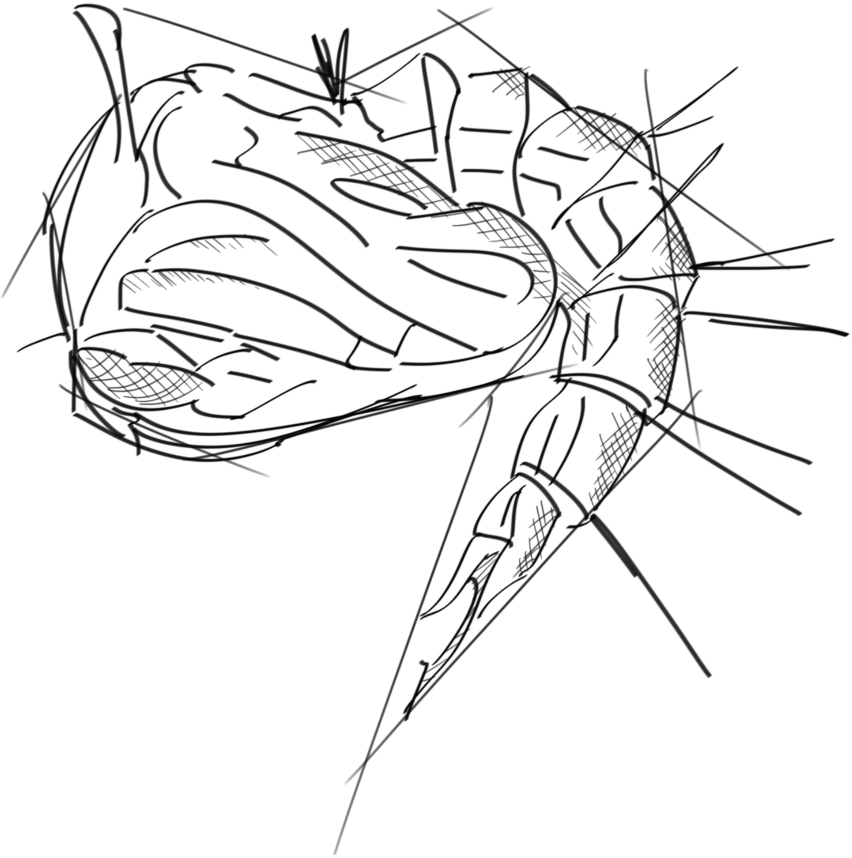
An egg is always an adventure: anthropogenic impacts on Culex pipiens population dynamics

Boerlijst, S.P.

Citation

Boerlijst, S. P. (2025, March 18). *An egg is always an adventure: anthropogenic impacts on Culex pipiens population dynamics*. Retrieved from https://hdl.handle.net/1887/4198436



Graphical illustration of a house mosquito pupa.

Chapter 6

Discussion

6.1 Introduction

The necessity of applying a One Health approach in emerging infectious disease challenges is increasingly acknowledged (Breitschwerdt, 2014; Destoumieux-Garzón et al., 2018; Ellwanger et al., 2022; Little, 2013). While One Health inherently implies interconnectedness of animal, human and environment health, many studies regarding the ecological side of One Health have largely resorted to simplified lab settings (De Brito Arduino et al., 2015; Ohba & Takagi, 2010; Saleeza et al., 2014) and/or focused on isolated effects of anthropogenic stressors (Kiarie-Makara et al., 2015; Sih, 1986). Such experiments fail to acknowledge that anthropogenic stressors, an omnipresent feature of most ecological systems these days, interact with the integrity of these ecological systems instead of acting purely as an external force, thus lacking ecological realism. Only recently there has been a shift towards less controlled environments allowing higher degree of ecological realism, such as field labs (Cano-Rocabayera et al., 2020; Silberbush et al., 2005). These experimental settings thereby allow for the inclusion of stressor interactions (Abrahams & Kattenfeld, 1997; Alcalay et al., 2018; Krol et al., 2019; Muturi et al., 2010), and environmentally realistic application of stressors (Beck-Johnson et al., 2017; Hall & Warner, 2020; Vajedsamiei et al., 2021).

