"M/+_Adult_mortality_rate"/Female_lifespan

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"M/+_Males"(t) = "M/+_Males"(t - dt) + ("M/+_Male_maturation" -
"M/+_Male_death") * dt
  INIT "M/+_Males" = 0
  INFLOWS:
    "M/+_Male_maturation" = "M/+_Adult_1"*Ratio_1
  OUTFLOWS:
    "M/+_Male_death" =
"M/+_Males"*"M/+_Adult_mortality_rate"/Male_lifespan
"M/M_Developmental_Stages"(t) = "M/M_Developmental_Stages"(t - dt) +
("M/M_Egg_lay" - "M/M_Pupation" - "M/M_Developmental_Death") * dt
  INIT "M/M_Developmental_Stages" = 0
  INFLOWS:
    "M/M_Egg_lay" =
E*Fertillity_Rate_per_adults*"M/M_Matings"*Developmental_Klimit
  OUTFLOWS:
    "M/M_Pupation" =
"M/M_Developmental_Stages"/Developing_lifespan*KLimit
    "M/M_Developmental_Death" =
"M/M_Developmental_Stages"*"M/M_Developmental_stages_mortality_rate"*
(1+"M/M_GD_fitness_cost")
"M/M_Females"(t) = "M/M_Females"(t - dt) + ("M/M_Female_maturation" -
"M/M_Female_death") * dt
  INIT "M/M_Females" = 0
  INFLOWS:
    "M/M_Female_maturation" = "M/MT_Adult"*(1-Ratio_2)
  OUTFLOWS:
    "M/M_Female_death" =
"M/M_Females"*"M/M_Adult_mortality_rate"/Female_lifespan
"M/M_Males"(t) = "M/M_Males"(t - dt) + ("M/M_Male_maturation" + Release
- "M/M_Male_death") * dt
  INIT "M/M_Males" = 0
  INFLOWS:
    "M/M_Male_maturation" = "M/MT_Adult"*Ratio_2
    Release = "No._released_individuals"*Time_of_release
  OUTFLOWS:
    "M/M_Male_death" =
"M/M_Males"*"M/M_Adult_mortality_rate"/Male_lifespan
"M/MT_Adult"(t) = "M/MT_Adult"(t - dt) + ("M/M_Pupation" -
"M/M_Male_maturation" - "M/M_Female_maturation") * dt
  INIT "M/MT_Adult" = 0
  INFLOWS:
    "M/M_Pupation" =
"M/M_Developmental_Stages"/Developing_lifespan*KLimit
  OUTFLOWS:
    "M/M_Male_maturation" = "M/MT_Adult"*Ratio_2
    "M/M_Female_maturation" = "M/MT_Adult"*(1-Ratio_2)
WT_Adult(t) = WT_Adult(t - dt) + (Pupation - WT_Male_maturation -
WT_Female_maturation) * dt
  INIT WT_Adult = 0
  INFLOWS:
    Pupation = WT_Developmental_Stages/Developing_lifespan*KLimit
  OUTFLOWS:
    WT_Male_maturation = WT_Adult*Ratio
    WT_Female_maturation = WT_Adult*(1-Ratio)
WT_Developmental_Stages(t) = WT_Developmental_Stages(t - dt) +
(WT_Egg_lay - Pupation - Developmental_Death) * dt
  INIT WT_Developmental_Stages = 0
  INFLOWS:

