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

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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>

Version: Publisher's Version

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Note: To cite this publication please use the final published version (if applicable).

Summary

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Anthropogenic change has significantly altered ecological systems. There is substantial worry that this will lead to increased transmission of infectious disease. As such, establishing relations between elements of anthropogenic change and infectious disease is a key challenge. In Europe, current morbidity caused by infectious diseases is comparatively low, which stands out in contrast to its history. Investigating current changes in land-use, water management and climate from a One Health perspective – which acknowledges the interconnectedness between human, animal and environmental health – aids in preparedness against (re-)emergence.

Among the various mechanisms through which infectious disease pathogens spread, arthropods, and mosquitoes specifically, play a crucial role as vector. These mosquito-borne diseases are dependent on their vector for transmission. Understanding how changes in the interplay between the environment, vector populations, and animal and human hosts relate to the increasing incidence and spread of pathogens, is crucial in anticipating and mitigating outbreaks.

Even though often complex, ecological processes are central to this understanding. Currently, most One Health studies rely on simplified lab experiments or isolated effects, thereby lacking ecological realism and relevance. In this thesis I aim to identify the main anthropogenic impacts on the cosmopolitan disease vector *Culex pipiens*' population dynamics, whilst demonstrating the importance of including ecological complexity. To this end, I limited myself to interactions between and within bottom-up, top-down and macro-environmental pressures impacting *Cx. pipiens* larvae.

Biting the hand that feeds

Anthropogenic impacts like altered climate, nutrient pollution and water management affect larval mosquito habitats via key abiotic pressures, most notably temperature, eutrophication and salinization. These pressures are widely assumed to affect subadult development and survival, and (perceived) habitat quality during egg-laying, but interactive impacts are poorly understood. To assess relevant interactions between these selected pressures, I performed a series of full-factorial mesocosm experiments. My results suggest large and positive impacts of anthropogenic impacts on mosquito populations, including accelerated growth and increased survival. Many of these impacts are exacerbated by increasing temperatures.

Furthermore, I show that stressor impacts rarely operate in a vacuum, and instead often co-occur, stressing the importance of evaluating their interactions. As the processes upon which the stressors and their interactions act should be comparable across species, but with potentially differing relative importance, experiments including stressor interactions should be the norm. Doing so will provide a basis for the ecological complexity needed to translate experimental findings to ecosystems.

When striving for ecological realism, it is essential to consider not only ecological complexity, but also realistic stressor application (including cyclic fluctuations), contextualization of test populations, and synergies with mitigation measures. The following sections explore these themes through investigation of selected pressures.

In the heat of the moment

Temperature is commonly acknowledged as one of the primary forces driving ectotherm vector populations. Although numerous experiments have been conducted on various species, the majority has been conducted using constant temperatures, while temperatures in nature follow diurnal fluctuations. Such temperature fluctuations have previously been described to impact metabolic processes at (sub)cellular level. In this thesis I developed an inexpensive method to study the impacts of temperature fluctuations in mesocosm experiments at the whole-organism level. By comparing the commonly used constant temperatures and block schemes to the simulated temperature fluctuation of an average day in June, I demonstrate that temperature fluctuations significantly decreased development time compared to constant temperatures. The majority of this difference occurs during the pupal development, when the mosquitoes do not eat, and interact the least with their environment.

Including natural fluctuations in temperature thus allows for a more accurate assessment of stressor impacts and its derived relative importance, which could have significant implications for the predicting the effects of large-scale disturbances in temperature regimes like climate-change.

Taking it with a grain of salt

Salinization, exacerbated by sea-level rise, land subsidence and freshwater extraction is a critical environmental cue affecting freshwater ecosystems. Salinization is commonly acknowledged to negatively affect biodiversity and

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ecosystem services, but little is known about the direct and indirect effects of animal populations under transitory conditions. Physiological and behavioral adaptations in mosquitoes exist, resulting in species specific tolerances, within which *Cx. pipiens* is commonly considered intolerant. Nevertheless, gradual exposure combined with their relatively short generation times may have driven local adaptation. To assess whether local adaptation to salinization exists, I quantified and compared tolerance of three populations along a gradient from coast to inland, expecting an improved tolerance near the coast. Mortality was considerably lower than expected at the currently described maximum lethal dose (LD100), with negligible impacts on development rates. Salinity tolerance was highest in the coastal and inland populations, with a significantly lower tolerance for the intermediate population.

These variations in tolerance can be partially attributed to geographic origin and historical context. This highlights the need to consider the population used in experiments and the following need to communicate and contextualize the geographic origin of the populations used in experiments.

Clashing in murky waters

There has been an increasing interest in One Health strategies like natural predation to combat mosquito-borne diseases, but at the same time we are witnessing widespread losses in biodiversity. In the last chapter of this thesis, I evaluated predator effectiveness against *Cx. pipiens*. We compared two European amphibians, the smooth newt (*Lissotriton vulgaris*) and middle green frog (*Pelophylax kl. Esculentus*) to the predatorial capacity of two ubiquitous invertebrates, including the two-spotted diving beetle (*Agabus bipustulatus*) and common backswimmer (*Notonecta glauca*). Results indicated that amphibians consumed 4-8 times more mosquito larvae than the selected invertebrates, with no significant differences between amphibian species, their sex, or eutrophication levels. Additionally, the presence of predators deterred mosquito egg-laying, suggesting a *landscape of fear*.

These findings underscore the potential of amphibians as natural mosquito control agents, both by limiting survival and preventing colonization altogether, emphasizing the need to prioritize conservation of these threatened species in both urban and rural landscapes.

Concluding remarks

Overall, results in this thesis emphasize the need to include ecological realism for accurate and relevant results that reach beyond their experimental setting. Especially within holistic and multidisciplinary frameworks like One Health, where interactions are the norm.

I have given an overview of the main anthropogenic drivers, and their interactions, acting on *Cx. pipiens* population dynamics. The findings thereby provide a foundation for predictions of how their populations will shift with our changing world. By furthering our understanding of the drivers that effective interventions should act on, this lays a basis for proactive mitigation of *Culex*-borne disease risk.