The realization that ecological realism is key when aiming for a One Health approach to VBD represents the basis upon which this thesis is built. This work which aims to identify the main anthropogenic drivers, their stressors and relevant interactions on *Culex pipiens* (*Cx. pipiens*) population dynamics, and how these feed through in a One Health approach to *Culex*-transmitted pathogens. To make this ambitious goal attainable, and enable a structured evaluation of stressors, I classified all stressors into a nested hierarchy of three levels of increasing spatial scale (section 6.3). In the previous chapters I discussed my findings on the importance of interactive effects between stressors, which are the norm rather than the exception **(Chapter 2)**, that realistic application of stressors massively affects stressor impacts **(Chapters 2 and 3)**, that the ecological context of the populations evaluated – such as prior (gradual) natural exposure to the stressors – affects stressor impact **(Chapter 4)** and that bottom-up and top-down stressors indirectly impact mosquito populations via habitat selection **(Chapters 2, 3, 4 and 5)**. Based on these findings, this concluding chapter will evaluate how systemic (bottom-up), external (top-down) and other stressors act and interact across ecological scales (Figure 6.1). This informs us how these should be applied in studies on experimental vector ecology, to make them more ecologically realistic. Furthermore, these insights offer a novel perspective on how higher understanding of ecological realism of *Cx. pipiens* contributes to (epidemiological) preparedness, via the processes of surveillance, risk-mapping and modelling and landscape design. Overall, this is an important step in the acknowledgement of the importance of ecological processes in the One Health approach.

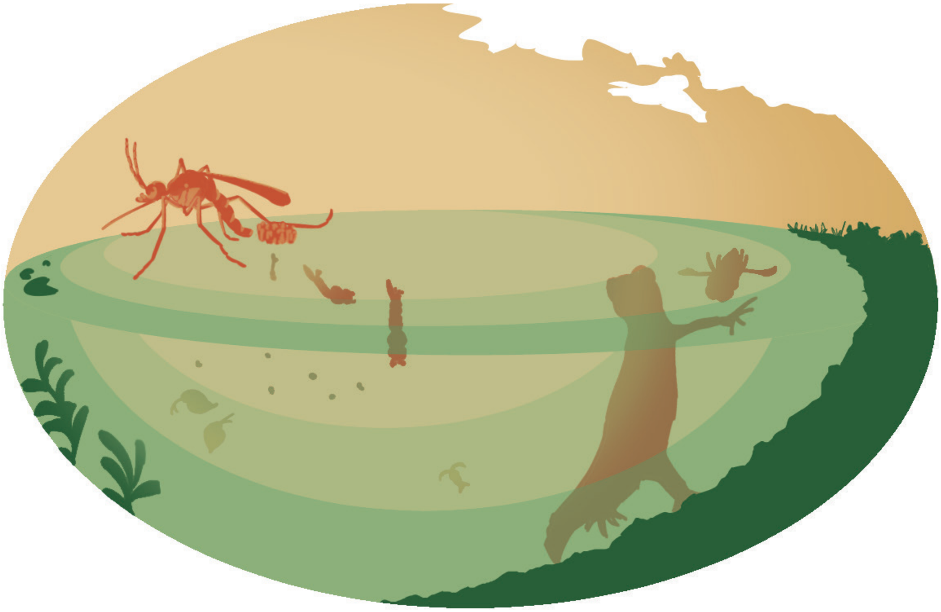


Figure 6.1 Overview of scales within which the drivers and pressures act on mosquito populations visualized as three concentric layers of increasing opacity. Bottom-up stressors, like eutrophication and salinization affect water quality and together with microclimate act on the microhabitat level, the innermost layer representing the direct surroundings of the larvae. External stressors, like competition and predation, impact community composition on the mesohabitat level, the intermediate layer. Finally, landscape characteristics, such as soil type and vegetation together with macroclimate act on the macrohabitat level, the outermost layer, representing the entire water body.

