An egg is always an adventure: anthropogenic impacts on Culex pipiens population dynamics

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Graphical illustration of an adult female house mosquito in mid-flight.

Chapter 1

General introduction

1.1 Infectious disease burden in Europe

In the past century the patterns of human trade and travel, climate conditions, and land and water usage, have undergone significant change (Lambin & Meyfroidt, 2011). The joint effect of these changes have caused rapid transmission of diseases to become more prevalent (Lühken et al., 2023), highlighting the interconnectedness of human well-being with earth's natural systems. The need for collaborative efforts and shared resources is therefore increasingly emphasized, to mitigate the spread of diseases, guarantee access to essential healthcare services, and promote equitable health outcomes worldwide, a concept known as planetary health (Whitmee et al., 2015).

Infectious diseases constitute a key challenge to planetary health (Myers, 2017), encompassing a wide array of pathogens including bacteria, viruses, and parasites. Among the various mechanisms through which these pathogens spread, arthropods play a crucial role as *vector** (WHO, 2014). Mosquito-borne pathogens, in particular, contribute significantly to the global disease burden, with a majority of arthropod-borne disease burden attributed to them (WHO, 2014).

Despite the interconnectedness facilitated by globalization and the changing environmental conditions, the current morbidity of infectious diseases, at least in Europe is still relatively low (Charalampous et al., 2023; Hilderink et al., 2020; Figure 1.1 exemplifying this for the Netherlands), especially when compared to tropical regions, where such diseases (e.g. Chikungunya, Dengue, malaria, West Nile fever and Zika) are increasingly prevalent (Global Burden of Disease Pediatrics Collaboration, 2016; Roser et al., 2021). These figures are in stark contrast to historical figures, e.g. in the 19th century, when Europe was more susceptible to outbreaks of infectious diseases than recently, with circulation of malaria (Boualam et al., 2021; Green, 2018), Cholera, typhoid and tuberculosis (Griffioen, 2024; Peters et al., 2021; Wolleswinkel-van den Bosch et al., 1997). Understanding whether the abovementioned global changes may eventually lead to the return of omnipresent vector borne diseases (hereafter VBD) in Europe as a key challenge, may aid the public-health infrastructure to prepare for potential re-emergence. For this, identifying changes in the *pathogenic landscape**, related to the disease ecology, is pivotal (Gratz, 1999).

*See textbox 1 for definitions.

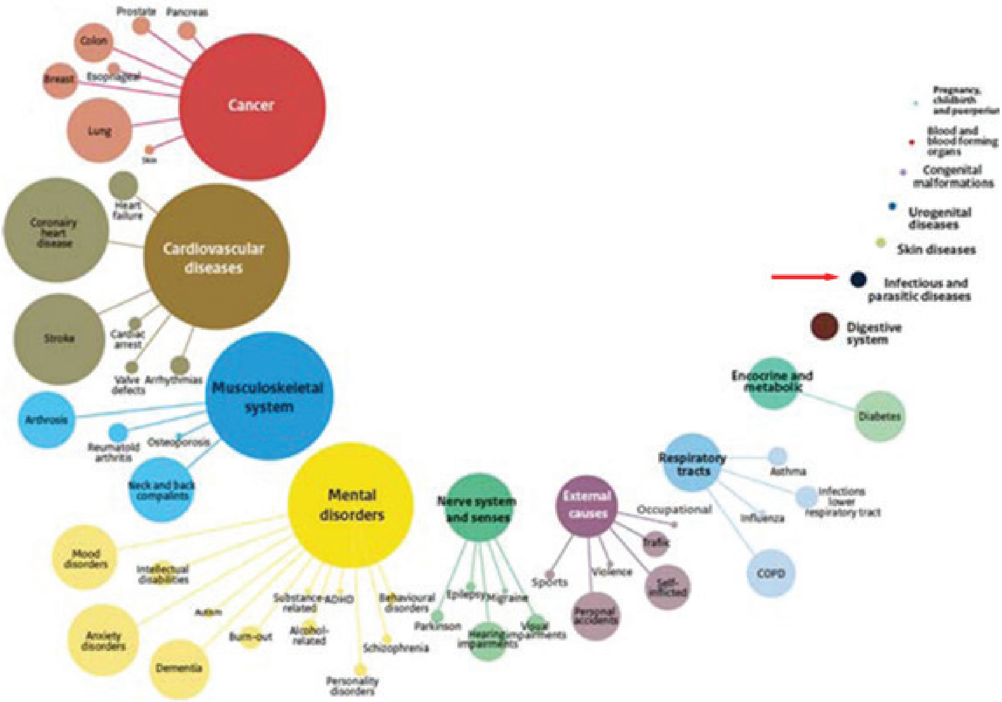

Figure 1.1 *Burden of Disease in the Netherlands in 2015, for 17 chapters of the international classification of diseases and associated health conditions. The size of the bubbles represents the burden of disease, expressed in disability-adjusted life years. Burden of disease for the combined infectious diseases is highlighted with a red arrow (Adapted from Hilderink et al., 2020).*

