

**THE BIO-ECOLOGY OF KEY MOSQUITO VECTOR SPECIES IN BOTSWANA:
IMPLICATIONS FOR SHIFTING ENVIRONMENTS**

by

MMABALEDI BUXTON

Reg. No: 14100540

BEd, MSc

Department of Biological Sciences and Biotechnology,
Faculty of Science,
Botswana International University of Science and Technology
mmabaledi.buxton@studentmail.biust.ac.bw, (+267) 75468974

A Dissertation Submitted to the Faculty of Science in Partial Fulfilment of the Requirements for
the Degree of Doctor of Philosophy (Biological Sciences) at the Botswana International
University of Science and Technology

Supervisors: Prof. Ryan John Wasserman and Prof. Casper Nyamukondiwa

Department of Biological Sciences and Biotechnology,

Faculty of Science, BIUST

R.Wasserman@ru.ac.za, (+27) 727385552

nyamukondiwac@biust.ac.bw, (+267) 75360701

March, 2021

Declaration and Copyright

I, **MMABALEDI BUXTON**, declare that this dissertation is my own original work and that it has not been presented anywhere and will not be presented to any other university for a similar or any other degree award.

Signature



This dissertation is copyright material protected under the Berne Convention, the Copyright Act of 1999 and other international and national enactments, in that behalf, on intellectual property. It must not be reproduced by any means, in full or in part, except for short extracts in fair dealing; for researcher private study, critical scholarly review or discourse with an acknowledgement, without the written permission of the office of the Provost, on behalf of both the author and the BIUST.

Certification

The undersigned certifies that they have read and hereby recommends for acceptance by the Faculty of Science a dissertation titled: **The Bio-Ecology of Key Mosquito Vector Species In Botswana: Implications For Shifting Environments**, in fulfilment of the requirements for the degree of Doctor of Philosophy in Biological Sciences at BIUST.



.....
Supervisors: Prof. Ryan John Wasserman

Date: 29/ 03/ 2021



.....
Prof. Casper Nyamukondiwa

Date: 29/03/2021

Dedication

This dissertation is dedicated to those who encouraged and supported me through challenges and hardships especially my family and friends.

Acknowledgements

I managed to complete this training programme through efforts of a number of individuals and collaborative institutions. Firstly, I would like to thank my supervisors, Prof. Ryan John Wasserman and Prof. Casper Nyamukondiwa, for their time, effort and skill in guidance towards my training and producing a high-quality dissertation. Their professionalism was always outstanding in giving timely feedback amidst their busy schedules. Great thanks go to my collaborators, Dr Ross Noel Cuthbert (GEOMAR, Helmholtz-Zentrum für Ozeanforschung Kiel, Germany) and Dr Tatenda Dalu (University of Venda, South Africa) and Dr Kebaneilwe Lebani (Botswana International University of Science and Technology (BIUST), Botswana) for sharing skills, guidance and ideas in the scientific process throughout my training programme.

I sincerely acknowledge assistance given by my colleagues from the Eco-physiological Entomology Laboratory (Honest Machezano, Murphy Tladi, Nonfo Gotcha, Vimbai Tarusikirwa, Lefang Chobolo, Bame Segaiso, Precious Mpofu, Virgil Joseph and Onalethata Keosentse). They assisted extensively in field work, laboratory work and writing skills. Also, I acknowledge the help of Tinashe Nyabako (University of Zimbabwe) who designed the response template for the survey. The greatest thanks also go to Mbako Joseph, Kitso Kombane, Obakeng Monei, Janet Mokwena, Allan Moikothai, Ogaufi Moikothai and Malebogo Portia Buxton who participated fruitfully in various laboratory experiments and field work activities. I also thank Patrick Reavell for identification of notonectids.

I thank the Ministry of Environment, Natural Resources Conservation and Tourism (Botswana) for issuing of a research permit for the study and other collaborative initiatives. The Ministry of Health and Wellness is also acknowledged as a collaborator in this project and in giving opportunity platforms to offer presentations on scientific findings in their annual malaria meetings and conferences. This has substantially improved my presentation skills and I have learnt a lot in the malaria programme.

Most importantly, I thank BIUST for offering me a training opportunity and providing funds and research facilities for the study. I also acknowledge the Department of Biological Sciences and Biotechnology (BIUST), Department of Physics and Astronomy (BIUST), Department of Chemistry and Forensic Sciences (BIUST) and the South African Environmental Observation Network (NRF-SAEON), South Africa, for utilisation of facilities, equipment and human resource.

Table of Contents	
Declaration and Copyright.....	ii
Certification	iii
Dedication.....	iv
Acknowledgements	v
List of Figures and Tables	x
General abstract.....	1
CHAPTER 1	2
General Introduction.....	2
1.1. Drivers of mosquito proliferation.....	2
1.1.1 <i>Human-mediated factors</i>	2
1.1.2. <i>Climate-mediated factors</i>	5
1.2. Mosquito control	9
1.2.1. <i>Chemical control measures</i>	10
1.2.2. <i>Genetic control measures</i>	11
1.2.3. <i>Biological control measures</i>	13
1.3. Problem statement.....	15
1.4. Justification of the study.....	16
1.5. Present study.....	17
1.6. Objectives.....	19
1.7. References.....	21
CHAPTER 2	33
Knowledge, attitude and practices of the communities from Serowe, Palapye and Bobirwa sub-districts on mosquito bio-ecology and burden	33
2.1. Introduction	34
2.2. Materials and Methods.....	38
2.2.1. <i>Knowledge Attitude and Practices</i>	38
2.2.2. <i>Study area</i>	38
2.2.3. <i>Sampling technique</i>	40
2.2.4. <i>Data analysis</i>	41
2.3. Results.....	41
2.3.1. <i>Socio-demographic characteristics</i>	41

2.3.2. <i>Knowledge</i>	44
2.3.3. <i>Attitude</i>	47
2.3.4. <i>Practices</i>	49
2.4. Discussion.....	51
2.5. References.....	58
CHAPTER 3	67
Mosquito species composition in Serowe, Palapye and Bobirwa sub-districts of Central district, Botswana	67
3.1. Introduction	68
3.2. Materials and Methods.....	71
3.2.1. <i>Preliminary larval collection and rearing in Palapye village</i>	71
3.2.2. <i>Mosquito community collection across sub-districts</i>	72
3.2.3. <i>Identification</i>	73
3.2.4. <i>Statistical analyses</i>	74
3.3. Results.....	75
3.4. Discussion.....	82
3.5. References.....	86
CHAPTER 4	96
Cattle-induced eutrophication favours disease-vector mosquitoes.....	96
4.1. Introduction	97
4.2. Materials and methods.....	99
4.2.1. <i>Study area and experimental design</i>	99
4.2.2. <i>Identification of mosquito larvae</i>	102
4.2.3. <i>Statistical analyses</i>	103
4.3. Results.....	104
4.4. Discussion.....	107
4.5. References.....	111
CHAPTER 5	116
Complementary impacts of heterospecific predators facilitate improved biological control of mosquito larvae	116
5.1. Introduction	117
5.2. Materials and methods.....	120

5.2.1. <i>Study area and experimental design</i>	120
5.2.2. <i>Statistical analyses</i>	122
5.3. Results.....	124
5.4. Discussion.....	130
5.5. References.....	134
CHAPTER 6	142
Predator density modifies mosquito regulation in increasingly complex environments.....	142
6.1. Introduction	143
6.2. Materials and Methods.....	148
6.2.1. <i>Animal collection and maintenance</i>	148
6.2.2. <i>Experimental design</i>	149
6.2.3. <i>Data analyses</i>	150
6.3. Results.....	152
6.4. Discussion.....	156
6.5. References.....	162
CHAPTER 7	171
Implications of increasing temperature stress for predatory biocontrol of mosquito vectors	171
7.1. Introduction	172
7.2. Materials and Methods.....	176
7.2.1 <i>Animal collection and maintenance</i>	176
7.2.2. <i>Experimental design</i>	177
7.2.3. <i>Microclimate data recordings</i>	178
7.2.4. <i>Statistical analysis</i>	179
7.3. Results.....	180
7.4. Discussion.....	184
7.5. References.....	192
CHAPTER 8	203
General Discussion.....	203
References	211
Appendix 1.....	217
Appendix 2.....	9

List of Figures and Tables

CHAPTER 1

- Figure 1.1.** Free range cattle roaming in (a) a semi-urban Palapye often searching for (b) pasture and (c) water from potential sources while subsequently depositing dung.....5
- Figure 1.2.** Thermo-biological scale of insect performance to temperature changes (extracted from Vannier, 1994).....8
- Figure 1.3.** Map of Botswana showing six malaria endemic and 10 non-endemic districts/sub-districts across the country. Malaria endemic are Okavango (1), Ngamiland (2), Chobe (3), Tutume (4), Boteti (5) and Bobirwa (6). Malaria non-endemic districts/sub-districts are Palapye (7), Serowe (8), Mahalapye (9), North-east (10), Kgatleng (11), South-east (12), Southern (13), Kweneng (14), Kgalagadi (15) and Ghanzi (16). Central district consists of six sub-districts: Tutume (4), Boteti (5), Bobirwa (6), Palapye (7), Serowe (8) and Mahalapye (9).....16

CHAPTER 2

- Figure 2.1.** Map showing (a) the location of Botswana within Africa, (b) the malaria endemic sub-districts and the study site in Botswana, and (c) surveyed villages of the Central district; Serowe, Palapye and Bobirwa sub-districts. 1= Okavango, 2= Ngamiland, 3= Chobe, 4= Tutume, 5= Boteti, 6= Bobirwa.....39
- Figure 2.2.** Details of the structure and diversity of water holding containers (outlined by respondents), that may serve as potential mosquito breeding sites found on the premises of respondents across sub-districts.....44
- Figure 2.3.** Pie charts summarising community's knowledge on (a) mosquito developmental stages, (b) HIV transmission by mosquitoes, (c) general mosquito trends over the last 10 years and (d) perceived effect of cattle-dung contaminated water on mosquito abundance.....47
- Figure 2.4.** A summary of community concerns on the public health significance of mosquitoes across localities.....48
- Figure 2.5.** Summary responses (%) on how respondents protect themselves from indoor mosquito bites across sub-districts.....50
- Table 2.1.** Summary results of the socio-demographic characteristics for non-endemic areas (Serowe and Palapye) and endemic (Bobirwa) sub-districts ($n = 611$).....43

CHAPTER 3

- Figure 3.1.** A map showing (a) the location of Botswana in Africa, (b) malaria endemic districts [Okavango: 1, Ngamiland: 2, Chobe: 3, Tutume: 4, Boteti: 5, and Bobirwa] with the Central district indicating (c) adult and (d) larval sampling sites across malaria endemic (Bobirwa) and non-endemic districts (Palapye and Serowe). For coordinates of sampling sites see Table S3.2.....72
- Figure 3.1.** Key morphological characteristics of a collected mosquito: (a): Mesepimeron, showing two well-separated white scale patches (arrow). (b): Thorax, showing scutum with a pair of submedian-longitudinal white stripes (top arrow), and white lyre-shaped markings (bottom arrow). (c): Midfemur, showing white knee-spot (bottom arrow) and anterior portion with a longitudinal white stripe (top arrow). (d): Clypeus, showing white scale patches (top arrow). Photographs by MB and RJW.....76
- Figure 3.3.** Dendrogram outline the level of mosquito community similarity between sub-districts (Bobirwa, Palapye, Serowe) across a malaria endemicity gradient. The dendrogram was constructed using by creating a Euclidean distance similarity matrix. Data used was for both larvae and adult samples collected.....80
- Figure 3.4.** Mean \pm 95% confidence dominant mosquito abundances (*Aedes aegypti*, *Culex pipiens*, *Culex sinaiticus*, *Aedes vittatus*) across malaria endemic (Bobirwa) and non-endemic (Palapye, Serowe) sub-districts.....81
- Table 3.1.** A summary of mosquito species identification information as sampled from across endemic and non-endemic malaria districts.....78
- Table 3.2.** Summary of community diversity measures from samples of communities in the Bobirwa, Palapye and Serowe sub-districts. S = total species number; N = total number of individuals; d = Margalef's species richness; H' = species diversity and J' = Pielou's evenness.....78
- Table 3.3.** SIMPER test results showing the level of pairwise dissimilarities between sub-districts.....79
- Table 3.4.** A summary of factorial ANOVA showing mosquito abundances across malaria endemic (Bobirwa) and non-endemic sub-districts (Serowe and Palapye).....81

CHAPTER 4

- Figure 4. 1.** Compound figure showing (a) the location of the study site in Central district, Botswana, (b) a typical rocky outcrop and temporary pond in the region and (c) the common site of cattle congregating on rocky outcrops given their ability to hold water (arrow indicates a dung deposit).....100
- Figure 4.2.** Illustration of the lateral view of the mesocosms showing (a) placement of mesh cloth during maturation stage to prevent colonisation and water use by organisms, and (b) placement of mesh cloth post treatment dung inoculation to facilitate oviposition by mosquitoes and enable later capture of larvae.....102

Figure 4.3. Temperature readings in the control and treatment mesocosms from Day 7 to Day 21. Dung was inoculated on Day 7 (shown by an arrow), prior to which water in the mesocosms were allowed to mature.....105

Figure 4.4. Weekly mean (± 1 SE) larval mosquito instar (1–4) abundance across different cattle dung concentration (grams of dung per liter water) treatments for anopheline and culicine species. C = Control (0 g L⁻¹), T 1 = Treatment 1 (1 g L⁻¹), T2 = Treatment 2 (2 g L⁻¹), T3 = Treatment 3 (4 g L⁻¹) and T4 = Treatment 4 (8 g L⁻¹).....106

CHAPTER 5

Figure 5.1. Mean larval mosquito prey mortality across different instar stages and predator treatments, irrespective of prey density. Error bars represent means \pm SE.....126

Figure 5.2. Functional responses towards larval mosquito prey instars 1 (a, b, c, d), 2/3 (e, f, g, h) and 4 (i, j) by different combinations of copepod and notonectid predators. Multiple predator predictions (p) result from simulations based on individual predator functional responses (a, e, i) using a population-dynamic approach. Shaded areas are 95% confidence intervals.....129

Table 5.1. First order terms resulting from logistic regression of the proportion of prey killed as a function of prey density across different predator and prey instar stage treatments. Attack rate and handling time parameter estimates result from Rogers' random predator equation.....127

CHAPTER 6

Figure 6.1. Mean \pm SE larval mosquito prey mortality across different predator treatments (*Anisops*, A; *Enithares*, E) and habitat complexities, irrespective of prey supply. Letters and asterisks indicate significant differences.....153

Figure 6.2. Functional responses towards larval mosquito prey under low (a, b, c), intermediate (d, e, f) and high (g, h, i) habitat complexities by different combinations of notonectid predators (*Anisops*, A; *Enithares*, E). Multiple predator predictions (p) result from simulations based on individual predator functional responses (a, d, g). Shaded areas are 95% confidence intervals.....156

Table 6.1. First order terms resulting from logistic regression of the proportion of prey killed as a function of prey density across different predator (*Anisops*, A; *Enithares*, E) and habitat complexity treatments. Attack rate and handling time parameter estimates result from Rogers' random predator equation.....154

CHAPTER 7

Figure 7.1. Critical thermal limits ([a] CT_{max} and [b] CT_{min}) of three mosquito predators (*Lovenula falcifera* [Lf], *Enithares chinai* [Ec] and *Anisops sardea* [As]) and three larval prey (*Aedes aegypti* [Aa], *Anopheles quadriannulatus* [Aq] and *Culex pipiens*

[Cp]) at 1st, 2/3rd and 4th instar stages. Group medians with different letters are statistically different from each other. (n = 20 per experimental group). In the boxplots, the box gives the interquartile ranges and the whiskers show the largest and smallest values up to 1.5 × interquartile range. The points in each box show the median.....181

Figure 7.2. Microclimatic data showing mean daily maximum (Max), minimum (Min) and average (Ave) temperature (°C) of (a) a clay lined pond and (b) a rock pool during their wet phase between August 2019 and February 2020.....184

Table 7.1. Summary of thermal breadths (warming and cooling tolerance) of predators and larval prey, calculated as temperatures between the critical thermal limits (CTLs) and the habitat temperature (T_{hab}). Means are shown alongside standard errors (SE).....183

Appendix 1

S1: Mosquito Knowledge, Attitude and Practices by Communities in the Serowe, Palapye and Bobirwa Sub-districts, Botswana.....216

Appendix 2

Table S3.1. Malaria cases and deaths between 2013 and 2018 transmission season for the Bobirwa, Palapye and Serowe sub-districts.....8

Table S3.2. Mosquito sample collection site information for adult (homesteads) and the larvae (water sources) from Bobirwa, Palapye and Serowe sub-districts.....8

Preface

This thesis comprises a general introduction (Chapter 1), followed by a series of data chapters (Chapters 2, 3, 4, 5, 6 and 7) and a general discussion chapter (Chapter 8). The data chapters are organised as scientific papers, all except one of which are published, as outlined below:

- Chapter 2: Buxton, M., Machezano, H., Gotcha, N., Nyamukondiwa, C., and Wasserman, R. J. (2020). Are vulnerable communities thoroughly informed on mosquito bio-ecology and burden? *International Journal of Environmental Research and Public Health*, 17: 8196.
- Chapter 3: Buxton, M., Nyamukondiwa, C., Kesamang, M. and Wasserman, R. J. (under review). Mosquito species composition: Insights from malaria endemic and non-endemic areas of Botswana. *African Entomology*. under review.
- Chapter 3 (Component): Buxton, M., Lebani, K., Nyamukondiwa, C., and Wasserman, R. J. (2019). First Record of *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) (Diptera: Culicidae) in Botswana. *BioInvasions Records*, 8: 551-557.
- Chapter 4: Buxton, M., Cuthbert, R. N., Dalu, T., Nyamukondiwa, C., and Wasserman, R. J. (2020). Cattle-induced eutrophication favours disease-vector mosquitoes. *Science of the Total Environment*, 715: 136952.
- Chapter 5: Buxton, M., Cuthbert, R. N., Dalu, T., Nyamukondiwa, C., and Wasserman, R. J. (2020). Complementary impacts of heterospecific predators facilitate improved biological control of mosquito larvae. *Biological Control*, 144: 104216.
- Chapter 6: Buxton, M., Cuthbert, R. N., Dalu, T., Nyamukondiwa, C., and Wasserman, R. J. (2020). Predator density modifies mosquito regulation in increasingly complex environments. *Pest Management Science*, 76: 2079-2086.
- Chapter 7: Buxton, M., Nyamukondiwa, C., Dalu, T., Cuthbert, R. N., and Wasserman, R. J. (2020). Implications of increasing temperature stress for predatory biocontrol of vector mosquitoes. *Parasites and Vectors*, 13: 1-11

General abstract

Mosquitoes account for significant morbidity and mortality globally, with the African region being most affected. In many arid regions, including Botswana, mosquitoes are increasingly becoming problematic, harbouring pathogens and parasites that cause debilitating infectious diseases to differential host species. The risk of mosquito proliferation and burden is accelerated when affected societies have knowledge gaps coupled with climatic mediated global change scenarios. In Botswana mosquito research is skewed towards malaria and associated anopheline vector species, neglecting other mosquito taxa, despite their economic significance as disease vectors for humans, domestic animals and wildlife. The national vector control programme in the country is mainly focused on adult mosquito species with management of juveniles (e.g., larvae) largely disregarded. Cognisant of these shortcomings, the thesis was thus aimed at bridging knowledge gaps in mosquito bio-ecology within the Central district, Botswana, a largely neglected region on vector mosquito bio-ecology. Knowledge, attitude and practices of local communities were assessed on mosquitoes across sub-districts of malaria endemic and non-endemicity status, with communities largely exhibiting limited knowledge on aspects of mosquito bio-ecology. Mosquito communities across the region were also assessed with key taxa species of medical (*Anopheles*, *Aedes* and *Culex* species) and veterinary (*Culiseta longiareolata*) importance identified throughout the study areas. The study also reports for the first time in the country, a globally important and medically significant invasive vector species, *Ae. aegypti* from Palapye sub-district. Using a mesocosm approach, the effects of anthropogenic activities were tested on mosquito breeding. The degradation associated with cattle dung nutrification in aquatic environments favoured oviposition by mosquito vector species. With the aim to assess natural predator (*Lovenula falcifera* and *Anisops sardea*) mosquito control potential, their predation on larvae of the abundant mosquito species (*Culex pipiens*) were quantified using a functional response (FR) approach. Both predators exhibited Type II FRs and typically contributed additively to prey risk, with synergistic impacts evident on prey of intermediate sizes. In complementing this approach, multiple predator effects by notonectid predators, *A. sardea* and *Enithares chinai*, were quantified across a habitat complexity gradient. *Enithares chinai* exhibited significantly greater consumption rates than *A. sardea*, both as individuals and conspecific pairs. Each predator type displayed Type II FRs across experimental treatments, with synergistic multiple predator effects displayed in the absence of habitat complexity. Effects of increasing habitat complexity modified multiple predator effects differentially between species given behavioral differences. Whilst the ecosystem services provided by natural predators for mosquito biological control are evident, understanding how climate change-induced environmental perturbations may corrode these services remains largely unknown. Here, critical thermal limits of key predator-prey participants across instar stages (as above) were explored. All predators generally had lower activity limits and narrower thermal windows compared to their larval mosquito prey. These results point to significant predator-prey mismatches under environmental change, potentially adversely affecting natural mosquito biocontrol given projected shifts in temperature fluctuations in the study region. The information gleaned from the various chapters will be useful for practitioners dealing with the various human, biological and ecological components of pest mosquito control and emerging threats in the region.

CHAPTER 1

General Introduction

1.1. Drivers of mosquito proliferation

Mosquito vectors account for significant annual human morbidity and mortality globally. Whilst intervention strategies are intensified and deployed against the burden of mosquitoes among societies, these vectors thrive successfully across varied landscapes. Societal knowledge on mosquito bio-ecology is key in either suppressing or elevating the effects associated with mosquitoes. Therefore, anthropogenic factors coupled with environmental factors are the major concern globally for mosquito vector proliferation (Lee *et al.*, 2020; Schrama *et al.*, 2020). This calls for global structures and systems to bridge knowledge gaps that may foster mosquito control initiatives and practices, especially in Africa where communities are mostly affected. The thesis presents and addresses knowledge gaps on mosquito vector species of Botswana (Central district) and how human activity may likely support proliferation with ultimate goal to establish an inherent and sustainable natural enemies' ecosystem service amidst challenges of the prevailing global change.

1.1.1 *Human-mediated factors*

Anthropogenic activities may negatively impact the biophysical environment, including biodiversity and associated ecosystem functioning and services (Sannigrahi *et al.*, 2020). Change of land use fostered by human population growth, is a major issue of concern in recruiting harmful mosquito vectors leading to debilitating disease burden upon communities (Lee *et al.*, 2020; Schrama *et al.*, 2020). Accordingly, man-made aquatic systems emanating from urbanisation and agriculture (subsistence or commercial) are readily colonised by mosquito

species (see Warchot *et al.*, 2020). Although mosquitoes are also known to be strong fliers with potential to migrate following seasonal variation and environmental niche exploration (Faiman *et al.*, 2020 but see Ciota *et al.*, 2012), terrestrial adults can possibly be relocated by several modes of human transportations and subsequently invade new areas (Eritja *et al.*, 2017). For instance, *Ae. aegypti* was believed to be first introduced to other parts of the world from Africa through transportation and trade systems (e.g., Ibañez-Justicia *et al.*, 2017). In addition, industrialisation directly influences negative impacts of global warming, favouring mosquito populations by shortening generation time due to temperature elevations, impacting on future vector-borne disease spread and projections (Mitton and Ferrenberg 2012; Tjaden *et al.*, 2017). Temperature also impacts parasite and/pathogen incubation times (e.g., Kamiya *et al.*, 2020). Thus, reduced vector generation time and parasite incubation periods may act synergistically favouring proliferation of vector-borne disease epidemics (Sukiato *et al.*, 2017).

Some human mediated factors leading to mosquito proliferation are often linked to degradation of natural aquatic breeding habitats (Azrag and Mohammed 2018; Wilke *et al.*, 2020). Agricultural activities are pertinent in this regard, and particularly so in water scarce areas as natural water bodies can become disproportionately important for agricultural needs (Dalu *et al.*, 2017; Mamai *et al.*, 2017). In the case of livestock, animals readily utilise natural water bodies, but typically also urinate and defecate in water sources (Mamai *et al.*, 2017). This, and other agricultural activities e.g., fertilization of crops result in nutrification of these environments with implications for water quality and the nature of food resources available for aquatic communities (Mamai *et al.*, 2017). Such human-mediated degradation of water sources can

compromise natural communities by favouring certain taxa (Dalu *et al.*, 2017; Mamai *et al.*, 2017).

In Botswana, agriculture forms the mainstay of the economy. In particular, cattle rearing is an important socio-economic activity practiced in all parts of the country's semi-arid environments. As cattle are almost entirely free-ranging in the country, they typically roam in and around human habitation, utilising available pasture, natural and artificial water bodies for watering purposes (Figure 1.1a,b,c). While prevalent and widespread throughout rural and even wildlife reserve areas (Wikan 2004; Wallgren *et al.*, 2009), cattle are even found within urban areas of major cities in Botswana (Figure 1.1a). It is highly likely that they contribute to the degradation of water sources at massive spatial scales. Given the importance of aquatic habitats for mosquito persistence, such human-mediated habitat disturbance may drive mosquito population success, although never assessed. The studies are necessary to inform policy makers about animal movement restriction and natural aquatic body management and conservation of native predators of mosquito larvae that help reduce disease vectors. Furthermore, given movement of people to and from malaria endemic areas, nationwide survey of mosquito population monitoring and evaluation is warranted in establishing the risk of vectors in novel environments.

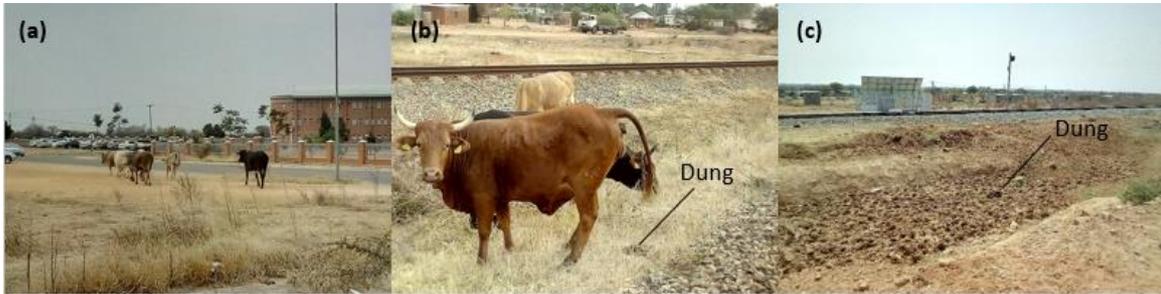


Figure 1. 1. Free range cattle roaming in (a) a semi-urban Palapye often searching for (b) pasture and (c) water from potential sources while subsequently depositing dung.

1.1.2. *Climate-mediated factors*

Mosquitoes are ectotherms and as such, their body temperature is always in equilibrium with their ambient environment (Chown and Nicolson 2004; Angilletta 2009). Thus, fluctuations in the bio-physical environmental may impact organismal function, altering their fitness and overall parity status (Ezeakacha and Yee 2019). To maintain organismal function and fitness, mosquitoes need to adjust to oscillating climatic conditions in the short, medium and long timescales (Parmesan 2006; Paaijmans *et al.*, 2013). Through compensatory modification (e.g., behavioral, physiological and molecular mechanisms), insects are able to remodel their resistance to biophysical environments phenotypes, allowing them to cope and maintain function during stressful environments (Chown and Nicolson 2004). This phenomenon, termed phenotypic plasticity, allows organisms to permanently shift their phenotypes upon change of environment, without change in genetic make-up (West-Eberhard 2003; Whitman and Ananthakrishnan 2009). Phenotypic plasticity (within a single generation) and transgenerational plasticity (across generations) is near ubiquitous in insects (e.g., West-Eberhard 2003; Sgro *et al.*, 2016). As such, this mechanism is likely utilised by mosquito species to survive episodes of stressful environments and also invading and establishing in novel environments (Whitman

2007; Gunderson *et al.*, 2017). Indeed, this phenomenon has been reported to aid establishment in novel environments for invasive species (see Nyamukondiwa and Terblanche 2010). Similarly, invertebrates seldom use seasonal or habitat carryover effects to survive harmful biophysical environment (Evans *et al.*, 2018). For instance, Evans *et al.* (2017) demonstrated that *Ae. albopictus* carry-over effects had a strong positive relationship with larval environmental temperature thus, exhibiting implications for mosquito-borne pathogen transmission. Consequently, mosquitoes are likely to expand their ranges, colonise and establish in new environments as a result of climate change (Iwamura *et al.*, 2020; Liu *et al.*, 2020).

As a strategy for population persistence, mosquitoes may transform within the short term (hardening) or medium (acclimation) owing to the fitness cost and/ or benefits brought about by phenotypic plasticity and climate change (Sgro *et al.*, 2016). Therefore, assessing and monitoring mosquito responses to environmental variability (e.g., thermal performance) remains paramount for predicting climate change related alterations in population abundance, biogeography and disease modelling. Thus, thermal performance curve (TPC) summarises thermal performance traits within certain ranges of temperature tolerance (Figure 1.2; Angilletta 2009). Consequently, TPCs describe effects of temperature on processes that affect insect biological rate processes (Schulte *et al.*, 2011), varying across space, taxa and ontogeny and having significant ecological relevance on the fitness of vector species and disease epidemiology (Kingsolver *et al.*, 2013). The effects of a temperature gradient on insect activity or function can be summarised using the thermo-biological scale (Figure 1.2; Vannier, 1994). When insects are subjected to increasing temperature, they reach a point of losing locomotory function (physiological failure) known as the heat stupor or the critical thermal maximum (CT_{max}) until irreversible injury and/ or death

(Figure. 1.2). In the process, insects can lose water and/ accumulate sugars (trehalose) as a buffer against dehydration and unwanted biochemical pathways to compensate for the heat stress (Watanabe *et al.*, 2002). As temperature is ramped down from the optimum (T_{opt}), insects undergo cold stupor, also referred to as the critical thermal minimum (CT_{min}) and subsequently experiences a chill coma. At sub-zero temperatures, insects may reach a point where their bodily fluids freeze, termed supercooling points (Sinclair *et al.*, 2003; Sinclair *et al.*, 2015). Some insects die upon freezing of their bodily constituents while others can tolerate freezing. As such, these organisms can be grouped as freeze tolerant and freeze intolerant insects (Sinclair *et al.*, 2003). A third group of insects, termed chill-susceptible die at more benign temperatures, and may succumb to periods of low stressful but non sub-zero temperatures (Lee 2012). Understanding these strategies of insect survival when exposed to extreme temperature may bring knowledge on insect abundance and population dynamics in the face of thermally shifting environments brought about by climate change. Indeed, several traits depicted in the thermobiological scale (e.g., supercooling points), CT_{max} and CT_{min} have been used in physiological experiments to accurately predict insects' responses to temperature and climate change (see Chown and Nicolson 2004; Angilletta 2009; Huey and Kearney 2020). However, information on mosquito thermal performance is currently limited (but see Lyons *et al.*, 2012; Verhulst *et al.*, 2020).

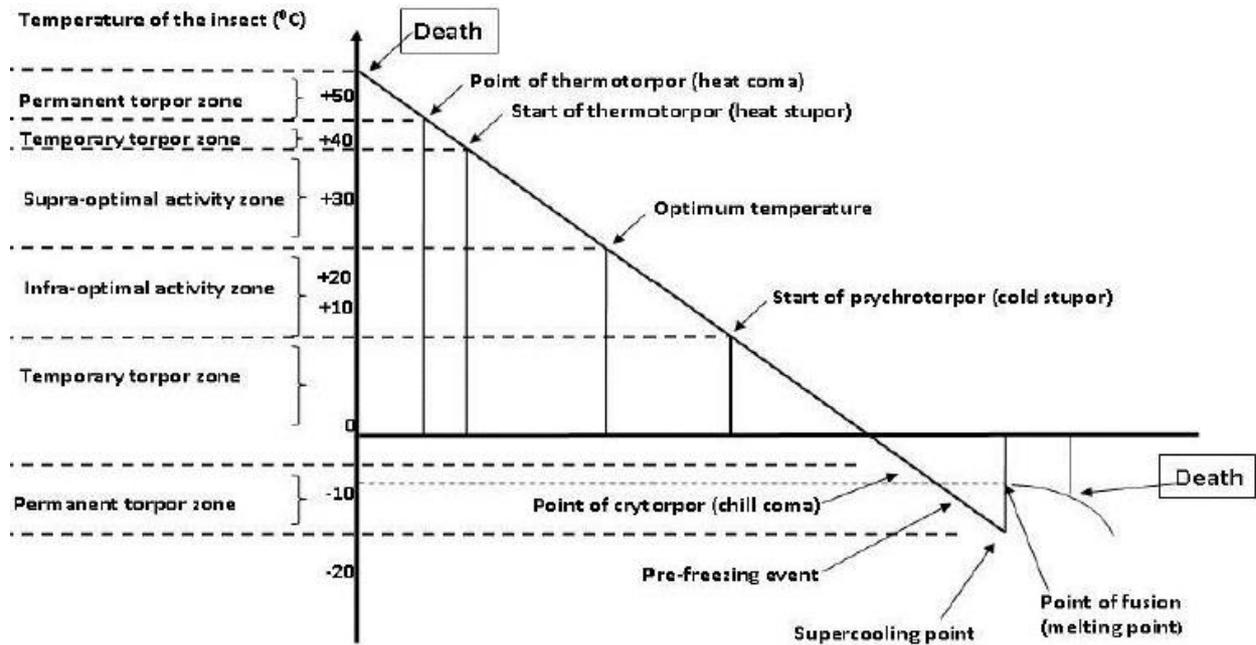


Figure 1. 2. Thermo-biological scale of insect performance to temperature changes (extracted from Vannier, 1994).

Botswana falls with the region of southern Africa where climatic change projections are currently prevalent (Daron 2018). Oscillation of climatic variability plays a critical role in the physiological responses of mosquito species across space (Sgro *et al.*, 2016). Owing to the factors affecting mosquito population dynamics (e.g., temperature and rainfall) (Wilke *et al.*, 2017), the country has recently exhibited a shift in climatic variability mainly due to effects of global warming (Nkemelang *et al.*, 2018). With little information available on key mosquito species performance to thermal variability in the country (see Buxton *et al.*, 2020), exploration of such is necessary. This has implications for climatic modelling for risk of vector disease epidemics countrywide. Availability of rainfall is a crucial factor in the survival of mosquito populations. Indeed, seasons that receive a lot of rains are prone to elevated risk of mosquito-borne infections (Whelan *et al.*, 2003). Although some mosquito species are not able to survive

the dry period, some have developed sustaining mechanisms to survive periods with little or no rain (e.g., *Ae. aegypti*) (Poh et al., 2019). In Botswana, no studies have investigated the effect of seasonality on mosquito population dynamics and the risk of disease spread.

1.2. Mosquito control

The global distribution of mosquito species has contributed to the socio-economic burden across continents (WHO 2014). Proliferation of these species and their interaction with competent pathogen, yields various dangerous diseases, progressively compromising the health of diverse hosts. Accordingly, vectors have the capacity to be susceptible to various pathogens with the risk of emerging and re-emerging infections (Mitchell *et al.*, 2015; Saijo 2020). To this universal threat, there is a need to manage vector population and their parasites, as a mitigation strategy towards reducing the burden of infections. In doing so, a holistic approach is necessary encompassing aspects of entomology, epidemiology and medicine. In particular, entomological protocols targeting mosquito ontogeny for species reduction are essential.

Understanding the foraging and resting behavior, makes control measures more targeted, less costly and more efficacious. Adult mosquitoes may prefer feeding (endophagic) and resting (endophilic) indoors, while others feed (exophagic) and rest (exophilic) outdoors (Govella *et al.*, 2009; Paaijmans *et al.*, 2011). However, reports of evolutionary behavioural modifications have been documented (Sougoufara *et al.*, 2014; Thomsen *et al.*, 2016). Furthermore, some mosquitoes are specific on feeding times, exploiting humans (anthropophilic), animals (zoophilic) and/ or both (Chaves *et al.*, 2010; Waite *et al.*, 2017; Stones and Gross 2018). In addition, the larval resting positions, feeding and behavioural adaptations may be unique across

taxa. Given these dynamics, control strategies need to be relevant and suitable, exploring diverse management approaches (Becker *et al.*, 2010). Control measures that are more sustainable (e.g., biocontrol agents and low risk substances) with minimal negative effects on biodiversity and ecosystem functioning, are rather desirable. Currently, mosquito control strategies are variable including; chemical, genetic and biological and interference methods.