6.2 Applying ecological realism to One Health

One Health, as an interdisciplinary approach, recognizes the interconnectedness between human, animal and environmental health. It thereby (albeit hitherto largely theoretically) acknowledges that changes in ecosystems may impact all domains and that holistic, collaborative efforts that touch on these changes may benefit everyone involved (Cunningham et al., 2017; Danasekaran, 2024; K. M. Smith et al., 2019). By considering ecological processes, One Health approaches hold the potential to develop more effective strategies for health issues, but to date an actual, practical implication of this potential is largely lacking. Breaking this deadlock necessitates accurate knowledge on how, often complex, ecological processes interact and impact us. Ecological complexity – the interconnectedness of living systems across multiple scales, processes and interactions – originates from the field of multitrophic interactions, and stem from the simple observation that organisms interact many other organisms in their environment (Anand et al., 2010; Harvey et al., 2015). Disease transmission similarly depends on complex interactions between organisms, and is influenced by various other abiotic and biotic factors (Su et al., 2022), across multiple spatial scales (Laporta & Sallum, 2014). More ecological realism, via the inclusion of higher levels of ecological complexity, should therefore be considered for infectious disease studies, as current approaches may be overly reductionist (de Garine-Wichatitsky et al., 2022; Maurer, 1999; Su et al., 2022).

6.3 The scales of ecological realism

Environmental complexity can be explored spatially, by dividing it into three (hierarchical) scales of spatial structure: microhabitat, habitat and (macrohabitat): the landscape. These scales distinctly affect habitat selection, adding to their relevance (Alcalay et al., 2019; Nikookar et al., 2015). The different scales separate the ecological interactions into bottom-up, top-down and macro-environmental pressures, respectively (Figure 6.1). Below I elaborate on how the ecological complexity that was included in this thesis plays out, and shapes ecological dynamics, at all three scales. Additionally, I discuss some of the immediate implications of my findings at each of these three scales.

6.3.1 microhabitat

Small, localized physical and chemical conditions of the immediate surroundings of a given organism are defined here as the microhabitat. This scale is comprised of effects from, and spatial distribution of, compounds acting on water quality. For mosquitoes, the main relevant pressures at this scale are eutrophication, salinization and micro-climate that shape the larval microhabitat, all of which are further elaborated below. Larvicidal compounds such as heavy metals (El-Sheikh et al., 2010), plant metabolites (Al-Doghairi et al., 2004; David et al., 2000, 2001) and insecticides (Hamaidia & Soltani, 2016; Li et al., 2002) act on this scale as well, but are not further evaluated as this thesis focuses on naturally occurring ecological pressures.

Eutrophication results in nutrient accumulation in aquatic system, and is an important regulator of the potential primary biomass production, primarily achieved by microbes (Edwards et al., 2006). Nutrient enrichment is therefore indicative of the direct (Aly & Dadd, 1989) and indirect (Merritt et al., 1992) food availability. The species-specific effects of eutrophication on mosquitoes have been well documented (Buxton et al., 2020a; Krol et al., 2019; Schrama et al., 2018), and *Cx. pipiens* has previously been described to prefer high eutrophication levels (Hasselschwert & Rockett, 1988; Rejmánková et al., 2013). However, these studies commonly simplify eutrophication as food availability, without considering its interactions with larval density and temperature (Edwards et al., 2006), which were tested in **Chapter 2**. The results of **Chapter 2** confirm that *Cx. pipiens* has a strong preference for the most eutrophic state if presented with a gradient from oligotrophic to hypertrophic conditions during oviposition behavior. While temperature did not interactively affect development and survival, eutrophication and larval density did interact inversely **(Chapter 2)**. This suggests that temperature and associated microbial activity did not reduce resource competition at the time scale tested. Depth of the habitat, affecting temperature fluctuations, will therefore be unlikely to affect food availability, meaning that permanent and ephemeral water bodies should be considered equally, based on their eutrophic level. As eutrophication continues to rise globally (Smith et al., 2006), it continues to be one of the most important underlying drivers of Cx pipiens abundance. Countering these effects should therefore receive priority if the goal is to lower the abundance of Cx pipiens.