1.2 Pathogenic landscapes

The pathogenic landscape – i.e., the interplay between environment, people, vectors, and animal *hosts** – is subject to diverse natural processes that operate along spatial and temporal axes. Spatially, infectious diseases are influenced by factors such as land use types, vector habitat preferences, wildlife habitats, and ultimately human interactions with vectors, all of which contribute to the geographic distribution and prevalence of diseases (Abella-Medrano et al., 2015; Day, 2016; Ferraguti et al., 2023). Temporally, disease dynamics exhibit variations driven by climate-induced seasonal changes, fluctuations in vector activity, patterns of host migration and movement, and global travel, impacting the likelihood of disease transmission and outbreak occurrence (Beck-Johnson, Nelson, Paaijmans, Read, & Bjørnstad, 2017; Gratz, 1999; Tatem et al., 2012).

*See textbox 1 for definitions.

There is a discernible trend of increasing incidence and spread of pathogen outbreaks over time, extending beyond natural oscillations (Lühken et al., 2023), suggesting that anthropogenic influences such as human activities and environmental changes underly these trends. These activities directly affect pathogens via encounter rates with vectors, and indirectly affect pathogens via climate change, land use change, and water management, thereby impacting habitat quantity and quality for *reservoir species**, vector species, and human populations (see section 1.4).

Changes in the pathogenic landscape may impact, and be impacted by, shifts in the distributions and abundances of reservoir species, vector species, and human populations, influencing disease transmission and outbreak potential (Lambin et al., 2010). Traditional assessments and interventions for infectious diseases often operate in separate disciplines (i.e. ecology, entomology and medical sciences) and measures tend to be reactive, initiated only after disease spillover into human populations has occurred, thereby often limiting their effectiveness (Laydon et al., 2023; Sacco et al., 2023).

Proactive measures that anticipate and mitigate outbreaks prior to occurrence might lead to more effective responses to disease outbreaks (Laydon et al., 2023; Meckawy et al., 2022; Sacco et al., 2023; Figure 1.2). By addressing the underlying factors driving changes in the pathogenic landscape, proactive measures can help prevent the emergence and spread of infectious diseases. A holistic assessment, integrating insights from various disciplines, is necessary to understand and manage infectious diseases effectively. A *One Health** approach offers a framework for comprehensive assessment and intervention, considering the complex interactions within the pathogenic landscape, to improve disease management in a changing world.

*See textbox 1 for definitions.

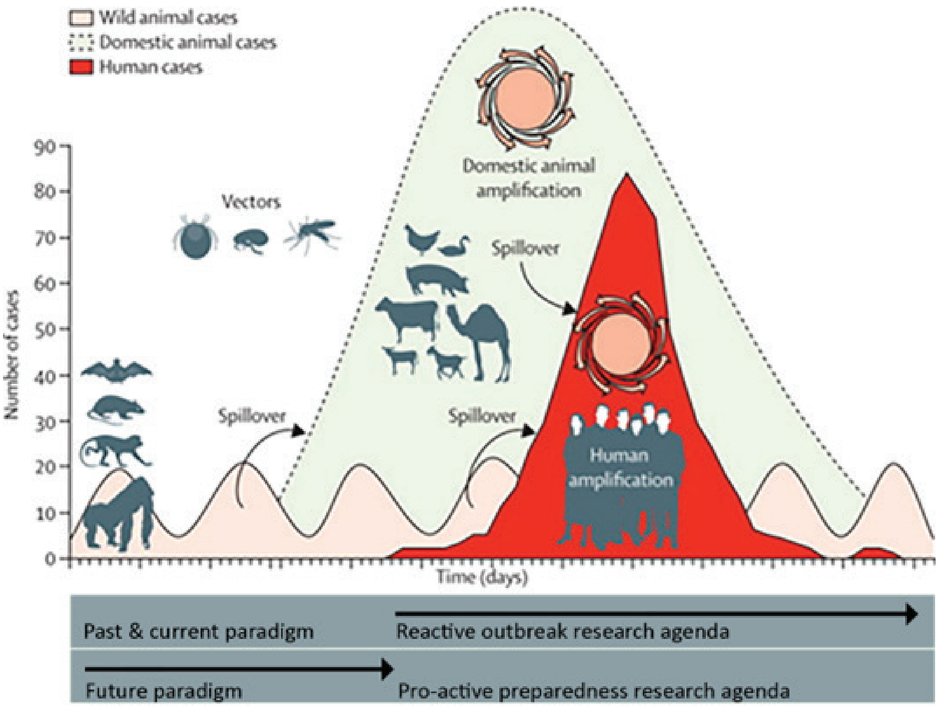

Figure 1.2 *Transmission of infection and amplification in people (bright red) occurs after a pathogen from wild animals (pink) moves into reservoir species (e.g., livestock) to cause an outbreak (light green) that amplifies the capacity for pathogen transmission to people. Proactive research may provide early warning indicators for changes for risk assessment and fitting interventions to limit spillover to people. Spillover arrows show cross-species transmission. (Adapted from Karesh et al., 2012).*

1.3 A one health approach towards preparedness

Early detection of infectious disease outbreaks is vital for timely and proactive response and effective interventions. Monitoring of early warning indicators, including changes in vector populations, wildlife behavior, and environmental conditions, can provide crucial insights into potential disease emergence (Becker et al., 2019; Childs et al., 2019; Montgomery et al., 2017). Effective interventions, however, require a comprehensive understanding of the factors that shape the pathogenic landscape which includes intricate interactions between different players, including hosts, vectors, and environmental factors. Information on the relative importance of these factors is currently, however, not available. Acquiring this information requires interdisciplinary expertise, as interventions can then be tailored to target specific vulnerabilities within the pathogenic landscape.