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        WT_Egg_lay =
E*Fertillity_Rate_per_adults*WT_Matings*Developmental_Klimit
    OUTFLOWS:
        Pupation = WT_Developmental_Stages/Developing_lifespan*KLimit
        Developmental_Death =
WT_Developmental_Stages*Developmental_stages_mortality_rate
WT_Females(t) = WT_Females(t - dt) + (WT_Female_maturation -
WT_Female_death) * dt
    INIT WT_Females = 1000
    INFLOWS:
        WT_Female_maturation = WT_Adult*(1-Ratio)
    OUTFLOWS:
        WT_Female_death =
WT_Females*WT_Adult_mortality_rate/Female_lifespan
WT_Males(t) = WT_Males(t - dt) + (WT_Male_maturation - WT_Male_death) *
dt
    INIT WT_Males = 1000
    INFLOWS:
        WT_Male_maturation = WT_Adult*Ratio
    OUTFLOWS:
        WT_Male_death = WT_Males*WT_Adult_mortality_rate/Male_lifespan
"No._released_individuals" = 15000
Annual_Temperature = GRAPH(TIME)
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(5, 4.444444444), (6, 4.444444444), (7, 6.666666667), (8, 4.444444444),
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Developing_lifespan = GRAPH(Filtered_Temperature)
(8.00, 90.0), (9.00, 87.0), (10.00, 79.3), (14.00, 28.8), (18.00,
18.2), (20.00, 16.0), (22.00, 14.0), (25.00, 11.0), (26.00, 10.8),
(28.00, 9.9), (30.00, 12.0), (31.00, 5.0)
Developmental_K = 100000000
Developmental_Klimit = 1-
(Total_Developmental_Population/Developmental_K)
Developmental_seasonality = IF(Seasons=1) THEN winter1_1 ELSE
IF(Seasons=2) THEN spring_1 ELSE IF(Seasons=3) THEN summer_1 ELSE
IF(Seasons=4) THEN highsummer_1 ELSE IF (Seasons=5) THEN fall_1 ELSE 0
Developmental_seasonality_1 = IF(Seasons=1) THEN winter1_2 ELSE
IF(Seasons=2) THEN spring_5 ELSE IF(Seasons=3) THEN summer_3 ELSE
IF(Seasons=4) THEN highsummer_3 ELSE IF (Seasons=5) THEN fall_3 ELSE 0
Developmental_seasonality_2 = IF(Seasons_2=1) THEN winter1_4 ELSE
IF(Seasons_2=2) THEN spring_4 ELSE IF(Seasons_2=3) THEN summer_5 ELSE
IF(Seasons_2=4) THEN highsummer_5 ELSE IF (Seasons_2=5) THEN fall_5
ELSE 0
Developmental_stages_mortality_rate = Developmental_seasonality
E = 1.95840456587876
F = 10.4540472683207
fall = 0.0035
fall_1 = 0.011
fall_2 = 0.0035
fall_3 = 0.011
fall_4 = 0.0035
fall_5 = 0.011
Female_lifespan = GRAPH(Filtered_Temperature)
(10.00, 35.00), (14.00, 27.30), (18.00, 18.20), (22.00, 10.50), (26.00,
12.80), (28.00, 10.70), (30.00, 2.00)
Fertillity_Rate_per_adults = GRAPH(Filtered_Temperature)
(9.00, 0.000), (10.00, 0.100), (15.00, 0.290), (20.00, 1.600), (25.00,
2.090), (30.00, 0.490), (31.00, 0.000)
G = 7.28613762570794
highsummer = 0.007
highsummer_1 = 0.056
highsummer_2 = 0.007
highsummer_3 = 0.056
highsummer_4 = 0.007
highsummer_5 = 0.056
K = 10000000
K1 = 0.38
Klimit = 1-(Total_Adult_Population/K)
"M/+_Adult_mortality_rate" = "M/+_Adult_seasonality"*G
"M/+_Adult_seasonality" = IF(Seasons_1=1) THEN winter1_5 ELSE
IF(Seasons_1=2) THEN spring_2 ELSE IF(Seasons_1=3) THEN summer_2 ELSE
IF(Seasons_1=4) THEN highsummer_2 ELSE IF (Seasons_1=5) THEN fall_2
ELSE 0
"M/+_Developmental_stages_mortality_rate" = Developmental_seasonality_1
"M/+_GD_fitness_cost" = 0.28
"M/+_Matings" = ((0.5*MIN("M/+_Males",
WT_Females))+(0.5*MIN("M/+_Females",
WT_Males)))+(0.5*MIN("M/+_Females", "M/+_Males"))+(1*MIN("M/M_Males",
WT_Females))+(1*MIN(WT_Males, "M/M_Females"))+(0.5*MIN("M/+_Females",
"M/M_Males"))+(0.5*MIN("M/M_Males", "M/+_Females"))))

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"M/+_Population" = "M/+_Males"+"M/+_Females"
"M/M_Adult_mortality_rate" = WT_Adult_seasonality_2*G
"M/M_Developmental_stages_mortality_rate" = Developmental_seasonality_2
"M/M_GD_fitness_cost" = 0.65
"M/M_Matings" = (0.25*MIN("M/+_Females",
"M/+_Males")+0.5*MIN("M/+_Females", "M/M_Males")+0.5*MIN("M/M_Females",
"M/+_Males")+1*MIN("M/M_Females", "M/M_Males"))
"M/M_Population" = "M/M_Males"+"M/M_Females"
Male_lifespan = GRAPH(Filtered_Temperature)
(10.00, 31.0), (14.00, 20.7), (18.00, 16.8), (22.00, 13.2), (26.00,
12.8), (28.00, 10.1), (30.00, 1.0)
Ratio = 0.5
Ratio_1 = 0.5
Ratio_2 = 0.5
Seasons = GRAPH(TIME)
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spring = 0.007
spring_1 = 0.011
spring_2 = 0.007
spring_3 = 0.007
spring_4 = 0.011
spring_5 = 0.011
summer = 0.015
summer_1 = 0.002
summer_2 = 0.015
summer_3 = 0.002
summer_4 = 0.015
summer_5 = 0.002
Time_of_release = IF (TIME=435) OR (TIME=445) OR (TIME=455) THEN 1 ELSE 0
Total_Adult_Population =
"M/+_Population"+"M/M_Population"+WT_Population
Total_Developmental_Population =
WT_Developmental_Stages+"M/+_Developmental_Stages"+"M/M_Developmental_S
tages"
winter1 = .025*X
winter1_1 = 0.029*F
winter1_2 = 0.029*F
winter1_3 = .025*X
winter1_4 = 0.029*F
winter1_5 = .025*X
WT_Adult_mortality_rate = WT_Adult_seasonality*G
WT_Adult_seasonality = IF(Seasons=1) THEN winter1 ELSE IF(Seasons=2)
THEN spring ELSE IF(Seasons=3) THEN summer ELSE IF(Seasons=4) THEN
highsummer ELSE IF (Seasons=5) THEN fall ELSE 0
WT_Adult_seasonality_2 = IF(Seasons_2=1) THEN winter1_3 ELSE
IF(Seasons_2=2) THEN spring_3 ELSE IF(Seasons_2=3) THEN summer_4 ELSE
IF(Seasons_2=4) THEN highsummer_4 ELSE IF (Seasons_2=5) THEN fall_4
ELSE 0
WT_Matings = 1* MIN( WT_Females, WT_Males)+0.5*MIN(WT_Females,
"M/+_Males")+0.5*0*MIN("M/+_Females",
WT_Males)+0.25*0*MIN("M/+_Females", "M/+_Males")
WT_Population = WT_Males+WT_Females
X = 3.96731030852217
{ The model has 111 (111) variables (array expansion in parens).

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In 1 Modules with 0 Sectors.  
Stocks: 13 (13) Flows: 24 (24) Converters: 74 (74)  
Constants: 37 (37) Equations: 61 (61) Graphicals: 8 (8)  
}
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