Unlike eutrophication, salinization is typically considered detrimental to freshwater organisms (Dahl, 1956). While several physical adaptations in salt water tolerant mosquitoes exist (Akhter et al., 2017; Donini et al., 2007), *Cx. pipiens* is mostly limited to regulation of drinking rate and active secretion (Aly & Dadd, 1989; Bradley, 1987; Bradley & Phillips, 1976). Consequently, *Cx. pipiens* is relatively vulnerable to salinization (Figueroa et al., 2016; Kengne et al., 2019). Analysis on the Dutch populations in **Chapter 4**, however, revealed a higher tolerance than expected, which I hypothesize is caused by gradual historical exposure (Oude Essink et al., 2010; van Baaren & Oude Essink, 2009). Additionally, an interaction between temperature and salinity delayed development under brackish conditions **(Chapter 2)**, implying that the increased active expulsion of ions under higher temperatures may have impacted larval energy budget. As a result, salinization is unlikely to affect *Cx. pipiens* populations directly, unless in ephemeral water bodies which may experience higher maximum temperatures. Overall, these findings suggest that abundances of *Cx. pipiens* will likely (locally) increase with increasing salinization, but more work is needed to understand the full implications of this stressor.

Micro-climate is a result of the interplay between the landscape characteristics macro-climate, vegetation cover and spatial habitat structure. From the point of view of mosquito larvae, several elements of the microclimate are rather unimportant, including light intensity and dissolved oxygen (Muturi et al., 2008; Vezzani & Albicócco, 2009) while (fluctuations in) temperature are considered very important (Beck-Johnson et al., 2017; De Majo et al., 2019; Vajedsamiei et al., 2021). In this work, we show that including temperature fluctuations, both natural and through block-schemes, similarly reduced development time by approximately one third compared to a constant temperature of the same mean **(Chapters 2 and 3)**. Additionally, the majority of this difference arises during pupation **(Chapter 3)**. As pupae do not eat (Becker et al., 2010), suboptimal development under static temperatures may then result in mosquitoes of decreased fitness.

This highlights the importance of including natural fluctuations, in experimental work and modelling practices on climatic effects in vector ecology.

Overall, the ecological interactions at the micro habitat scale underscore the importance of these regulating factors, and potential interactions with larval density but no interactions with the facets of micro-climate, such as temperature. Moreover, these findings underscore that, at the micro-habitat scale, the handling perspective should be geared towards reducing the impact of isolated stressors by mitigating anthropogenic disturbance (e.g. limiting eutrophication by decoupling the water table near agriculture).

6.3.2 mesohabitat

The biotope – the system of biotic interactions within the larvae their spatial range – is here defined as the mesohabitat . This scale is defined by the impact of biotic interactions, for example the impact of competitors and predators (Braks et al., 2004; Knight et al., 2004; Murrell & Juliano, 2008).

Due to the surface-dwelling nature of mosquito larvae, reliance on atmospheric instead of dissolved oxygen (Muturi et al., 2008), and the preference of *Cx. pipiens* for semi-temporary water bodies (Becker et al., 2010), most competition originates from other mosquito larvae (Becker et al., 2010). This may be simplified to density dependency effects for competition within the same species (Beck-Johnson et al., 2013; Couret & Benedict, 2014). Such intraspecific competition mainly impacts development, as extremely small amounts of resources are needed for survival **(Chapter 2)**. Competition mostly delays development **(Chapter 2)**, but high densities may promote early maturation (Knight et al., 2004; Meyabeme Elono et al., 2018), predominantly in males (Alcalay et al., 2018), to escape competition. However, since mosquitoes actively select for eutrophic habitats (Buxton et al., 2020a; Hasselschwert & Rockett, 1988), with low competition (Mwingira et al., 2020), such conditions are unlikely to exist naturally.

Predation is one of the strongest selective forces, directly limiting survival (Culler & Lamp, 2009; DuRant & Hopkins, 2008; Knight et al., 2004; Kovács et al., 2014). Although many predators potentially consider mosquito larvae as prey, the range of actual predators on *Cx. pipiens* may be far more limited, due to the preference of *Cx. pipiens* for ephemeral water bodies. This implies that any potential predator needs to be able to disperse over land or through the air. The preference of *Cx. pipiens* for highly eutrophic water, which is generally related to higher turbidity and low concentrations of dissolved oxygen (Coffin et al., 2021), may further limit the range of predators to those able to breathe atmospheric oxygen and hunt with decreased vision. Based on these notions, **Chapter 5** dove into the species that may be successful predators of mosquito larvae. All selected predator species were competent antagonists of *Cx. pipiens*. Among these common predator groups, amphibians outperformed all other groups on an individual level, irrespective of eutrophication levels **(Chapter 5)**. Facilitating (amphibian) predator presence may therefore prove to be a valuable nature-based solution to limit mosquito presence, as the living community affect habitat suitability both directly and indirectly.