To address the interdisciplinary challenges regarding this approach to study infectious disease, the One Health PACT consortium was assembled in 2020 (www.onehealthpact.org), a partnership between (Dutch) national and international partners, to address shared health challenges collectively. The project is a collaboration of experts ranging from ecological and climate modelling to medical entomology, virology and public health, and aims to determine large-scale changes (tipping points) in disease transmission, which could lead to outbreaks of VBD. To this end, the project i) generates data on the dynamics of humans, vectors, and reservoirs, ii) characterizes arbovirus infections, iii) models the complex interplay that drive arbovirus dynamics, and iv) develops interventions and surveillance strategies. This thesis plays a central role in this endeavor by investigating the ramifications of the effects of land use and change therein on mosquito vector populations.

By investigating how anthropogenic activities impact ecosystem dynamics, proactive measures, including a surveillance network, can be developed for timely interventions to minimize disease risks. Identifying factors that limit disease outbreaks, such as vector presence and abundance, further informs these interventions by identifying and predicting potential risk sites. Vector ecology plays a crucial role in this process, as vector presence and abundance directly influence disease dynamics (Kolimenakis et al., 2021), and since effective interventions often target mosquitoes (Aryaprema et al., 2023; Benelli, 2015; Benelli et al., 2016; Floore, 2006; Pilger et al., 2010). Thus, integrating insights from ecological studies on critical facets of disease transmission, including vector dynamics, enables a comprehensive approach to mitigating the impacts of infectious diseases as a result of anthropogenic changes in the environment.

1.4 Mosquito ecology in a changing world

Anthropogenic impacts on disease transmission can occur in a number of ways 1) through changes in land use, 2) through changes in water management, 3) through changes in climatic conditions, and joined effects of these on overall biodiversity. These factors are especially relevant to the transmission of mosquito borne diseases, which are responsible for a major part of vector borne disease incidences (van den Berg & Schaffner, 2016; WHO, 2017).

Vector borne diseases may be affected by fragmentation of ecosystems resulting from the conversion of natural habitats, e.g. for agriculture and urbanization (Ferraguti et al., 2016, 2023). Deforestation, in particular, disturbs wildlife habitats, leading to increased human-wildlife interactions and observed subsequent spillover of pathogens (Gottwalt, 2013; Gratz, 1999; Ortiz et al., 2022). Changes in land use can modify vector habitat suitability, promoting pioneer species, such as disease-transmitting mosquitoes (Abella-Medrano et al., 2015; Steiger et al., 2012). Agricultural practices, including irrigation and deforestation, can create nutrient rich breeding grounds for disease vectoring mosquitoes, amplifying disease transmission cycles (Buxton et al., 2020; Rasmussen, 1998; Waters et al., 2021). Moreover, urbanization concentrates human populations, facilitating the rapid spread of infectious diseases in densely populated areas (Romeo-Aznar et al., 2018).

Water management, and changes therein, may impact pathogen transmission as well (Akanda et al., 2020). Changes in water flow patterns, for instance, can create favorable conditions for mosquito breeding, leading to increased transmission of VBD (Elias et al., 2022). Poor sanitation and wastewater management further exacerbate the risk of disease transmission by creating high quality breeding habitats (Lines et al., 1994).

Climate change-induced alterations in precipitation patterns and water availability can further influence disease dynamics by affecting water permeability of soil, thereby creating mosquito breeding sites (Caplan et al., 2019; Krol et al., 2023). Rising temperatures can expand the geographic range of disease vectors, allowing them to thrive in previously inhospitable areas (Kraemer et al., 2015; Lühken et al., 2023; Medlock et al., 2012). Ongoing salinization on the other hand may limit their geographic range (Jardine et al., 2008; Roberts & Irving-Bell, 1997). Finally, climate change can disrupt seasonal patterns, prolonging the transmission seasons of VBD (Romeo-Aznar et al., 2018) and increasing the frequency and intensity of extreme weather events (Stott, 2016), which can facilitate disease outbreaks and exacerbate health risks (Esser et al., 2019). Although all these facets can impact mosquito ecology, it is currently unknown what their relative importance is, as this is highly context dependent. Their relevance, particularly in the Dutch landscape, should therefore be more closely examined.