1.2.1. *Chemical control measures*

Chemical based interventions have been used over decades for successful mosquito control globally (Benelli 2015). Insecticides can be broadly grouped as simpler compounds of mineral origin (inorganic) and the complex carbon-based molecular structure (organic). They interact with the organism, to trigger behavioural or physiological responses targeting mainly (i) the nervous and hormonal system, (ii) pH and water balance, (iii) enzyme and electron transfer inhibition, (iv) cuticle formation and energy production (Das 2013). There are four main classes of organic insecticides *vis* organochlorines, organophosphates, pyrethroids and carbamates (Zhu *et al.*, 2014). Organophosphates and carbamates inhibit the enzyme acetyl cholinesterase in the synaptic cleft whilst organochlorines and pyrethroids target the sodium ion channels (Sparks and Nauen 2015). Although chemical control is highly effective, insecticides have persistent detrimental effects to the bio-physical environment (Thompson *et al.*, 2017), mostly to the non-target plants and animals (Sanchez-Bayo 2012; Chaturvedi *et al.*, 2013). In particular, insecticides have been implicated in the reduction of biodiversity (Brühl and Zaller 2019; Li *et al.*, 2020), e.g., decline in pollinator species (Kumar *et al.*, 2018) and essential ecosystem services (Ndakidemi *et al.*, 2016). Furthermore, there is increasing evidence linking insecticide exposure with public health risks (e.g., cancer, birth deformities) (Asghar *et al.*, 2016). Coupled

with resistance development in mosquito species (e.g., cross and multiple resistance) in Africa and elsewhere (Liu 2015; Djouaka *et al.*, 2016), this warrant exploring other benign and environmentally friendly low risk alternatives.

Vector control in Botswana is formally facilitated through a national mosquito monitoring and eradication programme (Kgoroebutswe *et al.*, 2020b). The programme has been mainly targeting adult mosquitoes using chemical intervention (pyrethroids and/or dichloro-diphenyl-trichloroethane [DDT]; having same mode of action) for indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLITNs). This approach has been used in the country since at least the 1950s (see Chihanga *et al.*, 2016; Makate 2016; Kgoroebutswe *et al.*, 2020b). However, recently (2019), pirimiphos-methyl (organophosphate) has been deployed to all malaria endemic districts (Kgoroebutswe *et al.*, 2020b). To this end, patterns of mosquito vector resistance status are not fully explored in Botswana given the persistence usage of same control intervention strategy (although see Kgoroebutswe *et al.* 2020a). Monitoring vector resistance in mosquito species and the use of other complementary efficacious methods that do not exacerbate selection pressure remains crucial as management strategies curbing resistance.

1.2.2. Genetic control measures

Several genetic approaches of mosquito control have been developed and used for population suppression globally (Harvey-Samuel *et al.*, 2017). These methods focus on mosquito gene manipulation, mass rearing and subsequent release, targeting wild populations species abundance reduction (e.g., Teem *et al.*, 2020) and this has further demonstrated to decreased mosquito vectorial capacity and disease burden in certain parts of the world (e.g., Qasim *et al.*, 2020). The most common is sterile insect technique (SIT), a form of birth control in insects. The

technique involves sterilisation of males using either radiation or chemicals and released into the wild to potentially mate with females resulting in non-viable F₁ progenies (Bourtzis 2016). For instance, the primary vectors of dengue virus, *Ae. aegypti* and *Ae. albopictus*, have been reduced using the SIT, significantly lessening the burden of the disease (Carvalho *et al.*, 2015).

In southern Africa, some SIT work has been explored on *Anopheles* vectors with promising output (e.g., Munhenga *et al.*, 2011). More recently, the precision guided Sterile Insect Technique (pgSIT) using the novel CRISPR-based technology has been developed and can also be utilised as a complementary method (see Kandul *et al.*, 2019). Other genetic methods involve gene manipulation and cloning techniques (e.g., gene drive, paratransgenesis, RNA interference) which most of them are being developed (Wilke and Marrelli 2015; Airs and Bartholomay 2017; Sim *et al.*, 2019) as promising tool to generate transgenic mosquitoes capable of eliminating disease vectors (Wise de Valdez *et al.*, 2011; Leftwich *et al.*, 2016). Furthermore, mosquito gut microbiota (e.g., *Pantoea agglomerans*) can be modified to regulate pathogen transmission (Wang *et al.*, 2012). These methods can be used in a holistic approach involving other methods of controlling the vector and pathogen to reduce vector-borne disease burden. Although numerous techniques have been employed to yield transgenic mosquitoes, impacts of genetically modified material transfer into wild population (e.g., through the release of insects with dominant lethal [RIDL] technologies) is currently a major concern (Evans *et al.*, 2019; Nazareth *et al.*, 2020). Furthermore, these approaches are more costly as opposed to the sustainable biological control methods. Nevertheless, they remain a significant and compatible component of the integrated management of mosquito vector species.

In Botswana, none of these genetic approaches have been explored for mosquito control. Although the negative effects of these methods have not been fully investigated regarding biodiversity, the country may need to integrate the existing vector control strategies with these innovative approaches. This may aid in mosquito species reduction reported in high numbers by Pachka *et al.* (2016) from the country's malaria 'hotspot' zone, the swampy Okavango region (Chirebvu *et al.*, 2016). Moreso, the current vector control is mainly focused on chemical intervention strategy towards the adult mosquitoes. As Botswana is getting ready to eliminate and sustain malaria free country, there is a need for holistic approach on the control of vectors thus utilising a variety of methods as complimentary strategies.

1.2.3. *Biological control measures*

Biological control is regarded as one of the most sustainable and eco-friendly approach of mosquito regulation (Benelli *et al.*, 2016). This method involves the use of natural organisms to suppress pest population and can target any life stage across pest insect ontogeny (e.g., eggs, larvae, pupae, adults) (e.g., Bukhari *et al.*, 2013). However, despite its ecological and economic soundness coupled by high and target specific efficacy, its use for mosquito control is often neglected. However, other approaches using semiochemicals such as push pull systems (e.g., using plants and/ or their derivatives) are not lethal and can only deter mosquito species away from targeted hosts (Menger *et al.*, 2014). However, pheromones can easily be employed in a lure and kill system (Benelli and Govindarajan 2017). A range of microbial approaches (e.g., *Wolbachia* infection) are also utilised as promising microbial tools against mosquito vectors (Caputo *et al.*, 2020). However, these techniques are very costly to elicit effective population reduction over a long period of time despite their negative effects to the natural environment

(Evans *et al.*, 2019). Biological control using natural enemies remains a viable option in mosquito regulation although non-native species are not recommended due to possibility of invasive potential (see e.g., Walton 2007; Chandra *et al.*, 2008). Therefore, the thesis promotes mosquito biological control of larvae by the use of native aquatic predatory species.

The use of natural enemies (e.g., predators) in mosquito control, is also widely studied in certain parts of the world and deployed in field setting for disease vector population reduction (e.g., Cuthbert *et al.*, 2018). Promotion and conservation of these predators (e.g., crustaceans and insect natural enemies) is crucial, especially in natural wetlands where mosquito vector species proliferate (Culler and Lamp 2009). Whilst many predators have been identified and assessed (Saha *et al.*, 2012; Cuthbert *et al.*, 2020a), more diverse native species need to be explored for further quantification of their predatory impacts against mosquito aquatic prey (larvae).

In Botswana, mosquito colonises a variety of larval habitats (e.g., Chirebvu and Chimbari *et al.*, 2015) of which their ecosystem functioning has not been fully understood. As the country's national vector control is focused mainly on adult mosquitoes, exploration of alternative and sustainable larval control approaches are warranted. Although some microbial complementary approaches (Bio-larviciding using *Bacillus thuringiensis israelensis* [Bti]) have been initiated in some malaria endemic districts (see Obopile *et al.*, 2018), this method may be very costly as needs to be repeated at short intervals for effective results (Mpofu *et al.*, 2016). Whilst malaria elimination in Botswana is eminent (Motlaleng *et al.*, 2018), a holistic approach of vector control strategies is essential targeting both the terrestrial and the neglected aquatic life stages. Therefore, unraveling predator prey interaction and wetland ecosystem functioning in the

country, remains crucial in suppressing mosquito population and reducing risks of disease burden.

1.3. Problem statement

Vector mosquitoes are increasing their natural ranges in response to changes in climate and human-mediated translocation of species. This has a consequence on the parasites/pathogens they vector, disease epidemics and development of control strategies. Currently, Botswana has labeled the northern transect malaria endemic with ~ 0.1- 1.0 % cases per 1000 population annually (Motlaleng *et al.*, 2018; Figure 1.3). Although the country is aiming for malaria elimination by the year 2023, annual sporadic malaria cases are often recorded in non-endemic districts (e.g., Central, Ghanzi, Kweneng, Mahalapye) of Botswana (Chihanga *et al.*, 2016; Figure 1.3). In particular, the Central district consists of three malaria endemic and three non-endemic sub-districts with Serowe and Palapye on the malarial invasion front (Figure 1.3). Wedged between South Africa and Zimbabwe, these sub-districts are also very close to regions of their bordering countries where mosquito-borne diseases are perpetually problematic (Gunda *et al.*, 2017; Adeola *et al.*, 2019), and even invasive vectorially-incriminated mosquito species have been detected (Rotz 2016). As such, the region is neglected on aspects of mosquito bio-ecology and needs mosquito research, monitoring and control prioritisation owing to the prevailing anthropogenic activities and climate mediated factors. This has implications for how local communities interact with mosquitoes, as differences in knowledge on mosquito biology may hinder or facilitate mosquito proliferation.

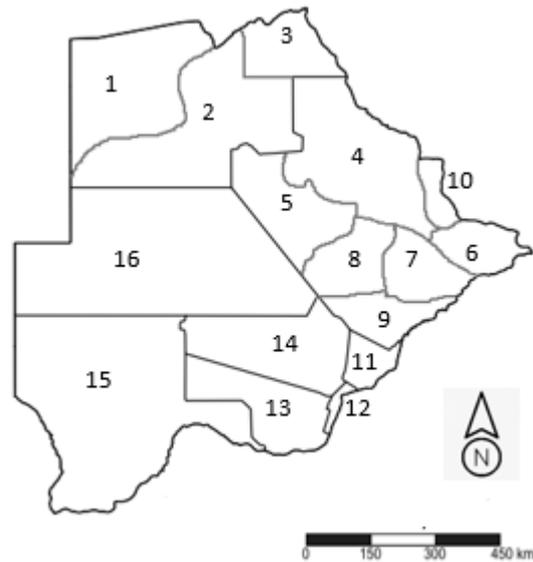


Figure 1. 3. Map of Botswana showing six malaria endemic and 10 non-endemic districts/ sub-districts across the country. Malaria endemic are Okavango (1), Ngamiland (2), Chobe (3), Tutume (4), Boteti (5) and Bobirwa (6). Malaria non-endemic districts/ sub-districts are Palapye (7), Serowe (8), Mahalapye (9), North-east (10), Kgatleng (11), South-east (12), Southern (13), Kweneng (14), Kgalagadi (15) and Ghanzi (16). Central district consists of six sub-districts: Tutume (4), Boteti (5), Bobirwa (6), Palapye (7), Serowe (8) and Mahalapye (9).

1.4. Justification of the study

The purpose of the study was to address knowledge gaps on broad themes of mosquito bio-ecology in the Central district of Botswana. Despite being the largest district in the country, it is highly neglected in aspects of mosquito surveillance and control strategies. The findings will strengthen community knowledge on overlooked areas that may curb and ease the burden of mosquito-borne infections. Many societies often do not regard anthropogenic activities as crucial when managing the impacts of mosquito vectors population dynamics. This brings down efforts in fighting the debilitating effects of mosquitoes and associated burdens. As such, the study will bridge knowledge gaps on how to sustainably suppress mosquito population amidst challenges of the prevailing global change scenarios. Therefore, policy makers and programme leaders in

the country will augment their current structures, processes and systems surrounding mosquito subject to themes explored under this study.

1.5. Present study

The national vector control programme in Botswana is mainly focused on chemical intervention as a form of control of terrestrial adult mosquitoes. In Botswana, officials are understandably cautious regarding exploration of biological control, given the potential unintended effects of certain biological control strategies (Todd *et al.*, 2020). Wildlife tourism comprises an important component of the economy and as such, in-tact ecosystems are deemed valuable for this industry. Threats to biodiversity are, therefore, not taken lightly. For example, using biological control measures such as *Wolbachia* infections, is deemed to be very risky, given the implications for other dipterans which are important for the ecological integrity of the Okavango Delta, a tourism ‘hotspot’ and socio-culturally important system (Mbaiwa 2004; Mopelwa and Blignaut 2006; Mbaiwa 2011).

The management of aquatic habitats as mosquito breeding sites is also largely overlooked. In particular, the role of natural early mosquito life-stage biocontrol ecosystems services, and their corrosion, has received little attention. Given the life-history dynamics of mosquitoes, aquatic early life-stages represent a period where very large numbers of individuals are highly concentrated, when compared to their relatively well dispersed adult stage. As such, considering early life-stage control could be highly useful as part of larger integrated control measures, with implications for mosquito proliferation at the landscape level. In particular, small temporary wetlands are of great relevance in this regard. These are by far the dominant aquatic

environments in arid Botswana, but are completely missed in the national vector control programme. Their role as potential mosquito breeding sites, particularly when ecologically compromised, is largely unacknowledged. Knowledge on inherent natural ecosystem services contributing to the control of mosquitoes is almost non-existent for the region (but see recent work by Cuthbert *et al.*, 2018; 2019a,b; 2020b, from the Eastern Cape of South Africa, around 1200 km away). This body of work is centered around aspects of mosquito ecology, focusing on their interactions with components of the aquatic environment.

The thesis addresses a number of key components of mosquito ecology, within the context of specific factors that may facilitate the proliferation or control of mosquito larvae in temporary wetland habitats. The study was conducted in the Central district of Botswana, and as such, has certain parochial components whereby local context has dictated rationale. The work has, however, been contextualised within the broader ecological context wherever possible, making the work more widely relevant. Included in the thesis are a basic assessment of local community knowledge, attitudes and practices on mosquito ecology and control, as well as a local descriptive mosquito diversity survey and first record of an invasive mosquito species in Botswana. The study then assessed the implications of a locally important temporary water body pollution issue on mosquito oviposition success. The biological control potential of natural temporary wetland invertebrate predators, under various environmental contexts was then assessed. Finally, the thesis highlights the mismatch in responses to temperature stress of aquatic predators and mosquito larvae, highlighting the possible role of projected increased temperatures in reducing natural biological control potential in temporary wetlands.

1.6. Objectives

The overall objective of this project was to bridge knowledge gaps on mosquito bio-ecology *vis* identifying sustainable biological control systems against larval mosquitoes amidst environmental factors driving vector proliferation.

In the second chapter, the knowledge, attitude and practices of the communities of Serowe, Palapye and Bobirwa sub-districts, towards mosquitoes were assessed. The specific objectives in this chapter were to determine if:

- communities are better informed on mosquito related issues iregardless of malaria endemicity status.
- communities across study sites protect themselves against mosquitoes appropriately.

In the third chapter, the study aimed to identify the diversity and abundance of mosquito species in malaria endemic Bobirwa and non-endemic Serowe and Palapye sub-districts. The specific objectives in this chapter were to:

- Identify the different and dominant mosquito species across endemic and non-endemic districts.
- Search for previously unrecorded potentially important mosquito species in the region.

The aim of the fourth chapter was to investigate the potential effect from cattle, a socio-culturally important livestock, on mosquito breeding success within temporary wetlands using mesocosms approach. The specific objectives in this chapter were to determine if:

- dung-enriched *in situ* mesocosms on rocky outcrops would be colonised by vector-mosquito species.
- cattle-dung eutrophication would drive similar proliferation rates between anopheline and non-anopheline species.

In the fifth chapter, the study aimed to quantify and contrast the predatory potential of dominant temporary wetland aquatic predators using a functional response approach. The specific objectives in this chapter were to determine if:

- natural enemies have the same predatory effects across mosquito larval ontogeny.
- multiple predator effects (MPEs) would be evident under heterospecific predator scenarios.

The aim of the sixth chapter was to quantify and contrast the predatory potential of two closely related temporary wetland aquatic predators under different habitat complexities, using a functional response approach. The specific objectives in this chapter were to determine if:

- increased habitat complexity would have a greater reduction effect on the predatory efficiencies of *A. sardea* than *E. chinai*.
- both predatory species would have relatively high efficiencies towards larval mosquito prey at low habitat complexity.

- *A. sardea* MPEs would result in reduction of predation efficiency with an increase in habitat complexity.
- *E. chinai* conspecific MPEs would increase prey vulnerability with an increase in habitat complexity.

In the seventh chapter, the aim was to assess the thermal fitness of predators and different mosquito larval prey for effective biological control. The specific objectives in this chapter were to determine if:

- larval thermal tolerance would vary across mosquito species.
- predators would have synchronised thermal windows with their mosquito larval prey species.
- the thermal window for the wholly aquatic copepod would match that of semi-aquatic notonectid predators.

1.7. References

- Abdulla-Khan, R., Coetzee, M., and Hunt, R. H. (1998). Description of *Anopheles* (*Cellia*) *seretsei* sp. nov. from Kasane, Botswana. *Journal of the American Mosquito Control Association-Mosquito News*, 14: 248-252.
- Adeola, A., Ncongwane, K., Abiodun, G., Makgoale, T., Rautenbach, H., Botai, J., and Botai, C. (2019). Rainfall trends and malaria occurrences in Limpopo province, South Africa. *International Journal of Environmental Research and Public Health*, 16: 5156.
- Airs, P. M., and Bartholomay, L. C. (2017). RNA interference for mosquito and mosquito-borne disease control. *Insects*, 8: 4.
- Angilletta, M. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press, Oxford, UK.

- Asghar, U., Malik, M. F., and Javed, A. (2016). Pesticide exposure and human health: a review. *Journal of Ecosystem and Ecography*, 5(5): 005.
- Azrag, R. S., and Mohammed, B. H. (2018). *Anopheles arabiensis* in Sudan: a noticeable tolerance to urban polluted larval habitats associated with resistance to Temephos. *Malaria Journal*, 17: 204.
- Bango, Z. A., Tawe, L., Muthoga, C. W., and Paganotti, G. M. (2020). Past and current biological factors affecting malaria in the low transmission setting of Botswana: A review. *Infection, Genetics and Evolution*, 85: 104458.
- Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M., Dahl, C., and Kaiser, A. (2010). *Mosquitoes and their control*. Springer Science and Business Media, Heidelberg, Germany.
- Benelli, G., and Govindarajan, M. (2017). Green-synthesized mosquito oviposition attractants and ovicides: towards a nanoparticle-based “lure and kill” approach? *Journal of Cluster Science*, 28: 287-308.
- Benelli, G., Jeffries, C. L., and Walker, T. (2016). Biological control of mosquito vectors: past, present, and future. *Insects*, 7: 52.
- Benelli, G. (2015). Research in mosquito control: current challenges for a brighter future. *Parasitology Research*, 114: 2801-2805.
- Bourtzis, K. (2016). The sterile insect technique as a tool for control of insect vectors and vector-borne diseases. *International Journal of Infectious Diseases*, 53: 24.
- Brühl, C. A., and Zaller, J. G. (2019). Biodiversity decline as a consequence of an inadequate environmental risk assessment of pesticides. *Frontiers in Environmental Science*, 7: 177.
- Bukhari, T., Takken, W., and Koenraadt, C. J. (2013). Biological tools for control of larval stages of malaria vectors—a review. *Biocontrol Science and Technology*, 23: 987-1023.
- Buxton, M., Wasserman, R. J., and Nyamukondiwa, C. (2020). Disease Vector Relative Spatio-Temporal Abundances to Water Bodies and Thermal Fitness Across Malaria Endemic Semi-Arid Areas. *Journal of Medical Entomology*, tjaa221, <https://doi.org/10.1093/jme/tjaa221>.
- Caputo, B., Moretti, R., Manica, M., Serini, P., Lampazzi, E., Bonanni, M., and Calvitti, M. (2020). A bacterium against the tiger: preliminary evidence of fertility reduction after

- release of *Aedes albopictus* males with manipulated *Wolbachia* infection in an Italian urban area. *Pest Management Science*, 76: 1324-1332.
- Carvalho, D. O., McKemey, A. R., Garziera, L., Lacroix, R., Donnelly, C. A., Alphey, L., and Capurro, M. L. (2015). Suppression of a field population of *Aedes aegypti* in Brazil by sustained release of transgenic male mosquitoes. *PLoS Neglected Tropical Diseases*, 9: e0003864.
- Chaturvedi, M., Sharma, C., and Chaturvedi, M. (2013). Effects of Pesticides on Human Beings and Farm Animals: A Case Study. *Research Journal of Chemical and Environmental Sciences*, 1: 14-19.
- Chandra, G., Bhattacharjee, I., Chatterjee, S. N., and Ghosh, A. (2008). Mosquito control by larvivorous fish. *Indian Journal of Medical Research*, 127: 13.
- Chaves, L. F., Harrington, L. C., Keogh, C. L., Nguyen, A. M., and Kitron, U. D. (2010). Blood feeding patterns of mosquitoes: random or structured? *Frontiers in Zoology*, 7: 3.
- Chihanga, S., Haque, U., Chanda, E., Mosweunyane, T., Moakofhi, K., Jibril, H. B., and Glass, G. E. (2016). Malaria elimination in Botswana, 2012–2014: achievements and challenges. *Parasites and Vectors*, 9: 99.
- Chirebvu, E., Chimbari, M. J., Ngwenya, B. N., and Sartorius, B. (2016). Clinical malaria transmission trends and its association with climatic variables in Tubu Village, Botswana: a retrospective analysis. *PloS ONE*, 11: e0139843.
- Chirebvu, E., Chimbari, M. J., and Ngwenya, B. N. (2013). Knowledge and practices on malaria in Tubu village, in a malaria-endemic area in northern Botswana: implications for interventions. *Malaria World Journal*, 4: 1-9.
- Ciota, A. T., C. L., Drummond, M. A., Ruby, J., Drobnack, G. D., Ebel, and L. D. Kramer. 2012. Dispersal of *Culex* mosquitoes (Diptera: Culicidae) from a wastewater treatment facility. *Journal of Medical Entomology*. 49: 35-42.
- Cornel, A. J., Lee, Y., Almeida, A. P. G., Johnson, T., Mouatcho, J., Venter, M., and Braack, L. (2018). Mosquito community composition in South Africa and some neighboring countries. *Parasites and Vectors*, 11: 1-12.
- Culler, L. E., and Lamp, W. O. (2009). Selective predation by larval *Agabus* (Coleoptera: Dytiscidae) on mosquitoes: support for conservation-based mosquito suppression in constructed wetlands. *Freshwater Biology*, 54: 2003-2014.

- Cuthbert, R. N., Callaghan, A., Sentis, A., Dalal, A., and Dick, J. T. (2020a). Additive multiple predator effects can reduce mosquito populations. *Ecological Entomology*, 45: 243-250.
- Cuthbert, R. N., Dalu T, and Wasserman, R. J. (2020b). Arid-adapted paradiaptomid copepods contribute to mosquito regulation. *African Zoology*, 55, 185-186.
- Cuthbert, R., Dalu, T., Wasserman, R. J., Weyl, O. L. F., Froneman, P. W., Callaghan, A., and Dick, J. T. A. (2019). Additive multiple predator effects of two specialist paradiaptomid copepods towards larval mosquitoes. *Limnologica*, 179, 125727.
- Cuthbert, R. N., Dalu, T., Wasserman, R. J., Dick, J. T. A., Callaghan, A., Froneman, P. W., and Weyl, O. L. F. (2019). Quantifying reproductive state and higher predator effects on copepod motility in ephemeral ecosystems. *Journal of Arid Environments*, 168, 59-61.
- Cuthbert, R. N., Dalu, T., Wasserman, R. J., Callaghan, A., Weyl, O. L., and Dick, J. T. (2018). Calanoid Copepods: An Overlooked Tool in the Control of Disease Vector Mosquitoes. *Journal of Medical Entomology*, 55: 1656-1658.
- Dalu, T., Wasserman, R. J., and Dalu, M. T. B. (2017). Agricultural intensification and drought frequency increases may have landscape-level consequences for ephemeral ecosystems. *Global Change Biology*, 23: 983-985.
- Daron, J. D. (2018). Regional climate message for Southern Africa. Scientific report from the CARIAA Adaptation at Scale in Semi-Arid Regions (ASSAR) Project, December 2014, Ottawa.
- Djouaka, R. J., Atoyebi, S. M., Tchigossou, G. M., Riveron, J. M., Irving, H., Akoton, R., and Wondji, C. S. (2016). Evidence of a multiple insecticide resistance in the malaria vector *Anopheles funestus* in South West Nigeria. *Malaria Journal*, 15: 1-10.
- Eritja, R., Palmer, J. R., Roiz, D., Sanpera-Calbet, I., and Bartumeus, F. (2017). Direct evidence of adult *Aedes albopictus* dispersal by car. *Scientific Reports*, 7: 1-15.
- Escobar, D., Ascencio, K., Ortiz, A., Palma, A., Sánchez, A., and Fontecha, G. (2020). Blood meal sources of *Anopheles* spp. in malaria endemic areas of Honduras. *Insects*, 11: 450.
- Evans, B. R., Kotsakiozi, P., Costa-da-Silva, A. L., Ioshino, R. S., Garziera, L., Pedrosa, M. C., and Powell, J. R. (2019). Transgenic *Aedes aegypti* mosquitoes transfer genes into a natural population. *Scientific Reports*, 9: 1-6.

- Evans, M. V., Shiau, J. C., Solano, N., Brindley, M. A., Drake, J. M., and Murdock, C. C. (2018). Carry-over effects of urban larval environments on the transmission potential of dengue-2 virus. *Parasites and Vectors*, 11: 426.
- Evans, M. V., Shiau, J. C., Solano, N., Brindley, M. A., Drake, J. M., and Murdock, C. C. (2017). Carry-over effects of larval microclimate on the transmission potential of a mosquito-borne pathogen. *bioRxiv*, 211037.
- Ezeakacha, N. F., and Yee, D. A. (2019). The role of temperature in affecting carry-over effects and larval competition in the globally invasive mosquito *Aedes albopictus*. *Parasites and Vectors*, 12: 1-11.
- Faiman, R., Diallo, M., Dao, A., Djibril, S., Sanogo, Z. L., Sullivan, M., and Lehmann, T. (2020). Quantifying flight aptitude variation in wild *Anopheles gambiae* in order to identify long-distance migrants. *Malaria Journal*, 19: 1-15.
- Govella, N. J., Chaki, P. P., Geissbuhler, Y., Kannady, K., Okumu, F., Charlwood, J. D., and Killeen, G. F. (2009). A new tent trap for sampling exophagic and endophagic members of the *Anopheles gambiae* complex. *Malaria Journal*, 8: 157.
- Gunda, R., Chimbari, M. J., Shamu, S., Sartorius, B., and Mukaratirwa, S. (2017). Malaria incidence trends and their association with climatic variables in rural Gwanda, Zimbabwe, 2005–2015. *Malaria Journal*, 16: 1-13.
- Gunderson, A. R., Dillon, M. E., and Stillman, J. H. (2017). Estimating the benefits of plasticity in ectotherm heat tolerance under natural thermal variability. *Functional Ecology*, 31: 1529-1539.
- Harvey-Samuel, T., Ant, T., and Alphey, L. (2017). Towards the genetic control of invasive species. *Biological Invasions*, 19: 1683-1703.
- Huey, R. B., and Kearney, M. R. (2020). Dynamics of death by heat. *Science*, 369: 1163-1163.
- Ibañez-Justicia, A., Gloria-Soria, A., Den Hartog, W., Dik, M., Jacobs, F., and Stroo, A. (2017). The first detected airline introductions of yellow fever mosquitoes (*Aedes aegypti*) to Europe, at Schiphol International airport, the Netherlands. *Parasites and Vectors*, 10: 603.
- Iwamura, T., Guzman-Holst, A., and Murray, K. A. (2020). Accelerating invasion potential of disease vector *Aedes aegypti* under climate change. *Nature Communications*, 11: 1-10.

- Kamiya, T., Greischar, M. A., Wadhawan, K., Gilbert, B., Paaijmans, K., and Mideo, N. (2020). Temperature-dependent variation in the extrinsic incubation period elevates the risk of vector-borne disease emergence. *Epidemics*, 30: 100382.
- Kandul, N. P., Liu, J., Sanchez, C. H. M., Wu, S. L., Marshall, J. M., and Akbari, O. S. (2019). Transforming insect population control with precision guided sterile males with demonstration in flies. *Nature Communications*, 10: 84.
- Kgoroebutswe, T. K., Ramatlho, P., Reeder, S., Makate, N., and Paganotti, G. M. (2020a). Distribution of *Anopheles* mosquito species, their vectorial role and profiling of knock-down resistance mutations in Botswana. *Parasitology Research*, 119: 1201-1208.
- Kgoroebutswe, T. K., Makate, N., Fillinger, U., Mpho, M., Segoea, G., Sangoro, P. O., and Mosweunyane, T. (2020b). Vector control for malaria elimination in Botswana: progress, gaps and opportunities. *Malaria Journal*, 19: 1-12.
- Kingsolver, J. G., Diamond, S. E., and Buckley, L. B. (2013). Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Functional Ecology*, 27: 1415-1423.
- Kumar, S., Joshi, P. C., Nath, P., and Singh, V. K. (2018). Impacts of Insecticides on Pollinators of Different Food Plants. *Entomology, Ornithology and Herpetology*, 7: 7.
- Leftwich, P.T., Bolton, M., and Chapman, T. (2016). Evolutionary biology and genetic techniques for insect control. *Evolutionary Applications*, 9: 212-230.
- Li, Y., Miao, R., and Khanna, M. (2020). Neonicotinoids and decline in bird biodiversity in the United States. *Nature Sustainability*, 1-9: <https://doi.org/10.1038/s41893-020-0582-x>.
- Liu, Z., Zhou, T., Lai, Z., Zhang, Z., Jia, Z., Zhou, G., and Lin, L. (2017). Competence of *Aedes aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus* mosquitoes as Zika virus vectors, China. *Emerging Infectious Diseases*, 23: 1085.
- Lyons, C. L., Coetzee, M., Terblanche, J. S., and Chown, S. L. (2012). Thermal limits of wild and laboratory strains of two African malaria vector species, *Anopheles arabiensis* and *Anopheles funestus*. *Malaria Journal*, 11: 226.
- Makate, N. M. (2016). A Review of Insecticide Resistance Status in Botswana. *Insecticides Resistance*, 2016: 263.
- Mamai, W., Hood-Nowotny, R., Maiga, H., Ali, A. B., Bimbile-Somda, N. S., Soma, D. D., and Gilles, J. R. (2017). Reverse osmosis and ultrafiltration for recovery and reuse of larval

- rearing water in *Anopheles arabiensis* mass production: Effect of water quality on larval development and fitness of emerging adults. *Acta Tropica*, 170: 126-133.
- Mbaiwa, J. (2011). The effects of tourism development on the sustainable utilisation of natural resources in the Okavango Delta, Botswana. *Current Issues in Tourism*, 14: 251-273.
- Mbaiwa, J. E. (2008). *Tourism development, rural livelihoods, and conservation in the Okavango Delta, Botswana*. Texas A & M University, Texas, USA.
- Mbaiwa, J. E. (2004). The socio-economic benefits and challenges of a community-based safari hunting tourism in the Okavango Delta, Botswana. *Journal of Tourism Studies*, 15: 37.
- Menger, D. J., Otieno, B., de Rijk, M., Mukabana, W. R., van Loon, J. J., and Takken, W. (2014). A push-pull system to reduce house entry of malaria mosquitoes. *Malaria Journal*, 13: 119.
- Mitchell, S. N., Kakani, E. G., South, A., Howell, P. I., Waterhouse, R. M., and Catteruccia, F. (2015). Evolution of sexual traits influencing vectorial capacity in anopheline mosquitoes. *Science*, 347: 985-988.
- Mitton, J. B., and Ferrenberg, S. M. (2012). Mountain pine beetle develops an unprecedented summer generation in response to climate warming. *The American Naturalist*, 179: E163-E171.
- Mopelwa, G., and Blignaut, J. N. (2006). The Okavango Delta. The value of tourism: economic. *South African Journal of Economic and Management Sciences*, 9: 113-127.
- Motlaleng, M., Edwards, J., Namboze, J., Butt, W., Moakofhi, K., Obopile, M., and Oumer, N. (2018). Driving towards malaria elimination in Botswana by 2018: progress on case-based surveillance, 2013–2014. *Public Health Action*, 8: S24-S28.
- Mpofu, M., Becker, P., Mudambo, K., and Jager, C. (2016). Field effectiveness of microbial larvicides on mosquito larvae in malaria areas of Botswana and Zimbabwe. *Malaria Journal*, 15: 586.
- Munhenga, G., Brooke, B. D., Chirwa, T. F., Hunt, R. H., Coetzee, M., Govender, D., and Koekemoer, L. L. (2011). Evaluating the potential of the sterile insect technique for malaria control: relative fitness and mating compatibility between laboratory colonised and a wild population of *Anopheles arabiensis* from the Kruger National Park, South Africa. *Parasites and Vectors*, 4: 1-11.

- Nazareth, T., Craveiro, I., Moutinho, A., Seixas, G., Gonçalves, C., Gonçalves, L., and Sousa, C. A. (2020). What happens when we modify mosquitoes for disease prevention? A systematic review. *Emerging Microbes and Infections*, 9: 348-365.
- Ndakidemi, B., Mtei, K., and Ndakidemi, P. A. (2016). Impacts of synthetic and botanical pesticides on beneficial insects. *Agricultural Sciences*, 7: 364.
- Nkemelang, T., New, M., and Zaroug, M. (2018). Temperature and precipitation extremes under current, 1.5 C and 2.0 C global warming above pre-industrial levels over Botswana, and implications for climate change vulnerability. *Environmental Research Letters*, 13: 065016.
- Nwachukwu, W. E., Yusuff, H., Nwangwu, U., Okon, A., Ogunniyi, A., Imuetinyan-Clement, J., and Dogunro, F. (2020). The response to re-emergence of yellow fever in Nigeria, 2017. *International Journal of Infectious Diseases*, 92: 189-196.
- Nyamukondiwa, C., and Terblanche, J. S. (2010). Phenotypic plasticity of thermal tolerance contributes to the invasion potential of Mediterranean fruit flies (*Ceratitidis capitata*). *Ecological Entomology*, 35: 565-575.
- Obopile, M., Segoea, G., Waniwa, K., Ntebela, D. S., Moakofhi, K., Motlaleng, M., and Manzi, M. (2018). Did microbial larviciding contribute to a reduction in malaria cases in eastern Botswana in 2012–2013? *Public Health Action*, 8: S50-S54.
- Paaijmans, K. P., Heinig, R. L., Seliga, R. A., Blanford, J. I., Blanford, S., Murdock, C. C., and Thomas, M. B. (2013). Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology*, 19: 2373-2380.
- Pachka, H., Annelise, T., Alan, K., Power, T., Patrick, K., Véronique, C., Janusz, P. and Ferran, J. (2016). Rift Valley fever vector diversity and impact of meteorological and environmental factors on *Culex pipiens* dynamics in the Okavango Delta, Botswana. *Parasites and Vectors*, 9: 434.
- Poh, K. C., Chaves, L. F., Reyna-Nava, M., Roberts, C. M., Fredregill, C., Bueno Jr, R., and Hamer, G. L. (2019). The influence of weather and weather variability on mosquito abundance and infection with West Nile virus in Harris County, Texas, USA. *Science of the Total Environment*, 675: 260-272.
- Powell, J. R., and Tabachnick, W. J. (2013). History of domestication and spread of *Aedes aegypti*-a review. *Memórias do Instituto Oswaldo Cruz*, 108: 11-17.

- Qasim, M., Xiao, H., He, K., Omar, M. A., Liu, F., Ahmed, S., and Li, F. (2020). Genetic engineering and bacterial pathogenesis against the vectorial capacity of mosquitoes. *Microbial Pathogenesis*, 147: 104391.
- Rotz, P. D. (2016). Sweetness and Fever? Sugar Production, *Aedes aegypti*, and Dengue Fever in Natal, South Africa, 1926–1927. *South African Historical Journal*, 68: 286-303.
- Saha, N., Aditya, G., Banerjee, S., and Saha, G. K. (2012). Predation potential of odonates on mosquito larvae: Implications for biological control. *Biological Control*, 63: 1-8.
- Saijo, M. (2020). Emerging and Re-emerging Virus Infections in Japan: Dengue Fever, Zika Virus Disease, and SFTS. *Yakugaku Zasshi: Journal of the Pharmaceutical Society of Japan*, 140: 895-899.
- Sanchez-Bayo, F. P. (2012). Insecticides mode of action in relation to their toxicity to non-target organisms. *Journal of Environmental and Analytical Toxicology*, S4: 1-11.
- Sannigrahi, S., Zhang, Q., Joshi, P. K., Sutton, P. C., Keesstra, S., Roy, P. S., and Paul, S. K. (2020). Examining effects of climate change and land use dynamic on biophysical and economic values of ecosystem services of a natural reserve region. *Journal of Cleaner Production*, 257: 120424.
- Schrama, M., Hunting, E. R., Beechler, B. R., Guarido, M. M., Govender, D., Nijland, W., and Gorsich, E. E. (2020). Human practices promote presence and abundance of disease-transmitting mosquito species. *Scientific Reports*, 10: 1-6.
- Schulte, P. M., Healy, T. M., and Fangué, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integrative and Comparative Biology*, 51: 691-702.
- Sgro, C. M., Terblanche, J. S., and Hoffmann, A. A. (2016). What can plasticity contribute to insect responses to climate change? *Annual Review of Entomology*, 61: 433-451.
- Simon, C., Moakofhi, K., Mosweunyane, T., Jibril, H. B., Nkomo, B., Motlaleng, M., and Haque, U. (2013). Malaria control in Botswana, 2008–2012: the path towards elimination. *Malaria Journal*, 12: 1-9.
- Sinclair, B. J., Alvarado, L. E. C., and Ferguson, L. V. (2015). An invitation to measure insect cold tolerance: methods, approaches, and workflow. *Journal of Thermal Biology*, 53: 180-197.