6.3.3 landscape

The landscape is defined here as one or multiple habitats and their immediate surroundings. Landscape characteristics may then consist of pressures over multiple habitats, including detritus type (Murrell & Juliano, 2008), soil type (Krol et al., 2023), vegetation (Laporta & Sallum, 2014; Medeiros-Sousa et al., 2017) and macro-climatic conditions such as temperature (Ciota et al., 2014).

Although effects of pressures at a landscape level, such as mean temperature and nitrogen deposition, may impact mosquito larvae directly **(Chapter 2)**, I propose that the majority of landscape-wide effects occur during ovipositing. Namely, we find that female mosquitoes actively evade habitats where their offspring has a low chance of survival, and select for environments where the contrary is the case **(Chapters 2, 4 and 5)**, contrasting literature on other mosquito species indicating that ovipositing may be decoupled from potential larval survival (Roberts, 1996; Roberts & Irving-Bell, 1997; Yee et al., 2020). Our findings show that habitat selection during egg-laying may be affected by poor water quality **(Chapter 4)**, limited resources **(Chapter 2)** or predation **(Chapter 5)**. In the latter case, habitats close to those deemed unfavorable are evaded as well **(Chapter 5)**. However, even though some habitats are avoided, ovipositing does take place at the comparatively 'most suitable' habitat (Kennedy, 1942). Perceived habitat quality thus seems to be dependent on water quality, resource availability and community structure (mainly competitors and predators) on a case-by-case basis. At the landscape level, these choices are affected by spatial context. I.e., larval habitats that may seem to be poor choices individually, may be the best option available, and therefore may still be colonized depending on the context.

6.4 Implementing realism in experimental design

Considering the abovementioned spatial scales of environmental complexity can help to determine how ecological realism may be incorporated into experiments, by providing context and relevance for its different facets. To address the complexities, ecological realism is here subdivided into stressor interactions, spatiotemporal ranges and treatment application. These aspects recognize the complexity of ecological systems, by acknowledging 1) the multifaceted nature of ecological systems, 2) the variability and often cyclic nature of ecological stress over time and space and 3) the unintended potential of experimental conditions modifying stressor impact.

6.4.1 Stressor interactions

Stressors have distinct direct impacts on mosquito populations (section 6.3), but there may be several indirect interactive effects as well. Stressors rarely operate in isolation **(Chapter 2)**, and often co-occur (Figure 6.2), highlighting the importance of considering potential interactions among stressors. These interactions may occur within a spatial scale, as with competition and predation (Knight et al., 2004), or act across them, e.g., temperature often interacts with pollutants like salts **(Chapter 2)** and pesticides (Muturi et al., 2011). Inter-scale interactions are particularly noteworthy when evaluating interspecies dynamics, where antagonists are often subject to the same stressors, but with different tolerances. For example, predation may interact with eutrophication (Krol et al., 2019), salinity levels (Silberbush et al., 2014), temperature (Lundkvist et al., 2003) and habitat complexity (Buxton et al., 2020c). Similarly, competition can be impacted by temperature (Costanzo et al., 2005), vegetation (Laporta & Sallum, 2014) and detritus (Murrell & Juliano, 2008).

Unintended stressor interactions may also occur, as many stressor impacts are density dependent. Alcalay (2018) found that larval density interacted with temperature and dissolved solute concentration – an indicator for evaporation – increasing development time and lowering survival rates. Alto (2012) found an additional interaction between density and nutrition, increasing male to female sex-ratio, consistent with our findings regarding food availability **(Chapter 2)**. Larval densities may therefore be an often implicit, but potential confounding factor. Chosen densities should therefore be consciously evaluated, even when effects of density dependence were not directly evaluated. The same holds true for interactive effects with temperature, for which effects of fluctuations remain underrepresented. Therefore, it is crucial to consider interactive effects, intended and unintended, for accurate assessment of processes acting on mosquito populations.