1.5 Shifting mosquito habitat dynamics in the pathogenic landscape

Understanding mosquito ecology is crucial to identify how anthropogenic influences impact mosquito population dynamics and behavior, and thereby associated disease transmission and eventually the pathogenic landscape. The quality and availability of larval habitats play a critical role in shaping mosquito populations (Beck-Johnson et al., 2013; Radrova et al., 2013; Rejmánková, Grieco, Achee, & Roberts, 2013). Evaluating *pressures** from environmental variation and human impacts (Lambin et al., 2001; Steiger et al., 2011) on habitat quality and availability therefore provides a framework for understanding the impacts on mosquito *oviposition** behavior and *life-history traits**.

Any one pressure may have multiple (interconnected) influences. For instance, habitat quality may directly shape populations, by influencing factors such as development rate, mortality and sex ratio (Beck-Johnson et al., 2013; Couret & Benedict, 2014; Knight et al., 2004). Additionally, habitat quality may alter ovipositioning behavior, thereby impacting whether a larval habitat gets colonized at all. Namely, changes in pressures, such as food availability, chemical pollution and density of other mosquito larvae, may alter cues that inform decision making on where and where not to put one's eggs (M. Akiner & Eksi,

*See textbox 1 for definitions.

2015; Dhileepan, 1997; J. Li et al., 2009; Mwingira et al., 2020; Rejmánková, Grieco, Achee, Roberts, et al., 2013). The identified *stressors** therefore provide insight in the dynamics and productivity of these habitats and can thus be used to model and predict adult abundance. This in turn can be used to inform interventions and thereby limit disease risk. Although most current literature focuses on a limited set of single stressors (Alcalay et al., 2018), more and more studies demonstrate the importance of synergetic and antagonistic effects on population dynamics (Couret & Benedict, 2014; Krol et al., 2019; Meyabeme Elono et al., 2018; Schrama et al., 2018; Sukiato et al., 2019), highlighting the need for a more holistic approach.

1.6 Quantifying effects of stressors on mosquito population dynamics

Mosquito population dynamics are characterized by several parameters dictating population size and structure, including development rate, survival rate, sex ratio, and oviposition behavior. None of these parameters occur in a vacuum and as a result they may impact each other. For instance, high conspecific densities may speed up development (Alcalay et al., 2018) and ovipositioning behavior may affect larval survival and population growth (Ikeshoji, 1966). These parameters and their interactions determine the size and distribution of mosquito populations, in turn influencing transmission of mosquito-borne diseases.

Development rate, defined as the duration it takes for mosquitoes to progress from egg to adult (Figure 1.3), is dependent on resource availability and expenditure (Beketov & Liess, 2007; Huxley et al., 2021; Rivero et al., 2011; Ukubuiwe et al., 2017), with potentially life stage specific effects (Clark et al., 2004). Generally limited by the amount of energy allocated for growth, development times may adaptively increase as maintenance and repair processes to maintain homeostasis become increasingly necessary as a result of stress. This stress can be due to various factors impacting metabolic rate (such as food availability and temperature) or toxicity (e.g., water quality, salinization and pesticides). Under exposure to accumulative stressors, however, there may be selection for early maturation (Alcalay et al., 2018; Jourdan et al., 2016). By entering metamorphosis with fewer resources, the individual then increases its chances to emerge, at the cost of decreased longevity and fecundity (Alcalay et al., 2018; Reisen et al., 1984).

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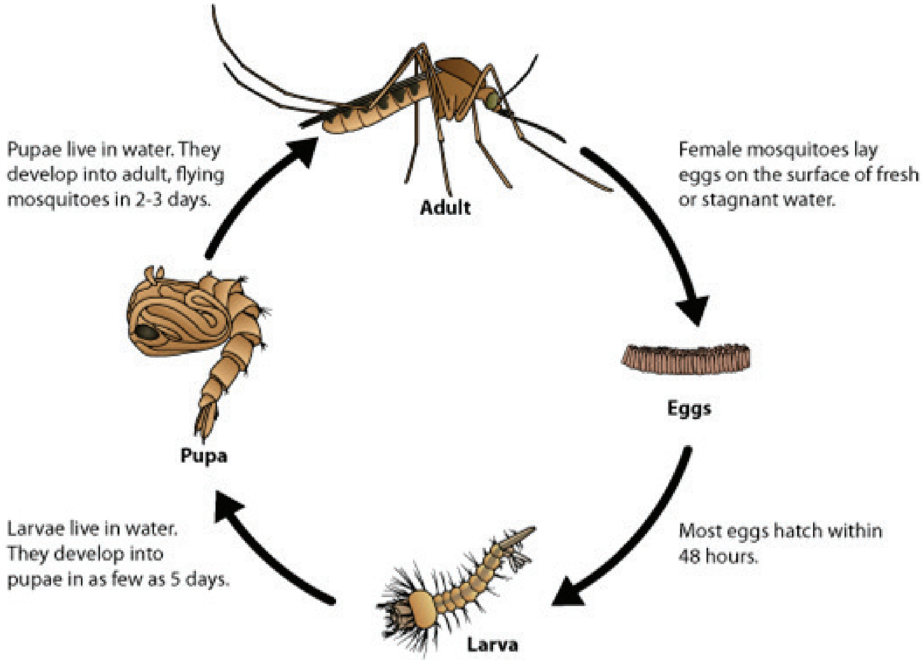