- Sinclair, B. J., Vernon, P., Klok, C. J., and Chown, S. L. (2003). Insects at low temperatures: an ecological perspective. *Trends in Ecology and Evolution*, 18: 257-262.
- Sim, S. B., Kauwe, A., Ruano, R. E., Rendon, P., and Geib, S. M. (2019). The ABCs of CRISPR in Tephritidae: Developing methods for inducing heritable mutations in the genera *Anastrepha*, *Bactrocera*, and *Ceratitis*. *Insect Molecular Biology*, 28: 277-289.
- Sougoufara, S., Diédhiou, S. M., Doucouré, S., Diagne, N., Sembène, P. M., Harry, M., and Ndiath, M. O. (2014). Biting by *Anopheles funestus* in broad daylight after use of long-lasting insecticidal nets: a new challenge to malaria elimination. *Malaria Journal*, 13: 125.
- Stone, C., and Gross, K. (2018). Evolution of host preference in anthropophilic mosquitoes. *Malaria Journal*, 17: 257.
- Sukiato, F., Wasserman, R.J., Wilson, R., Chern, S.F., Cuthbert, R.C., and Raman, S. (2019). The effects of temperature and shading effects on mortality and development rates of *Aedes aegypti* (Diptera: Culicidae). *Journal of Vector Ecology*, 44: 264-270.
- Teem, J. L., Alphey, L., Descamps, S., Edgington, M. P., Edwards, O., Gemmell, N., and Saah, J. R. (2020). Genetic Biocontrol for Invasive Species. *Frontiers in Bioengineering and Biotechnology*, 8: 452.
- Thompson, L. A., Ikenaka, Y., Yohannes, Y. B., Van Vuren, J. J., Wepener, V., Smit, N. J., and Ishizuka, M. (2017). Concentrations and human health risk assessment of DDT and its metabolites in free-range and commercial chicken products from KwaZulu-Natal, South Africa. *Food Additives and Contaminants: Part A*, 34: 1959-1969.
- Thomsen, E. K., Koimbu, G., Pulford, J., Jamea-Maiasa, S., Ura, Y., Keven, J. B., and Reimer, L. J. (2016). Mosquito behavior change after distribution of bednets results in decreased protection against malaria exposure. *The Journal of Infectious Diseases*, 215: 790-797.
- Tjaden, N. B., Caminade, C., Beierkuhnlein, C., and Thomas, S. M. (2017). Mosquito-borne diseases: advances in modelling climate-change impacts. *Trends in Parasitology*, 34: 227-245.
- Todd, J. H., Pearce, B. M., and Barratt, B. I. (2020). Using qualitative food webs to predict species at risk of indirect effects from a proposed biological control agent. *Biological Control*, 107: 1-14.

- van Lenteren, J. C., Bolckmans, K., Köhl, J., Ravensberg, W. J., and Urbaneja, A. (2018). Biological control using invertebrates and microorganisms: plenty of new opportunities. *Biological Control*, 63: 39-59.
- Vannier, G. (1994). The thermobiological limits of some freezing intolerant insects: the supercooling and thermostupor points. *Acta Oecologica*, 15: 31-42.
- Verhulst, N. O., Brendle, A., Blanckenhorn, W. U., and Mathis, A. (2020). Thermal preferences of subtropical *Aedes aegypti* and temperate *Ae. japonicus* mosquitoes. *Journal of Thermal Biology*, 102637.
- Waite, J. L., Swain, S., Lynch, P. A., Sharma, S. K., Haque, M. A., Montgomery, J., and Thomas, M. B. (2017). Increasing the potential for malaria elimination by targeting zoophilic vectors. *Scientific Reports*. 7: 40551.
- Wallgren, M., Skarpe, C., Bergström, R., Danell, K., Bergström, A., Jakobsson, T., and Strand, T. (2009). Influence of land use on the abundance of wildlife and livestock in the Kalahari, Botswana. *Journal of Arid Environments*, 73: 314-321.
- Walton, W. E. (2007). Larvivorous fish including *Gambusia*. *Journal of the American Mosquito Control Association*, 23:184-220.
- Wang, S., Ghosh, A. K., Bongio, N., Stebbings, K. A., Lampe, D. J., and Jacobs-Lorena, M. (2012). Fighting malaria with engineered symbiotic bacteria from vector mosquitoes. *Proceedings of the National Academy of Sciences*, 109: 12734-12739.
- Warchot, A., Whelan, P., Brown, J., Vincent, T., Carter, J., and Kurucz, N. (2020). The removal of subterranean stormwater drain sumps as mosquito breeding sites in Darwin, Australia. *Tropical Medicine and Infectious Disease*, 5: 9.
- Watanabe, M., Kikawada, T., Minagawa, N., Yukuhiro, F., and Okuda, T. (2002). Mechanism allowing an insect to survive complete dehydration and extreme temperatures. *Journal of Experimental Biology*, 205: 2799-2802.
- West-Eberhard, M. J. (2003). *Developmental Plasticity and Evolution*. Oxford University Press, New York, USA.
- Whelan, P. I., Jacups, S. P., Melville, L., Broom, A., Currie, B. J., Krause, V. L., and Porigneaux, P. (2003). Rainfall and vector mosquito numbers as risk indicators for mosquito-borne disease in Central Australia. *Communicable Diseases Intelligence*, 27: 110-116.

- Whitman, D. W. (2009). Acclimation. Phenotypic Plasticity of Insects. Mechanisms and Consequences (ed. By D.W. Whitman and T.N. Ananthakrishnan), Science Publishers Enfield, Boca Raton, USA.
- Whitman, D. W. and Ananthakrishnan, T. N. (2009). Phenotypic Plasticity of Insects. Mechanisms and Consequences. Science Publishers, Enfield, NH, USA.
- Wikan, G. (2004). The level of living in rural Botswana re-studied. *Norsk Geografisk Tidsskrift-Norwegian Journal of Geography*, 58: 1-10.
- Wilke, A. B. B., Medeiros-Sousa, A. R., Ceretti-Junior, W., and Marrelli, M. T. (2017). Mosquito population dynamics associated with climate variations. *Acta Tropica*, 166: 343-350.
- Wilke, A. B. B., and Marrelli, M. T. (2015). Paratransgenesis: a promising new strategy for mosquito vector control. *Parasites and Vectors*, 8: 342.
- Wise de Valdez, M. R., Nimmo, D., Betz, J., Gong, H. F., James, A. A., Alphey, L., and Black, W. C. (2011). Genetic elimination of dengue vector mosquitoes. *Proceedings of the National Academy of Sciences of the United States of America*, 108: 4772–4775.
- World Health Organization. (2014). A global brief on vector-borne diseases, Geneva, Switzerland.
- Zhu, W., Schmehl, D. R., Mullin, C. A., and Frazier, J. L. (2014). Four common pesticides, their mixtures and a formulation solvent in the hive environment have high oral toxicity to honey bee larvae. *PloS ONE*, 9: e77547.

CHAPTER 2

Knowledge, attitude and practices of the communities from Serowe, Palapye and Bobirwa sub-districts on mosquito bio-ecology and burden

Published as: **Buxton, M.**, Machekano, H., Gotcha, N., Nyamukondiwa, C., and Wasserman, R.

J. (2020). Are vulnerable communities thoroughly informed on mosquito bio-ecology and burden? *International Journal of Environmental Research and Public Health*, 17: 8196.

2.1. Introduction

Vector-borne infections contribute significantly to human morbidity and mortality globally (World Health Organisation [WHO] 2017), accounting annually for about one million deaths and ~17% of the overall infectious disease burden worldwide (WHO 2014). Whilst several arthropods are implicated in a wide range of public health epidemiological systems (de la Fuente *et al.*, 2008; Leitner *et al.*, 2015; Kweka *et al.*, 2017), mosquito vectors have accounted for the majority of disease burdens (Tolle 2009; Mutheneni *et al.*, 2017). Mosquitoes harbor pathogens (e.g., *Plasmodium* parasites, arboviruses, nematodes) that are causative agents for debilitating human, wildlife and livestock diseases (Brugman *et al.*, 2018; Paixão *et al.*, 2018; Antonini *et al.*, 2019). Although the distribution of vector mosquitoes and associated diseases is generally explored in Africa (e.g., Coetzee *et al.*, 2000; Braack *et al.*, 2018), some areas remain underexplored owing to the lag between unreported species and invasion potentials under current global change scenarios (Akiner *et al.*, 2016). Many of these vectors and pathogens exploit biotic and abiotic systems (e.g., hosts, climate, transportation systems and environmental modifications) across rural-urban landscapes to proliferate (Eritja *et al.*, 2017; Levine *et al.*, 2017; Ludwig *et al.*, 2019).

The extent to which these factors contribute to vector-parasite establishment in novel environments have been explored effectively in many parts of the world (Asigau and Parker 2018), however little attention has been given to arid and semi-arid environments (e.g., Botswana). This knowledge paucity hinders information dissemination in communities exposed to risks of emerging and re-emerging diseases although control efforts and educational programmes may be restricted to hotspot zones with little or no extension beyond these areas

(Chirebvu *et al.*, 2013). Amongst the mosquito-borne diseases, malaria is of most epidemiological concern globally (Howes *et al.*, 2016). Malaria cases are currently estimated at 228 million worldwide with most occurring in the African region (93%), followed by the South-East Asia region (3.4%) (WHO 2019). Although malaria incidences are known to be on the decline (Dhiman 2019), sub-Saharan Africa still exhibits the highest deaths rates with pregnant women and children under five as vulnerable groups (Simon-Oke 2019).

In the semi-arid Botswana, the only apparent mosquito-borne disease is malaria, endemic to the northern part of the country (Chirebvu *et al.*, 2014). Previous knowledge, attitude and practices (KAPs) studies have shown that communities from endemic areas (Okavango) were well informed on the dynamics of malaria transmission (Chirebvu *et al.*, 2013). Currently, the country is at an elimination stage with intensified surveillance and vector control intervention strategies (Motlaleng *et al.*, 2018), however the level of information dissemination, aimed at empowering communities in both endemic and non-endemic areas, is unknown. Non-endemic regions adjacent to endemic regions are vulnerable to spread of diseases associated with climate change and vector invasion mechanisms (Le Menach *et al.*, 2011; Shi *et al.*, 2017). Whilst research on dynamics of mosquito-borne infections is evident in the country's malaria endemic areas (Chirebvu and Chimbari 2015; Pachka *et al.*, 2016; Tawe *et al.*, 2017), Serowe, Palapye and other non-endemic region across the country remain neglected. Nevertheless, assessment of mosquito KAPs and regular monitoring of vector-pathogen burden capable of extending ranges are also critical in these regions. Given the proximity of these non-endemic sub-districts to the endemic zones, against the backdrop of anthropogenic climate change (Meehl and Tebaldi 2004; Thuiller 2007), shifting vector-pathogens (Kilpatrick and Randolph 2012) and increased

emerging re-emerging disease incidences (Short *et al.*, 2017), more locally contextual work on mosquitoes and associated infection baseline studies are warranted.

The country's national vector control programme deployed chemical-mediated approaches for vector management since the 1950s (Makate 2016). As this intervention has been going on in endemic zones for decades, no published work reports vector status on mosquitoes although some studies have reported insecticide resistance to commonly used insecticides worldwide (WHO 2018). Apart from a few studies based only on the northern part of the country (Tawe *et al.*, 2017; Cornel *et al.*, 2018), the knowledge on diversity and distribution of vector mosquitoes across the country is not well documented. In light of the national mosquito diversity spectrum, non-endemic areas remain a priority considering the recent changing environments (Thuiller 2007) and presence of highly invasive species (Akiner *et al.*, 2016).

Mosquitoes selectively breed in diverse aquatic systems that may even consist of compromised water quality (e.g., polluted, highly turbid, bogs, marshes or brackish waters) across different habitats (Kengluecha *et al.*, 2005). Given this behavioral adaptation, colonisation in Botswana's rural-urban arid and semi-arid landscapes may be possible. This may be encouraged by natural, modified and artificial wetland structures that hold water, serving as 'breeding hotspots'. Most of these have been implicated locally as aquatic ecosystems that potentially promote larval mosquito infestation and adult proliferation. The country's national vector control strategic plan mainly targets the mosquito adults through indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLITNs) (Simon *et al.*, 2013) and largely neglect

the control of aquatic life stages (eggs, larvae, pupae). However, previous work has suggested that compromised aquatic habitats, support fewer aquatic predators and favour larval mosquito proliferation (Leisnham *et al.*, 2005; Wilke *et al.*, 2020). As such, more work is essential in managing the mosquito aquatic stages from wetland systems through desirable and sustainable eco-friendly approaches (e.g., natural enemies) (Quiroz-Martínez and Rodríguez-Castro 2007; Shaalan and Canyon 2009). This may be a pivotal alternative, used in an integrated approach for mosquito population reduction in the country. In addition, the KAPs on mosquito ontogeny and life-history traits, mosquito-borne disease prevention and control dynamics is not yet assessed in many communities across the endemic and non-endemic zones (although see Chirebvu *et al.*, 2013). In this regard, KAPs evaluate public knowledge level and have the potential to strengthen individuals, households and communities against escalating disease burdens at various scales. Furthermore, KAPs are key in empowering end users on ways to deal with issues that directly concern public health through community engagement for the management of disease prevention and spread. Overall, KAPs play a pivotal role in optimising community health programme planning and serve as points in health system reforms (Launiala 2009).

This work was aimed at assessing mosquito KAPs on communities of endemic and non-endemic semi-arid Botswana, exploring areas that may help bridging knowledge gaps in vector and associated disease dynamics. The results of this study benchmark community level KAPs serving as baseline for building future research and interventions aimed at reducing disease burden through empowering communities. Here, it was hypothesised that (1) communities in malaria endemic areas are better informed on mosquito related issues than those in the non-

endemic zones and that (2) communities across study sites protect themselves against mosquitoes differentially, due to disease burden bias in malaria endemic incriminated sub-districts.

2.2. Materials and Methods

2.2.1. Knowledge Attitude and Practices

The use of KAPs approach was employed to assess what human communities know about mosquito bio-ecology and mosquito-borne infections, attitude towards the vectors and associated pathogens and their knowledge on control measures. For this study, “Knowledge” refers to what members of the communities know about mosquito biology, taxonomy, ecology and control. “Attitude” refers to individual’s feelings and preconceived ideas (perceptions) about mosquitoes and mosquito-borne infections whilst “practices” refer to what respondents do to curb mosquito impacts and manage associated disease(s). The interactions between the dependent variables (KAPs) were developed and measured against the independent variables (sub-district, village, sex, age, education and profession) targeting communities in non-endemic (Serowe and Palapye) and endemic (Bobirwa) zones of the Central district, Botswana (Figure 2.1). Quantification of KAPs was achieved using ordinal and rating scales, following modifications from Machekano *et al.* (2020).

2.2.2. Study area

Botswana has 10 major districts, six of which are characterised as malaria endemic (Chihanga *et al.*, 2016; Figure 2.1a,b). Each district is comprised of various sub-districts, with some districts housing both malaria endemic and non-endemic sub-districts. Three endemic and three non-endemic sub-districts make up the Central district (Figure 2.1c), offering an

opportunity to assess within-district KAP's in relation to malaria endemicity status. The study was conducted in three areas within the semi-arid Central district of Botswana namely: Serowe, Palapye and Bobirwa (Batisani and Yarnal 2010; Figure 2.1c). The area is dominated by savanna vegetation with temporary clay lined and rockpool wetlands (Hulsmans *et al.*, 2008) as potential breeding sites for mosquitoes. Mosquito diversity in the Central district is poorly explored, especially non-anopheline species, due to skewed focus towards malaria vectors. As such, only vectors belonging to *Anopheles gambiae* and *funestus* complexes have been the focus of mosquito studies in the region (Tawe *et al.*, 2017). Serowe and Palapye sub-districts are deemed non-endemic malaria areas, whilst Bobirwa falls within the malaria endemic zone of the country (Simon *et al.*, 2013). Seven villages were identified for the KAP survey in each sub-district according to their geographic distribution.

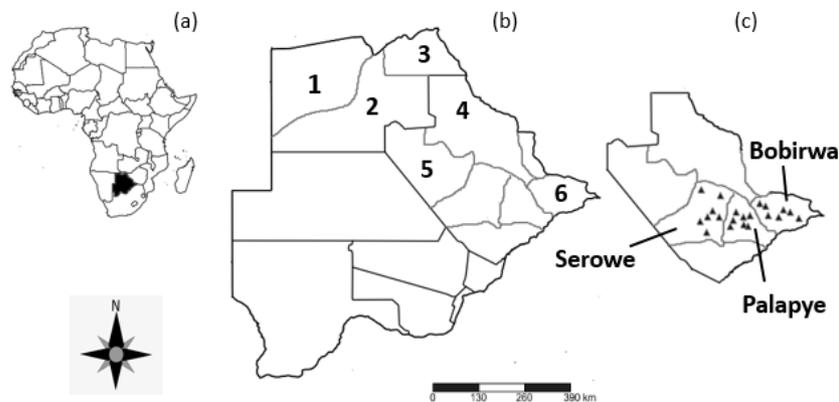


Figure 2. 1. Map showing (a) the location of Botswana within Africa, (b) the malaria endemic sub-districts and the study site in Botswana, and (c) surveyed villages of the Central district; Serowe, Palapye and Bobirwa sub-districts. 1= Okavango, 2= Ngamiland, 3= Chobe, 4= Tutume, 5= Boteti, 6= Bobirwa.

2.2.3. Sampling technique

A structured questionnaire was used to conduct face-to-face interviews with residents of Serowe ($n = 206$), Palapye ($n = 202$) and Bobirwa ($n = 203$) between July and August 2019 using fourth household approach to standardise data collection across study sites ($n = 611$). The questionnaire had four sections namely the socio-demographics, knowledge, attitude and practices on mosquitoes and their infectious diseases (Appendix S1). Although live specimens/pictures were not used as interview guides, the questionnaire had direct questions used to assess baseline information from the respondents. Sections had nominal scales, closed and open-ended answer questions on mosquito KAPs. The open questions were post-coded to quantify the responses for analysis. A pre-run trial of questionnaires ($n = 20$) was conducted in Palapye village to revise and refine the questionnaire. Following pre-run and questionnaire ‘debugging’, interviews were conducted by trained enumerators using both English and Setswana (local language) (Bowling 2005; Machezano *et al.*, 2020).

Prior to questionnaire administration, permission was sought from the Ministry of Environment, Natural Resources Conservation and Tourism (Botswana). In addition, the Department of Biological Sciences and Biotechnology, Botswana International University of Science and Technology, approved the survey and provided a covering support letter to seek verbal permission from village leaders (di Kgosi). A written consent was established with participants explaining the survey background, aim, method of data collection and subsequent usage, issues of anonymity and confidentiality.

2.2.4. *Data analysis*

Census and Survey Processing System software (CSPro 7.2) (United States Census Bureau) was used for data entry while, IBM Statistical Package for Social Sciences (SPSS) version 23 was used for statistical analyses. Data were reported in percentages, frequencies and statistically significant variables were separated at 95% confidence interval. Interactions between dependent and independent variables were enunciated using the Pearson Chi-square test of association, and Pearson's correlation coefficient was used to test for correlation.

2.3. Results

2.3.1. *Socio-demographic characteristics*

The majority of the respondents were literate (88.7%), females (71.5%), able-bodied (91.7%), single (70.4%) and middle aged (30- 39 years; [23.6%]) (Table 2.1). Education level was mostly junior certificate (2 years post-secondary education) (29.5%) or primary school (29%); very few respondents had attended senior secondary (five years post-secondary education) (14.5%), vocational training (8.7%) or tertiary education (4.7%) (Table 2.1). Information on mosquitoes and mosquito-borne infections was mostly accessed through audio-visual media (e.g., radio/ television) (50.9%), local health facilities (33.9%), family/ friends (4.7%), own experience (4.1%), print media (e.g., newspapers, magazines) (1.8%), electronic sources (0.3%) and other unspecified means (4.3%).

Fewer households had proper drainage systems (28.9%) compared to those without (71.1%). Conversely, the majority of households had pit latrines (84.5%) exhibiting signs of mosquitoes (e.g., presence of adults, flight or sound) (71.3%) as opposed to those without pit

latrines (15.5%) (Table 2.1). Moreover, households with pit latrines across sub-districts were not significant ($\chi^2 = 450$, $df = 2$, $p = 0.799$). Potential vector breeding habitats found in respondent's property were mainly indoor containers (28.6%), old tyres (22.6%), flowerpots (16.9%), bulk water tanks (Jojo) (14.9%), gutters (3.3%) and other artificial containers (2%) (Figure 2.2).

Table 2. 1. Summary results of the socio-demographic characteristics for non-endemic areas (Serowe and Palapye) and endemic (Bobirwa) sub-districts ($n = 611$).

Variables	Category	Number of Respondents	Proportion (%)
Gender	Male	174	28.5
	Female	437	71.5
Marital Status	Single (never married)	430	70.4
	Married	122	20
	Divorced	9	1.5
	Widowed	50	8.2
Age (years)	18- 29	135	22.1
	30-39	144	23.6
	40- 49	110	18.0
	50- 59	89	14.6
	≥60	133	21.8
Disability	Yes	49	8.0
	No	560	91.7
	Prefer not to say	2	0.3
Literacy	Literate	542	88.7
	Illiterate	65	10.6
	Prefer not to say	4	0.7
Education	None	83	13.6
	Primary	177	29.0
	Junior Certificate	180	29.5
	Form 4- 5 (Senior)	90	14.7
	Vocational	50	8.2
	Tertiary	29	4.7
	Prefer not to say	1	0.2
	Other	1	0.2
	Information access	Radio/ TV	311
	Health professionals	207	33.9
	Printed media	11	1.8
	Electronic sources	2	0.3
	Family/ Friends	29	4.7
	Own experience	25	4.1
	Other	26	4.3
Family size	1-2	84	13.7
	3-5	197	32.2
	6-10	229	37.5
	>10	101	16.5
Pit latrine (toilet)	Yes	516	84.5
	No	95	15.5
Drainage system	Yes	176	28.9
	No	433	71.1
Stagnant water	Yes	82	13.4
	No	524	85.8
	Not sure	4	0.7
	Don't know	1	0.2

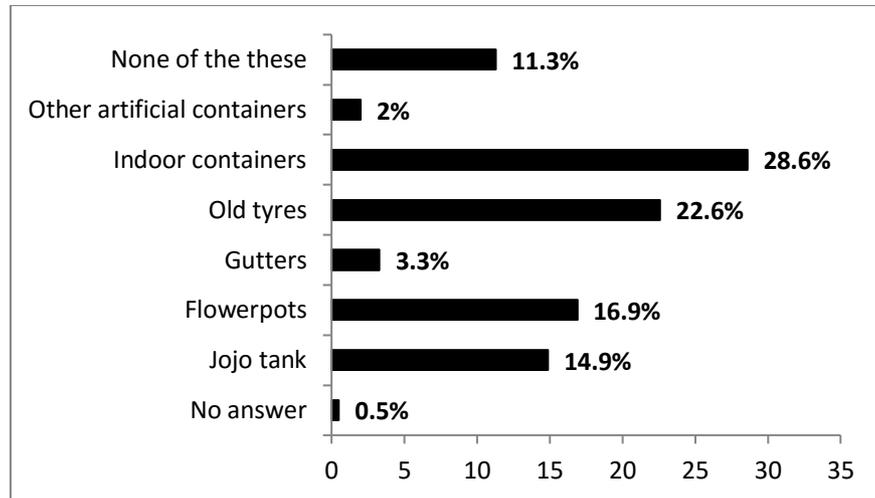


Figure 2. 2. Details of the structure and diversity of water holding containers (outlined by respondents), that may serve as potential mosquito breeding sites found on the premises of respondents across sub-districts.

2.3.2. Knowledge

The majority of the respondents knew adult mosquitoes (98.9%) as opposed to those who did not (1.1%). This result was the same across all three sub-districts ($\chi^2 = 3.081$, $df = 2$, $p = 0.214$). A total of 99%, 96.5%, 99% had not seen mosquito eggs, larvae and pupae respectively, and many (70.5%) did not know that mosquitoes go through different developmental stages regardless of the sub-district ($\chi^2 = 1.288$, $df = 6$, $p = 0.972$, Figure 2.3a). During austral winter (April, May, June 2019), precedent to the current study, the majority of respondents indicated to have seen mosquitoes within their properties (54.3%). Similarly, the austral summer prior to our survey (October 2018 to March 2019), the majority (87.4%) indicated to have seen and been bitten by mosquitoes too often to be counted (40.0%). Conversely, in austral winter (April to July 2019), the majority of respondents (58.1%) reported no bites while relatively few received very few bites (42.0%) and this differed significantly across the sub-districts ($\chi^2 = 63.812$, $df = 6$, $p < 0.001$) with many having received no bites in non-endemic areas. Spatially, the bites were

either received both indoors and outdoors (52.2%); indoors (38.8%), outdoors (8.5%) or respondents were not sure (0.5%). Temporally, bites were reported more intensive in the evenings (dusk) (56.3%) followed by night times (36.7%) when in bed. Respondents reported being bitten mostly on the face (39.3%) followed by the arms (34.2%). Furthermore, based on the plurality of the respondents, 46.3% believed that mosquito bites could transmit human immunodeficiency virus (HIV) to human beings (Figure 2.3b) and was not significantly different across sub-districts ($\chi^2 = 9.511$, $df = 8$, $p = 0.301$). The majority of respondents did not know mosquito-borne diseases such as yellow fever (83.3%), dengue (100%), avian malaria (97.5%), and elephantiasis (88.1%), as opposed to those who did not know malaria (2.1%). A total of 72.7% knew the signs and symptoms of malaria but this differed significantly across sub-district ($\chi^2 = 42.744$, $df = 12$, $p < 0.001$) with endemic area more knowledgeable than non-endemic. Relatively few respondents had personally suffered malaria (9.3%) but this also differed significantly across sub-districts ($\chi^2 = 28.724$, $df = 6$, $p < 0.001$) with non-endemic having less individuals. Although the majority of respondents (67.4%) did not know anyone within their area who had suffered from malaria, a significant proportion (30.8%) did.

Within localities, the majority of respondents (73.6%) confirmed that there were no new types of mosquitoes known to them. The association between the knowledge of mosquitoes and the observation of any new types of mosquitoes was significant ($\chi^2 = 18.827$, $df = 9$, $p = 0.027$). Although the majority did not know if certain parts of their village had more mosquitoes than others (40.9%), a considerable number of respondents (33.6%) reported distribution disparity with certain areas housing more mosquitoes than others. The association between knowledge of mosquitoes in certain parts of the village and the sub-district was, however, significant ($\chi^2 =$

14.108, $df = 6$, $p = 0.028$). Thus, localities with institutions such as school, clinic, camp sites and central business district (CBD), were believed to attract mosquitoes mainly through availability of stagnant waters (41.0%), dirty environments (8.8%), drainage systems (8.3%), dense vegetation (6.8%) amongst others. Most respondents did not know natural methods of controlling mosquito population (e.g., use of predators) in adults (82.2%) and juveniles (90.5%).

The majority of the respondents (49.3%) perceived mosquito abundance to be increasing over the last 10 years (Figure 2.3c), particularly during summer when temperatures were very high (90.5%). Some responders reported that mosquitoes were present regardless of temperature (6.5%), whilst others were either not sure (1.1%) or did not know (0.5%) about mosquito population trends within their localities. Similarly, the majority identified temperature as the main contributing factor to mosquito proliferation (35.9%).

The majority confirmed cattle to be roaming around their areas (91.5%), particularly free-range cattle reared in the unfenced communal areas (93.8%). As such, most respondents (47.5%) associated the interaction between cattle and the environment as the key contributor to high numbers of mosquitoes. Cattle waste products (e.g., dung and/urine) in water sources were believed to attract mosquitoes by the majority (72.3%). Similarly, 74.3% also believed that cattle-induced eutrophication through dung could modulate the increase of mosquito breeding and abundance (Figure 2.3d). However, this perception was not significantly associated with specific sub-districts ($\chi^2 = 10.428$, $df = 8$, $p = 0.236$).

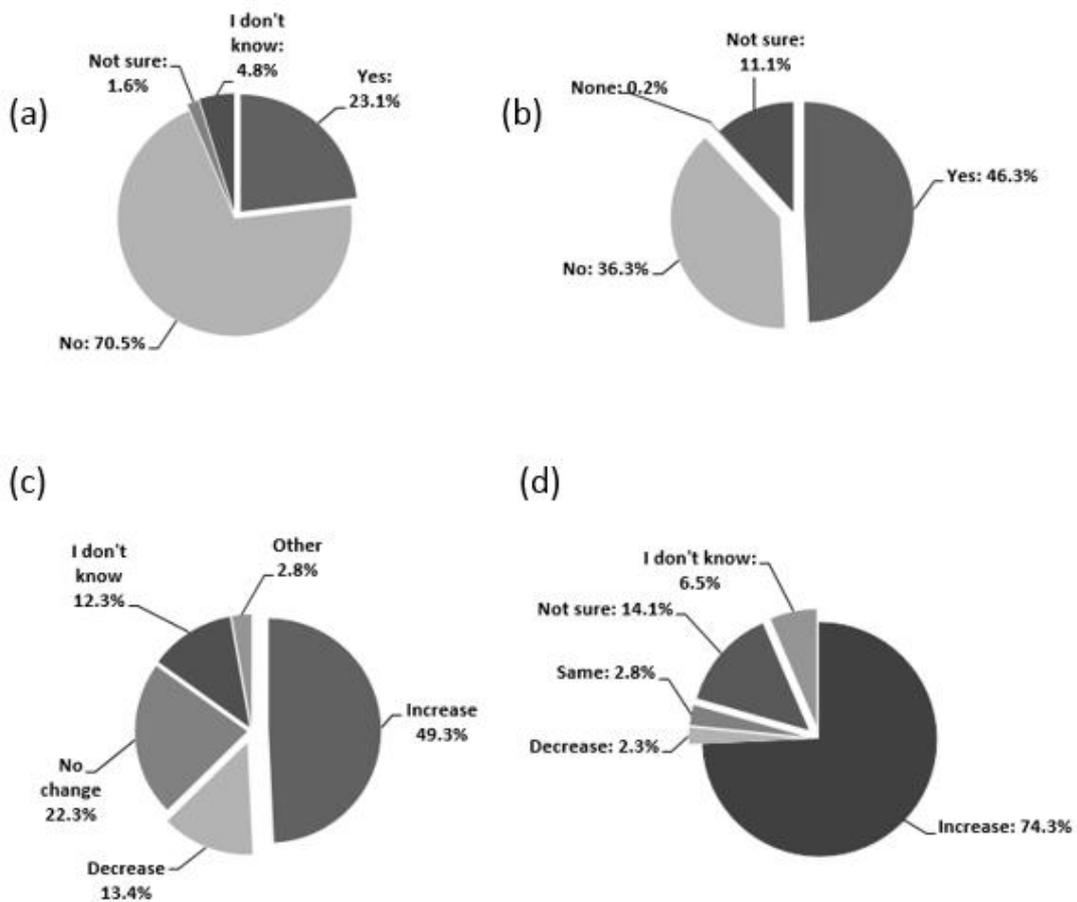


Figure 2. 3. Pie charts summarising community’s knowledge on (a) mosquito developmental stages, (b) HIV transmission by mosquitoes, (c) general mosquito trends over the last 10 years and (d) perceived effect of cattle-dung contaminated water on mosquito abundance.

2.3.3. Attitude

A significant proportion of respondents (49.6%) perceived mosquitoes as health risks to the community; 44.4% considered them to be just a biting nuisance while some (5.2%) were not concerned with mosquitoes at all (Figure 2.4).

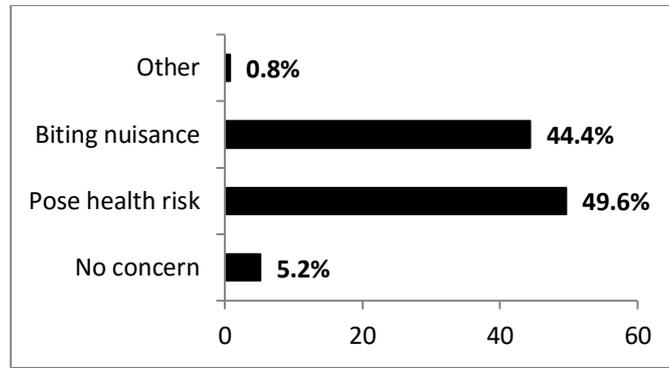


Figure 2. 4. A summary of community concerns on the public health significance of mosquitoes across localities.

The association between public health significance of mosquitoes and sub-district was, however, significant ($\chi^2 = 20.323$, $df = 6$, $p = 0.002$) with non-endemic area of Palapye mostly (51.8%) concerned about health risks. Whilst most of the respondents affirmed that one mosquito bite could pose a health risk (52.9%), 35.8% did not believe this notion. A small portion of respondents (10.5%) were not sure while even fewer respondents (0.8%) said mosquito bites never pose a health risk with overall no significant differences across sub-districts ($\chi^2 = 4.973$, $df = 6$, $p = 0.547$). However, this belief was not linked with respondent's level of education ($\chi^2 = 20.790$, $df = 21$, $p = 0.472$). In rating concerns about mosquito-borne infections, 23.9% were strongly concerned, 28.5% concerned, 28.2% less concerned, while 19.5% were not concerned. The level of individual concerns over mosquito-borne infections varied significantly across sub-districts ($\chi^2 = 52.632$, $df = 6$, $p < 0.001$) with greatest concerns emanating from the non-endemic area of Serowe. Most respondents from the non-endemic area (Palapye) strongly agreed with the notion that cross-border trading traversing sub-districts had the risk of importation of mosquito-borne infections (48.3%) with highly significant association at sub-district level ($\chi^2 = 53.625$, $df = 8$, $p < 0.001$).

2.3.4. Practices

There was some variation on how respondents regarded mosquito bite prevention strategies. Most respondents reported wearing clothes that cover much of the body (38.0%); 22% did nothing; 20% stayed indoors when mosquitoes were active (e.g., from dusk); 10% used repellent products while 9.4% used unspecified means. To discourage mosquito breeding and resting habitats within household properties, the majority of respondents cleared their premises of any grasses/resting plants (73.3%) with the endemic area having the highest response (90.1%) on this practice. Stagnant water in premises was marginally significant across sub-districts ($\chi^2 = 12.495$, $df = 6$, $p = 0.052$) and was reported in fewer yards (13.4%) as opposed to those without (85.8%). Moreover, activity of mosquitoes on stagnant water was not significant across sub-districts ($\chi^2 = 4.314$, $df = 6$, $p = 0.634$). For indoor intervention, the majority used insecticidal sprays (50.2%) (Figure 2.5). The endemic area used insecticidal sprays less compared to the non-endemic. Most respondents regarded the use of bed nets as the most effective method of indoor protection (59.9%). Nonetheless, the association between bed net usage and the sub-district was highly significant ($\chi^2 = 72.127$, $df = 12$, $p < 0.001$) with the endemic area displaying their highest usage.

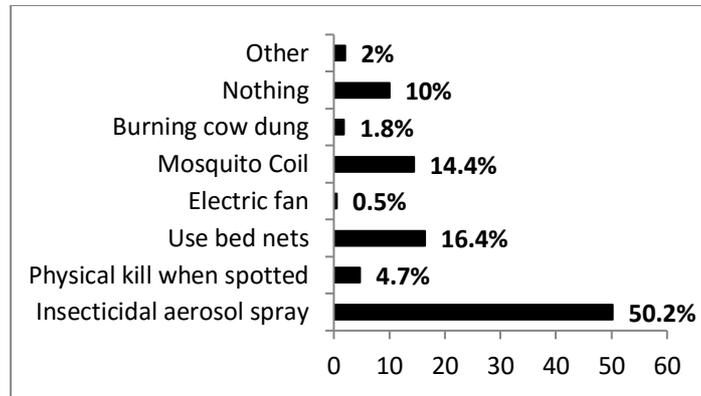


Figure 2. 5. Summary responses (%) on how respondents protect themselves from indoor mosquito bites across sub-districts.