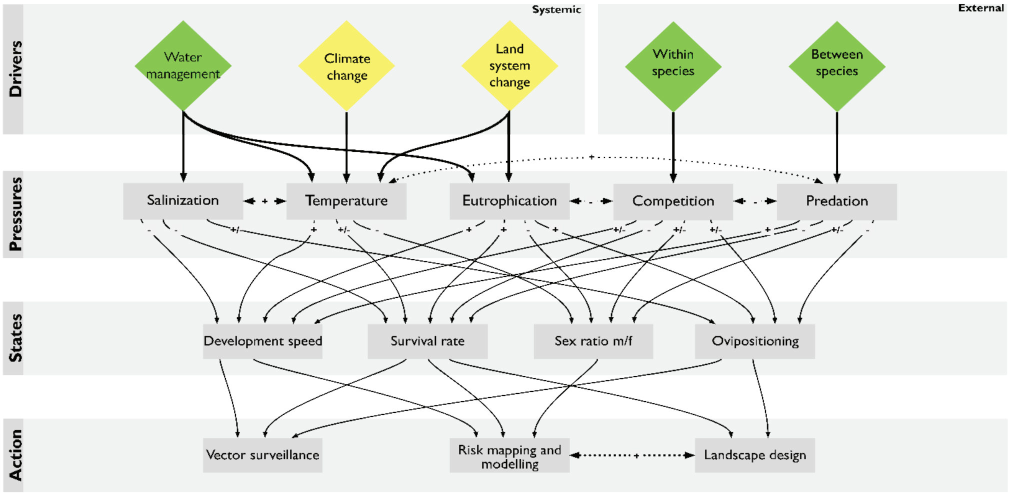


Figure 6.2 *Overview of systemic and external drivers on abiotic pressures and its impacts on downstream population parameters of mosquitoes. For each of the established impacts between pressures and states, it is indicated whether the association is positive (+), negative (−) or both (+/−).*

6.4.2 *Spatiotemporal ranges*

Including spatial and temporal variation in stressor levels is crucial for realistic experimental outcomes. Temporally, different types of stressors act within differing time frames. Ranges of top-down stressors acting on habitat level may be assessed relatively quickly. For instance, predation pressure acts over several days **(Chapter 5)**, while competitive and micro-climatic effects impact mosquito larvae over several weeks **(Chapter 2)**. Microhabitat stressors, such as chemical pollutants, however, may affect mosquitoes differently across various life stages (Clark et al., 2004, **Chapters 2 and 4**), and potentially across generations, as demonstrated for ecotoxicological effects on other organisms (Nederstigt et al., 2022; Tran et al., 2018). However, most current mosquito experiments evaluate direct toxicity for single stressors on single life stages (Kengne et al., 2019), while multigenerational experiments with changing or transient stressors remain scarce. This is problematic, as valuable information on stressor impact is then lost. Both for life stage dependent sensitivity, and for temporal changes in toxicity, due to gradual increases in exposure (Mottram et al., 1994), changes in bio-availability (Schleier III & Peterson, 2013) and degradation of pollutants (David et al., 2001).

Spatially, chemical and climatic stressors display stratification. Effective treatments and measurements should therefore be tailored to the location in the water column that mosquito larvae predominantly inhabit, typically the water surface **(Chapters 2, 3 and 4)**. Consequently, measuring stressors like temperature at higher depths, or using air temperature as indicator, misrepresents actual surface water temperatures, and may thereby cause inaccurate results (Asare et al., 2016; Paaijmans et al., 2008). However, up until recently, using air temperatures has been the norm for modelling practices (Asare et al., 2016; Erraguntla et al., 2021). Biotic interactions are similarly stratified, as effects of competition or predation may be limited if the antagonist resides in a different layer (section 6.3.2), or when a competitor uses different feeding methods (Merritt et al., 1992). Chemical stressors may express differing levels of bioavailability depending on the place in the water column, as polar substances like pyrethrins can bind to sediment (Schleier III & Peterson, 2013), depending on the (controlled-release) formulation used (Andriessen et al., 2015; Mapossa et al., 2021). As such, relevant timeframes and spatio-temporal processes should be evaluated to inform realistic stressor application.