Figure 1.3 *Life stages of Culex species mosquitoes (Adapted from CDC, 2022)*

Responses in development rate are relatively well known for single stressors like temperature, eutrophication, pesticides and conspecific density under static conditions (Agnew et al., 2000; Ciota et al., 2014; Couret & Benedict, 2014; Kiarie-Makara et al., 2015), but increasing evidence suggests that including natural fluctuations, particularly for temperature, may exacerbate the effects of a stressor (Beck-Johnson, Nelson, Paaijmans, Read, Thomas, et al., 2017; Colinet et al., 2015; Hagstrum & Milliken, 1991). While including natural oscillations may yield more accurate and relevant results, it is underrepresented, likely as small-scale and affordable experimental systems were until recently unavailable (Hagstrum & Milliken, 1991; Hermann et al., 2022). This highlights the need for an inexpensive fieldable temperature control system and validation comparing commonly used temperature regimes for mosquitoes.

The impacts of the environmental variable salinization are less well known, but nevertheless of increasing relevance (Lassiter, 2021). While numerous studies have examined mortality associated with salinity (Kengne et al., 2019; Thamer & Abdulsamad, 2005; Van Schie et al., 2009), and occasionally sublethal effects such as development rates (Schuler & Relyea, 2018), little is known on sublethal effects for species considered particularly vulnerable to salinity, like *Cx. pipiens* (Kengne et al., 2019). This lack of understanding is especially true for interactive stressors like temperature, which could modify ion uptake via increased metabolism. Information on the impacts of salinity is even scarcer in regions experiencing ongoing salinization, such as the Netherlands (Oude Essink et al., 2010; Pauw et al., 2012). Gradual exposure near the coast in such regions may have caused adaptations (Ramasamy & Surendran, 2012), possibly developing more rapidly than in their predators, due to differences in generation time, which may cause alleviation in predation pressure (Schuler & Relyea, 2018). Understanding the impacts of salinization is therefore crucial, as this may inform us whether to regard areas with ongoing salinization as potential mosquito hotspots.

Mosquito survival, referring to the percentage of individuals that develop from one life stage to the next, is impacted similarly, through effects of resource availability (and lack thereof) and expenditure. For instance, minor stress may be overcome if sufficient resources are available (Huxley et al., 2021). If limited resources are available, survival may be prioritized over reproduction, as described above. As a result, combined and synergistic stressor effects may cause increased mortality compared to single stressor experiments (Mottram et al., 1994). One such synergistic interaction may involve larval competition (Agnew et al., 2000), translating to availability of food resources per capita. This interaction is known to depend on larval density (Alto et al., 2012), larval habitat size (Wynn & Paradise, 2001) and microbial abundance (Peck & Walton, 2006). A potential link with temperature exists via bacterial production and larval competition (Adams et al., 2010; Ezeakacha & Yee, 2019), but has not been explored in this context. These impacts on mortality may be sex-specific, thereby causing shifts in sex ratio. Female mosquitoes on average take longer to emerge as a result of their larger body size (Loetti et al., 2011; Virginio et al., 2015). As a result, they may be exposed to (accumulative) stress for longer periods than male individuals, which may cause increased mortality resulting in a skewed sex ratio (Alcalay et al., 2018).

There are many ways in which individuals may deal with stress, such as adaptations in morphology and behavior, or by avoiding high-stress locations altogether. Egg-laying behavior actively affects the impacts of stressors to which the eggs, larvae and pupae are subjected. For instance, gravid females provide their brood with favorable conditions such as food availability and lowered competition (Bruno & Laurence, 1979; Hasselschwert & Rockett, 1988; Mwingira et al., 2020; Silberbush et al., 2014). As a result, conditions under which mosquito larvae theoretically could be present, may not be colonized (M. M. Akiner & Eksi, 2015; Day, 2016; Ikeshoji & Mulla, 1970). It is therefore essential to determine both the effects of single stressors as well as antagonistic and synergetic effects on egg-laying behavior to contextualize the results of the experiments of *systemic** and *external drivers**.

*See textbox 1 for definitions.

1.7 Interventions

Interventions to mitigate mosquito-borne disease transmission primarily focus on mosquito control (Aryaprema et al., 2023; Pilger et al., 2010). Among these interventions, a substantial proportion concentrates on the control of mosquito larvae, as immature stages are confined in relatively high densities in small habitats and allow for increased efficacy (Becker et al., 2013). Such interventions historically often involved the use of chemicals like biocides (Nazni et al., 2005; Rochlin et al., 2016). More recently, use of toxic secondary metabolites from microorganisms such as Bti has become prevalent (Kroeger et al., 2013; Poopathi & Abidha, 2010; Setha et al., 2007). However, these chemical methods are short-term solutions, leading to resistance (Hamdan et al., 2005; Li et al., 2002) and creating a pesticide treadmill. As competing species are often impacted similarly, chemical control may result in rapid recolonization (Meyabeme Elono et al., 2018) and overcompensation (Neale & Juliano, 2019) by mosquitoes and subsequent ecological imbalances (Allgeier et al., 2019; Brühl et al., 2020; Meyabeme Elono et al., 2018), potentially posing risks to conservation areas. This combined with the limited information on food-web effects (Brühl et al., 2020), and the subsequent unknown fate of the substances, makes its use risky, especially in conservation areas. As a result, development of alternative interventions is increasingly considered.