The control of mosquitoes through IRS was noted in communities of the endemic Bobirwa sub-district as a national intervention strategy where the majority did not know the chemical used for IRS in their structures (88.3%). However, the majority (79.6%) testified that IRS was effective in controlling indoor resting mosquitoes. Accordingly, after IRS activity, many (76.9%) observed reductions in mosquito population indoors and subsequent decrease in biting intensity (71.5%). Lack of knowledge on mosquito predators was not linked to overall access to information on mosquitoes ($\chi^2 = 16.219$, $df = 12$, $p = 0.181$). Most respondents (55.3%) regarded the pepper tree (*Schinus molle*) as an effective repellent for adult mosquitoes while many (75.8%) did not know any indigenous/exotic plant capable of treating mosquito-borne diseases. Accordingly, many in the endemic area confirmed sufficient diagnosis and treatment of mosquito-borne infections (59.4%) within their villages by the clinics/hospitals but this varied significantly among sub-districts ($\chi^2 = 50.991$, $df = 6$, $p < 0.001$). A total of 65.1% indicated that they had travelled to malaria endemic districts mostly for more than two weeks (74.1%). The majority of respondents (90.2%) did not take any prescribed prophylactic medication against mosquito-borne infections, either as residents in, or when travelling to malaria-endemic areas.

2.4. Discussion

This study showed that the majority of household respondents were females. According to the Afro-cultural norm, men go out to work to generate income for family support while women stay home and engage in day-to-day household activities (Menjívar and Agadjanian 2007); Yabiku *et al.*, 2010). Women are therefore regarded vulnerable to vector-borne diseases although they are strong drivers of rural household welfare (Gunn *et al.*, 2018). The most common source of information on mosquitoes and mosquito-borne infections was through audio-visual media (e.g., radio/ television) with non-endemic areas having greater information access than endemic areas. Information access remains a challenge to the remote and unserved (e.g., no electricity, mobile/ telephone network range and television reception) endemic areas, particularly with individuals who cannot afford acquiring audio-visual media especially the poor and socially vulnerable (Bonifay *et al.*, 2017). This is highly critical, although the knowledge gap on mosquito-borne diseases was not linked to how information was accessed ($\chi^2 = 15.841$, $df = 18$, $p = 0.604$). Endemic areas may constitute groups highly vulnerable to vector-borne diseases, necessitating the need for intensive education and communication across urban-rural gradients (Malhotra *et al.*, 2014).

Surveyed premises revealed uniform usage of pit latrines across sub-districts. Furthermore, the majority of respondents confirmed that pit latrines facilitated mosquito presence. Although pit latrines are the most cost-effective ablution facilities in low income rural settlements, they significantly contribute to direct and indirect compromised household hygiene. For example, they provide mosquito refugia, oviposition sites and food resources for juveniles (e.g., larvae) (Nakagiri *et al.*, 2015). Most nuisance homestead mosquitoes (e.g., *Anopheles and*

Culex spp.), utilise these pit latrine as ‘hotspots’ for successful propagation (Emidi *et al.*, 2017) and further leverage other human-health complications (see discussions in Nakagiri *et al.* [2015]). Given this scenario, modified mosquito proofing structures that limit mosquito entry into the pit latrines and minimise potential breeding may be better explored (Nathan *et al.*, 1996; Chaggu 2004; Sivagnaname *et al.*, 2005). Similarly, drainage systems contribute significantly as a habitat for mosquito species providing diverse nutrient inputs to developing juveniles (Castro *et al.*, 2010). Although few respondents had proper drainage systems within their properties, caution is needed on their regular maintenance (e.g., overflow avoidance, entry point elimination) across sub-districts. In addition, old tyres had a considerable proportion (22.6%) of potential water holding within premises mostly in endemic areas. These microhabitats are highly favourable to aedine species (Bennett *et al.*, 2019) given that their colonisation and abundance in differential landscapes mainly revolve around human-mediated environmental modifications (Medlock and Vaux 2014).

Although the majority of respondents reported knowledge of adult mosquitoes, lack of recognition on mosquito juveniles was evident with similar trend across endemic and non-endemic areas irrespective of location (sub-district) ($p = 0.972$), education level ($p = 0.094$) or literacy ($p = 0.681$). The knowledge of these bionomic processes may deepen the community’s understanding and enhance life-stage specific intervention strategies especially on the larvae that colonise many container-type microhabitats (Parker *et al.*, 2019) that are common at most visited homesteads. Most respondents who confirmed knowledge of adult mosquitoes, identified them through their ‘sound’ as opposed to gross morphology and simplified taxonomic identification keys (0.2%). Regardless of the endemicity status, the local communities need these identification

keys and skills to identify mosquito species capable of transmitting debilitating diseases in humans, wildlife and livestock (Rueda 2004; Tolle 2009; Tangena *et al.*, 2017; Braack *et al.*, 2018). The current study relied on respondent's assumptions that indeed they know and/or have seen mosquitoes within their homesteads. Therefore, to err on the side of caution, the conclusions drawn from this study should be interpreted within the framework of this limitation. Moreover, the current study did not perform independent assessment of mosquito habitats and mosquitoes at each residence. Similar future work should thus make independent assessments of information (e.g., presence and absence of mosquito larval habitats, mosquitoes and developmental stages) to complement questionnaire data. Furthermore, improved approaches of administering questionnaires using pictorial aids to effectively assess knowledge are recommended.

With the exception of malaria, all sub-districts exhibited very limited knowledge on other mosquito-borne infections. The result is in keeping with previous studies to date (Braack *et al.*, 2018; Rivero and Gandon 2018). This could be due to the bias of the burden of malaria under prevailing climatological impacts locally (Chirebvu *et al.*, 2016) and its over-representation at global scale (Karunamoorthi 2012; Snow 2014). The results also showed mosquito summer bites increment with a similar trend across sub-districts, although highly variable in winter. Again, the endemic area experienced the greatest bites in winter compared to the non-endemic. Thus, studies reported active mosquito-borne transmission shifts that are climate modulated (Park 2011) necessitating spatio-temporal supplementary vector control interventions. Further research is, therefore, needed on seasonal mosquito population and biting dynamics at national level. Moreso respondents reported misconception on the possibility of HIV

transmission by mosquito bites. This warrants extensive and urgent community public health education on issues that may even involve socio-cultural stigmatisation (Okoror *et al.*, 2016).

Moreover, modelling disease and vector status remains crucial given the increased mosquito population trends over the last 10 years (49.3%) as mostly reported in non-endemic areas (Palapye). Global change may increase mosquito numbers and parasite virulence through climate-mediated influences (Short *et al.*, 2017). Temperature plays a crucial role in facilitating shortened mosquito life cycle and increased generations/year (Beck-Johnson *et al.*, 2017). Furthermore, consistent with respondents' observations, there is need to investigate the contribution of cattle-induced eutrophication on the aquatic ecosystem regarding impacts on mosquito proliferation and implication on natural predators. Aquatic predators may potentially play a critical role in community structure assemblage as a sustainable biocontrol tool if utilised effectively in the local context (Raffel *et al.*, 2010; Weis 2011).

The majority of the respondents confirmed mosquitoes pose health risks within their communities, although different sub-districts varied significantly on this assertion. The non-endemic area of Palapye had the highest concerns of health risks necessitating special attention. The study areas were all affected differentially by mosquito-borne infections, e.g., malaria ($p = 0.019$). The burden of malaria gained ground especially in the endemic area (Bobirwa) more than any other mosquito-borne infection. Recently, areas outside the endemic areas previously not known to have indigenous cases of malaria, reported more sporadic cases including the imported cases across the country (Chihanga *et al.*, 2016). Moreover, through travel and networking, imported cases may be guaranteed given the prevalence of other life-threatening mosquito-borne infections in the neighbouring countries regionally (Moi *et al.*, 2010) and elsewhere (Monath

and Vasconcelos 2015; Pan *et al.*, 2019). Accordingly, non-endemic study sites are at risk since they are at intersection of many risk factors (Tatem *et al.*, 2006; Barnett and Walker 2008). This calls for community mobilisation in both endemic and non-endemic zones in raising awareness not only for malaria but all other mosquito-borne related illnesses (Shafie *et al.*, 2016). While it is encouraging that most communities asserted that one mosquito bite could lead to health risk, there is more to be done in ensuring further development and bridging knowledge gaps in parasite transmission blockage dynamics (Satapathy and Taylor-Robinson 2016) moreso in light of the respondents' request to be trained on mosquito biology (38.5%). In addition, the results of the survey showed that mosquito bites were mostly received both indoors and outdoors (52.2%) possibly relating to human behaviour (Finda *et al.*, 2019) and the resting patterns indoors (endophilic) and outdoors (exophilic) influenced by microclimatic (Paaijmans and Thomas 2011). Thus, more work is also needed in investigating mosquito biting-patterns, useful in determining appropriate interventions against mosquito vectors (Pates and Curtis 2005). Further, community willingness to effectively explore both indoor and outdoor interventions is highly desirable in arresting mosquito life-stage development and survival mechanisms.

Although the majority of respondents use clothes that cover much of the body for personal protection, this practice differed significantly across sub-districts ($p < 0.001$), with endemic area exhibiting the highest personal protection compared to the non-endemic areas. A higher proportion reported face bites (39.3%) suggesting that there is a need to explore other protection measure that may cater for exposed area (e.g., head). Thus, further knowledge and awareness regarding other methods of personal protection other than long sleeved clothing (e.g., acoustic and electric devices) are essential (Breisch *et al.*, 2012; Lapshin and Vorontsov 2018).

The bed nets were regarded as an overall effective indoor intervention against adult mosquitoes, although mostly used in the endemic area. Conversely, insecticidal aerosol spray use dominated more than bed nets in the non-endemic areas. The use of aerosol sprays together with the national intervention strategies (e.g., IRS and LLITNs in endemic areas) may exacerbate resistance in mosquito species both in the endemic and the non-endemic areas. Whilst the ongoing ‘more than one’ chemical-based intervention (Okumu and Moore 2011) and prolonged pesticide use on mosquitoes are practiced, susceptibility status assessing potential resistance development remains eminent. The communities need to understand issues of induced resistance at household level through organised campaigns and public education as a way of managing resistance in disease vectors. This will augment onto the overall holistic approach of appropriate practices involving personal, indoor and environmental manipulations (e.g., clearing resting and foraging vegetation) as demonstrated by most respondents (73.3%) in the endemic area (Muema *et al.*, 2017).

The respondents reported cattle within their areas across all sub-districts highlighting that more work may be needed to determine the role of animal-induced nutrification in wetland systems as a factor driving mosquito abundance. Degraded aquatic habitats are known to negatively impact useful natural enemies of mosquito populations (Schrama *et al.*, 2018). As such, further research on degraded aquatic ecosystems may be useful in advising communities on cattle movement, herd size and managing watering points. This may reduce mosquito proliferation in villages that have natural ponds and numerous other water-collecting structures while conserving natural enemies as a tool for ecosystem service (Roux and Robert 2019). Exotic and indigenous plant species outlined as attractants and/ or repellents for mosquitoes need further

exploration. The outcomes may optimise traditional knowledge systems (locally available plants) in sustainable vector control measures and advise the communities on their utilisation (e.g., push-pull vector control systems) (Wagman *et al.*, 2015). The majority of the respondents reported having travelled to the endemic areas without prophylactic treatment (Schoepke *et al.*, 1998) mostly those in the endemic area (Bobirwa). Communities should be constantly advised to take medication regardless of endemicity status as drugs strengthen immunity and further block mechanisms of parasite replication (Long and Zavala 2016).

Results of this survey indicate that the majority of the community had limited knowledge in many areas of mosquito bio-ecology. Although most were concerned about contracting mosquito-borne infections, capacitating the communities on awareness of personal, structural and environmental control strategies through public education is needed. Furthermore, it is essential to educate communities on practices that exacerbate mosquito population without harming the environment. These include management of water holding structures (e.g., pit latrines, drainage), chemical intervention and promoting and conservation of natural enemies needed for sustainable integrated control of mosquito populations, with consequent reduction of associated disease burdens. The knowledge paucity reported here points to a need for training of local communities in mosquito bio-ecology, especially identification of key developmental life stages for efficient vector and disease management enhanced by modern real-time ‘citizen science’ application as a reporting system for prompt vector surveillance initiatives. This may help in early warning systems against the spread of vectors and associated pathogens and the management of emerging and re-emerging mosquito-borne infections under anthropogenic changing environments.

2.5. References

- Akiner, M. M., Demirci, B., Babuadze, G., Robert, V., and Schaffner, F. (2016). Spread of the invasive mosquitoes *Aedes aegypti* and *Aedes albopictus* in the Black Sea region increases risk of chikungunya, dengue, and Zika outbreaks in Europe. *PLoS Neglected Tropical Diseases*, 10: e0004664.
- Asigau, S., and Parker, P. G. (2018). The influence of ecological factors on mosquito abundance and occurrence in Galápagos. *Journal of Vector Ecology*, 43: 125-137.
- Barnett, E. D., and Walker, P. F. (2008). Role of immigrants and migrants in emerging infectious diseases. *Medical Clinics of North America*, 92: 1447-1458.
- Batisani, N., and Yarnal, B. (2010). Rainfall variability and trends in semi-arid Botswana: implications for climate change adaptation policy. *Applied Geography*, 30: 483-489.
- Bennett, K. L., Martínez, C. G., Almanza, A., Rovira, J. R., McMillan, W. O., Enriquez, V., and Gittens, R. A. (2019). High infestation of invasive *Aedes* mosquitoes in used tires along the local transport network of Panama. *Parasites and Vectors*, 12: 1-10.
- Beck-Johnson, L. M., Nelson, W. A., Paaijmans, K. P., Read, A. F., Thomas, M. B., and Bjørnstad, O. N. (2017). The importance of temperature fluctuations in understanding mosquito population dynamics and malaria risk. *Royal Society Open Science*, 4: 160969.
- Bonifay, T., Douine, M., Bonnefoy, C., Hurpeau, B., Nacher, M., Djossou, F., and Epelboin, L. (2017). Poverty and arbovirus outbreaks: when chikungunya virus hits more precarious populations than dengue virus in French Guiana. In *Open forum Infectious Diseases*, 4: ofx247.
- Bowling, A. (2005). Mode of questionnaire administration can have serious effects on data quality. *Journal of Public Health*, 27: 281-291.
- Breisch, N. L., Golden, D. B., Feldweg, A. M. (2012). Prevention of arthropod and insect bites: Repellents and other measures. Up To Date, Waltham, MA: [https://www.uptodate.com/contents/prevention-of-arthropod-and-insect-bites-repellents-and other measures](https://www.uptodate.com/contents/prevention-of-arthropod-and-insect-bites-repellents-and-other-measures) [accessed November 18, 2019].

- Castro, M. C., Kanamori, S., Kannady, K., Mkude, S., Killeen, G. F., and Fillinger, U. (2010). The importance of drains for the larval development of lymphatic filariasis and malaria vectors in Dar es Salaam, United Republic of Tanzania. *PLoS Neglected Tropical Diseases*, 4: e693.
- Chaggu, E. J. (2004). Sustainable environmental protection using modified pit-latrines. Ph.D Thesis, Wageningen University, Wageningen, The Netherlands.
- Chihanga, S., Haque, U., Chanda, E., Mosweunyane, T., Moakofhi, K., Jibril, H. B., and Glass, G. E. (2016). Malaria elimination in Botswana, 2012–2014: achievements and challenges. *Parasites and Vectors*, 9: 99.
- Chirebvu, E., Chimbari, M. J., Ngwenya, B. N., and Sartorius, B. (2016). Clinical malaria transmission trends and its association with climatic variables in Tubu Village, Botswana: a retrospective analysis. *PloS ONE*, 11: e0139843.
- Chirebvu, E., and Chimbari, M. J. (2015). Characteristics of *Anopheles arabiensis* larval habitats in Tubu village, Botswana. *Journal of Vector Ecology*, 40: 129-138.
- Chirebvu, E., Chimbari, M. J., and Ngwenya, B. N. (2014). Assessment of risk factors associated with malaria transmission in Tubu village, northern Botswana. *Malaria Research and Treatment*, 403069: <https://doi.org/10.1155/2014/403069>.
- Chirebvu, E., Chimbari, M. J., and Ngwenya, B. N. (2013). Knowledge and practices on malaria in Tubu village, in a malaria-endemic area in northern Botswana: implications for interventions. *Malaria World Journal*, 4: 1-9.
- Cornel, A. J., Lee, Y., Almeida, A. P. G., Johnson, T., Mouatcho, J., Venter, M., and Braack, L. (2018). Mosquito community composition in South Africa and some neighboring countries. *Parasites and Vectors*, 11: 1-12.
- de la Fuente, J., Estrada-Pena, A., Venzal, J. M., Kocan, K. M., and Sonenshine, D. E. (2008). Overview: ticks as vectors of pathogens that cause disease in humans and animals. *Frontiers in Bioscience*, 13: 6938-6946.
- Dhiman, S. (2019). Are malaria elimination efforts on right track? An analysis of gains achieved and challenges ahead. *Infectious Diseases of Poverty*, 8: 1-19.

- Emidi, B., Kisinza, W. N., Stanley, G., and Mosha, F. (2017). Seasonal variation of *Culex quinquefasciatus* densities emerged from Pit-Latrines in rural settings, Muheza, Tanzania. *SM Journal of Public Health and Epidemiology*, 3: 1040.
- Eritja, R., Palmer J. R, Roiz, D., Sanpera-Calbet, I., and Bartumeus, F. (2017) Direct evidence of adult *Aedes albopictus* dispersal by car. *Scientific Reports*, 7: 14399.
- Finda, M. F., Moshi, I. R., Monroe, A., Limwagu, A. J., Nyoni, A. P., Swai, J. K., and Coetzee, M. (2019). Linking human behaviours and malaria vector biting risk in south-eastern Tanzania. *PloS ONE*, 14: e0217414.
- Gunn, J. K., Ernst, K. C., Center, K. E., Bischoff, K., Nuñez, A. V., Huynh, M., and Hayden, M. H. (2018). Current strategies and successes in engaging women in vector control: a systematic review. *BMJ Global Health*, 3: e000366.
- Howes, R. E., Mioramalala, S. A., Ramiranirina, B., Franchard, T., Rakotorahalahy, A. J., Bisanzio, D., and Ratsimbaoa, A. (2016). Contemporary epidemiological overview of malaria in Madagascar: operational utility of reported routine case data for malaria control planning. *Malaria Journal*, 15: 1-18.
- Hulsmans, A., Vanschoenwinkel, B., Pyke, C., Riddoch, B. J., and Brendonck, L. (2008). Quantifying the hydroregime of a temporary pool habitat: a modelling approach for ephemeral rock pools in SE Botswana. *Ecosystems*, 11: 89-100.
- Karunamoorthi, K. (2012). Global malaria burden: socialomics implications. *Journal of Socialomics*, 1: e108.
- Kengluecha, A., Singhasivanon, P., Tiensuwan, M., Jones, J. W., and Sithiprasasna, R. (2005). Water quality and breeding habitats of anopheline mosquito in northwestern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, 36: 46-53.
- Kilpatrick, A. M., and Randolph, S. E. (2012). Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. *The Lancet*, 380: 1946-1955.
- Kweka, E. J., Kimaro, E. E., Kimaro, E. G., Nagagi, Y. P., and Malele, I. I. (2017). Major Disease Vectors in Tanzania: Distribution, Control and Challenges. *Biological Control of Pest and Vector Insects*, 12: 257.

- Lapshin, D. N., and Vorontsov, D. D. (2018). Low-frequency sounds repel male mosquitoes *Aedes diaantaeus* NDK (Diptera, Culicidae). *Entomological Review*, 98: 266-271.
- Launiala, A. (2009). How much can a KAP survey tell us about people's knowledge, attitudes and practices? Some observations from medical anthropology research on malaria in pregnancy in Malawi. *Anthropology Matters*, 11: <https://doi.org/10.22582/am.v11i1.31>.
- Leisnham, P. T., Slaney, D. P., Lester, P. J., and Weinstein, P. (2005). Increased larval mosquito densities from modified landuses in the Kapiti region, New Zealand: vegetation, water quality, and predators as associated environmental factors. *EcoHealth*, 2: 313-322.
- Leitner, W. W., Wali, T., Kincaid, R., and Costero-Saint Denis, A. (2015). Arthropod vectors and disease transmission: translational aspects. *PLoS Neglected Tropical Diseases*, 9: e0004107.
- Le Menach, A., Tatem, A. J., Cohen, J. M., Hay, S. I., Randell, H., Patil, A. P., and Smith, D. L. (2011). Travel risk, malaria importation and malaria transmission in Zanzibar. *Scientific Reports*, 1: 93.
- Levine, R. S., Hedeem, D. L., Hedeem, M. W., Hamer, G. L., Mead, D. G., and Kitron, U. D. (2017). Avian species diversity and transmission of West Nile virus in Atlanta, Georgia. *Parasites and Vectors*, 10: 62.
- Long, C. A., and Zavala, F. (2016). Malaria vaccines and human immune responses. *Current Opinion in Microbiology*, 32: 96-102.
- Ludwig, A., Zheng, H., Vrbova, L., Drebot, M. A., Iranpour, M., and Lindsay, L. R. (2019). Increased risk of endemic mosquito-borne diseases with climate change. *Canada Communicable Disease Report*, 45: 4.
- Machekano, H., Mvumi, B. M., and Nyamukondiwa, C. (2020). *Plutella xylostella* (L.): pest status, control practices, perceptions and knowledge on existing and alternative management options in arid small-scale farming environments. *International Journal of Pest Management*, 66: 48-64.
- Makate, N. M. (2016). A Review of Insecticide Resistance Status in Botswana. *Insecticides Resistance*, 263: <https://doi.org/10.5772/61513>.

- Malhotra, G., Yadav, A., and Dudeja, P. (2014). Knowledge, awareness and practices regarding dengue among rural and slum communities in North Indian city, India. *International Journal of Medical Science and Public Health*, 3: 295-299.
- Medlock, J. M., and Vaux, A. G. (2014). Colonization of a newly constructed urban wetland by mosquitoes in England: implications for nuisance and vector species. *Journal of Vector Ecology*, 39: 249-260.
- Meehl, G. A., and Tebaldi, C. (2004). More intense, more frequent, and longer lasting heat waves in the 21st century. *Science*, 305: 994-997.
- Menjívar, C., and Agadjanian, V. (2007). Men's migration and women's lives: views from rural Armenia and Guatemala. *Social Science Quarterly*, 88: 1243-1262.
- Moi, M. L., Takasaki, T., Kotaki, A., Tajima, S., Lim, C. K., Sakamoto, M., and Kurane, I. (2010). Importation of dengue virus type 3 to Japan from Tanzania and Côte d'Ivoire. *Emerging Infectious Diseases*, 16: 1770.
- Monath, T. P., and Vasconcelos, P. F. (2015). Yellow fever. *Journal of Clinical Virology*, 64: 160-173.
- Motlaleng, M., Edwards, J., Namboze, J., Butt, W., Moakofhi, K., Obopile, M., and Oumer, N. (2018). Driving towards malaria elimination in Botswana by 2018: progress on case-based surveillance, 2013–2014. *Public Health Action*, 8: S24-S28.
- Muema, J. M., Bargul, J. L., Njeru, S. N., Onyango, J. O., and Imbahale, S. S. (2017). Prospects for malaria control through manipulation of mosquito larval habitats and olfactory-mediated behavioural responses using plant-derived compounds. *Parasites and Vectors*, 10: 1-18.
- Mutheni, S. R., Morse, A. P., Caminade, C., and Upadhyayula, S. M. (2017). Dengue burden in India: recent trends and importance of climatic parameters. *Emerging Microbes and Infections*, 6: 1-10.
- Nakagiri, A., Niwagaba, C. B., Nyenje, P. M., Kulabako, R. N., Tumuhairwe, J. B., and Kansime, F. (2015). Are pit latrines in urban areas of Sub-Saharan Africa performing? A review of usage, filling, insects and odour nuisances. *BMC Public Health*, 16: 1-16.

- Nathan, M. B., Toney, S., Bramble, S., and Reid, V. (1996). Control of *Culex quinquefasciatus* in pit latrines, using shredded, waste polystyrene. *Annals of Tropical Medicine and Parasitology*, 90: 207-212.
- Okoror, T. A., Falade, C. O., Walker, E. M., Olorunlana, A., and Anaele, A. (2016). Social context surrounding HIV diagnosis and construction of masculinity: a qualitative study of stigma experiences of heterosexual HIV positive men in southwest Nigeria. *BMC Public Health*, 16: 507.
- Okumu, F. O., and Moore, S. J. (2011). Combining indoor residual spraying and insecticide-treated nets for malaria control in Africa: a review of possible outcomes and an outline of suggestions for the future. *Malaria Journal*, 10: 1-13.
- Paaijmans, K. P., and Thomas, M. B. (2011). The influence of mosquito resting behaviour and associated microclimate for malaria risk. *Malaria Journal*, 10: 183.
- Pachka, H., Annelise, T., Alan, K., Power, T., Patrick, K., Véronique, C., and Ferran, J. (2016). Rift Valley fever vector diversity and impact of meteorological and environmental factors on *Culex pipiens* dynamics in the Okavango Delta, Botswana. *Parasites and Vectors*, 9: 434.
- Pan, J., Fang, C., Yan, J., Yan, H., Zhan, B., Sun, Y., and Zhang, Y. (2019). Chikungunya fever outbreak, Zhejiang province, China, 2017. *Emerging Infectious Diseases*, 25: 1589.
- Park, J. W. (2011). Changing transmission pattern of *Plasmodium vivax* malaria in the Republic of Korea: relationship with climate change. *Environmental Health and Toxicology*, 26: e2011001.
- Parker, C., Garcia, F., Menocal, O., Jeer, D., Alto, B. A. (2019). Mosquito Workshop and Community Intervention: A Pilot Education Campaign to Identify Risk Factors Associated with Container Mosquitoes in San Pedro Sula, Honduras. *International Journal of Environmental Research and Public Health*, 16: 2399.
- Pates, H., and Curtis, C. (2005). Mosquito behavior and vector control. *Annual Review of Entomology*, 50: 53-70.

- Quiroz-Martínez, H., and Rodríguez-Castro, A. (2007). Aquatic insects as predators of mosquito larvae. *Journal of the American Mosquito Control Association*, 23: 110-117.
- Raffel, T. R., Hoverman, J. T., Halstead, N. T., Michel, P. J., and Rohr, J. R. (2010). Parasitism in a community context: trait-mediated interactions with competition and predation. *Ecology*, 91: 1900-1907.
- Rivero, A., and Gandon, S. (2018). Evolutionary ecology of avian malaria: past to present. *Trends in Parasitology*, 34: 712-726.
- Roux, O., and Robert, V. (2019). Larval predation in malaria vectors and its potential implication in malaria transmission: an overlooked ecosystem service? *Parasites and Vectors*, 12: 217.
- Rozendaal, J. A. (1997). Vector control: methods for use by individuals and communities. World Health Organization, Geneva, Switzerland.
- Rueda, L. M. (2004). Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission. Walter Reed Army Institute Of Research Washington Dc, Department Of Entomology, Silver Springs, MD, USA.
- Satapathy, S., and Taylor-Robinson, A. W. (2016). Bridging the Knowledge Gap in Transmission-Blocking Immunity to Malaria: Deciphering Molecular Mechanisms in Mosquitoes. *Advances in Infectious Diseases*, 6: 33.
- Schoepke, A., Steffen, R., and Gratz, N. (1998). Effectiveness of personal protection measures against mosquito bites for malaria prophylaxis in travelers. *Journal of Travel Medicine*, 5: 188-192.
- Schrama, M., Gorsich, E. E., Hunting, E. R., Barmantlo, S. H., Beechler, B., and van Bodegom, P. M. (2018). Eutrophication and predator presence overrule the effects of temperature on mosquito survival and development. *PLoS Neglected Tropical Diseases*, 12: e0006354.
- Shalan, E. A. S., and Canyon, D. V. (2009). Aquatic insect predators and mosquito control. *Tropical Biomedicine*, 26: 223-261.
- Shafie, A., Roslan, M. A., Ngui, R., Lim, Y. A. L., and Sulaiman, W. Y. W. (2016). Mosquito biology and mosquito-borne disease awareness among island communities in Malaysia. *Journal of the American Mosquito Control Association*, 32: 273-281.

- Shi, B., Zheng, J., Qiu, H., Yang, G. J., Xia, S., and Zhou, X. N. (2017). Risk assessment of malaria transmission at the border area of China and Myanmar. *Infectious Diseases of Poverty*, 6: 1-9.
- Short, E. E., Caminade, C., and Thomas, B. N. (2017). Climate change contribution to the emergence or re-emergence of parasitic diseases. *Infectious Diseases: Research and Treatment*, 10: 1178633617732296.
- Simon-Oke, I. A. (2019). Prevalence of Malaria Parasites among Pregnant Women and Children under Five years in Ekiti State, Southwest Nigeria. *Journal of Biomedicine and Translational Research*, 5: 5-10.
- Sivagnaname, N., Amalraj, D. D., and Mariappan, T. (2005). Utility of expanded polystyrene (EPS) beads in the control of vector-borne diseases. *Indian Journal of Medical Research*, 122: 291.
- Snow, R. W. (2014). Sixty years trying to define the malaria burden in Africa: have we made any progress? *BMC Medicine*, 12: 227.
- Tangena, J. A. A., Thammavong, P., Malaithong, N., Inthavong, T., Ouanesamon, P., Brey, P. T., and Lindsay, S. W. (2017). Diversity of mosquitoes (Diptera: Culicidae) attracted to human subjects in rubber plantations, secondary forests, and villages in Luang Prabang province, Northern Lao PDR. *Journal of Medical Entomology*, 54: 1589-1604.
- Tatem, A. J., Rogers, D. J., and Hay, S. I. (2006). Global transport networks and infectious disease spread. *Advances in Parasitology*, 62: 293-343.
- Tawe, L., Ramatlho, P., Waniwa, K., Muthoga, C. W., Makate, N., Ntebela, D. S., and Paganotti, G. M. (2017). Preliminary survey on *Anopheles* species distribution in Botswana shows the presence of *Anopheles gambiae* and *Anopheles funestus* complexes. *Malaria Journal*, 16: 1-7.
- Thuiller, W. (2007). Climate change and the ecologist. *Nature*, 448: 550-552.
- Tolle, M. A. (2009). Mosquito-borne diseases. *Current Problems in Pediatric and Adolescent Health Care*, 39: 97-140.

- Wagman, J. M., Grieco, J. P., Bautista, K., Polanco, J., Briceño, I., King, R., and Achee, N. L. (2015). The field evaluation of a push-pull system to control malaria vectors in Northern Belize, Central America. *Malaria Journal*, 14: 184.
- Weis, J. S. (2011). Invasion and predation in aquatic ecosystems. *Current Zoology*, 57: 613-624.
- WHO (World Health Organization) (2019). World malaria report 2019. World Health Organization, Geneva, Switzerland.
- WHO (World Health Organization) (2018). Global report on insecticide resistance in malaria vectors: 2010–2016. World Health Organization, Geneva, Switzerland.
- WHO (World Health Organization) (2017). Vector-Borne Diseases Factsheet. World Health Organization, Geneva, Switzerland.
- WHO (World Health Organization) (2014). A global brief on vector-borne diseases. World Health Organization, Geneva, Switzerland.
- Wilke, A. B., Carvajal, A., Vasquez, C., Petrie, W. D., and Beier, J. C. (2020). Urban farms in Miami-Dade county, Florida have favorable environments for vector mosquitoes. *PLoS One*, 15: e0230825.
- Yabiku, S. T., Agadjanian, V., and Sevoyan, A. (2010). Husbands' labour migration and wives' autonomy, Mozambique 2000–2006. *Population Studies*, 64: 293-306

CHAPTER 3

Mosquito species composition in Serowe, Palapye and Bobirwa sub-districts of Central district, Botswana

Component published as: **Buxton, M.**, Lebani, K., Nyamukondiwa, C., and Wasserman, R. J. (2019). First Record of *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) (Diptera: Culicidae) in Botswana. *BioInvasions Records*, 8: 551-557.

Buxton, M., Nyamukondiwa, C., Kesamang, M. and Wasserman, R. J. Mosquito species composition; Insights from malaria endemic and non-endemic areas of Botswana. *African Entomology*. In review.

3.1. Introduction

Through the transmission of parasites such as protozoans, helminths and viruses, mosquitoes facilitate incapacitating diseases such as malaria, Zika, dengue, West Nile fever, chikungunya (Lee *et al.*, 2018; Ralapanawa and Kularatne 2020). Globally, the introduction of invasive vector mosquitos in new environments are on the rise (Akiner *et al.*, 2016; Wilke *et al.*, 2020) and so are the population numbers for other vector incriminated mosquito species. This has led to a concomitant surge in cases of vector-borne diseases (WHO 2014), with disease vector mosquitoes contributing increasingly to human morbidity and mortality worldwide (Ahmed *et al.*, 2020; Boyer *et al.*, 2020). Similarly, although less well documented, vector mosquitoes also contribute significantly to livestock and wildlife disease transmission (Mroz *et al.*, 2017). The distribution of mosquitoes has been well studied in tropical and temperate regions worldwide (Atoni *et al.*, 2019; Foster and Walker 2019; Martinet *et al.*, 2019) with biotic and abiotic factors shaping mosquito community composition (Biteye *et al.*, 2018; Akpan *et al.*, 2019; Cane *et al.*, 2020; Mouatasseem *et al.*, 2020). There is, however, continued scope for further exploration in this regard, given that changing environmental conditions and biological invasions are resulting in shifts in distribution of known vectors, and the presence of new vector mosquito species and associated pathogens (Weyl *et al.*, 2019; Pereira dos Santos *et al.*, 2018).

Climatic and human-mediated factors are regarded as crucial drivers of mosquito population dynamics (Monaghan *et al.*, 2018; Lee *et al.*, 2020; Wang *et al.*, 2020). Given the recent increase in climate and anthropogenic activities facilitating vector mosquito species (Schrama *et al.*, 2020; Wang *et al.*, 2020), it is highly likely certain mosquitoes may proliferate and translocate to novel environments (Liu-Helmersson *et al.*, 2019). Many mosquitoes have

also been reported as being invasive (Vaux *et al.*, 2019; Fălcuță *et al.*, 2020) with potential of introductions and distribution across different landscapes (Kampen *et al.*, 2017; Strickman 2020). This necessitates the need for continual and revised spatio-temporal monitoring of known and potential vector species (Ludwig *et al.*, 2019; El-Sayed and Kamel 2020). The geographic distribution and composition of mosquito communities is well documented in Africa (Jupp and McIntosh 1990; Njabo *et al.*, 2009; Alves *et al.*, 2010; Johnson *et al.*, 2020) albeit biased towards malaria-burdened areas (Coetzee *et al.*, 2000; Wiebe *et al.*, 2017). As such, anopheline mosquitoes are the focus of much of the biological, autecological and even community ecology studies in the region, particularly in southern Africa (Ferguson *et al.*, 2010; Zengenene *et al.*, 2020). As a result, little work has focused on culicine mosquito species in southern Africa (but see Matowo *et al.*, 2019; Kahamba *et al.*, 2020). In particular, apart from the most abundant taxa (e.g., *Culex* spp) (e.g., Pachka *et al.*, 2016), little work has explored culicines.

Botswana mosquito research is largely reflective of that of the region, with mosquito work mainly explored within malaria endemic part of the country (Chirebvu and Chimbari 2015; Pachka *et al.*, 2016; Tawe *et al.*, 2017; Cornel *et al.*, 2018, Kgoroebutswe *et al.*, 2020). Malaria is currently the most apparent mosquito-borne infection in the country resulting in anopheline species prioritised for research. As such, the non-endemic malaria areas and non-anopheline mosquitoes are largely neglected (however see Bango *et al.*, 2020). Furthermore, the vector control monitoring programmes are also focused on anopheline species within the endemic areas. Areas that were previously known to be malaria free (e.g., Palapye, Serowe, Ghanzi, Kweneng) have, however, recently recorded both indigenous and exported malaria cases (Chihanga *et al.*, 2016; Bango *et al.*, 2020). For example, in recent years, malaria cases and deaths have been

reported persistently across the Central district, with Bobirwa having the highest cases, Serowe exhibiting the least while Palapye region remained intermediate (Table S3.1). *Culex* spp. are also known vectors of diseases for livestock and wild animals, although very little work has been conducted on this in the region. Furthermore, globally invasive vector species have been detected in surrounding countries (Masaninga *et al.*, 2014; Noden *et al.*, 2014; Weyl *et al.*, 2019), but the investigation of their presence in Botswana has yet to be explored. As such, vector mosquito monitoring as a whole across the country is rather scant. This has hindered understanding of species distribution and the implication of disease risk in areas or for taxa regarded of 'less concern'. An understanding of available mosquito species would facilitate development of policy for a move from the *Anopheles*-centric approach to a more inclusive vector management strategy, including screening for associated human, domestic and wildlife associated pathogens and parasites.