6.4.3 Treatment application and relevance

Realistic application of treatments relies on the principles from realistic stressor interactions and ranges as well as the relevant spatial scale. For instance, landscape characteristics such as vegetation should be considered when evaluating predation, as vegetation may interact by providing a resting spot for predators **(Chapter 5)**, as well as by creating a predator refuge (Bond et al., 2005). When simulating the micro-climate, natural fluctuations should be considered. For example, natural temperatures are cyclic in nature, and their inclusion may severely impact population parameters **(Chapter 3)**. Such spatiotemporal changes in exposure are important for chemical stress as well, as reported by Viaene (2016).

When considering the relevance of particular stressor treatments, it is important to consider the populations used. The historical context of populations should therefore be evaluated and reported when assessing effects of stressors which may have caused local adaptations. For instance, Kengne (2019) reported on salinity tolerance of multigenerational lab populations originating from Cévenne, France, explicitly mentioning potential bias as a result of local adaptations and inbreeding depression. Differences in historical exposure may indeed partially explain the large discrepancy in tolerance found for Dutch populations **(Chapter 4)**, stressing the importance of communicating and contextualizing geographic origin. Finally, the relevance of each treatment should be evaluated by assessing colonization by the relevant species **(Chapters 2, 4 and 5)**. Information on habitat preferences for colonization and its potential discrepancies with other population parameters may inform whether certain conditions may create population sinks, potential hotspots, cause dispersal or will simply not affect populations.

6.5 Future strategic research areas

In my analysis of the main anthropogenic drivers of *Cx. pipiens* population dynamics, I have strived to be as complete as possible within the limits of a 4-year PhD project. However, some areas of research were not evaluated, even though they do require further testing. These include an evidence-based link between mosquito population dynamics and disease risk, community evaluation of salinization impacts and generalization of the found impacts for other mosquito species. In doing so, the current findings could more easily and accurately be applied to mosquito-borne disease preparedness.

A major and consistent assumption in the field of vector ecology, mine including, regards the translation of population dynamics to disease risk. Namely, it is assumed that these stressors not only affect mosquito abundances, but thereby also modify the probability of transmission, and hence influence disease dynamics and ultimately disease risk. There is some emerging evidence that modifying mosquito abundances indeed influence transmission (Kolimenakis et al., 2021), but a lot on this topic is still unknown. Vector-borne diseases, by definition, are limited to transmission by their vector, but information on the minimum viable vector density, is scarce. This is caused by a disconnect with vector-host dynamics, for which a framework has only recently been established (Thongsripong et al., 2021), which highlights that additional information is needed on (stressor impacts on) human-mosquito contact rates. Such information should include stressor impact on dispersal capacity, biting rates and biting behavior (Thongsripong et al., 2021). This could then be combined with information on the impacts of growth conditions on vector competence (Esser et al., 2019) to assess vector-borne disease risk semi-quantitatively (Petrić et al., 2014).

This thesis provides some indications that coastal areas experiencing ongoing salinization may be potential risk sites for disease transmission. Central to this idea are the assumptions that predation might become lower in these areas and that *Cx. pipiens* willingly colonizes saline water bodies. These factors should be evaluated further. Firstly, the impacts of ongoing salinization on mosquito predator communities should be assessed either experimentally or by field survey to determine whether freshwater predators show similar plasticity or adaptations or whether alternative predator species take their place. Secondly, egg-laying behavior during the salinization experiments used freshwater bacterial communities, which were self-selected during acclimatization. Although these self-selected community and their medium attracted gravid mosquitoes up to moderate salinities, bacterial activity may have been limiting egg laying behavior under the most saline conditions. In the light of ongoing salinization, further evaluation is needed to understand this mechanism, as even higher salinities may prove to be sufficiently 'attractive' when using bacterial communities natural to these areas.

And last but not least, the hypotheses in this thesis should be evaluated for other mosquito species to test their generality. Although the processes on which the stressors and their interactions act should be comparable across species, whether their relative importance remains the same, remains to be tested.