In recent years, proactive measures have gained considerable attention as alternatives to traditional larvicides. Notably, natural control – i.e., the release or facilitation of predator species – has emerged as a promising alternative (Benelli et al., 2016). Many mosquito breeding sites, however, especially those in urban and peri-urban areas, lack natural predators. Facilitating predatory organisms in these breeding sites therefore presents an environmentally sustainable, long-term and potentially effective means of mosquito control. This method deters egg-laying (Rubbo et al., 2011; Sougué et al., 2021) and limits immature survival whilst lowering growth rates, fecundity and delaying reproduction (S. Fischer et al., 2012; Lundkvist et al., 2003; Schrama et al., 2018). Additionally, stressful conditions – including predator presence – promote a more heterogeneous mix of developmental stages, as a result of different growth strategies among the mosquito larvae (S. Fischer et al., 2012; Knight et al., 2004), which could enable cannibalistic behavior (El Husseiny et al., 2018; Koenraadt & Takken, 2003).

The potential predator species for mosquito larval control encompass a diverse array of organisms, including aquatic beetles, true bugs, amphibians, and fishes (Covaciu-Marcov, 2010; DuRant & Hopkins, 2008; Fischer et al., 2012; Lundkvist et al., 2003; Saha et al., 2010; Saleeza et al., 2014). These predators exhibit species-specific degrees of effectiveness in controlling mosquito larvae, depending on factors such as habitat type, mosquito species, and predator-prey interactions. Current information on predator efficiency is, however, mainly limited to groups of invertebrate species (Benelli, 2015; Lacey & Orr, 1994; McDonald & Buchanan, 1981), while amphibians are hypothesized to be far more efficient (DuRant & Hopkins, 2008). As such, despite the growing interest in employing natural predators for mosquito control, a critical gap exists in our understanding of which species are most suitable for this purpose.

Additionally, there is a scarce information indicating interactions between predation and the environment (Krol et al., 2019; Schrama et al., 2018). Murky – nutrient-rich – waters affect larval mosquito feeding behavior (Dadd, 1975; Merritt et al., 1992), and may therefore impact predator encounter rates. Eutrophication's impact on predators is uncertain (Schmutzer et al., 2008), potentially affecting their oxygen acquisition (Coffin et al., 2021) and hunting efficiency due to increased anti-predator behavior (Tuno et al., 2004) and reduced vision-based hunting (Abrahams & Kattenfeld, 1997), increasing ways for the prey to seek shelter. This highlights the need for a comparison of amphibian and invertebrate predator groups, and an assessment of predator effectiveness across the eutrophic range.

1.8 Aims of this thesis

The primary goal of this thesis is to identify the key drivers of *Culex pipiens* population dynamics in response to anthropogenic drivers (objective f). To gain a comprehensive understanding of how the drivers shape *Culex pipiens* its population dynamics, this thesis will assess the main systemic and external pressures (objective a and d) of selected stressors, their interactions, and how they interact with the life-history traits and egg laying behavior (objective e; Figure 1.4). Additionally, this thesis will assess a potential adaptation to ongoing coastal salinization (objective c) and evaluate the impacts of temperature fluctuations (objective b), to bring these results into their ecological context.

To address these objectives, the current thesis will focus on *Culex pipiens* s.l.. This species complex, more commonly referred to as the house mosquito, is a cosmopolitan assemblage consisting of the species *Culex australicus*, *Culex globocoxitus*, *Culex pipiens* and *Culex quinquefasciatus*. *Culex pipiens* s.l. is the most abundant mosquito species assemblage in the Netherlands, (Ibanez-Justicia et al., 2015) and locally consists of *Cx. pipiens pipiens*, *Cx. pipiens* molestus and *Cx. torrentium*. This thesis will treat this assemblage as a whole, as the complex is extremely tedious to separate these three species by morphological and molecular methods (Harbach, 2012). The species can hibernate and is therefore frequently found during winter (Becker et al., 2013; Cianci et al., 2015). It has a wide tolerance for temperature and eutrophication levels and is known to occupy almost every kind of water source, making it a common nuisance in urban environments (Becker et al., 2013).

Culex as a genus represents the predominant vectors of West Nile virus (WNV), of which the *Culex pipiens* assemblage is the most widespread (Harbach, 2012). Wild populations of the common house mosquito have also been confirmed to transmit avian malaria, Batai, Sindbis and Usutu (Ibanez-Justicia et al., 2015). An additional aptitude for transmission of *Dirofilaria immitis*, Tahyna and Zika was verified in lab experiments (Guo et al., 2016; Ibanez-Justicia et al., 2015). Due to its high abundance, wide range and its aptitude for transmission of a wide variety of diseases, it is important to understand the population dynamics of the species. Even more so considering the recent findings of WNV in the north-west of Europe, including the United Kingdom and the Netherlands (Folly et al., 2020; Vlaskamp et al., 2020). Both for assessment of vector competence under different scenarios and as a competitor with invasive species.