The Central district of Botswana straddles a malaria-endemicity gradient, consisting of three malaria- endemic and three -non-endemic zones (Figure 3.1). It has been identified that the Central district is an area requiring research prioritisation in Botswana given its primacy and centrality, thus attracting national developmental attention. In this component of the thesis, the aim was to conduct a basic assessment of the mosquito species composition in human inhabited areas in the district. More specifically, species composition was contrasted across the malaria endemic (Bobirwa) and non-endemic (Serowe, Palapye) sub-districts. It was predicted that (i) amongst other mosquito genera, the abundance of *Culex* spp. would be more prominent across sub-districts (ii), anopheline species would be present in higher numbers in the malaria endemic than in non-endemic areas, and that (iii) invasive *Aedes* spp. would be present across sampled

sub-districts given their increased urban size and proximity to South Africa where this species has been reported (Weyl *et al.*, 2019). To this end, this chapter is comprised of preliminary screening for *Aedes* spp. in urban Palapye, given anecdotal reports of the presence of invasive species (Kemp and Jupp 1991; Jupp 2005), and built on by a more thorough investigation of Bobirwa, Serowe and Palapye sub-districts. The results of this study will serve as baseline findings for future work for these understudied regions (Tawe *et al.*, 2017; Bango *et al.*, 2020; Kgorobutswe *et al.*, 2020) and enlighten the communities of potential risk vectors, and associated disease transmission. The findings will also have implications for vector proliferation due to anthropogenic and climate driven factors prevailing in the region. In particular, global change and degradation (e.g., mosquito breeding habitats) by human activity, are likely to favour the success of harmful mosquito species (Schrama *et al.*, 2020).

3.2. Materials and Methods

3.2.1. Preliminary larval collection and rearing in Palapye village

Larval specimens were collected in January 2019 in Palapye village, Botswana (22° 32.976S; 027° 11.504E), from three open 20 L paint containers (300 mm diameter) that collected ~ 10 L rain water adjacent to buildings. A representation of 4th instar larvae were collected using a 200 µm mesh sieve, placed in two small plastic containers (300 ml) filled with rain water and housed in a rearing cage (Bugdorm-BD43030F, 240 mm³, Megaview Science Co., Ltd, Taiwan). Fish food (Lopis: Tropical fish food flakes) was used to feed the larvae *ad libitum*, reared at 27°C ± 2 and 75% ±10 RH, under a 12:12 light:dark photocycle in a climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany) housed in the laboratory.

3.2.2. *Mosquito community collection across sub-districts*

Mosquito samples were collected from both the malaria endemic (Bobirwa) and the non-endemic sub-districts (Palapye and Serowe) between November and January austral summer of 2019/ 2020 (Figure 3.1; Table S3.2). Sampling was conducted during this period as it falls within the rainy season for the area, the time of year when mosquito abundances are highest and where communities identified risk of bites to be the greatest. Nine collection points were identified per sub-district based on proximity to human habitation (six for adults) and the presence of breeding sites (three for larvae) mainly from standing rain water, drying river bays and artificial container systems (see Table S3.2 for specific details). Adult and larval mosquito species were sampled from a total of nine collection points per sub-district, monitored repeatedly over 3 days (adults). The adults were trapped using a Biogents BG-GAT (Model: 10719, Biogents AG, Germany) and stored individually in 2 mL eppendorf tubes with desiccating silica gel beads. Mosquito larvae were sampled with a 200 µm mesh net (40 cm × 60 cm) from three separate aquatic habitats within each sub-district and standardised for 10 minutes per sampling site. Each larval specimen was preserved in 2 mL eppendorf tubes using 80% alcohol.

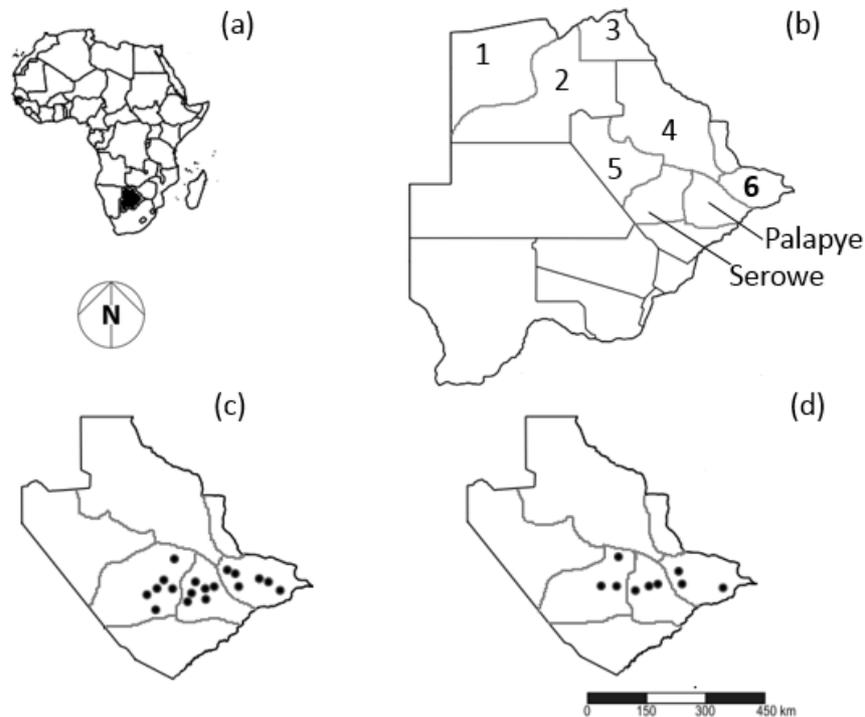


Figure 3. 1. A map showing (a) the location of Botswana in Africa, (b) malaria endemic districts [Okavango: 1, Ngamiland: 2, Chobe: 3, Tutume: 4, Boteti: 5, and Bobirwa] with the Central district indicating (c) adult and (d) larval sampling sites across malaria endemic (Bobirwa) and non-endemic districts (Palapye and Serowe). For coordinates of sampling sites see Table S3.2.

3.2.3. Identification

All mosquito specimens were first identified using morphological features (Gillies and Coetzee 1987; Jupp 1996; Rueda *et al.*, 2004) and subsequently confirmed with molecular techniques following modified protocols of Makanda *et al.* (2019). Five individuals of both adults and larvae were randomly selected across species and subjected to polymerase chain reaction (PCR) for the amplification of the cytochrome oxidase subunit I (COI) using the universal primers LCO 1490 and HCO 2198. For adults, a leg was used in the amplification whilst for larval specimens, a portion of the abdomen was utilised (Bass *et al.*, 2007). Dissected

mosquito parts were added directly to the 50 μL PCR reaction mix consisting of 25 μL of 2 \times Phusion U Multiplex PCR Mix (Thermo Fisher Scientific, USA), 9 μL nuclease free water (VWR International LLC, USA) and 0.8 μM of each primer. The PCR conditions were; initial denaturation at 98 $^{\circ}\text{C}$ for 30 seconds, 35 cycles of denaturation at 98 $^{\circ}\text{C}$ for 10 seconds, annealing at 48 $^{\circ}\text{C}$ for 30 seconds and extension at 72 $^{\circ}\text{C}$ for 30 seconds. Subsequently, a final extension at 72 $^{\circ}\text{C}$ for 7 minutes was performed. Visualisation of amplicons were done under 1 % agarose gel stained with ethidium bromide. Purification of 40 μL PCR product was performed using a JET PCR purification kit (Thermo Fisher Scientific, USA) according to manufacturer's recommended protocols, followed by elution with 20 μL nuclease free water (VWR International LLC, USA). Amplicons were sequenced in Inqaba Biotechnical Industries (Pretoria, South Africa). The sequences were trimmed and contigs were assembled using a CodonCode Aligner 8.0.2 software package and subjected to nucleotide blast for species homology identification. Sequences were further deposited in Genbank to obtain species accession numbers.

3.2.4. *Statistical analyses*

Data on mosquito community composition were analysed using the Primer version 6 statistical software package, within which the total species number (S), total number of individuals (N), Margalef's species richness (d), species diversity (H) and Pielou's evenness (J') were determined per site. The same software package was then used to assess similarities in community composition, whereby a Euclidean distance similarity matrix was used to construct a dendrogram to assess similarities across sub-districts. Although mixed methods were employed, given that the effort was standardised across all three sub-districts (6 and 3 sites each for adult and larval collection, respectively), a combined species list along with abundances per

species was created per sub-district. The Simper analysis in Primer version 6 was then used to assess the levels of dissimilarity between the sites (sub-districts) and the species contributing the greatest towards these differences.

Mosquito abundances (adults) were determined across sub-districts to establish any significant differences. These data were analysed in STATISTICA, version 13.5.0.17 (Statsoft Inc., Tulsa, Oklahoma). The data was checked for normality using Shapiro-Wilk test and fulfilled assumptions of analysis of variance (ANOVA). Factorial ANOVA was then used to analyse data, with abundances as dependent variables while the species, sex, sub-district were the independent factors.

3.3. Results

3.3.1. Preliminary sampling from Palapye village

The mosquitos were identified as domestic *Ae. (Stegomyia) aegypti* (Linnaeus, 1762) by diagnostic morphological features on the head, thorax and legs (Figure 3.2). Four distinct diagnostic features were used, namely: (a) the mesepimeron with two well-separated white scale patches; (b) white lyre-shaped markings on a black scutum and a pair of submedian-longitudinal white stripes; (c) the anterior portion of midfemur having a longitudinal white stripe, and; (d) clypeus on the head with two white separate scale patches (Rueda 2004).

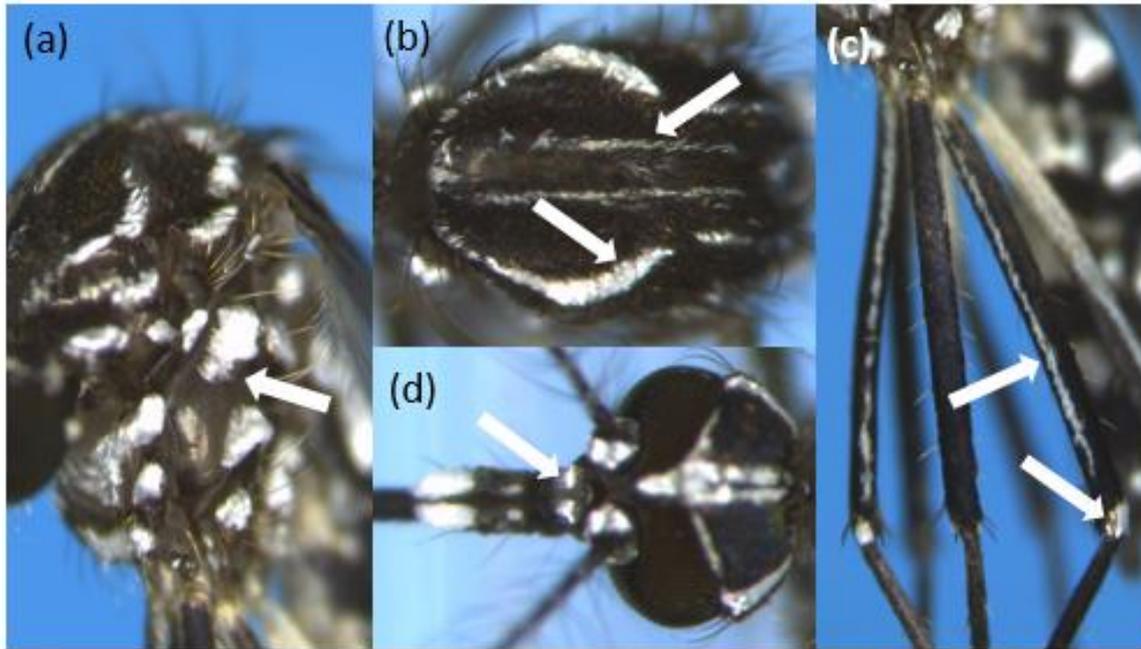


Figure 3. 2. Key morphological characteristics of a collected mosquito: (a): Mesepimeron, showing two well-separated white scale patches (arrow). (b): Thorax, showing scutum with a pair of submedian-longitudinal white stripes (top arrow), and white lyre-shaped markings (bottom arrow). (c): Midfemur, showing white knee-spot (bottom arrow) and anterior portion with a longitudinal white stripe (top arrow). (d): Clypeus, showing white scale patches (top arrow). Photographs by MB and RJW.

All specimens had identical nucleotide sequences. A single consensus sequence was formed. Results confirmed that the specimens were *Ae. aegypti*. The nucleotide sequence had 99.64% homology to a partial cytochrome oxidase subunit I (COI) gene from an isolate of *Ae. aegypti* (GenBank, Accession number: MK300221.1). When the nucleotide sequence was translated to a protein sequence using a compensatory invertebrate mitochondrial genetic code, the translated sequence had 100% homology to the cytochrome oxidase subunit I protein of *Ae. aegypti*.

3.3.2. Mosquito community survey from the sub-districts

A total of 1013 mosquito samples were collected across the three sub-districts sampled. Of these, ten species from 4 genera (*Aedes*, *Anopheles*, *Culex*, *Culiseta*) were identified in either adult, larval stage, and/or both (Table 3.1). Aedine species identified similar to the partial COI gene isolate were *Ae. vittatus* and *Ae. aegypti* with the nucleotide sequence homology of 99.4 and 99.64% respectively. *Aedes aegypti* were identified and reported in all sub-districts however, *Ae. vittatus* was only found in Bobirwa. The anopheline species were identified as *An. rhodesiensis* (93%), *An. quadriannulatus* (98.51%), *An. gambiae* sensu stricto (s.s) (98.95%), *An. rufipes* (95.53%) and an unidentified *Anopheles* sp. with homology of 100%. Bobirwa had one anopheline species (*An. quadriannulatus*) as compared to more species reported for the non-endemic sub-districts of Serowe (*An. quadriannulatus* and *An. rufipes*) and Palapye (*An. rhodesiensis*, *An. gambiae* s. s and *Anopheles* sp.). Thus, Palapye documented the highest number of anopheline spp. from sampled sub-districts. *Culex* mosquito species identified were *Cx. pipiens* and *Cx. sinaiticus* with 99.85 and 99.23% homology respectively while *Cs. longiareolata* were identified at homology of 96.59% (Table 3.1). Mosquito species identified as adults were *Ae. aegypti*, *Ae vittatus*, *Cx. sinaiticus* and *Cx pipiens*. For the larval samples, *Ae. aegypti*, *Ae. vittatus*, *An. rhodesiensis*, *An. quadriannulatus*, *An. gambiae* s. s, *An. rufipes*, *Anopheles* sp., *Cx. sinaiticus*, *Cx. pipiens* and *Cs. longiareolata* were identified. Overall, larval sampling yielded more species and genera (*Aedes*, *Anopheles*, *Culex* and *Culiseta*) compared to adult (*Aedes* and *Culex*) collection. Subsequently, the sequences of all species identified here, were deposited in the Genbank and accession numbers confirmed for each species (Table 3.1).

Table 3. 1. A summary of mosquito species identification information as sampled from across endemic and non-endemic malaria districts.

Mosquito species	Accession No	Homology ID	ID %
<i>Aedes vittatus</i>	MT741508	MG242527.1	99.4
<i>Culex sinaiticus</i>	MT741509	KU380450.1	99.23
<i>Culex pipiens</i>	MT741514	MK300247.1	99.85
<i>Anopheles quadriannulatus</i>	MT741513	DQ792581.1	98.51
<i>Anopheles gambiae</i> sensu stricto	MT741510	MK300234.1	98.95
<i>Anopheles</i> sp.	MT741511	MF372931.1	100
<i>Culiseta longiareolata</i>	MT741512	JQ388785.1	96.59
<i>Anopheles rhodesiensis</i>	MT830930	MK047667.1	93.00
<i>Aedes aegypti</i>	MK571449	MK300221.1	99.64
<i>Anopheles rufipes</i>	MT741515	MK586028.1	95.53

Out of the 1013 total mosquito samples, Bobirwa had the highest number of mosquitoes (429) while Serowe produced the least (228), with 356 individuals collected from Palapye (Table 3.2). A total of 4, 5 and 6 different species were reported in Serowe, Bobirwa and Palapye respectively (Table 3.2). The highest species richness was reported for Palapye (0.8511), the least Serowe (0.5526) while Bobirwa was intermediate (0.6599) (Table 3.2). Palapye exhibited the highest species diversity (1.304), while Serowe reported the least (0.7554) with Bobirwa intermediate (0.9433). The highest evenness was observed for Palapye while both Bobirwa and Serowe reported the least species evenness (Table 3.2).

Table 3. 2. Summary of community diversity measures from samples of communities in the Bobirwa, Palapye and Serowe sub-districts. S = total species number; N = total number of individuals; d = Margalef's species richness; H' = species diversity and J' = Pielou's evenness.

	S	N	d	H'	J'
Bobirwa	5	429	0.6599	0.9433	0.5861
Palapye	6	356	0.8511	1.304	0.7281
Serowe	4	228	0.5526	0.7554	0.5449

Average pairwise dissimilarity between Bobirwa and Serowe was 31.8 %, Bobirwa and Palapye was 30.7 % while Palapye and Serowe dissimilarity was 27.7% (Table 3.3). The dominant species contributing to dissimilarity between Bobirwa and Palapye was *Cx. pipiens* at 40.3%. Similarly, *Cx. pipiens* contributed to dissimilarity (59.8%) between Bobirwa and Serowe. For Palapye and Serowe sub-districts, *Ae. aegypti* contributed to the dissimilarity (21%) (Table 3.3). Overall, Palapye and Serowe (non-endemic) exhibited similar level of resemblance as opposed to Bobirwa (malaria endemic) (Figure 3.3).

Table 3. 3. SIMPER test results showing the level of pairwise dissimilarities between sub-districts.

Districts	Average dissimilarity (%)	Dominant dissimilarity Contributing species	Dominant dissimilarity contributions (%)
Bobirwa vs Palapye	30.7	<i>Culex pipiens</i>	40.3
		<i>Anopheles quadriannulatus</i>	17.8
		<i>Culiseta longiareolata</i>	11.2
		<i>Anopheles gambiae</i> s. s	10.8
Bobirwa vs Serowe	31.8	<i>Culex pipiens</i>	59.8
		<i>Aedes aegypti</i>	15.8
		<i>Culiseta longiareolata</i>	14.4
		<i>Culex sinaiticus</i>	4.3
Palapye vs Serowe	27.7	<i>Aedes aegypti</i>	21.0
		<i>Culex pipiens</i>	17.3
		<i>Culiseta longiareolata</i>	16.7
		<i>Anopheles rhodesiensis</i>	16.1

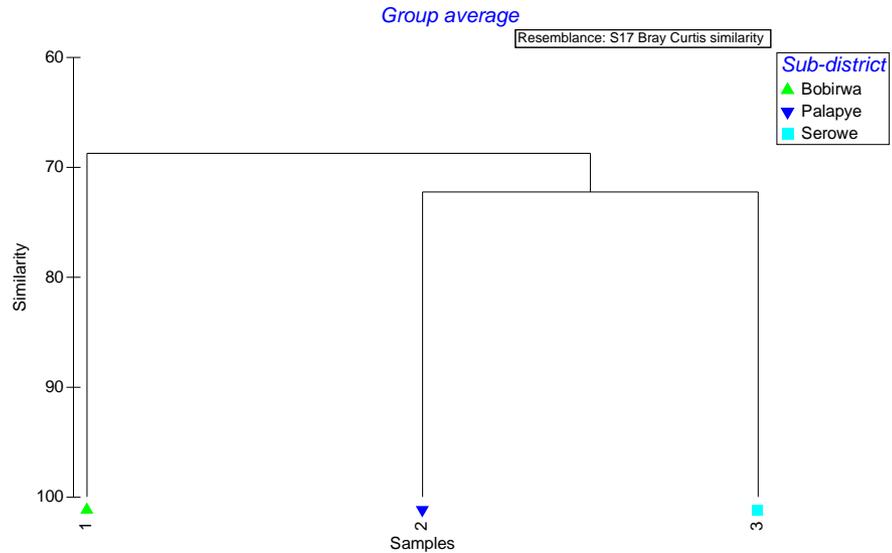


Figure 3. 3. Dendrogram outline the level of mosquito community similarity between sub-districts (Bobirwa, Palapye, Serowe) across a malaria endemicity gradient. The dendrogram was constructed using by creating a Euclidean distance similarity matrix. Data used was for both larvae and adult samples collected.

For the adults sampled, the abundances were significantly different for each species across sub-districts (Figure 3.4; Table 3.4). Bobirwa exhibited the highest abundances of *Cx. pipiens* mosquitoes compared to the Serowe and Palapye. In addition, neither sex or districts were statistically significant factors (Table 3.4). Similarly, the interaction between Sex \times Species, Sex \times District, Species \times District and Species \times Sex \times District were not significant (Table 3.4).

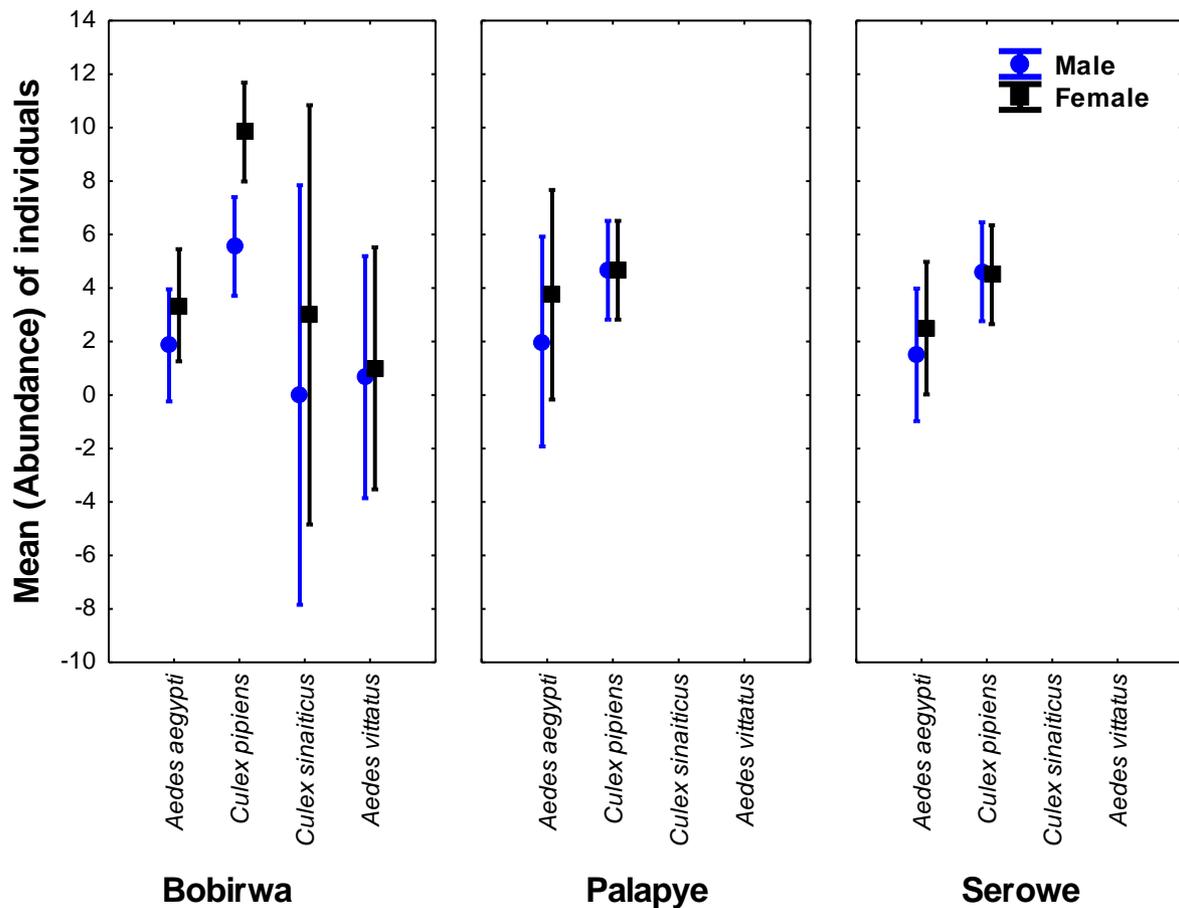


Figure 3. 4. Mean \pm 95% confidence dominant mosquito abundances (*Aedes aegypti*, *Culex pipiens*, *Culex sinaiticus*, *Aedes vittatus*) across malaria endemic (Bobirwa) and non-endemic (Palapye, Serowe) sub-districts.

Table 3. 4. A summary of factorial ANOVA showing mosquito abundances across malaria endemic (Bobirwa) and non-endemic sub-districts (Serowe and Palapye).

Effect	SS	df	MS	F	P
Sex	54.22	1	54.22	2.845	= 0.094
Species	302.7	1	302.7	19.2	< 0.001
District	55.75	2	27.87	1.462	= 0.235
Sex \times Species	2.9	3	1.0	0.057	= 0.982
Sex \times District	67.17	2	33.58	1.762	= 0.175
Species \times District	70.2	2	35.1	2.23	= 0.111
Species \times Sex \times District	37.1	2	18.5	1.18	= 0.311

3.4. Discussion

Although anecdotally reported from the region, no published collection information on *Aedes aegypti* from Botswana was found. Here, the preliminary survey presented *Aedes aegypti* for the first time in Palapye village and the country. Although the distribution of *Ae. aegypti* has been studied in Africa and globally (Kraemer *et al.*, 2015; Ngoagouni *et al.*, 2017), surveillance of the species remains essential given its invasion potential. Modelling of the vector species and associated arboviral infections demonstrated the capacity for geographic range expansion through correlation with environmental niches and current global change (Tjaden *et al.*, 2017). Recently, southern Africa produced new reports of *Ae. aegypti* in Namibia, Zambia, Angola and Mozambique (Weetman *et al.*, 2018). In particular, Namibia, Angola and South Africa, have close proximity to the neighbouring Botswana with potential risks of cross-border invasion. However, the origins of *Ae. aegypti* in Botswana are not yet known. The presence of *Ae. aegypti* in Botswana and its formal documentation in the primary literature, contributes to its current global re-distribution and mapping under climate change scenarios.

The survey across other sub-districts (Central district) revealed that the highest number of mosquitoes were from malaria endemic area (Bobirwa) although the species richness was highest in a non-endemic sub-district (Palapye). This study also showed that *Culex* spp. were significantly more abundant than other mosquito species sampled. Anopheline species instead, dominated in the non-malaria endemic sub-districts while the malaria endemic Bobirwa reported only one Anopheline species (*An. quadriannulatus*). The sub-districts bordering South Africa (Bobirwa and Palapye) as well as Serowe, which is further way, reported invasive *Aedes aegyptii*. *Aedes vittatus* was, however, only reported from Bobirwa. Overall, the abundances of male and female mosquitoes were the same across sub-districts. Key mosquito taxa (*Aedes*,

Anopheles and *Culex*) were recorded across all the sub-districts with *Culiseta* spp. reported only in Palapye. The species reported here, have vectorial capacity for infectious diseases such as dengue, yellow fever, Zika, chikungunya (*Aedes*), malaria (*Anopheles*) and Rift Valley, West Nile fevers, filariasis, various encephalitis (*Culex*) and avian malaria (*Culiseta* and *Culex*) (Braack *et al.*, 2018; Lee *et al.*, 2018; Ralapanawa and Kularatne 2020). The presence of these incriminating vectors represents a significant threat to biosecurity in the District. In particular, if associated pathogens and parasites are present in the region, this may threaten the health of the affected populations through associated vector-borne disease burdens (Ewen *et al.*, 2012).

The three districts reported *Ae. aegypti*, a novel invasive species mainly across tropical and sub-tropical regions and originally from West Africa (Powell *et al.*, 2018). The species colonises artificial water holding systems or microenvironments (e.g., used tyres and tins) at disposal of human habitation (Powell and Tabachnick 2013). Although *Ae. aegypti* was sampled from homesteads of rural-urban lifestyle, the species has never been assessed for transmission of pathogens to humans in the country. However, future studies are recommended to explore pathogen incrimination to assess the risk of infection. Currently, it is distributed worldwide (e.g., Díez-Fernández *et al.*, 2018) as an invasive species (Alarcón-Elbal *et al.*, 2020) breeding in a variety of habitats (e.g., rock pools, artificial containers, tree holes) (Eshag *et al.*, 2019). The species has the potential to spread a wide variety of arboviruses to humans, livestock and wildlife (Díez-Fernández *et al.*, 2018). With regard to its role as an emerging threat to public health (see Sudeep and Shil 2017), a fine-scale distribution of the species countrywide and regionally, is highly warranted. Botswana is a tourism hotspot contributing to the country's second largest economic sector (e.g., ~8.5% of GDP in 2014) after diamonds, receiving an increasing influx of

tourists annually (see Mbaiwa 2017). Should tourists arrive from regions where dengue and other *Ae. aegypti* associated diseases are prevalent, there is a chance of importing these pathogens and parasites. The presence of healthy populations of *Ae. aegypti* should be a concern for authorities. *Aedes vittatus* was also reported in the endemic Bobirwa sub-district, although in fewer abundances compared to the invasive congeneric *Ae. aegypti*.

The study further reported *Cx. pipiens* as the most abundant and widely distributed species from sampled areas, congruent with Karki *et al.* (2016). This may be mainly due to the ability of the species to colonise various habitats of diverse water quality (Geoffrey *et al.*, 2017; Amusan and Ogbogu 2020), making them near omnipresent. Although environmental parameters play a crucial role in *Cx. pipiens* development and growth (Pachka *et al.*, 2016), life stage physiological traits (e.g., aestivation and diapause) and seasonal variability (Ewing *et al.*, 2019) promoting population exacerbation is less explored in the country. Despite the vectorial role of *Cx. pipiens* in humans (Mavridis *et al.*, 2018) and livestock (Pachka *et al.*, 2016) transmission cycles, it is a key carrier of avian malaria parasite (e.g., *Plasmodium* and *Haemoproteus*) (Schoener *et al.*, 2019). To this end, no study on the local context has explored host-parasite interaction to ascertain the role of *Cx. pipiens* in avian vertebrate host species. This is crucial since the country benefits from wildlife through tourism schemes. In addition, *Cs. longiareolata* reported here, is associated with the spread of avian malaria parasites (see Brahim *et al.*, 2019). Botswana is a country well-known for its wetlands of importance to migratory bird species (Ramberg *et al.*, 2006) likely to translocate parasites and pathogens in time and space. Therefore, there it is essential to disentangle and understand the functioning of the tripartite ecological interaction in curbing emerging and reemerging disease associated with wildlife.

Anopheles spp. were reported from all districts regardless of the malaria endemicity status. This has potential for the burden of malaria (Janko *et al.*, 2018), to further extend to the non-endemic areas given the possibility of parasite introduction. Moreso, *Anopheles* spp. were reported in Palapye (*An. gambiae* s. s, *An. rhodesiensis*, *Anopheles* sp.) and Serowe (*An. rufipes*, *An. quadriannulatus*), both formally designated as non-malarious zones. The prevalence of *An. rufipes* and *An. quadriannulatus* reported here, confirm previous studies documenting the same species in the Central district (Tawe *et al.*, 2017; Bango *et al.*, 2020). Although *An. quadriannulatus* is regarded non-malaria vector (Lobo *et al.*, 2008), in the current study, it was sampled along the river near homesteads, likely to exploit human habitation for dietary and resting needs, in corroboration with reports by Pates *et al.* (2006). This has implications for its current susceptibility status to *Plasmodium* parasites (see Takken *et al.*, 1999). The study further reports unidentified *Anopheles* sp. in non-endemic area (Palapye). This warrants taxonomic classification and investigation regarding vector incrimination. In the country, the malaria pathogen (*Plasmodium*) is mainly vectored by *An. arabiensis* (Tawe *et al.*, 2017) which was not reported although other studies documented the species within the Central district and elsewhere in the country (see Abdulla-Khan 1998; Bango *et al.*, 2020; Kgorobutswe *et al.*, 2020).

The species abundances varied across the sub-districts in the current study with *Cx. pipiens* dominated the dissimilarity. In particular, *Cx. pipiens* abundances were high in the malaria endemic area. Although seasonal abundance and fine scale distribution of this species is not investigated in Bobirwa, it remains a threat not only as a biting nuisance but also as a potential disease vector in the region. The study also highlights that mosquito sex ratios are similar across the sub-districts. However, other studies have demonstrated a skewed collection of significantly

high numbers of females compared to males mainly associated with trapping methodology (McPhatter and Gerry 2017).

In conclusion, this basic mosquito diversity survey of human inhabited areas outlined the presence of key vector mosquito taxa (*Aedes*, *Anopheles*, *Culex*) across all sampled districts regardless of malaria endemicity status, including the presence of a previously unrecorded invasive species (*Ae. aegypti*). More mosquito species were sampled from aquatic habitats (during early life stages) than were adults in the terrestrial environment, highlighting the importance of the incorporation of mosquito breeding sites into monitoring programmes. Surveillance of mosquito species remains essential in the Central district to strengthen vector management and reduce disease spread. Therefore, more varied mosquito sampling techniques and analysis are recommended to enhance effective collection and representation of numerous species of public health concern. In addition, further studies should focus on sampling across differential seasonality, space and diverse habitats.

3.5. References

- Abdulla-Khan, R. (1998). *A survey of the Anopheline mosquito fauna of Botswana, with special reference to the malaria vectors* (Doctoral dissertation). University of the Witwatersrand, Johannesburg, South Africa.
- Ahmed, A. M., Mohammed, A. T., Vu, T. T., Khattab, M., Doheim, M. F., Ashraf Mohamed, A., and Kassem, M. A. (2020). Prevalence and burden of dengue infection in Europe: A systematic review and meta-analysis. *Reviews in Medical Virology*, 30: e2093.
- Akiner, M. M., Demirci, B., Babuadze, G., Robert, V., and Schaffner, F. (2016). Spread of the invasive mosquitoes *Aedes aegypti* and *Aedes albopictus* in the Black Sea region increases

- risk of chikungunya, dengue, and Zika outbreaks in Europe. *PLoS Neglected Tropical Diseases*, 10: e0004664.
- Alarcón-Elbal, P. M., Rodríguez-Sosa, M. A., Newman, B. C., and Sutton, W. B. (2020). The First Record of *Aedes vittatus* (Diptera: Culicidae) in the Dominican Republic: Public Health Implications of a Potential Invasive Mosquito Species in the Americas. *Journal of Medical Entomology*. tjaa128, <https://doi.org/10.1093/jme/tjaa128>.
- Alves, J., Gomes, B., Rodrigues, R., Silva, J., Arez, A. P., Pinto, J., and Sousa, C. A. (2010). Mosquito fauna on the Cape Verde Islands (West Africa): an update on species distribution and a new finding. *Journal of Vector Ecology*, 35: 307-312.
- Amusan, B., and Ogbogu, S. (2020). Surveillance of mosquito larvae in various microhabitats in a University Campus in Southwestern Nigeria. *UNED Research Journal*, 12: e2605-e2605.
- Atoni, E., Zhao, L., Karungu, S., Obanda, V., Agwanda, B., Xia, H., and Yuan, Z. (2019). The discovery and global distribution of novel mosquito-associated viruses in the last decade (2007-2017). *Reviews in Medical Virology*, 29: e2079.
- Bango, Z. A., Tawe, L., Muthoga, C. W., and Paganotti, G. M. (2020). Past and current biological factors affecting malaria in the low transmission setting of Botswana: A review. *Infection, Genetics and Evolution*, 85: 104458.
- Biteye, B., Fall, A. G., Ciss, M., Seck, M. T., Apolloni, A., Fall, M., and Gimonneau, G. (2018). Ecological distribution and population dynamics of Rift Valley fever virus mosquito vectors (Diptera, Culicidae) in Senegal. *Parasites and Vectors*, 11: 27.
- Boyer, S., Marcombe, S., Yean, S., and Fontenille, D. (2020). High diversity of mosquito vectors in Cambodian primary schools and consequences for arbovirus transmission. *PloS one*, 15: e0233669.
- Braack, L., de Almeida, A. P. G., Cornel, A. J., Swanepoel, R., and De Jager, C. (2018). Mosquito-borne arboviruses of African origin: review of key viruses and vectors. *Parasites and Vectors*, 11: 29.