6.6 Implications in a one health context

One of the aims of this thesis was to identify the main anthropogenic drivers impacting *Cx. pipiens* its population dynamics (Figure 6.2), to support the development of practical tools, approaches and risk-assessment in One Health PACT to intervene in, and enhance preparedness against, mosquito-borne outbreaks in a changing world. The work presented in this thesis describes anthropogenic impacts at three habitat scales. Each scale is impacted by a combination of stressors that will shape the size and structure of mosquito populations. Similarly, each scale contains a set of these parameters that could be acted on as potential interventions for risk of pathogen transmission, either directly harnessing stressor impact, or by using relevant interactions.

Most traditional interventions act as bottom-up stressors at the microhabitat scale, encompassing common insecticides, either as chemical compound (i.e. DDT), or as bacterium producing larvicidal compounds (Bti) (Geetha & Manonmani, 2008; Rochlin et al., 2016). However, these are short-term interventions, as they impact the population size, but not the habitat quality, thereby allowing for recolonization as soon as the effectivity has faded (Benelli et al., 2016). Because of this, as well as concerns with increasing insecticide resistances, interventions at the mesohabitat scale are gaining increasing attention (Abagli & Alavo, 2019; Chen et al., 2005; Kumar & Hwang, 2006). By employing mosquito predators as biocontrol agents as reinforcement or standalone. In doing so mosquito populations may be suppressed for longer periods (Talaga et al., 2020). Using predator assemblages further improve effectivity (Buxton et al., 2020b), potentially limiting confounding effects of habitat complexity as well (Buxton et al., 2020c). These effects at landscape scale should inform which (combination of) predator species could most effectively limit mosquito populations, by assessing their mobility within the water body (Alto et al., 2005; Buxton et al., 2020c) as well as dispersal capacity across water bodies (Brodman & Dorton, 2006; Raghavendra et al., 2007; **Chapter 5**). In doing so, natural dispersal may be facilitated so that mosquito habitat suitability is definitively decreased. This may provide a more long-term and less challenging method to be exhaustively achieved compared to local interventions like draining standing water, or luring gravid mosquitoes, as has been proposed by Wong (2012).

Although the current thesis insinuates that predator free, (anthropogenically) disturbed habitats are the most conducive to mosquito populations **(Chapters 2-5)**, it is unclear how well these results translate to more ecologically complex habitats. The currently used mesocosms are the preferred habitat for *Cx. pipiens* (Becker et al., 2010), but more complex habitats are known to be colonized as well (Krol et al., 2023). Similarly, without information on the link between mosquito population dynamics and disease transmission (Section 6.5), it is impossible to make any definitive statements on future disease risk based on the current results. The information in this thesis can however give indications on how to identify potential hotspots by their abiotic conditions and ecological community, thereby allowing effective interventions when outbreaks do occur. Similarly, it is currently impossible to state the extent to which mosquito populations should be managed to prevent outbreaks. Nevertheless, the findings within this thesis do support the notion that potential hotspots may be preempted by facilitating healthy ecosystems at or near the site.

6.7 Conclusion

In this thesis, I argue that integrating ecological realism into infectious disease studies, is crucial to develop more effective strategies. This includes recognizing interactions across microhabitat, habitat and landscape scales, and incorporating realistic stressor interactions, ranges and treatment application, as described in this thesis. To this end, I have chosen a spatial and systems-based approach, following from the principles of One Health. I have shown that stressors interact across all three spatial scales, affecting *Cx. pipiens* in its natural habitat, not in isolation from its habitat. I have shown that several stressors have impacts reaching further than commonly evaluated impacts, especially when including realistic application. Additionally, I have shown that changes in climate often interact with other anthropogenic impacts and should therefore be evaluated conjunctly. This thesis shows that more care should be taken to include ecological realism for accurate and relevant results that reach further than their experimental setting. Especially within holistic and multidisciplinary frameworks like One Health, where interactions are the norm.

These results give an overview of the main anthropogenic drivers, and their interactions, acting on *Cx. pipiens* population dynamics, thereby laying the foundation for predictions of how their populations will shift with our changing world. Similarly, this thesis provides insight in the drivers that effective interventions should act on. This lays a basis for proactive mitigation of *Culex*-borne disease risk, by furthering our understanding of human impacts on *Cx. pipiens*. Further scientific endeavors are needed to extend these findings to other species.