This thesis examines the main external and systemic drivers of population parameters for *Culex pipiens* (Diptera: *Culicidae*), the common house mosquito in a mesocosm set-up. For this, the current thesis focuses on the interactions between and within i) systemic pressures impacting larval habitat, such as climate variables and nutrient availability and ii) external pressures impacting *Cx. pipiens* larvae, such as predation and density dependence.

Specifically, the work in this thesis has the following objectives:

a. Assess the effects of salinization, temperature, eutrophication, larval density and relevant interactions on mosquito populations.
b. Develop a temperature control system to study the significance of thermal variation on mosquito development.
c. Evaluate local adaptation of house mosquito populations to salinization along a coastal-to-inland gradient by quantifying their tolerance to varying chloride concentrations.
d. Assess the effectiveness of common amphibian species in controlling mosquito populations.
e. Determine the influence of selected stressors and their interactions on egg-laying behavior.
f. Identify the main anthropogenic drivers on *Culex pipiens* population dynamics.

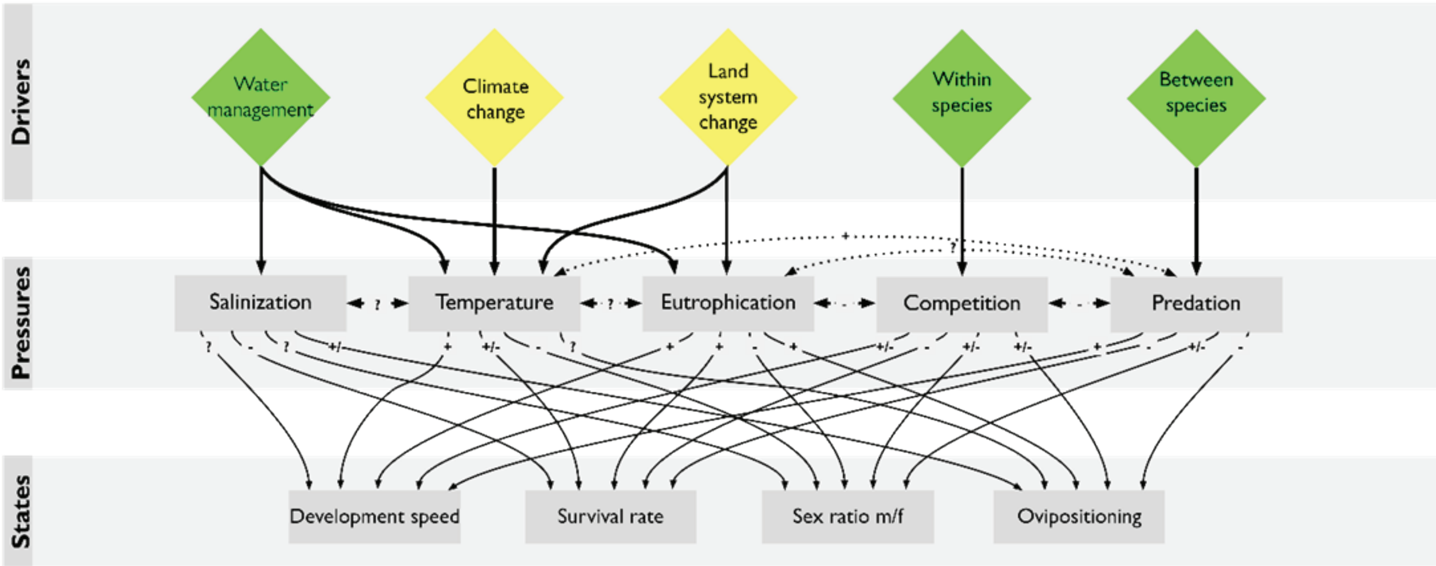

Figure 1.4 *Overview of systemic and external drivers on abiotic pressures and its impacts on downstream population parameters of mosquitoes. For each of the established impacts between pressures and states, it is indicated whether the association is positive (+), negative (−) or both (+/−). Question marks indicate hypothesized links and interactions of pressures where further work is needed.*

1.9 Thesis outline

Chapter 2 investigates the impact of anthropogenic pressures on mosquito populations and the potential implications for the transmission of mosquito-borne diseases. Specifically, the text aims to explore the effects of salinization, temperature, and food availability on mosquito populations, as well as the interactions between these factors (objectives a and e). This chapter aims to provide a comprehensive assessment of the effects of these environmental stressors on mosquito population parameters. The presented study provides an overview of the main stressors and interactions on Culex pipiens population dynamics, thereby providing a basis for predictive models, prevention and mitigation strategies.

Chapter 3 further examines the effects of temperature via the development of an inexpensive, easily reproducible, and open-source Arduino-based temperature control system that can emulate natural sinusoidal fluctuations in temperature above ambient levels (objective b). The ultimate goal is to provide a more accurate assessment of the relative importance of thermal variation on ectotherm development, which could have implications for predicting the effects of large-scale disturbances like climate change on these organisms.