- Brahim, M., Manel, M., Imen, M., and Laid, O. M. (2019). Responses of the four larval stages (11 to 14) of the avian malaria vector *Culiseta longiareolata* exposed to spinosad (naturally derived insecticide). *Advances in Animal and Veterinary Sciences*, 7: 599-603.
- Cane, R. P., Hartley, S., Gradwell, B., and Singe, M. (2018). Spatial and temporal distribution, environmental drivers and community structure of mosquitoes in the Kaipara Harbour, New Zealand. *Bulletin of Entomological Research*, 108: 305-313.
- Chirebvu, E., and Chimbari, M. J. (2015). Characteristics of *Anopheles arabiensis* larval habitats in Tubu village, Botswana. *Journal of Vector Ecology*, 40: 129-138.
- Coetzee, M., Craig, M., and Le Sueur, D. (2000). Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitology Today*, 16: 74-77.
- Cornel, A. J., Lee, Y., Almeida, A. P. G., Johnson, T., Mouatcho, J., Venter, M., and Braack, L. (2018). Mosquito community composition in South Africa and some neighboring countries. *Parasites and Vectors*, 11: 1-12.
- Díez-Fernández, A., Martínez-de la Puente, J., Ruiz, S., Gutiérrez-López, R., Soriguer, R., and Figuerola, J. (2018). *Aedes vittatus* in Spain: current distribution, barcoding characterization and potential role as a vector of human diseases. *Parasites and Vectors*, 11: 1-6.
- El-Sayed, A., and Kamel, M. (2020). Climatic changes and their role in emergence and re-emergence of diseases. *Environmental Science and Pollution Research International*, 27: 22336-22352.
- Eshag, O. S., Bashir, N. H., and Dukeen, M. Y. (2019). Prevalence, habitat and productivity profiles of *Aedes* mosquitoes (Diptera: Culicidae) in Sennar state, Sudan. *International Journal of Mosquito Research*, 6: 102-108.
- Ewen, J. G., Bensch, S., Blackburn, T. M., Bonneaud, C., Brown, R., Cassey, P., and Pérez-Tris, J. (2012). Establishment of exotic parasites: the origins and characteristics of an avian malaria community in an isolated island avifauna. *Ecology Letters*, 15: 1112-1119.

- Ewing, D. A., Purse, B. V., Cobbold, C. A., Schäfer, S. M., and White, S. M. (2019). Uncovering mechanisms behind mosquito seasonality by integrating mathematical models and daily empirical population data: *Culex pipiens* in the UK. *Parasites and Vectors*, 12: 1-19.
- Fălciuță, E., Prioteasa, L. F., Horváth, C., Păstrav, I. R., Schaffner, F., and Mihalca, A. D. (2020). The invasive Asian tiger mosquito *Aedes albopictus* in Romania: towards a country-wide colonization? *Parasitology Research*, 119: 841–845.
- Ferguson, H. M., Dornhaus, A., Beeche, A., Borgemeister, C., Gottlieb, M., Mulla, M. S., and Killeen, G. F. (2010). Ecology: a prerequisite for malaria elimination and eradication. *PLoS Medicine*, 7: e1000303.
- Foster, W. A., and Walker, E. D. (2019). Mosquitoes (Culicidae). In *Medical and Veterinary Entomology* (pp. 261-325). Academic press.
- Geoffrey, K. K., Ngure, N. V., Kamau, L., Bet, D., Lugali, R., Wangila, A., and Kiarie, W. M. (2017). Survey of urban mosquitoes species (Diptera: Culicidae) with focus on waste water channels as larval habitats in Nairobi industrial area, Kenya. *African Journal of Health Sciences*, 30: 120-138.
- Gillett, J. D. (1972). *Common African mosquitos and their medical importance*. London, UK, William Heinemann.
- Gillies, M. T., and M. Coetzee. (1987). A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical region). *South African Institute for Medical Research*. 1987: 55.
- Habtewold, T., Povelones, M., Blagborough, A. M., and Christophides, G. K. (2008). Transmission blocking immunity in the malaria non-vector mosquito *Anopheles quadriannulatus* species A. *PLoS Pathogens*, 4: e1000070.
- Janko, M. M., Irish, S. R., Reich, B. J., Peterson, M., Doctor, S. M., Mwandagalirwa, M. K., and Emch, M. E. (2018). The links between agriculture, *Anopheles* mosquitoes, and malaria risk in children younger than 5 years in the Democratic Republic of the Congo: a population-based, cross-sectional, spatial study. *The Lancet Planetary Health*, 2: e74-e82.
- Jayakrishnan, L., Sudhikumar, A. V., and Aneesh, E. M. (2018). Role of gut inhabitants on vectorial capacity of mosquitoes. *Journal of Vector Borne Diseases*, 55: 69.

- Johnson, T., Braack, L., Guarido, M., Venter, M., and Gouveia Almeida, A. P. (2020). Mosquito community composition and abundance at contrasting sites in northern South Africa, 2014–2017. *Journal of Vector Ecology*, 45: 104-117.
- Jupp, P. G. (2005). Mosquitoes as vectors of human disease in South Africa. *South African Family Practice*, 47: 68-72.
- Jupp, P. G., and McIntosh, B. M. (1990). *Aedes furcifer* and other mosquitoes as vectors of chikungunya virus at Mica, northeastern Transvaal, South Africa. *Journal of the American Mosquito Control Association*, 6: 415-420.
- Kahamba, N. F., Limwagu, A. J., Mapua, S. A., Msugupakulya, B. J., Msaky, D. S., Kaindoa, E. W., and Okumu, F. O. (2020). Habitat characteristics and insecticide susceptibility of *Aedes aegypti* in the Ifakara area, south-eastern Tanzania. *Parasites and Vectors*, 13: 53.
- Kampen, H., Schuhbauer, A., and Walther, D. (2017). Emerging mosquito species in Germany—a synopsis after 6 years of mosquito monitoring (2011–2016). *Parasitology Research*, 116: 3253-3263.
- Karki, S., Hamer, G. L., Anderson, T. K., Goldberg, T. L., Kitron, U. D., Krebs, B. L., and Ruiz, M. O. (2016). Effect of trapping methods, weather, and landscape on estimates of the *Culex* vector mosquito abundance. *Environmental Health Insights*, 10: EHI-S33384.
- Kemp, A., and Jupp, P. G. (1991). Potential for dengue in South Africa: mosquito ecology with particular reference to *Aedes aegypti*. *Journal of the American Mosquito Control Association*, 7: 574-583.
- Kgoroebutswe, T. K., Ramatlho, P., Reeder, S., Makate, N., and Paganotti, G. M. (2020). Distribution of *Anopheles* mosquito species, their vectorial role and profiling of knock-down resistance mutations in Botswana. *Parasitology Research*, 119: 1201-1208.
- Kraemer, M. U., Sinka, M. E., Duda, K. A., Mylne, A. Q., Shearer, F. M., Barker, C. M., and Hendrickx, G. (2015). The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *Elife* 4: e08347.

- Lee, J. M., Wasserman, R. J., Gan, J. Y., Wilson, R. F., Rahman, S., and Yek, S. H. (2020). Human activities attract harmful mosquitoes in a tropical urban landscape. *EcoHealth*, 17: 52-63.
- Lee, H., Halverson, S., and Ezinwa, N. (2018). Mosquito-borne diseases. *Primary Care: Clinics in Office Practice*, 45: 393-407.
- Lemine, A. M. M., Lemrabott, M. A. O., Ebou, M. H., Lekweiry, K. M., Salem, M. S. O. A., Brahim, K. O., and Basco, L. (2017). Mosquitoes (Diptera: Culicidae) in Mauritania: a review of their biodiversity, distribution and medical importance. *Parasites and Vectors*, 10: 35.
- Liu-Helmersson, J., Rocklöv, J., Sewe, M., and Brännström, Å. (2019). Climate change may enable *Aedes aegypti* infestation in major European cities by 2100. *Environmental Research*, 172: 693-699.
- Ludwig, A., Zheng, H., Vrbova, L., Drebot, M. A., Iranpour, M., and Lindsay, L. R. (2019). Increased risk of endemic mosquito-borne diseases with climate change. *Canada Communicable Disease Report*, 45: 90–97.
- Makanda, M., Kemunto, G., Wamuyu, L., Bargul, J., Muema, J., and Mutunga, J. (2019). Diversity and Molecular Characterization of Mosquitoes (Diptera: Culicidae) in selected ecological regions in Kenya. *F1000Research*, 8: 262.
- Martinet, J. P., Ferté, H., Failloux, A. B., Schaffner, F., and Depaquit, J. (2019). Mosquitoes of North-Western Europe as potential vectors of arboviruses: a review. *Viruses*, 11: 1059.
- Masaninga, F., Muleba, M., Masendu, H., Songolo, P., Mweene-Ndumba, I., Mazaba-Liwewe, M. L., and Babaniyi, O. A. (2014). Distribution of yellow fever vectors in Northwestern and Western Provinces, Zambia. *Asian Pacific Journal of Tropical Medicine*, 7: S88-S92.
- Matowo, N. S., Abbasi, S., Munhenga, G., Tanner, M., Mapua, S. A., Oullo, D., and Utzinger, J. (2019). Fine-scale spatial and temporal variations in insecticide resistance in *Culex pipiens* complex mosquitoes in rural south-eastern Tanzania. *Parasites and Vectors*, 12: 413.

- Mavridis, K., Fotakis, E. A., Kioulos, I., Mpellou, S., Konstantas, S., Varela, E., and Vontas, J. (2018). Detection of West Nile Virus–Lineage 2 in *Culex pipiens* mosquitoes, associated with disease outbreak in Greece, 2017. *Acta Tropica*, 182: 64-68.
- Mbaiwa, J. E. (2017). Poverty or riches: who benefits from the booming tourism industry in Botswana? *Journal of Contemporary African Studies*, 35: 93-112.
- McPhatter, L., and Gerry, A. C. (2017). Effect of CO₂ concentration on mosquito collection rate using odor-baited suction traps. *Journal of Vector Ecology*. 42: 44-50.
- Monaghan, A. J., Sampson, K. M., Steinhoff, D. F., Ernst, K. C., Ebi, K. L., Jones, B., and Hayden, M. H. (2018). The potential impacts of 21st century climatic and population changes on human exposure to the virus vector mosquito *Aedes aegypti*. *Climatic Change*, 146: 487-500.
- Mouatassef, T. F., El Ouali Lalami, A., Faraj, C., Rais, N., and Guemmouh, R. (2020). Study of Abiotic and Biotic Parameters Affecting the Abundance of Mosquito Larvae (Diptera: Culicidae) in the Region of Fez (Morocco). *International Journal of Zoology*, 2020.
- Mroz, C., Gwida, M., El-Ashker, M., El-Diasty, M., El-Beskawy, M., Ziegler, U., and Groschup, M. H. (2017). Seroprevalence of Rift Valley fever virus in livestock during inter-epidemic period in Egypt, 2014/15. *BMC Veterinary Research*, 13: 87.
- Ngoagouni, C., Kamgang, B., Kazanji, M., Paupy, C., and Nakouné, E. (2017). Potential of *Aedes aegypti* and *Aedes albopictus* populations in the Central African Republic to transmit enzootic chikungunya virus strains. *Parasites and Vectors*, 10: 164.
- Njabo, K. Y., Cornel, A. J., Sehgal, R. N., Loiseau, C., Buermann, W., Harrigan, R. J., and Smith, T. B. (2009). Coquillettidia (Culicidae, Diptera) mosquitoes are natural vectors of avian malaria in Africa. *Malaria Journal*, 8: 193.
- Noden, B. H., Musuuu, M., Aku-Akai, L., van der Colf, B., Chipare, I., and Wilkinson, R. (2014). Risk assessment of flavivirus transmission in Namibia. *Acta tropica*, 137, 123-129.
- Ogunmodede, A. F. (2020). Mosquitoes and their Medical Importance. *Western Journal of Medical and Biomedical Sciences*, 1: 115-120.

- Pachka, H., Annelise, T., Alan, K., Power, T., Patrick, K., Véronique, C., and Ferran, J. (2016). Rift Valley fever vector diversity and impact of meteorological and environmental factors on *Culex pipiens* dynamics in the Okavango Delta, Botswana. *Parasites and Vectors*, 9: 434.
- Pates, H. V., Takken, W., Curtis, C. F., and Jamet, H. (2006). Zoophilic *Anopheles quadriannulatus* species B found in a human habitation in Ethiopia. *Annals of Tropical Medicine and Parasitology*, 100: 177-179.
- Pereira dos Santos, T., Roiz, D., Santos de Abreu, F. V., Luz, S. L. B., Santalucia, M., Jiolle, D., and Paupy, C. (2018). Potential of *Aedes albopictus* as a bridge vector for enzootic pathogens at the urban-forest interface in Brazil. *Emerging Microbes and Infections*, 7: 1-8.
- Powell, J. R., Gloria-Soria, A., and Kotsakiozi, P. (2018). Recent history of *Aedes aegypti*: Vector genomics and epidemiology records. *Bioscience*, 68: 854-860.
- Powell, J. R., and Tabachnick, W. J. (2013). History of domestication and spread of *Aedes aegypti*-A Review. *Memórias do Instituto Oswaldo Cruz*, 108: 11-17.
- Ralapanawa, U., and Kularatne, S. (2020). *Aedes*-One Mosquito Species, A Few Serious Diseases. *SF Internal Medicine*, 1: 1002.
- Ramberg, L., Hancock, P., Lindholm, M., Meyer, T., Ringrose, S., Sliva, J., and Vander Post, C. (2006). Species diversity of the Okavango Delta, Botswana. *Aquatic Sciences*, 68: 310-337.
- Schoener, E. R., Harl, J., Himmel, T., Fragner, K., Weissenböck, H., and Fuehrer, H. P. (2019). Protozoan parasites in *Culex pipiens* mosquitoes in Vienna. *Parasitology Research*, 118: 1261-1269.
- Schrama, M., Hunting, E. R., Beechler, B. R., Guarido, M. M., Govender, D., Nijland, W., and Gorsich, E. E. (2020). Human practices promote presence and abundance of disease-transmitting mosquito species. *Scientific Reports*, 10: 1-6.

- Strickman, D. (2020). Invasive Mosquito Species and Potential Introductions. In *Mosquitoes, Communities, and Public Health in Texas* (pp. 307-315). Academic Press, <https://doi.org/10.1016/B978-0-12-814545-6.00010-9>.
- Sudeep, A. B., and Shil, P. (2017). *Aedes vittatus* (Bigot) mosquito: An emerging threat to public health. *Journal of Vector-borne Diseases*, 54: 295.
- Takken, W., Eling, W., Hooghof, J., Dekker, T., Hunt, R., and Coetzee, M. (1999). Susceptibility of *Anopheles quadriannulatus theobald* (Diptera: Culicidae) to *Plasmodium falciparum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 93: 578-580.
- Tawe, L., Ramatlho, P., Waniwa, K., Muthoga, C. W., Makate, N., Ntebela, D. S., and Paganotti, G. M. (2017). Preliminary survey on *Anopheles* species distribution in Botswana shows the presence of *Anopheles gambiae* and *Anopheles funestus* complexes. *Malaria Journal*, 16: 1-7.
- Tjaden, N. B., Suk, J. E., Fischer, D., Thomas, S. M., Beierkuhnlein, C., and Semenza, J. C. (2017). Modelling the effects of global climate change on Chikungunya transmission in the 21st century. *Scientific Reports*, 7: 3813.
- Vaux, A. G. C., Dallimore, T., Cull, B., Schaffner, F., Strode, C., Pflüger, V., and Catton, M. (2019). The challenge of invasive mosquito vectors in the UK during 2016-2018: a summary of the surveillance and control of *Aedes albopictus*. *Medical and Veterinary Entomology*, 33: 443-452.
- Wang, Y., Yim, S. H. L., Yang, Y., and Morin, C. W. (2020). The effect of urbanization and climate change on the mosquito population in the Pearl River Delta region of China. *International Journal of Biometeorology*, 64: 501-512.
- Weetman, D., Kamgang, B., Badolo, A., Moyes, C., Shearer, F., Coulibaly, M., and McCall, P. (2018). *Aedes* mosquitoes and *Aedes*-borne arboviruses in Africa: Current and future threats. *International Journal of Environmental Research and Public Health*, 15: 220.
- Weyl, O. L. F., Ellender, B. R., Wasserman, R. J., Truter, M., Dalu, T., Zengeya, T., and Smit, N. (2019). *Alien Freshwater Fauna in South Africa*. In Measy J, van Wilgen B (Eds) *South African Biological Invasions and their Management*. (pp. 153-183). Springer, Cham.

- Wiebe, A., Longbottom, J., Gleave, K., Shearer, F. M., Sinka, M. E., Massey, N. C., and Smith, D. L. (2017). Geographical distributions of African malaria vector sibling species and evidence for insecticide resistance. *Malaria Journal*, 16: 85.
- Wilke, A. B., Benelli, G., and Beier, J. C. (2020). Beyond frontiers: On invasive alien mosquito species in America and Europe. *PLoS Neglected Tropical Diseases*, 14: e0007864.
- Zengenene, M. P., Soko, W., Brooke, B. D., Koekemoer, L. L., Govere, J., Mazarire, T. T., and Munhenga, G. (2020). *Anopheles* species composition and breeding habitat characterisation in Chiredzi District, Zimbabwe. *African Entomology*, 28: 84-94.

CHAPTER 4

Cattle-induced eutrophication favours disease-vector mosquitoes

Published as: **Buxton, M.**, Cuthbert, R. N., Dalu, T., Nyamukondiwa, C., and Wasserman, R. J.

(2020). Cattle-induced eutrophication favours disease-vector mosquitoes. *Science of the Total Environment*, 715: 136952.

4.1. Introduction

Livestock, particularly cattle, play a significant global economic and socio-cultural role in the livelihoods of farmers, especially those based in rural settlements (Hebinck and Faku 2013). Over the years, cattle production has improved human population wellbeing as a source of income, food supply, employment and a sustainable agricultural diversification model (Enahoro *et al.*, 2018). Moreover, livestock is often used as a ‘safety net’ and buffers farmers in the event of crop production failure (Tolera and Abebe 2007). However, despite the value of cattle in small-to-large scale farming communities, there is limited consideration for subsequent direct and indirect ecosystem disservices by both free-range (communal) and fenced area cattle production (Lange *et al.*, 1998). Studies that have assessed environmental degradation associated with cattle are largely restricted to terrestrial landscapes, dealing predominantly with overgrazing and its effects (Stavi *et al.*, 2016). However, the implications of cattle on aquatic ecosystem degradation are less well-known.

In many arid regions around the world, aquatic landscapes are characterised by both semi-permanent and temporary wetland systems (Arntzen 2016). Temporary wetland systems are typically characterised by a unique assemblage of invertebrates (Bird *et al.*, 2019). Within landscapes that contain cattle, however, these systems typically serve as watering points, particularly following intermittent rainfall periods (Scoones 1991). The known propensity for cattle to urinate and defecate in bodies of water while drinking (Mesa *et al.*, 2015) may have consequences for aquatic faunal groups and nutrient input dynamics in these ecosystems, with concurrent implications for mosquito pest species that utilise these environments as breeding sites (Batzer and Boix 2016). Although the duration of hydroperiods in these habitats may be a

limiting factor to many species, mosquitoes are well adapted to utilise such habitats given their rapid development, particularly under nutrient-rich conditions (Marinho *et al.*, 2016). While climate-mediated factors have been explored within the context of mosquito ecology (Asigau and Parker 2018), aquatic habitat degradation by animal-induced eutrophication is less explored. Specifically, no work has been done concerning the impacts of cattle dung on mosquito proliferation in temporary wetland systems, despite the ecological significance of these wetlands, and epidemiological importance of mosquitoes (Raj *et al.*, 2014).

Mosquitoes harbour pathogens which cause human, wildlife and livestock diseases, driving high rates of morbidity and mortality worldwide (Kapesa *et al.*, 2018). For instance, *Anopheles* sp. (malaria causing *Plasmodium*) and *Culex* sp. (West Nile virus, Rift Valley fever, Usutu virus, encephalitis viruses, filarioid worms, Haemoproteus) form an important tripartite ecological component of vector-parasite and host disease transmission dynamics (Schmid *et al.*, 2017; Braack *et al.*, 2018). Importantly, their oviposition site selection can be contingent on patterning of risk and reward among habitat patches (Pintar *et al.*, 2018). For example, many mosquito species detect cues and subsequently disregard breeding sites containing predators (Eveland *et al.*, 2016). Alternatively, some mosquitoes select sites with low larval density to reduce competition (Himeidan *et al.*, 2013), although these sites may further enhance their vectorial capacity (Juliano *et al.*, 2014). Availability of food may be prioritised over predation risk (Albeny-Simões *et al.*, 2014). In this context, stagnant and slow-moving waters with high organic resources (nutrients) are preferentially selected to sustain the development of mosquito juveniles (Turnipseed *et al.*, 2018). Nutrient-enriched habitats might thus promote mosquito colonisation and proliferation with increased risk of associated diseases. As such, exploring the

implications of cattle-induced eutrophication and rapidly increasing human population growth for vector mosquitoes is essential in a public health context.

In the tropics, there is an urgent need for improved understanding of factors facilitating increased mosquito abundance, particularly as human-mediated activities and climate change ensue. Here, I test for such effects using an in situ experimental mesocosm approach in a semi-arid African environment, to investigate the implications of cattle-induced eutrophication in aquatic habitats utilised by mosquitoes. Given the prevalence and contribution to temporary aquatic wetland environments, and their extensive utilisation by cattle and other wildlife, the experiment was carried out in a rocky outcrop landscape characterised by temporary rockpool habitats. Particular interest was in medically-important mosquito species given their distribution and prevalence in these diverse landscapes (Kamal *et al.*, 2018). The aim of the study was to investigate the effect of cattle dung concentration loadings on larval mosquito species colonisation and abundance using an in situ mesocosm approach. It was hypothesised that: (1) dung-nitrified mesocosms on rocky outcrops would be colonised by vector-mosquito species, and (2) cattle-dung eutrophication would drive similar proliferation rates between culicine and anopheline species.

4.2. Materials and methods

4.2.1. Study area and experimental design

The study was conducted on a rocky outcrop (22° 35' 46.7S; 27° 07' 30.3E) on Botswana International University of Science and Technology Palapye campus (Figure 4.1a,b) in the austral winter between June and July (2019). The large campus (2500 ha), situated in a semi-arid

environment (Batisani and Yarnal 2010), is characterised by numerous rocky outcrops, but is fenced and therefore excludes livestock. Outside the campus premises, however, such rocky outcrop landscapes are heavily utilised by free-range cattle, and particularly during the rainy season when rockpools fill up with water (Figure 4.1c).

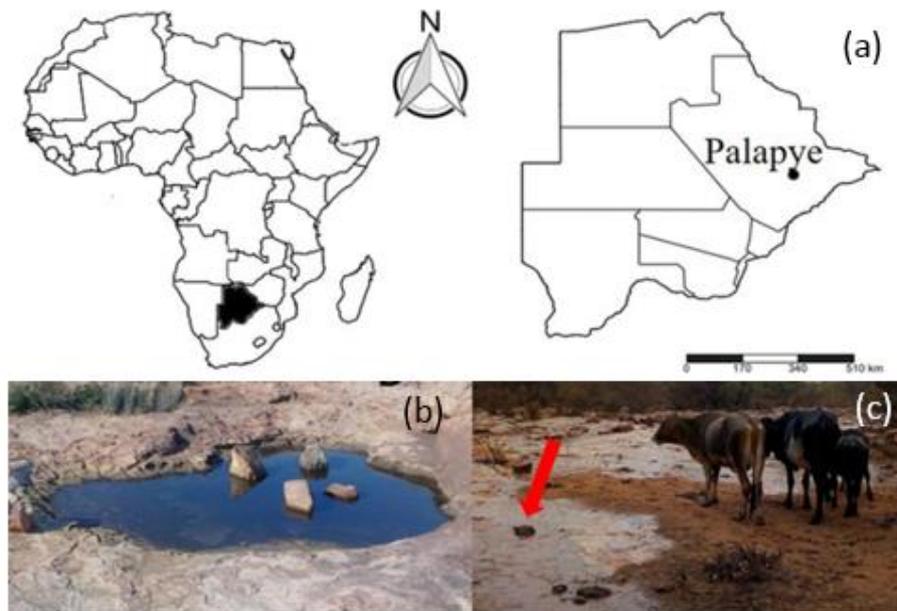


Figure 4. 1. Compound figure showing (a) the location of the study site in Central district, Botswana, (b) a typical rocky outcrop and temporary pond in the region and (c) the common site of cattle congregating on rocky outcrops given their ability to hold water (arrow indicates a dung deposit).

Twenty five oval-shaped mesocosms (105 cm length \times 72 cm width \times 40 cm depth; Figure 4.2) were distributed across the selected outcrop on campus, covering an area of approximately 1300 m². Mesocosms were placed such that they were at least 3 m away from the tree canopy (to avoid leaf litter in-fall) and 5 m away from adjacent mesocosms to avoid spatial contagion. Once in place, each mesocosm was filled with 50 L (to a depth of \sim 14.5 cm) of tap water (Day 1), and left to mature for 7 days. To avoid colonisation by other invertebrates or use

by vertebrates during this time, all mesocosms were covered with 500 μm mesh cloth (Figure 4.2a). Mesocosms were kept at 50 L by topping up with matured tap water every second day for the duration of the experiment.

After one week of water maturation, treatments were established in the 25 mesocosms (Day 7). Approximately 6 kg of fresh dung was collected from two cows in Palapye village. These two cows were monitored over the course of the day to allow immediate dung collection following defecation. Fresh dung from the two cows was homogenised by hand for 30 min in a large 100 L plastic container. Five dung treatments were established. In control mesocosms (i.e., C), no dung was inoculated. In treatments 1, 2, 3 and 4 (i.e., T1-T4), mesocosms were inoculated with dung at 1 g L^{-1} , 2 g L^{-1} , 4 g L^{-1} and 8 g L^{-1} , respectively. Treatment assignment to mesocosms was fully randomised, with 5 replicates assigned to each treatment. Post-dung inoculation, the 500 μm mesh cloth that was placed over each mesocosm for insect exclusion (Figure 4.2a), was weighted and submerged at the center within the 50 L of water (Figure 4.2b), thus allowing for mosquito colonisation. The experiment was then run for two weeks (allowing for oviposition and larval development) post-dung inoculation (Day 7 to Day 21), with temperature variation in each mesocosm measured at least every 3 days (at mid-day) during this period, using a multi-probe (Aquameter, Aquaread Ltd., Kent, UK).

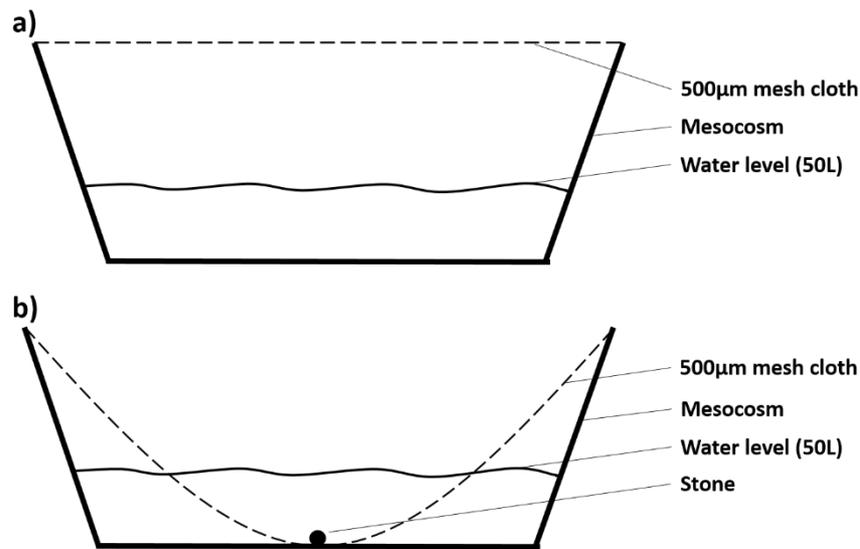


Figure 4. 2. Illustration of the lateral view of the mesocosms showing (a) placement of mesh cloth during maturation stage to prevent colonisation and water use by organisms, and (b) placement of mesh cloth post treatment dung inoculation to facilitate oviposition by mosquitoes and enable later capture of larvae.

Mosquito larvae were collected from all mesocosms 7 days after treatment establishment (Day 14) and again on Day 21. On each collection day, all mosquito larvae were removed from each mesocosm using a small (10 cm × 15 cm) 200 µm mesh net. All mosquito larvae were exhaustively collected through repeated scooping of the net through the water column. Following collection, each mesocosm was inspected in random order by three different researchers to ensure that no larvae remained in the mesocosm. All collected mosquito larvae were transferred to 80% ethanol within an hour of collection, for later identification.

4.2.2. Identification of mosquito larvae

Mosquito larvae were first broadly identified to genus level using morphological features, mainly of the siphon and head, following recommendations by Jupp (1996). The organisms were

then sorted according to size as first (1.3 ± 0.2 mm), second (2.5 ± 0.2 mm), third (4.2 ± 0.2 mm) and fourth (5.9 ± 0.3 mm) instars from all the mesocosm treatment collections. Further, a representation of six randomly-selected anopheline and culicine larval specimens (across life-histories) were each subjected to DNA extraction using Quick-DNA Tissue/Insect Miniprep Kit (Zymo Research, USA) followed by amplification of the mitochondrial cytochrome oxidase subunit I (COI) using polymerase chain reaction (PCR). This was achieved using species identification universal primers HCO 2198 and LCO 1490 following protocols of Makanda *et al.* (2019). The PCR products of all amplicons were purified using the GeneJET PCR purification kit (Thermo Fisher Scientific, USA) and sequenced at Inqaba Biotechnical Industries (Pretoria, South Africa). Obtained raw sequences were trimmed and contigs assembled using CodonCode Aligner 8.0.2 software package and subsequently subjected to the basic local alignment search tool (BLAST) for comparison with already identified species.

4.2.3. Statistical analyses

Differences in water temperatures were examined according to treatment over time using linear mixed effects models with individual mesocosms included as a random effect. Residual normality and homoscedasticity were assured using diagnostic plotting following \log_{10} transformation of temperatures. Generalised linear mixed effects models were used to examine counts of larval mosquitoes with treatment (5 levels), species (2 levels), instar stage (4 levels) as fixed and two-way interacting effects. Observation day (2 levels) was also included as an individual term. A random effects structure accounted for repeated measures within each experimental mesocosm. Owing to evidence for zero inflation (number of zeros exceeded those expected from simulations; Hartig 2019), a zero inflation parameter was applied to all

observations (Brooks *et al.*, 2017). There was no evidence for residual overdispersion in the model. The significance of main effects was inferred via analyses of deviance using Wald chi-square statistics with type III sums of squares (Fox and Weisberg 2019). Selected replicate mesocosms (C3, T1R1, T1R5, T3R5, T4R4) accumulated leaf-litter during the study, with potential to either inhibit or facilitate increased oviposition (Cuthbert *et al.*, 2019). As such, these mesocosms were discarded from the analysis. All statistical analyses were performed in R v3.4.4 (R Core Development Team 2017).

4.3. Results

Temperatures did not differ significantly among experimental treatments overall (treatment: $F_{4,20} = 0.37$, $p = 0.83$), yet displayed significant daily undulations over the monitoring period (time: $F_{7,168} = 542.03$, $p < 0.001$; Figure 5.3), ranging from 17 to 31 °C overall. All randomly selected specimens within each anopheline and culicine group had identical sequences. The result confirmed the culicine larvae as *Culex pipiens* with 99% homology to a partial COI gene of isolate *Cx. pipiens* (GenBank, Accession number: MK300250.1). Contrastingly, anopheline nucleotide sequences did not match any previously identified *Anopheles* species within the database. However, the sequences had 93% homology to a partial COI gene of a ‘taxonomically undescribed’ *Anopheles* species (later identified as *An. rhodesiensis*, see chapter 3) isolate from Kacheliba, Kenya (GenBank, Accession number: MK047667.1; see Ogola *et al.*, 2019).

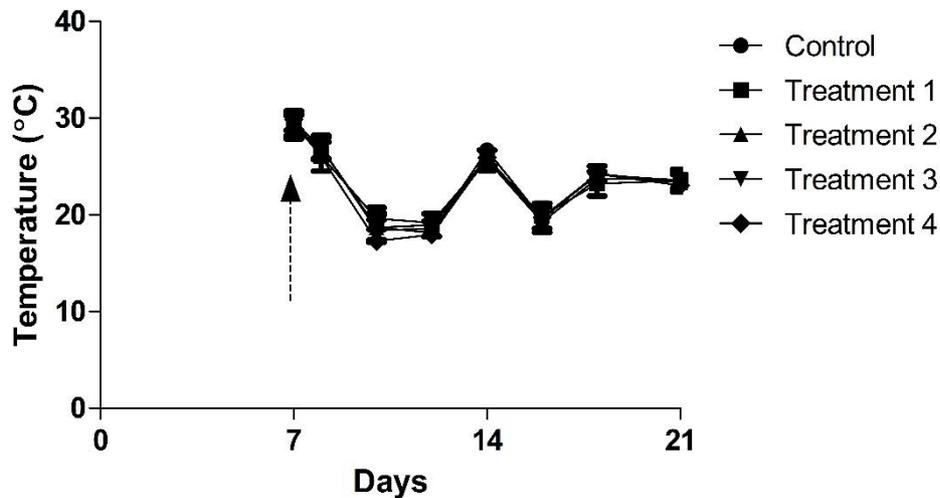


Figure 4. 3. Temperature readings in the control and treatment mesocosms from Day 7 to Day 21. Dung was inoculated on Day 7 (shown by an arrow), prior to which water in the mesocosms were allowed to mature.

Culicine larvae were, on average, 26 times more abundant than anopheline larvae across the sampling period (Figure 4.4). Nutrient treatment significantly influenced colonisation between mosquito species (treatment \times species: $\chi^2 = 13.86$, $df = 4$, $p = 0.008$). While mosquitoes of both genera never colonised control mesocosms (C) and negligible numbers colonised 1 g L⁻¹ treatments (T1), abundances increased significantly under nutrient loadings for both species (Figure 5.4). Mean culicine abundances were 192-times higher under the highest (T4, 8 g L⁻¹) compared to lowest (T1, 1 g L⁻¹) dung concentrations, whereas anopheline numbers increased from zero but remained relatively low (Figure 4.4). First instar stages were most abundant across increasing nutrient inputs (treatment \times instar stage: $\chi^2 = 166.07$, $df = 12$, $p < 0.001$), and instar stage responses did not differ significantly between species (species \times instar stage: $\chi^2 = 4.92$, $df = 3$, $p = 0.18$) (Figure 4.4). Larval mosquito abundances were significantly (321%) higher in the second observation week as compared to the first (day: $\chi^2 = 134.16$, $df = 1$, $p < 0.001$). Neither

species was present beyond the first instar stage at day 14 (Figure 4.4). At day 21, up to second instar anopheline larvae were present under 2 g L⁻¹ (T2) and 4 g L⁻¹ (T3) nutrient treatments. Contrastingly, in the second week for *Culex*, up to fourth instar stages were present both in 4 g L⁻¹ (T3) and 8 g L⁻¹ (T4) nutrient loadings (Figure 4.4).

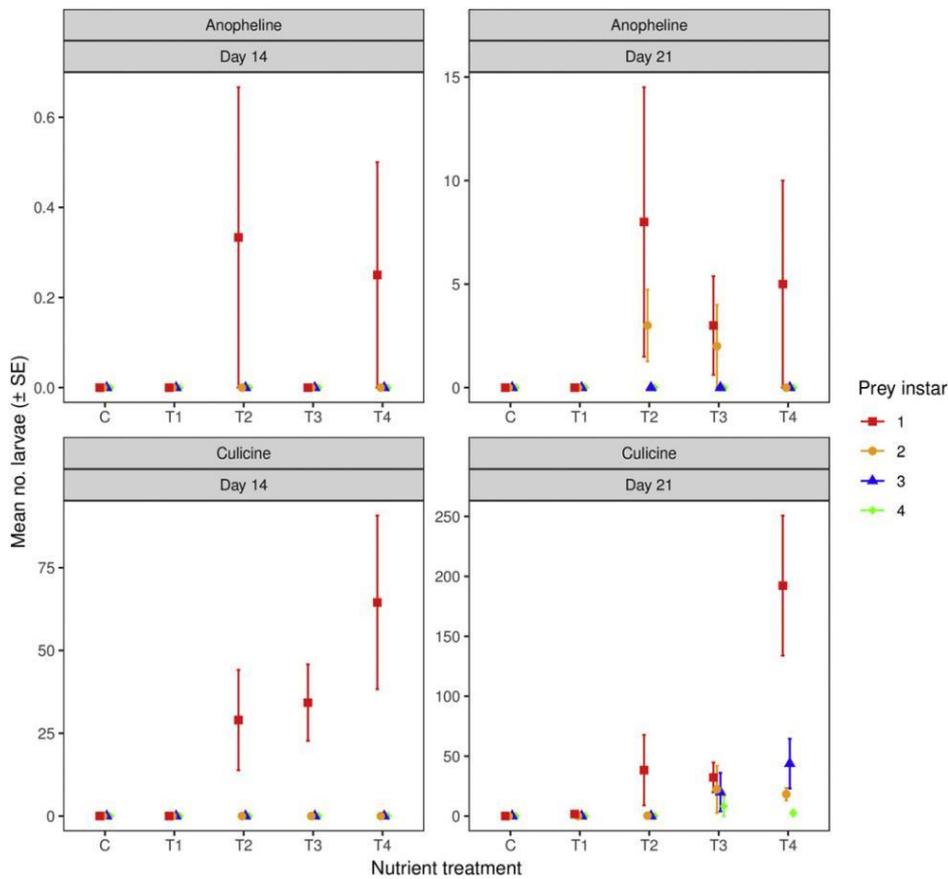


Figure 4. 4. Weekly mean (± 1 SE) larval mosquito instar (1–4) abundance across different cattle dung concentration (grams of dung per liter water) treatments for anopheline and culicine species. C = Control (0 g L⁻¹), T 1 = Treatment 1 (1 g L⁻¹), T2 = Treatment 2 (2 g L⁻¹), T3 = Treatment 3 (4 g L⁻¹) and T4 = Treatment 4 (8 g L⁻¹).