Chapter 4 evaluates whether (local) adaptation to salinization has occurred in populations of the house mosquito along a gradient from coast to inland (objective c and e). The study aims to quantify and compare the tolerance of Cx. pipiens populations to varying concentrations of chloride in mesocosms, ranging from freshwater to the predicted maximum inland surface water concentration of 7.5 g/L Cl- or the brackish-marine transition zone at almost half the concentration of seawater.

Chapter 5 explores the effectiveness of various predator species in controlling mosquito populations, with a focus on the European amphibian species smooth newt and edible frog as well as the invertebrate species two-spot water beetle and backswimmer. The study aims to compare the predator competence of these species in different ecological contexts, including levels of turbidity, and to determine whether there is a sex-specificity in predator competence among the amphibian species (objectives d and e). Additionally, the study aims to examine the effect of predator presence on mosquito oviposition behavior and to validate predatory behavior on other mosquito genera. In doing so this chapter aims to provide insights into the potential of natural predators as a means of controlling mosquito populations, particularly in light of concerns about the effectiveness and environmental impact of chemical interventions.

Chapter 6 discusses the obtained insights into main anthropogenic effects on Culex pipiens populations, examining their effects and interactions across ecological scales (objective f). It is further demonstrated how this understanding may enhance ecological realism in experimental vector ecology. A perspective is then provided on how these insights into the population dynamics of Culex pipiens contribute to epidemiological preparedness, via the processes of surveillance, risk-mapping and modelling and landscape design.

Textbox 1. Terminology surrounding vector ecology

Consistent terminology helps to avoid ambiguity in scientific and regulatory discourse. Definitions of terms surrounding vector ecology and anthropogenic pressures as adhered to in this thesis are provided below.

Anthropogenic pressures: Human-induced changes or disturbances to natural ecosystems.
Community composition: The relative abundance and diversity of species in a particular community.
Driver: refers to a process that directly or indirectly influences an ecosystem or population. Drivers may include climate change, land use changes, or freshwater use. These drivers can have both direct and indirect effects on mosquito populations, such as affecting food sources or inducing mortality.
External driver: biotic influences on an ecosystem or population as a result of interactions between and within species, such as predation, density dependence, and competition.
Host: an organism that harbors and provides a habitat for a pathogen, allowing it to live, grow, and potentially cause harm or disease within the host organism. Hosts can be humans, animals, plants, or even microorganisms such as bacteria or fungi.
Infectious diseases: illnesses caused by pathogenic microorganisms, such as bacteria, viruses, fungi, or parasites, which can spread directly or indirectly from one person to another, typically through contact with contaminated surfaces, bodily fluids, respiratory droplets, or vector species.
Life-history traits: characteristics of an organism that influence its survival and reproduction, encompassing traits such as age at maturity, reproductive output, and lifespan.
Mesocosm: a controlled environment used to simulate natural conditions. Mesocosms are used in ecological research to study the effects of various factors, such as temperature, salinity, or pollution, on populations or species communities. Mesocosms can vary in size and complexity, but they typically consist of a container or enclosure that contains a sample of an ecosystem, such as a pond or a forest, along with (a selection of) the organisms that inhabit it. Conditions within the mesocosm, such as by changing the temperature or adding pollutants, may be manipulated to observe how the species or population respond. Mesocosms allow us to conduct experiments in a controlled setting while still capturing some of the complexity of natural ecosystems, making them a valuable tool for studying ecological processes.

Natural control: The use of natural predators or other biological agents to control pest populations.

One Health: a collaborative interdisciplinary framework embracing the interconnectedness between human, animal and environmental health by recognizing that addressing health challenges requires an effort across multiple disciplines.

Oviposition: the process of laying eggs.

Pathogenic landscape: environment concerning the presence, distribution, and characteristics of pathogens (disease-causing microorganisms) within a particular geographical area or population.

Population parameters: measurable characteristics or attributes of a population. These parameters provide information about the size, structure, and dynamics of a population and may include, but are not limited to, sex ratio, mortality rate, growth rate and density.

Pressure: refers to an external forces or process that may affect ecosystems or populations. These pressures can be natural, such as soil type, or anthropogenic, meaning they are caused by human activities like land use practices. These pressures can shape ecosystems and influence the abundance and composition of species.

Reservoir: a population of organisms in which a pathogen reproduces, often without causing disease for the host. Reservoir species play a crucial role in the lifecycle of certain infectious agents by serving as a source of infection for other susceptible hosts, including humans. Reservoir species may actively participate in transmission to other hosts and may amplify or simply maintain the pathogen.

Stressor: refers to any biotic or abiotic factor that can cause stress to organisms in an ecosystem. This stress can be i) physical, such as changes in temperature, ii) biological, such as predation, or iii) chemical, such as salinization. Stressors can directly and indirectly affect various aspects of an organism's life, such as its egg-laying behavior, growth, and survival.

Systemic driver: anthropogenically induced influences on an ecosystem or population via for instance changes in climate, land use, and water management.

Vector: an organism, typically an invertebrate arthropod like a mosquito, tick, or flea, which transmits pathogens from one host to another.