4.4. Discussion

Quantitative understanding of facilitators of medically-important mosquito groups is imperative as risks of particular diseases are contingent on prevailing vector species. The present study shows that cattle dung-induced eutrophication drives significant mosquito proliferations, which may have implications for the vectoring of pathogens and parasites that cause diseases in humans, wildlife and livestock (Cox 2010; Braack *et al.*, 2018). These results demonstrate a previously unquantified but significant ecological disservice associated with free-range livestock rearing. While mosquitoes have been previously shown to colonise polluted aquatic environments with enriched nutrient levels (Pires and Gleiser 2010), no study has previously explored the role of cattle dung for mosquito proliferations in aquatic environments. Given that it is common practice for rural farmers to accumulate unsustainably high numbers of livestock (Caviglia-Harris 2005), and that cattle in particular require access to drinking water daily, these results are highly relevant within the context of public health risk.

While livestock farming has socio-economic and cultural significance, often providing for diversity in farmers' income sources, trade-offs between benefits and ecosystem disservices are inevitable. However, little work has assessed the implications of cattle-induced eutrophication on aquatic ecosystem dynamics. Moreover, the focus of previous studies has been narrowed solely on cattle-derived fecal microbial input into drinking water supply systems (Smolders *et al.*, 2015; Korajkic *et al.*, 2019). The current study, however, documents that increasing livestock rearing may have an ecological cost/disservice associated with increased vector mosquito abundance, through inconspicuous nutrient inputs into aquatic environments. Given that mosquito larvae filter-feed on plant debris, bacteria, protozoa, and algae (Duguma *et*

al., 2017), cattle dung presence likely facilitates the availability of such food items. Ovipositing mosquitoes are attracted to habitats depending on various factors such as predator density, competition and food resources (Wasserberg *et al.*, 2013). Pristine temporary wet-land systems often hold large abundances of invertebrates, including predatory crustaceans (O'Neill and Thorp 2014; Wasserman *et al.*, 2016). Many of these crustaceans hatch from dormant eggs in the sediment (Wasserman *et al.*, 2016), while semi-aquatic flying insects (such as mosquitoes) colonise these sites from externally (O'Neill and Thorp 2014).

The present study only found two species that colonised the mesocosms. While the regional diversity is much higher (Jupp 1996), the experiment was run on a specific localised landscape that is likely favoured by few mosquito species given the absence of permanent water bodies. *Culex pipiens* complex mosquitoes are key vector species for emerging and burdening infections (Phumee *et al.*, 2019). Species within this complex are mainly involved in pathogen and parasite transmission to humans, wildlife and livestock such as avian malaria, Rift Valley, West Nile and encephalitis (see Brugman *et al.*, 2018). Similarly, *Anopheles* species utilise diverse habitats, including temporary ponds in arid landscapes, to effectively transmit malaria causing parasites to humans. The study records an undescribed potential malaria vector likely to exacerbate the disease burden locally due to lack of knowledge on mosquito community, although previously detected regionally (Ogola *et al.*, 2019). While the *Culex pipiens* complex larval development have been found to be competitively superior to other species (Müller *et al.*, 2018), competition dynamics are largely species and microhabitat specific (Kweka *et al.*, 2012). Competition dynamics between the two sampled species likely had implications for community dynamics. However, this was not assessed in the present study. Nevertheless, with no mosquitoes

colonising dung-free water (controls), but colonising dung-inoculated mesocosms, the findings suggest a potential significant role of cattle-induced eutrophication in driving mosquito oviposition. These induced preferences in temporary wetlands with dung inputs may contribute to the shifting patterns of habitat selection for oviposition particularly by anopheline species as demonstrated in previous research (Gunathilaka *et al.*, 2013). Given the human-livestock drivers that modify aquatic systems in arid environments, it is evident that both anopheline and culicine mosquitoes may be well-adapted to exploit these as primary or alternative breeding habitats. This warrants further investigation into mosquito site selection beyond the container-based systems examined here, through monitoring of temporary wetlands (e.g., rockpools) across a gradient of degradation by animal waste. In addition, interaction between eutrophication in aquatic ecosystems and climate warming and increased variability projections for colonisation behaviour should further be explored. Such factors may have implications for the oviposition preferences of mosquito vectors across diverse aquatic habitats influenced by both biotic and abiotic ecosystem drivers.

Free-ranging cattle have potential access to vast landscapes, even those in close proximity to human dwellings, in turn depositing excess nutrients into standing waters. Aquatic systems with physico-chemical parameters that favour mosquito breeding can also potentially promote disease transmission cycles across urban and peri-urban environments with implications for risks to human, wildlife and livestock populations. While changes in physico-chemistry associated with cattle-induced eutrophication events were not measured, it was shown that cattle dung has the potential to change aquatic habitat features to favour mosquito proliferation. Future studies should consider investigating changes in physico-chemical properties following

dung eutrophication and the mechanisms behind the observations made in this study, to better understand the specific drivers of the process. The results of this study still, however, have relevance for many tropical and subtropical landscapes where free-range cattle practices are prevalent. The potential role of cattle in facilitating medically important pest species has implications for livestock management practices such as herd size, uncontrollable movement and tenure systems. In addition, since climate change is currently a driver of mosquito abundance and the risk of associated diseases worldwide (Ludwig *et al.*, 2019), it is likely that other human-mediated factors may synergistically interact with direct climate effects.

The current study documents the role of free-range cattle farming systems in optimising aquatic breeding habitats and associated vector mosquito disservices. Specifically, it was showed that cattle dung eutrophication promotes mosquito colonisation and abundance in temporary aquatic ecosystems. This has risk implications for emerging and re-emerging mosquito-borne infections, which may be exacerbated by livestock-induced degradation of temporary wetland breeding hotspots utilised by mosquito species in agro-landscapes. To circumvent associated dung eutrophication problems, further research on livestock-induced nitrification implications for vector mosquito proliferation is recommended, and engagement with local authorities for the exploration of adequate mitigation measures. This will contribute to our understanding of facilitators of medically-important mosquito groups with the end goal of minimising disservices linked to livestock associated eutrophication. Further, future work on water quality dynamics associated with livestock induced nitrification is recommended, using both in-and ex -situ approaches, to determine the specific drivers of mosquito facilitation in affected aquatic habitats. Similarly, the effect of different dung types (including both domestic and wildlife) on mosquito

species' colonisation would be useful to inform on if and how shifts from wildlife to domesticated animals may favour certain species through niche segregation dynamics.

4.5. References

- Albeny-Simões, D., Murrell, E. G., Elliot, S. L., Andrade, M. R., Lima, E., Juliano, S. A., and Vilela, E. F. (2014). Attracted to the enemy: *Aedes aegypti* prefers oviposition sites with predator killed conspecifics. *Oecologia*, 175: 481–492.
- Arntzen, J. (2016). Makgadikgadi wetlands (Botswana): planning for sustainable use and conservation. In: Finlayson, C., Milton, G., Prentice, R., Davidson, N. (Eds.), *The Wetland Book: II: Distribution, Description and Conservation*. Springer, Dordrecht.
- Asigau, S., and Parker, P. G. (2018). The influence of ecological factors on mosquito abundance and occurrence in Galápagos. *Journal Vector Ecology*, 43: 125-137.
- Batisani, N., and Yarnal, B. (2010). Rainfall variability and trends in semi-arid Botswana: implications for climate change adaptation policy. *Applied Geography*, 30: 483-489.
- Batzer, D., Boix, D. (2016). An introduction to freshwater wetlands and their invertebrates. *Invertebrates in Freshwater Wetlands*. Springer, Cham, pp. 1–23.
- Bird, M. S., Mlambo, M. C., Wasserman, R. J., Dalu, T., Holland, A. J., Day, J. A., and Brendonck, L. (2019). Deeper knowledge of shallow waters: reviewing the invertebrate fauna of southern African temporary wetlands. *Hydrobiologia*, 827: 89-121.
- Braack, L., de Almeida, A. P. G., Cornel, A. J., Swanepoel, R., and De Jager, C. (2018). Mosquito-borne arboviruses of African origin: review of key viruses and vectors. *Parasites and Vectors*, 11: 29.
- Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., and Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R journal*, 9: 378-400.
- Brugman, V., Hernández-Triana, L., Medlock, J., Fooks, A., Carpenter, S., and Johnson, N. (2018). The role of *Culex pipiens* L. (Diptera: Culicidae) in virus transmission in Europe. *International Journal Environmental Research Public Health*, 15: 389.

- Caviglia-Harris, J. L. (2005). Cattle accumulation and land use intensification by households in the Brazilian Amazon. *Journal of Agricultural and Resource Economics*, 34: 145-162.
- Cox, F. E. (2010). History of the discovery of the malaria parasites and their vectors. *Parasites and Vectors*, 3: 5.
- Cuthbert, R. N., Dalu, T., Mutshekwa, T., and Wasserman, R. J. (2019). Leaf inputs from invasive and native plants drive differential mosquito abundances. *Science of the Total Environment*, 689: 652-654.
- Duguma, D., Kaufman, M. G., and Simas Domingos, A. B. (2017). Aquatic microfauna alter larval food resources and affect development and biomass of West Nile and Saint Louis encephalitis vector *Culex nigripalpus* (Diptera: Culicidae). *Ecology and Evolution*, 7: 3507-3519.
- Enahoro, D., Lannerstad, M., Pfeifer, C., and Dominguez-Salas, P. (2018). Contributions of livestock-derived foods to nutrient supply under changing demand in low-and middle-income countries. *Global Food Security*. 19: 1-10.
- Eveland, L. L., Bohenek, J. R., Silberbush, A., and Resetarits, W. J. (2016). Detection of fish and newt kairomones by ovipositing mosquitoes. In: Schulte, B.A., Goodwin, T. (Eds.), *Chemical Signals in Vertebrates 13*. Springer, Cham.
- Fox, J., and Weisberg, S., 2019. *An R Companion to Applied Regression*. 3rd ed. Sage, Thousand Oaks, CA <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>.
- Gunathilaka, N., Fernando, T., Hapugoda, M., Wickremasinghe, R., Wijeyerathne, P., and Abeyewickreme, W. (2013). *Anopheles culicifacies* breeding in polluted water bodies in Trincomalee District of Sri Lanka. *Malaria Journal*, 12: 285.
- Hartig, F. (2019). DHARMA: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models (R package).
- Hebinck, P. G. M., and Faku, N. (2013). Cattle and rural development in the Eastern Cape, South Africa: the Nguni project revisited. In *the Shadow of Policy: Everyday Practices in South Africa's Land and Agrarian Reform*. Wits University press, Johannesburg.

- Himeidan, Y. E., Temu, E. A., Rayah, E., Amin, E., Munga, S., and Kweka, E. J. (2013). Chemical cues for malaria vectors oviposition site selection: challenges and opportunities. *Journal of Insects*, 2013: 685182.
- Juliano, S. A., Ribeiro, G. S., Maciel-de-Freitas, R., Castro, M. G., Codeço, C., Lourenço-de-Oliveira, R., and Lounibos, L. P. (2014). She's a femme fatale: low-density larval development produces good disease vectors. *Memorias do Instituto Oswaldo Cruz*, 109: 1070-1077.
- Jupp, P. G. (1996). Mosquitoes of Southern Africa: Culicinae and Toxorhynchitinae. Ekogilde Publishers, Johannesburg.
- Kamal, M., Kenawy, M. A., Rady, M. H., Khaled, A. S., and Samy, A. M. (2018). Mapping the global potential distributions of two arboviral vectors *Aedes aegypti* and *Ae. albopictus* under changing climate. *PloS ONE*, 13: e0210122.
- Kapesa, A., Kweka, E. J., Atieli, H., Afrane, Y. A., Kamugisha, E., Lee, M. C., and Yan, G. (2018). The current malaria morbidity and mortality in different transmission settings in Western Kenya. *PloS ONE*, 13: e0202031.
- Korajkic, A., McMinn, B. R., Ashbolt, N. J., Sivaganesan, M., Harwood, V. J., and Shanks, O. C. (2019). Extended persistence of general and cattle associated fecal indicators in marine and freshwater environment. *Science of the Total Environment*, 650: 1292-1302.
- Kweka, E. J., Zhou, G., Beilhe, L. B., Dixit, A., Afrane, Y., Gilbreath, T. M., and Yan, G. (2012). Effects of co-habitation between *Anopheles gambiae* ss and *Culex quinquefasciatus* aquatic stages on life history traits. *Parasites and Vectors*, 5: 33.
- Lange, G. M., Barnes, J. I., and Motinga, D. J. (1998). Cattle numbers, biomass, productivity and land degradation in the commercial farming sector of Namibia, 1915 to 1995. *Development Southern Africa*, 15: 555-572.
- Ludwig, A., Zheng, H., Vrbova, L., Drebot, M. A., Iranpour, M., and Lindsay, L. R. (2019). Increased risk of endemic mosquito-borne diseases with climate change. *Canada Communicable Disease Report*, 45: 4.

- Makanda, M., Kemunto, G., Wamuyu, L., Bargul, J., Muema, J., and Mutunga, J. (2019). Diversity and Molecular Characterization of Mosquitoes (Diptera: Culicidae) in selected ecological regions in Kenya. *F1000Research*, 8: 262.
- Marinho, R. A., Beserra, E. B., Bezerra-Gusmão, M. A., Porto, V. D. S., Olinda, R. A., and dos Santos, C. A. (2016). Effects of temperature on the life cycle, expansion, and dispersion of *Aedes aegypti* (Diptera: Culicidae) in three cities in Paraíba, Brazil. *Journal of Vector Ecology*, 41: 1-10.
- Mesa, L., Mayora, G., Saigo, M., and Giri, F. (2015). Nutrient dynamics in wetlands of the middle Paraná River subjected to rotational cattle management. *Wetlands*, 35: 1117-1125.
- Müller, R., Knautz, T., Vollroth, S., Berger, R., Kreß, A., Reuss, F., and Kuch, U. (2018). Larval superiority of *Culex pipiens* to *Aedes albopictus* in a replacement series experiment: prospects for coexistence in Germany. *Parasites and Vectors*, 11: 80.
- Ogola, E. O., Chepkorir, E., Sang, R., and Tchouassi, D. P. (2019). A previously unreported potential malaria vector in a dry ecology of Kenya. *Parasites and Vectors*, 12: 80.
- O'Neill, B. J., and Thorp, J. H. (2014). Untangling food web structure in an ephemeral ecosystem. *Freshwater Biology*, 59: 1462-1473.
- Phumee, A., Chomposri, J., Intayot, P., Boonserm, R., Boonyasuppayakorn, S., Buathong, R., and Siriyasatien, P. (2019). Vertical transmission of Zika virus in *Culex quinquefasciatus* Say and *Aedes aegypti* (L.) mosquitoes. *Scientific Reports*, 9: 5257.
- Pintar, M. R., Bohenek, J. R., Eveland, L. L., and Resetarits, W. J. (2018). Colonization across gradients of risk and reward: nutrients and predators generate species-specific responses among aquatic insects. *Functional Ecology*, 32: 1589-1598.
- Pires, D. A., and Gleiser, R. M. (2010). Mosquito fauna inhabiting water bodies in the urban environment of Córdoba city, Argentina, following a St. Louis encephalitis outbreak. *Journal of Vector Ecology*, 35: 401-409.
- Raj, A., Jhariya, M. K., and Toppo, P. (2014). Cow dung for eco-friendly and sustainable productive farming. *Environmental Science*, 3: 201-202.

- R Core Development Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Schmid, S., Dinkel, A., Mackenstedt, U., Tantely, M. L., Randrianambinintsoa, F. J., Boyer, S., and Woog, F. (2017). Avian malaria on Madagascar: bird hosts and putative vector mosquitoes of different Plasmodium lineages. *Parasites and Vectors*, 10: 6.
- Scoones, I. (1991). Wetlands in drylands: key resources for agricultural and pastoral production in Africa. *Ambio*, 20: 366-371.
- Smolders, A., Rolls, R. J., Ryder, D., Watkinson, A., and Mackenzie, M. (2015). Cattle-derived microbial input to source water catchments: an experimental assessment of stream crossing modification. *Journal of Environmental Management*. 156: 143-149.
- Stavi, I., Barkai, D., Knoll, Y. M., and Zaady, E. (2016). Livestock grazing impact on soil wettability and erosion risk in post-fire agricultural lands. *Science of the Total Environment*, 573: 1203-1208.
- Tolera, A., and Abebe, A. (2007). Livestock production in pastoral and agro-pastoral production systems of southern Ethiopia. *Livestock Research for Rural Development*, 19: 4-7.
- Turnipseed, R. K., Moran, P. J., and Allan, S. A. (2018). Behavioral responses of gravid *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles quadrimaculatus* mosquitoes to aquatic macrophyte volatiles. *Journal of Vector Ecology*, 43: 252-260.
- Wasserberg, G., White, L., Bullard, A., King, J., and Maxwell, R., (2013). Oviposition site selection in *Aedes albopictus* (Diptera: Culicidae): are the effects of predation risk and food level independent? *Journal of Medical Entomology*, 50: 1159-1164.
- Wasserman, R. J., Alexander, M. E., Barrios-O'Neill, D., Weyl, O. L., and Dalu, T. (2016). Using functional responses to assess predator hatching phenology implications for pioneering prey in arid temporary pools. *Journal of Plankton Research*, 38: 154-158.

CHAPTER 5

Complementary impacts of heterospecific predators facilitate improved biological control of mosquito larvae

Published as: **Buxton, M.**, Cuthbert, R. N., Dalu, T., Nyamukondiwa, C., and Wasserman, R. J. (2020). Complementary impacts of heterospecific predators facilitate improved biological control of mosquito larvae. *Biological Control*, 144: 104216.

5.1. Introduction

Natural regulatory mechanisms such as predation, play a significant role in the dynamics of aquatic ecosystem functioning and stabilisation (De Bernardi 1981; Wasserman *et al.*, 2013; Hammerschlag *et al.*, 2019). Predators are, however, often among the first trophic groups to disappear when natural systems are degraded through anthropogenic activities (de Sá *et al.*, 2018; Priawandiputra *et al.*, 2018). The maintenance and facilitation of natural predator communities can be highly desirable, particularly in environments where pest species proliferate in the absence of predation pressure (Dida *et al.*, 2015; Little *et al.*, 2017). As such, understanding predatory effects on pest prey populations remains a critical component of pest control. For insect pest species, the use of native natural enemies through conservation and biocontrol is often desirable (Lacey and Orr 1994; Chatterjee *et al.*, 2007). Moreover, it is considered a more sustainable and efficacious approach than using exogenous species or chemicals, with relatively little or no detrimental effects to the environment (Lacey and Orr 1994; Hayasaka 2014; Azevedo-Santos *et al.*, 2016). Numerous insect pest species are also of public health importance, driving immense morbidity and mortality in human populations and enormous associated economic costs (Bradshaw *et al.*, 2018; WHO 2017). This necessitates quantitative understandings of intrinsic population regulation processes which impact upon such pest species.

Mosquitoes are major pests globally, with many species serving as vectors of numerous human, wildlife and livestock diseases (Braack *et al.*, 2018). It is estimated that over 700,000 humans die from mosquito-borne diseases annually, while more than one billion people are infected (WHO 2017). *Culex* spp. are among the most important vectors of pathogens and

parasites which cause diseases such as avian malaria, Rift Valley fever, elephantiasis and other infections (Braack *et al.*, 2018). These vector species are widely distributed globally and are known to successfully colonize and proliferate in a range of aquatic habitat types (Azari-Hamidian 2007). Aquatic environments are used by mosquitoes across their life-history stages (Crans 2004), and the larval life history stage is particularly vulnerable to predation (Marten and Reid 2007; Shaalan and Canyon 2009; Roux and Robert 2019). As such, there is high potential for mosquito biocontrol through the conservation or augmentation of natural mosquito predator communities in aquatic habitats. Biological control of mosquitoes using predators has been effective in management strategies (Kay and Nam 2005). Aquatic predators such as insects, crustaceans and fish, have been identified as potentially effective biocontrol agents (Benelli *et al.*, 2016). However, natural enemy impacts often remain unquantified and are inherently context-dependent, with environmental and ecological community variabilities potentially altering enemy efficacies at the population level (Cuthbert *et al.*, 2018a,b). In particular, owing to rapid growth during the larval stage (Gomes *et al.*, 1995), size-related differences in larval ontogeny may facilitate refuge effects associated with prey body sizes which are difficult to capture or handle (Cuthbert *et al.*, 2018c; Dalal *et al.*, 2019a,b). Indeed, predator–prey body-size scaling of trophic interactions is pervasive and often non-linear across ecosystems, with predator efficiencies often reduced towards prey which are relatively large or small (Brose 2010; McCoy *et al.*, 2011).

Further to body size scaling in natural communities, diverse predator communities are typically present, with prey often simultaneously exposed to multiple predators (Sih *et al.*, 1998). Predators therefore frequently compete for shared resources, and these predator-predator

interactions may not combine additively in a prey risk context (Soluk 1993; Sih *et al.*, 1998; Barrios-O'Neill *et al.*, 2015; Wasserman *et al.*, 2016a). That is, prey risk may be enhanced through predator-predator synergism or be alleviated through antagonistic predatory interactions (Schmitz 2007; Woodcock and Heard 2011). In a mosquito control context, predator synergy or additivity is thus desirable (Cuthbert *et al.*, 2019a), as heightening of prey risk associated with positive multiple predator effects (MPEs) may impede density- or size-dependent refuge effects and reduce recruitment to the vectorially-important adult stage. In particular, in diverse heterospecific predator communities, different species may exhibit optimal foraging efficiencies towards particular prey sizes (Barrios-O'Neill *et al.*, 2016). In turn, to-wards mosquitoes, this feeding niche separation may bolster total predatory impacts through simultaneous targeting of different larval life history stages by multiple predators. Despite this, MPEs have not been well-explored within the context of aquatic predator communities and their implications for mosquito control across different ontogenic stages.

Botswana is characterised by arid landscapes with temporary wetlands dominating aquatic ecosystems throughout much of the region during the wet season (UNFCCC 2007; Kulongoski *et al.*, 2004). Temporary aquatic ecosystems are highly relevant for mosquito pest control considerations given their potential utilisation by many mosquito vector genera (Pires and Gleiser 2010). In these ecosystems, predation can take on the form of wholly-aquatic crustacean predators that emerge from dormant eggs in the sediment, or from predaceous semi-aquatic insects that colonize from other environments (Cuthbert *et al.*, 2019b; O'Neill and Thorp 2014). However, there is currently limited understanding of how top-down processes such as species-specific and emergent predation impact mosquitoes across their life-history. Both

paradiaptomid copepods and notonectids are known to play a key role in the structuring of temporary wetland communities through predation (Wasserman *et al.*, 2016b,c; Wasserman *et al.*, 2018; Cuthbert *et al.*, 2019c). Whilst both taxonomic groups are known to consume a range of mosquito larvae instars (Cuthbert *et al.*, 2018a; Dalal *et al.*, 2019a), predation efficiencies likely vary substantially across larval mosquito ontogenic stages.

A functional responses approach was used to explore prey size effects in relation to two common temporary wetland predators, and tested for any emergent MPEs. To this end, predation efficacy and MPEs of the wholly-aquatic predatory copepod *Lovenula falcifera* (Lovén, 1845) and semi-aquatic predatory notonectid *Anisops sardea*, Kirkaldy 1904 was quantified towards different larval instar stages of the mosquito *Culex pipiens* (Linnaeus 1758). All three species are widespread and prolific in temporary aquatic habitats of the region, but anecdotal evidence suggests that, in the wild, *L. falcifera* and *A. sardea* presence is an indication of low culicid absences. It was hypothesised that the natural enemies have differing predatory effects across mosquito larval ontogeny, and that MPEs would be evident under heterospecific predator scenarios owing to either antagonistic or synergistic interactions.

5.2. Materials and methods

5.2.1. Study area and experimental design

The prey, larvae of *Cx. pipiens*, originated from egg rafts sampled from water bodies situated on the Botswana International University of Science and Technology campus (022° 35' 05.7S; 027° 06' 58.7E). Using tap water left uncovered for 72 h (matured tap water), larvae were reared to the desired instars (see below) in climate chambers (HPP 260, Memmert GmbH +

Co.KG, Germany) set at $20\text{ }^{\circ}\text{C} \pm 2$ and $65\% \pm 10$ relative humidity, and housed in a temperature controlled laboratory ($20\text{ }^{\circ}\text{C} \pm 2$) on a diet of crushed rabbit food pellets (Westerman's Premium, Durban, South Africa). The predators, *L. falcifera* (hereafter copepod) and *A. sardea* (hereafter notonectid) were collected from similar habitats to where the prey were sampled (i.e. temporary pools) near Lerala village, Central district, Botswana ($022^{\circ} 52'16.0\text{S}$; $027^{\circ} 47' 42.7\text{E}$). Once in the laboratory the predators were separated into aerated 5 L plastic containers holding a 50:50 ratio of pond water and matured tap water. Predators were housed for five days prior to experimentation and fed *ad libitum* on a mixture of *Daphnia* spp., *Chironomus* sp. and *Cx. pipiens* larvae for acclimation. All predators were fasted for 24 h before being subjected to any experimental treatment.

A fully randomised factorial experimental design was employed with respect to predators (5 levels: 1 copepod, 1 notonectid, 2 copepods, 2 notonectids, 1 copepod + 1 notonectid), prey (3 levels: instar 1, instar 2/3, instar 4) and prey density (6 levels: 2, 4, 8, 16, 32, 64). Under each predator level, three discrete size classes of *Culex* prey were used (Mean \pm SD excluding the siphon; instar 1 = 1.4 ± 0.1 mm, instar 2/3 = 2.9 ± 0.2 mm, instar 4 = 4.9 ± 0.2 mm). Prey were supplied under six densities (2, 4, 8, 16, 32, 64; $n \geq 4$ per experimental group) per predator treatment in 350 mL glass experimental arenas (diameter 73.5 mm \times height 122.6 mm) holding 80 mL matured tap water from a continuously aerated source, and allowed to settle for 2 h. Corresponding predator treatments were then carefully transferred to arenas containing prey and allowed to feed for 4 h during light conditions, before being removed. Upon removal of predators, the remaining live larval mosquito prey were counted. Controls consisted of three replicates at each instar and prey supply, but in the absence of any predators.

5.2.2. Statistical analyses

Statistical analyses were performed in R v3.4.2 (R Core Development Team 2018). Poisson generalised linear models with log links were used to analyse counts of overall raw prey mortality at the end of the experimental feeding period with respect to predator (4 levels: 1 copepod, 1 notonectid, 2 copepods, 2 notonectids), prey (3 levels: instar 1, instar 2/3, instar 4) and density (6 levels: 2, 4, 8, 16, 32, 64). The single heterospecific predator group treatment was excluded here as it was not ubiquitous across all prey sizes (i.e., absent from prey instar stage 4). Initially, all two- and three-way interaction terms were included in a full model, which was reduced backward stepwise by removing non-significant terms and evaluating model deviance via likelihood ratio tests. Effects in the top model were discerned through analysis of deviance with Type III sums of squares where interaction terms were present. In the presence of an interaction term, constituting main effects were not considered further but were retained in the model. Estimated marginal means were used for Tukey comparisons *post-hoc*. Significance was considered at the 95 % confidence level. There was no evidence for zero inflation or residual overdispersion through comparison of observed residuals and model simulations.

Functional response analyses were undertaken phenomenologically, with logistic regression used to categorise functional response types. Heterospecific copepod-notonectid pairs were included here, and thus I fit functional response models separately for each predator group under all specific prey stages (i.e., 5 *predator* levels \times 3 *prey* levels). A Type II functional response is characterised by a significantly negative first-order term, whilst a Type III functional response is indicated by a significantly positive first-order term followed by a significantly negative second-order term (Juliano 2001; Pritchard *et al.*, 2017). Where functional response

Types were equivocal based on these criteria, the most appropriate functional response model (i.e., Type II, Type III, generalised) was selected *via* AIC (see Pritchard *et al.*, 2017). Owing to the non-replacement of mosquito prey during the experiment, Rogers' random predator equation was employed (Rogers 1972):

$$N_e = N_0(1 - \exp(a(N_e h - T))) \quad (1)$$

where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack rate, h is the handling time and T is the total experimental period. The *Lambert W* function was implemented due to the recursive characteristics of the random predator equation (Bolker 2008). Negligible consumption by copepods towards 4th instar mosquito prey negated formal functional response analyses for these experimental groups, and thus these groups were not considered further.

The population-dynamic approach was followed to simulate multiple predator consumption rates based on functional responses of individual predators (McCoy *et al.*, 2012; Sentis and Boukal 2018). This approach accounts for prey depletion (i.e., non-replacement) and non-linear feeding rates, and has been shown to be more robust than other approaches in the determination of multiple predator effects (Sentis and Boukal 2018). Predictions of consumption in paired predator groups were generated (i.e., 2 copepods, 2 notonectids, 1 copepod + 1 notonectid) based on the a and h estimates from the empirically-observed single predator treatments for both species across corresponding prey instar stages:

$$\frac{dN}{dt} = - \sum_{i=1}^n f_i(N)P_i \quad (2)$$

where N is the prey population density, P_i ($i = 1, 2, \dots, n$) are the population densities of predators i and $f_i(N)$ is the functional response of predator i (i.e. Equation 1) that was observed experimentally. Predictions from Equation 2 were subsequently compared to observed feeding rates from the experiment to quantify multiple predator effects across all prey densities. A global sensitivity analysis was followed to generate 95 % confidence intervals around predictions, using a and h parameter estimates, standard errors and covariance-variance matrices observed from single predator groups. Accounting for the total experimental time, a Latin hypercube sampling algorithm was employed to generate multiple parameter sets (Soetaert and Petzoldt 2010). Confidence intervals were extracted from 2.5 % and 97.5 % prey survival quantiles for both predicted and observed functional responses, with lower confidence limits capped at 0 (given negative values are not biologically feasible). Visual examination of 95 % confidence intervals was then used to compare functional responses across appropriate experimental groupings, and thus determine the occurrence of multiple predator effects (Sentis and Boukal 2018). Antagonistic multiple predator effects would be evidenced by significantly lower experimental observations than predicted, whilst synergistic effects would be determined by significantly greater experimental observations than predicted. Accordingly, this approach enables examination of whether the intensity of multiple predator effects shifts across con- and heterospecific predator combinations, and whether this is in turn dependent on the ontogenic stage of prey and prey density.

5.3. Results

All 378 mosquito prey survived in controls, and so prey mortality was attributed to predation by the copepods and notonectids, which was also observed. Consumptive differences

among predator treatments differed significantly across prey stages (*predator* × *prey*: $\chi^2 = 208.93$, $df = 6$, $p < 0.001$) (Figure 5.1). Towards 1st instar prey, single copepods consumed significantly more than single notonectids ($p < 0.001$). Similarly, copepod pairs consumed significantly more than notonectid pairs ($p < 0.001$). In turn, conspecific pairs of both species consumed significantly more than individual predators of the same species (both $p < 0.01$). Towards 2nd and 3rd instar prey, differences between copepod and notonectid predation were less statistically clear for both individual and paired treatments (both $p > 0.05$). Predation by conspecific pairs was, again, significantly greater than individual predators (both $p < 0.01$). Contrastingly, towards 4th instar prey, consumption by copepods was significantly lower than notonectids, for both individual and paired predator groupings (all $p < 0.001$). Predation by two copepods did not differ significantly from individuals ($p = 0.98$), whilst, conversely, consumption by two notonectids significantly exceeded that of one ($p < 0.001$). Accordingly, copepod predation was most efficacious towards early instar prey, whilst notonectids were consumptively superior towards late instars which copepods could not handle (Figure 5.1). In turn, fourth instar larval mosquitoes appeared to benefit from a copepod size refuge effect. A significant '*prey* × *density*' interaction also indicated greater consumptive differences among prey densities as prey size decreased ($\chi^2 = 25.48$, $df = 10$, $p = 0.005$), with higher magnitude functional responses reached towards earlier instar prey overall (Figure 5.2). Remaining two- and three-way interaction terms were removed stepwise from the top model owing to non-significance.

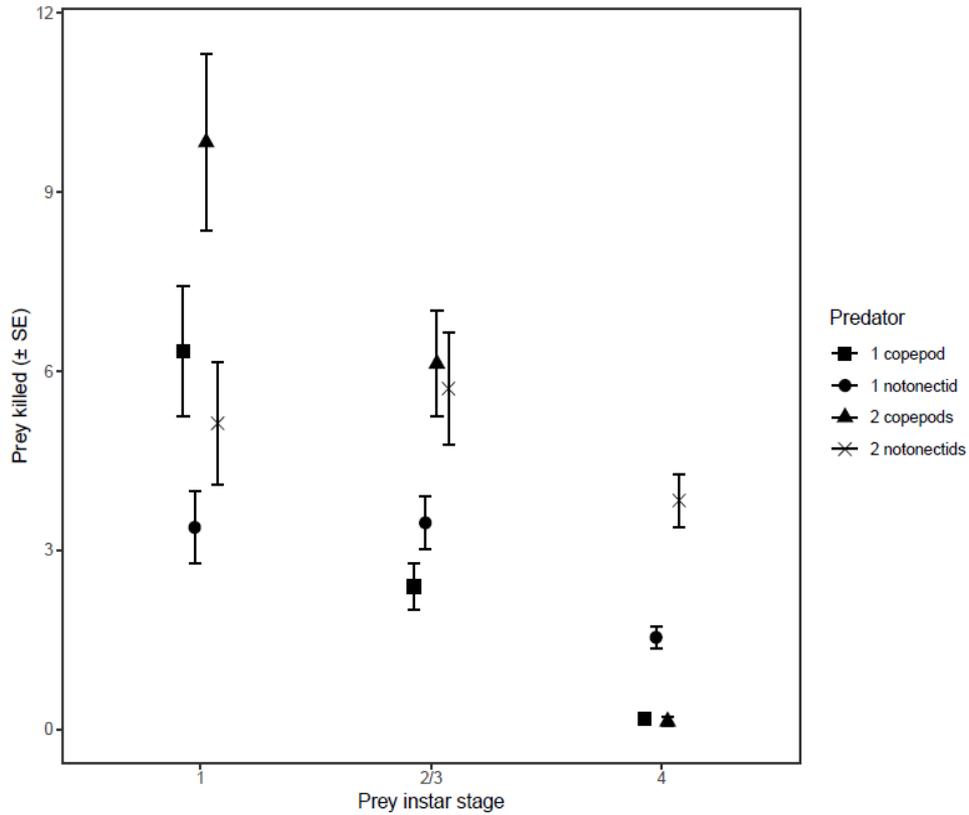


Figure 5. 1. Mean larval mosquito prey mortality across different instar stages and predator treatments, irrespective of prey density. Error bars represent means \pm SE.

Functional responses were categorised as Type II given significantly negative linear coefficients (Table 5.1). The individual notonectid treatment towards 1st instar prey was an exception to this ($p = 0.14$); however, the Type II model minimised information loss as compared to Type III or generalised functional response forms *via* Δ AIC comparisons (see Pritchard *et al.*, 2017). Towards 1st instars, copepods exhibited greater attack rates compared to notonectids as both individuals as conspecific pairs (Table 5.1). Attack rates tended to peak in the heterospecific predator pair here. As individuals, notonectid handling times were shorter than copepods; yet, the notonectid estimate was not significant and should thus be treated with caution ($p = 0.14$). Conspecific paired copepod handling times were shorter than those of notonectid pairs (Table

5.1). Towards 2nd and 3rd instar stages, notonectid attack rates were greater than those of copepods under individual predator treatment whilst, reciprocally, paired copepod attack rates were greater than estimates for paired notonectids. As with first instars, attack rates peaked under the heterospecific predator pair treatment. Handling times were more similar between copepods and notonectids as individuals and pairs here (Table 5.1). Towards 4th instar stages, attack rates were higher, and handling times shorter, by two notonectids as compared to individual notonectid estimates (Table 5.1).

Table 5. 1. First order terms resulting from logistic regression of the proportion of prey killed as a function of prey density across different predator and prey instar stage treatments. Attack rate and handling time parameter estimates result from Rogers’ random predator equation.

Predator	Prey instar stage	First-order term, p	Attack rate, p	Handling time, p
1 copepod	1	-0.02, < 0.001	0.77, < 0.001	0.05, < 0.001
1 notonectid	1	-0.01, 0.14	0.22, < 0.001	0.03, 0.14
2 copepods	1	-0.03, < 0.001	1.90, < 0.001	0.04, < 0.001
2 notonectids	1	-0.02, < 0.001	0.53, < 0.001	0.05, < 0.001
1 copepod + 1 notonectid	1	-0.03, < 0.001	1.98, < 0.001	0.04, < 0.001
1 copepod	2/3	-0.02, 0.005	0.32, 0.01	0.17, 0.004
1 notonectid	2/3	-0.03, < 0.001	0.82, 0.001	0.15, < 0.001
2 copepods	2/3	-0.03, < 0.001	1.35, < 0.001	0.08, < 0.001
2 notonectids	2/3	-0.02, < 0.001	0.86, < 0.001	0.07, < 0.001
1 copepod + 1 notonectid	2/3	-0.04, < 0.001	3.85, < 0.001	0.07, < 0.001
1 notonectid	4	-0.04, < 0.001	0.71, 0.01	0.39, < 0.001
2 notonectids	4	-0.03, < 0.001	0.99, < 0.001	0.14, < 0.001

For 1st instar prey, individual copepod functional responses were significantly higher than notonectids at low-intermediate prey densities, further indicating significant differences in attack rates (i.e., initial slope). Handling times (i.e., inversely maximum feeding rates) were more similar here (Figure 5.2a), owing to high prey density convergence. As illustrated by the

population-dynamic models (dashed lines), irrespective of predator pairing, multiple predator consumption rates towards 1st instars were well-predicted from those of individuals as confidence intervals overlapped across prey densities, indicating additive multiple predator effects (Figure 5.2b, c, d). Towards 2nd and 3rd instar prey, functional responses between individual copepods and notonectids did not significantly differ as confidence intervals overlapped across prey densities (Figure 5.2e). Similarly, conspecific paired copepod and notonectid functional responses were additive here (Figure 5.2f, g). For the heterospecific pairing, however, observed functional responses were significantly higher than those simulated by the population-dynamic model, indicating enhanced prey risk and synergism between predator types at low prey densities. This suggests significant differences in attack rates between heterospecific observations and simulations. Functional responses again converged at intermediate-high densities (Figure 5.2h). Towards 4th instar stages, paired notonectid functional responses were additive given their predictability from single notonectid consumption rates using the population-dynamic model (Figure 5.2i, j).

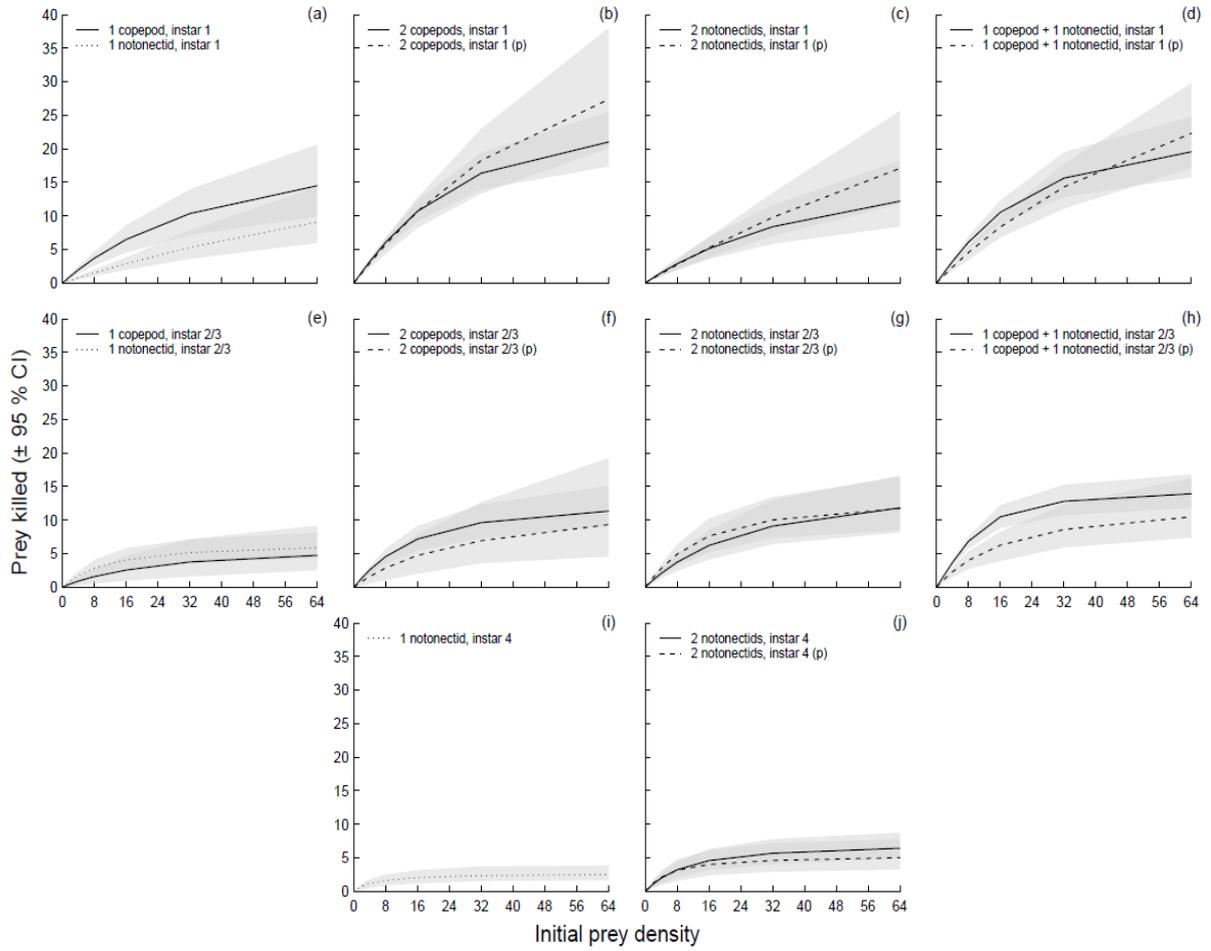


Figure 5. 2. Functional responses towards larval mosquito prey instars 1 (a, b, c, d), 2/3 (e, f, g, h) and 4 (i, j) by different combinations of copepod and notonectid predators. Multiple predator predictions (p) result from simulations based on individual predator functional responses (a, e, i) using a population-dynamic approach. Shaded areas are 95% confidence intervals.

5.4. Discussion

Predation is a pervasive force in aquatic communities (Hammerschlag *et al.*, 2019), yet biotic contexts such as prey size and predator-predator interactions can complicate predictions of interaction strength within ecosystems (Juliano 2009; Cooper Jr and Stankowich 2010; Wasserman *et al.*, 2016a). As resident natural enemies may provide important ecosystem services through regulation of medically important pest species such as mosquitoes (Zuharah and Lester 2010), robust quantifications of their contribution towards pest population regulation is imperative in the context of public health. In this study, it was demonstrated that an efficient predatory role of calanoid copepods and notonectids in potential regulating the disease vector mosquito *Cx. pipiens*. However, predation effects between species were dependent on the ontogenic stage of larval mosquitoes, with copepods exhibiting significantly higher efficacy towards 1st instar mosquito prey and notonectids more efficacious towards 4th instar mosquito prey, which copepods could not handle due to size refuge effects. Given predatory impacts increased with greater predator abundance and richness, and multiple predator effects combined at least additively, promotion of such predators in aquatic systems where vector mosquitoes breed could contribute to effective synergistic population-level regulation across various prey ontogenic stages.

Predator-prey interaction strengths are known to scale unimodally across predator-prey body size ratios, with search efficiencies peaking at intermediate size classes and predators often less efficient at handling relatively large or small prey (McCoy and Bolker 2008; Barrios-O'Neill *et al.*, 2016). These generalities are likely key in explaining the

consumptive propensities revealed in the present study, with copepod predation optimised towards early instars (stage 1) and falling concurrently with increases in prey size. Conversely, predation by larger notonectids tended to peak towards intermediate prey size classes (stages 2/3), before again reducing in strength towards late instar (stage 4) mosquito prey. These notonectid preferences also corroborate with studies on congenics, where intermediate instar stages of larval mosquitoes were preferred as prey (Dalal *et al.* 2019a,b). Differences in optimal prey size classes suggest that diverse predator communities provide intensive top-down pest species regulation across ontogenic prey stages in temporary ponds. Whilst other predators are known to colonise these ecosystems (O'Neill and Thorpe 2014; Dalu *et al.*, 2016), copepods and notonectids are representative groups which numerically dominate in early and late hydroperiod stages, respectively (Wasserman *et al.*, 2018). Furthermore, whilst predatory impacts of notonectids such as *A. sardea* are well-known (Eitam *et al.*, 2002), this study is the first to consider the predatory potential of *L. falcifera* in a pest control context, adding to a limited body of work suggesting this ecosystem service by temporary pond specialists (Cuthbert *et al.*, 2018d, 2019d). Whilst *L. falcifera* appears to impart size refuge to the largest mosquito instar stages, large calanoids such as this species are able to handle a greater diversity of prey sizes than cyclopoids, which have been comparatively well-studied for vector control (Marten and Reid 2007; Cuthbert *et al.* 2018e). When considered in combination with increasing predation rates under greater predator density and richness, the results suggest that co-existence of certain taxonomic group in natural aquatic habitats may facilitate effective control of vector mosquito populations.

All predator groupings in the present study displayed saturating Type II FRs towards all size classes of mosquito prey, characterised by high predation rates at low prey densities. Therefore, both predator types have the potential to drive local extirpations of mosquito prey, although other factors such as structural complexity within habitats are known to further modulate FR types (e.g., to sigmoidal Type III; Alexander *et al.*, 2012). Attack rates (i.e., search efficiency) correspond to predatory impact at low prey densities, and these results align with the consumptive patterns presented. That is, attack rates by copepods were generally highest towards early instar (stage 1) prey, whilst notonectid attack rates were higher towards later instar stages. Considering grouped predator treatments, when assessed, heterospecific copepod-notonectid pairs always exhibited the highest attack rates, and thus increased predator diversity may exacerbate interaction strength towards mosquito prey at low densities. Indeed, the study found evidence for emergent positive multiple predator effects in heterospecific predator groupings, indicating synergism among predators that enhances prey risk. Handling times (reciprocally maximum feeding rates) also reflected size-related differences in interaction strengths between predator species. Handling times by copepods were generally lowest, and thus maximum feeding rates highest, towards early instar (stage 1) mosquitoes. Contrastingly, handling times by notonectids were lower towards later instar stages compared to copepods, driving greater maximum feeding rates here. Notably, whilst copepods imparted size refuge to late instars (stage 4), high attack rates by predators towards earlier stages could drastically reduce early development stage mosquitoes in temporary ponds, negating potential size refugia in later instar classes.

Using a FR approach, multiple predator interactions tended to combine additively in the present study, as described by Příbylová and Berc (2015), with prey risk not alleviated by interference among predators. Although applications of FRs in assessments of multiple predator effects are relatively scarce (Cuthbert *et al.*, 2019a), several models have been used to quantify emergent multiple predator effects using a FR approach, as demonstrated by Sentis and Boukal (2018). The population-dynamic approach applied here has been identified as more robust than other methods of quantifying multiple predator effects (e.g., multiplicative risk model; see Sentis and Boukal 2018), and thus demonstrates high utility for pest management. These results suggest that increasing predator diversity and richness (Hamer and Brendonck 1997) manifests additively in increasing predatory effects on mosquito populations (Greenop *et al.*, 2018). Therefore, resident natural enemies within these systems should be recognised as an important public health ecosystem service, and communities containing such predators should be conserved and promoted in order to regulate vector species proliferations and the diseases they may spread (Cuthbert *et al.*, 2018a).

Here, it was demonstrated that *L. falcifera* and *A. sardea*, both widely distributed species in southern Africa, are efficient predators which exhibit optimal predatory performance towards different ontogenic stages of larval mosquitoes. Predator-predator interactions combined additively, suggesting that increasing predator diversity and richness can drive effective complimentary regulation of pest mosquitoes in aquatic habitats. It is thus, proposed that predator communities should be conserved in temporary pond ecosystems where vector mosquitoes can proliferate, given the high threat of degradation

that these habitats face (Dalu *et al.*, 2017). Future studies should examine MPEs in these systems using more complex community modules; however, in this study and others (e.g., Wasserman *et al.*, 2016a), predatory pairs were sufficient to deduce powerful quantitative insights into non-trophic interactions and context-dependencies. Further, the utility of other predators in these systems for mosquito population control should be explored, alongside the examination of more complex assemblages containing a variety of prey types and trophic groups to quantify interaction strengths. Future studies should also include field components, examining how habitat degradation alters predator diversity and abundance in temporary aquatic ecosystems, what such predator community alterations mean for larval mosquito abundances and the sustainability of this biocontrol in natural systems and applied in an integrated approach. Aquatic predator species loss associated with human-mediated aquatic habitat alteration is largely unexplored in temporary wetland ecosystems, particularly within the context of its contribution to aquatic pest species proliferation. Finally, engagement with local authorities and communities regarding vector mosquito disservices associated with aquatic habitat degradation should be considered.

5.5. References

- Alexander, M. E., Dick, J. T., O'Connor, N. E., Haddaway, N. R., and Farnsworth, K. D. (2012). Functional responses of the intertidal amphipod *Echinogammarus marinus*: effects of prey supply, model selection and habitat complexity. *Marine Ecology Progress Series*, 468: 191-202.
- Azari-Hamidian, S. (2007). Larval habitat characteristics of mosquitoes of the genus *Culex* (Diptera: Culicidae) in Guilan Province, Iran. *Journal of Arthropod-borne Diseases*, 1: 9-20.

- Azevedo-Santos, V. M., Vitule, J. R., García-Berthou, E., Pelicice, F. M., and Simberloff, D. (2016). Misguided strategy for mosquito control. *Science*, 351: 675-675.
- Barrios-O'Neill, D., Kelly, R., Dick, J. T., Ricciardi, A., MacIsaac, H. J., and Emmerson, M. C. (2016). On the context-dependent scaling of consumer feeding rates. *Ecology Letters*, 19: 668-678.
- Barrios-O'Neill, D., Dick, J. T., Emmerson, M. C., Ricciardi, A., and MacIsaac, H. J. (2015). Predator-free space, functional responses and biological invasions. *Functional Ecology*, 29: 377-384.
- Benelli, G., Jeffries, C., and Walker, T. (2016). Biological control of mosquito vectors: past, present, and future. *Insects*, 7: 52.
- Bolker, B. M. (2008). *Ecological models and data in R*. Princeton University Press. New Jersey, USA.
- Braack, L., de Almeida, A. P. G., Cornel, A. J., Swanepoel, R., and De Jager, C. (2018). Mosquito-borne arboviruses of African origin: review of key viruses and vectors. *Parasites and Vectors*, 11: 29.
- Bradshaw, C. J., Leroy, B., Bellard, C., Roiz, D., Albert, C., Fournier, A., and Courchamp, F. (2016). Massive yet grossly underestimated global costs of invasive insects. *Nature communications*, 7: 1-8.
- Brose, U. (2010). Body-mass constraints on foraging behaviour determine population and food-web dynamics. *Functional Ecology*, 24: 28-34.
- Chatterjee, S. N., Ghosh, A., and Chandra, G. (2007). Eco-friendly control of mosquito larvae by *Brachytron pratense* nymph. *Journal of Environmental Health*, 69: 44-49.
- Cooper Jr, W. E., and Stankowich, T. (2010). Prey or predator? Body size of an approaching animal affects decisions to attack or escape. *Behavioral Ecology*, 21: 1278-1284.
- Crans, W. J. (2004). A classification system for mosquito life cycles: life cycle types for mosquitoes of the northeastern United States. *Journal of Vector Ecology*, 29: 1-10.
- Cuthbert, R. N., Callaghan, A., Sentis, A., Dalal, A., and Dick, J. T. (2019a). Additive multiple predator effects can reduce mosquito populations. *Ecological Entomology*, 45: 243-250.

- Cuthbert, R. N., Dalu, T., Wasserman, R. J., Callaghan, A., Weyl, O. L., and Dick, J. T. (2019b). Using functional responses to quantify notonectid predatory impacts across increasingly complex environments. *Acta Oecologica*, 95: 116-119.
- Cuthbert, R. N., Dalu, T., Wasserman, R. J., Weyl, O. L., Callaghan, A., Froneman, W., and Dick, J. T. (2019c). Sex-skewed trophic impacts in ephemeral wetlands. *Freshwater Biology*, 64: 359-366.
- Cuthbert, R. N., Weyl, O. L., Wasserman, R. J., Dick, J. T., Froneman, P. W., Callaghan, A., and Dalu, T. (2019d). Combined impacts of warming and salinisation on trophic interactions and mortality of a specialist ephemeral wetland predator. *Freshwater Biology*, 64: 1584-1592.
- Cuthbert, R. N., Dalu, T., Wasserman, R. J., Dick, J. T., Mofu, L., Callaghan, A., and Weyl, O. L. (2018a). Intermediate predator naïveté and sex-skewed vulnerability predict the impact of an invasive higher predator. *Scientific Reports*, 8: 1-8.
- Cuthbert, R. N., Dick, J. T., Callaghan, A., and Dickey, J. W. (2018b). Biological control agent selection under environmental change using functional responses, abundances and fecundities; the Relative Control Potential (RCP) metric. *Biological Control*, 121: 50-57.
- Cuthbert, R. N., Dalu, T., Wasserman, R. J., Callaghan, A., Weyl, O. L., and Dick, J. T. (2018c). Calanoid copepods: an overlooked tool in the control of disease vector mosquitoes. *Journal of Medical Entomology*, 55: 1656-1658.
- Cuthbert, R. N., Dalu, T., Wasserman, R. J., Coughlan, N. E., Callaghan, A., Weyl, O. L., and Dick, J. T. (2018d). Muddy waters: efficacious predation of container-breeding mosquitoes by a newly-described calanoid copepod across differential water clarities. *Biological Control*, 127: 25-30.
- Cuthbert, R. N., Dick, J. T., and Callaghan, A. (2018e). Interspecific variation, habitat complexity and ovipositional responses modulate the efficacy of cyclopoid copepods in disease vector control. *Biological Control*, 121: 80-87.
- Dalal, A., Cuthbert, R. N., Dick, J. T., and Gupta, S. (2019a). Water depth-dependent notonectid predatory impacts across larval mosquito ontogeny. *Pest Management Science*, 75: 2610-2617.

- Dalal, A., Cuthbert, R. N., Dick, J. T., and Gupta, S. (2019b). Prey preferences of notonectids towards larval mosquitoes across prey ontogeny and search area. *Pest Management Science*, 76: 609-616.
- Dalu, T., Weyl, O. L., Froneman, P. W., and Wasserman, R. J. (2016). Trophic interactions in an austral temperate ephemeral pond inferred using stable isotope analysis. *Hydrobiologia*, 768: 81-94.
- Dalu, T., Wasserman, R. J., and Dalu, M. T. (2017). Agricultural intensification and drought frequency increases may have landscape-level consequences for ephemeral ecosystems. *Global Change Biology*, 23: 983-985.
- De Bernardi, R. (1981). Biotic interactions in freshwater and effects on community structure. *Italian Journal of Zoology*, 48: 353-371.
- De Sá, L. C., Oliveira, M., Ribeiro, F., Rocha, T. L., and Fütter, M. N. (2018). Studies of the effects of microplastics on aquatic organisms: what do we know and where should we focus our efforts in the future? *Science of the Total Environment*, 645: 1029-1039.
- Dida, G. O., Gelder, F. B., Anyona, D. N., Abuom, P. O., Onyuka, J. O., Matano, A. S., and Ofulla, A. V. (2015). Presence and distribution of mosquito larvae predators and factors influencing their abundance along the Mara River, Kenya and Tanzania. *SpringerPlus*, 4: 136.
- Eitam, A., Blaustein, L., and Mangel, M. (2002). Effects of *Anisops sardea* (Hemiptera: Notonectidae) on oviposition habitat selection by mosquitoes and other dipterans and on community structure in artificial pools. *Hydrobiologia*, 485: 183-189.
- Gomes, A. D. C., Gotlieb, S. L. D., Marques, C. C., Paula, M. B. D., and Marques, G. R. A. (1995). Duration of larval and pupal development stages of *Aedes albopictus* in natural and artificial containers. *Revista de Saude Publica*, 29: 15-19.
- Greenop, A., Woodcock, B. A., Wilby, A., Cook, S. M., and Pywell, R. F. (2018). Functional diversity positively affects prey suppression by invertebrate predators: a meta-analysis. *Ecology*, 99: 1771-1782.
- Hamer M. L., and Brendonck, L. (1997). Distribution, diversity and conservation of Anostraca (Crustacea: Branchiopoda) in southern Africa. In *Studies on Large Branchiopod Biology and Conservation*. Springer, Dordrecht, Netherlands.

- Hammerschlag, N., Schmitz, O. J., Flecker, A. S., Lafferty, K. D., Sih, A., Atwood, T. B., and Cooke, S. J. (2019). Ecosystem function and services of aquatic predators in the Anthropocene. *Trends in Ecology and Evolution*, 34: 369-383.
- Hayasaka, D. (2014). Study of the impacts of systemic insecticides and their environmental fate in aquatic communities of paddy mesocosms. *Journal of Pesticide Science*, 39: 172-173.
- Hutchinson, G. E. (1927). *A revision of the Notonectidae and Corixidae of South Africa*. Trustees of the South African museum, Cape Town, South Africa.
- Juliano, S. A. (2009). Species interactions among larval mosquitoes: context dependence across habitat gradients. *Annual Review of Entomology*, 54: 37-56.
- Juliano, S. A. (2001). Non-linear curve fitting: predation and functional response curve. Design and Analysis of Ecological Experiment. *Oxford University Press*, Oxford, UK.
- Jupp, P. G. (1996). Mosquitoes of Southern Africa: Culicinae and Toxorhynchitinae. *Ekogilde Publishers*, Johannesburg, South Africa.
- Kay, B. H., and Nam, V. S. (2005). New strategy against *Aedes aegypti* in Vietnam. *Lancet*, 365: 613-617.
- Kulongoski, J. T., Hilton, D. R., and Selaolo, E. T. (2004). Climate variability in the Botswana Kalahari from the late Pleistocene to the present day. *Geophysical Research Letters*, 31: L10204.
- Lacey, L. A., and Orr, B. K. (1994). The role of biological control of mosquitoes in integrated vector control. *The American Journal of Tropical Medicine and Hygiene*, 50: 97-115.
- Little, E., Biehler, D., Leisnham, P. T., Jordan, R., Wilson, S., and LaDeau, S. L. (2017). Socio-ecological mechanisms supporting high densities of *Aedes albopictus* (Diptera: Culicidae) in Baltimore, MD. *Journal of Medical Entomology*, 54: 1183-1192.
- Marten, G. G., and Reid, J. W. (2007). Cyclopoid copepods. *Journal of the American Mosquito Control Association*, 23: 65-92.

- McCoy, M. W., Stier, A. C., and Osenberg, C. W. (2012). Emergent effects of multiple predators on prey survival: the importance of depletion and the functional response. *Ecology Letters*, 15: 1449-1456.
- McCoy, M. W., Bolker, B. M., Warkentin, K. M., and Vonesh, J. R. (2011). Predicting predation through prey ontogeny using size-dependent functional response models. *The American Naturalist*, 177: 752-766.
- McCoy, M. W., and Bolker, B. M. (2008). Trait-mediated interactions: influence of prey size, density and experience. *Journal of Animal Ecology*, 77: 478-486.
- O'Neill, B. J., and Thorp, J. H. (2014). Untangling food-web structure in an ephemeral ecosystem. *Freshwater Biology*, 59: 1462-1473.
- Pires, D. A., and Gleiser, R. M. (2010). Mosquito fauna inhabiting water bodies in the urban environment of Córdoba city, Argentina, following a St. Louis encephalitis outbreak. *Journal of Vector Ecology*, 35: 401-409.
- Priawandiputra, W., Zakaria, F. R. N., and Prawasti, T. S. (2018). Aquatic Insect Community as Indicator of Water Quality Assessment in Situ Gede System, Bogor, Indonesia. In *IOP Conference Series: Earth and Environmental Science*. IOP Publishing. 197: 012016.
- Přibylová, L., and Berec, L. (2015). Predator interference and stability of predator-prey dynamics. *Journal of Mathematical Biology*, 71: 301-323.
- Pritchard, D. W., Paterson, R., Bovy, H. C., and Barrios-O'Neill, D. (2017). Frair: an R package for fitting and comparing consumer functional responses. *Methods in Ecology and Evolution*, 8: 1528-1534.
- Rogers, D. (1972). Random search and insect population models. *The Journal of Animal Ecology*, 41: 369-383.
- Roux, O., and Robert, V. (2019). Larval predation in malaria vectors and its potential implication in malaria transmission: an overlooked ecosystem service? *Parasites and Vectors*, 12: 217.
- Schmitz, O. J. (2007). Predator diversity and trophic interactions. *Ecology*, 88: 2415-2426.
- Sentis, A., and Boukal, D. S. (2018). On the use of functional responses to quantify emergent multiple predator effects. *Scientific Reports*, 8: 1-12.

- Shaalán, E. A. S., and Canyon, D. V. (2009). Aquatic insect predators and mosquito control. *Tropical Biomedicine*, 26: 223-261.
- Sih, A., Englund, G., and Wooster, D. (1998). Emergent impacts of multiple predators on prey. *Trends in Ecology and Evolution*, 13: 350-355.
- Soetaert, K., and Petzoldt, T. (2010). Inverse modelling, sensitivity and Monte Carlo analysis in R using package FME. *Journal of Statistical Software*, 33: 1-28.
- Soluk, D. A. (1993). Multiple predator effects: predicting combined functional response of stream fish and invertebrate predators. *Ecology*, 74: 219-225.
- UNFCCC (2007). Climate Change Impacts, Vulnerabilities and Adaptation in Developing Countries. <http://unfccc.in/resources/docs/publications>.
- Wasserman, R. J., Weston, M., Weyl, O. L., Froneman, P. W., Welch, R. J., Vink, T. J., and Dalu, T. (2018). Sacrificial males: the potential role of copulation and predation in contributing to copepod sex-skewed ratios. *Oikos*, 127: 970-980.
- Wasserman, R. J., Alexander, M. E., Dalu, T., Ellender, B. R., Kaiser, H., and Weyl, O. L. (2016a). Using functional responses to quantify interaction effects among predators. *Functional Ecology*, 30: 1988-1998.
- Wasserman, R. J., Alexander, M. E., Weyl, O. L., Barrios-O'Neill, D., Froneman, P. W., and Dalu, T. (2016b). Emergent effects of structural complexity and temperature on predator-prey interactions. *Ecosphere*, 7: e01239.
- Wasserman, R. J., Alexander, M. E., Barrios-O'Neill, D., Weyl, O. L., and Dalu, T. (2016c). Using functional responses to assess predator hatching phenology implications for pioneering prey in arid temporary pools. *Journal of Plankton Research*, 38: 154-158.
- Wasserman, R. J., Noyon, M., Avery, T. S., and Froneman, P. W. (2013). Trophic level stability-inducing effects of predaceous early juvenile fish in an estuarine mesocosm study. *PLoS ONE*, 8: e61019.
- Woodcock, B. A., and Heard, M. S. (2011). Disentangling the effects of predator hunting mode and habitat domain on the top-down control of insect herbivores. *Journal of Animal Ecology*, 80: 495-503.
- World Health Organization. (2017). Factsheet: Vector-borne disease. World Health Organization, Geneva, Switzerland.

Zuharah, W. F., and Lester, P. J. (2010). The influence of aquatic predators on mosquito abundance in animal drinking troughs in New Zealand. *Journal of Vector Ecology*, 35: 347-353.

CHAPTER 6

Predator density modifies mosquito regulation in increasingly complex environments

Published as: **Buxton, M.**, Cuthbert, R. N., Dalu, T., Nyamukondiwa, C., and Wasserman, R. J. (2020). Predator density modifies mosquito regulation in increasingly complex environments. *Pest Management Science*, 76: 2079-2086.

6.1. Introduction

Predation plays a critical role in ecosystem functioning and community composition (Griffin *et al.*, 2008; Vonesh *et al.*, 2009; Wasserman *et al.*, 2013). In a natural enemy context, predators exhibit top-down pressures on prey and may aid regulation of target organisms through both trophic and non-trophic interactions (McCoy *et al.*, 2009; Raffel *et al.*, 2009; Weis 2011; Heithaus *et al.*, 2012). This natural control can keep pest numbers below problematic thresholds, unless efficacy is disturbed through anthropogenic interference (Van Driesche and Hoddle 2009). However, the strength of predatory interactions may also be mediated through biotic and abiotic context-dependencies, which are often poorly understood (Shears *et al.*, 2008; Chamberlain *et al.*, 2014; Paterson *et al.*, 2015). In particular, emergent effects associated with changeable densities of antagonists may reduce or enhance the efficacy of natural enemy populations in controlling pest populations (Cardinale *et al.*, 2003; Ghosh *et al.*, 2005). Multiple predator interactions may manifest in three broad forms: (1) additively, where predators interact independently with their prey irrespective of predator density, (2) antagonistically, where predator-predator interference reduces impact and thus alleviates prey risk and (3), synergistically, where multiple predator effects enhance predatory impacts and therefore increase prey risk (Skalski and Gilliam 2001; Wasserman *et al.*, 2016a; Cuthbert *et al.*, 2019a,b,c). In addition, habitat complexity is also known to alter predatory interactions Cuthbert *et al.*, 2019a; Grabowski 2004). In particular, emergent multiple predator effects may be further mediated by the structuring of habitats according to specific predatory tactics. Depending on the nature of predator-prey capture tactics, increasing habitat complexities may reduce predatory impacts through provisioning of prey refuge via predator-free space (Barrios-

O'Neill *et al.*, 2015). Conversely, the presence of habitat complexity could intensify predatory efficiencies, such as for ambush predators which are less conspicuous in structured environments (Mandal *et al.*, 2008), or through prey clumping effects (Alexander *et al.*, 2012). However, there is a lack of information on how multiple context-dependencies interact to mediate natural enemy efficiencies.

The intensity of trophic interactions also differs according to densities of resources within environments (Anholt and Werner 1998; Nicolle *et al.*, 2011). In a predator-prey context, functional responses (FRs) have been applied in multiple ecological fields to quantify the density-dependence of interaction strengths (Solomon 1949; Holling 1959; Oaten and Murdoch 1975). Three forms of FRs have been commonly characterised: the linear Type I, hyperbolic Type II and sigmoidal Type III, with each form theoretically associated with different prey population stability outcomes (Alexander *et al.*, 2013; Dick *et al.*, 2014). For natural enemies, Type II FRs are desirable given characteristically high rates of target resource consumption at low densities. In turn, these interactions could destabilise target populations owing to a lack of low-density refugia (Cuthbert *et al.*, 2018a; 2019c). Conversely, Type III FRs can impart refuge to low density prey populations and may thus allow for population persistence (Leeuwen *et al.*, 2007; Thorp *et al.*, 2018). Functional response quantifications, importantly, also allow for the explicit assessment of context-dependency effects on interaction strengths (Barrios-O'Neill *et al.*, 2014; Cuthbert *et al.*, 2019b). In particular, recent studies have applied FRs to quantify the prey density-dependences of emergent multiple predator effects (McCoy *et al.*, 2012; Wasserman *et al.*, 2016a; Sentis and Boukal 2018), and others have demonstrated the effects of habitat

complexity for interaction strength (Robson 1996; Michel and Adams 2009). In addition, studies have examined the interactive complexities of multiple environmental factors on predator-prey dynamics, which often combine non-linearly (Wasserman *et al.*, 2016b; Cuthbert *et al.*, 2019b).

Temporary aquatic habitats are known to contain temporally dynamic predator densities and diversities (Vonesh and Blaustein 2010; O'Neill and Thorp 2014; Wasserman *et al.*, 2018). Moreover, the vegetative habitat structure therein can vary substantially over the hydroperiod, with implications for habitat complexity dynamics (Hulsmans *et al.*, 2008; Vanschoenwinkel *et al.*, 2009). Eutrophication processes facilitate increased submerged, emergent macrophyte and filamentous algal densities (Daldorph and Thomas 1991). These aquatic ecosystems are also often quickly colonised by mosquitoes, some of which potentially vector pathogens and parasites that cause disease in humans and wildlife (Jackman and Olson 2002). The habitat complexity dynamics of such aquatic habitats may thus modulate predatory impacts towards larval mosquito prey (Grabowski 2004). Moreover, external recruitment dynamics which drive predator density and diversity changes may alter natural enemy effects over time, with many higher insect predators recruited during the hydroperiod in high abundances (Baber *et al.*, 2004). Accordingly, the combined effects of increasing habitat complexity and predator density on the population regulation potential of mosquitoes is of key importance for pest and disease vector regulation. Furthermore, given natural enemies of mosquitoes utilise different prey capture strategies (e.g., active *vs.* sit-and-wait) (Ross and Winterhalder 2015), effects of habitat complexity may manifest differently across predator species. For example, habitat

complexity may drive negative multiple predator effects that reduce mosquito prey risk from certain predator species or, conversely, could reduce predator-predator interference which increases prey risk. In turn, the relative contributions of predator-predator interactions to prey risk may differ across habitat complexities.

Notonectids colonise a wide range of aquatic habitats where they are often top predators, with the capability to drive trophic cascades (Ellis and Borden 1970; Blaustein 1998; Svensson *et al.*, 2000). In Botswana, *Anisops sardea* (Anisopinae) and *Enithares chinai* (Notonectinae) are both found in temporary wetlands, although *A. sardea* are typically found in much higher abundances than *E. chinai* in these habitats (RJ Wasserman, *pers. obs.*). The role of notonectids in the regulation of vector mosquito populations has been well-documented (Scott and Murdoch 1983; Streams 1992; Domingos and Arcifa 2017), however studies contrasting the predatory impacts of Notonectinae and Anisopinae sub-families within the context of habitat complexity are lacking. This is relevant given behavioural (Matthews and Seymour 2008) and habitat selection (Miller 1966) differences between members of these sub-families which often co-occur in temporary wetlands (Dimentman and Margalit 1981; Wasserman *et al.*, 2018). In particular, members of the Anisopinae are capable of maintaining neutral buoyancy at depth, given that they contain haemoglobin, and can thus control the partial pressure of oxygen in their air bubble while diving (Matthews and Seymour 2008). As such, they are often found in open-water environments free from vegetation structure (Miller 1966; Kaiser 1940). Members of the Notonectinae, however, have been observed to be positively buoyant upon diving (Matthews and Seymour 2006). Rather than neutral buoyancy processes, maintenance of

position at depth by this group is facilitated through active movement or the grasping of structure at depth (Wasserman *et al.*, 2016b). Indeed, members of the Notonectinae are often found in close association with submerged aquatic plants (Kaiser 1940; Wasserman *et al.*, 2016b). It is highly likely that the Notonectinae are more efficient at performing in and around structure than the Anisopinae, presumably with implications for prey acquisition. While differences in predatory behavior between the two types of notonectids will likely have implications for predator-prey interaction outcomes, prey behaviour will also be important in determining levels of predation success. Larval mosquitoes typically rest at the surface, and may swim downwards if exposed to antagonistic interactions, such as an interaction with a predator. However, the nature of anti-predator responses is known to vary among species depending on coevolutionary context (Ohba *et al.*, 2012; Zuharah and Lester 2011). This anti-predator response may increase and decrease vulnerability depending on the position of the predators. Under multiple predator scenarios, risk effects may even become emergent as encounter rates will likely increase, particularly if individual predators are positioned at differing depths. These considerations are particularly pertinent for habitat complexity dynamics in Notonectinae and Anisopinae predator systems, given general differences in their habitat preference and behaviour. However, studies have yet to examine the influence of habitat complexity on the predatory impacts of notonectids as predator densities change or contrast such differences between these two widespread notonectid sub-families.

The present study thus uses a FR approach to quantify and contrast predator-prey interactions between two locally-abundant notonectids, *Anisops sardea* (Anisopinae) and

Enithares chinai (Notonectinae), towards larvae of *Culex pipiens* vector mosquito. Multiple predator effects between conspecifics of the two notonectid species were quantified and examined the influence of a habitat complexity gradient on the strength of notonectid-mosquito interactions. Given differences in the behavior of the predators and the escape behaviour of the prey, it was predicted that: (1) both predatory species would show high attack rates towards larval mosquito prey at low habitat complexity, but that; (2) increased habitat complexity would have a greater reduction effect on predatory impacts of *A. sardea* than *E. chinai*. It was further hypothesised that: (3) *A. sardea* MPEs would result in reduction of predation with an increase in habitat complexity, but that; (4) *E. chinai* conspecific MPEs would increase prey vulnerability with an increase in habitat complexity. Experimental and population dynamic modelling approaches were combined, providing novel insights into the emergent effects of predator density and habitat complexity for the population regulation of pest and vector species. This information may help improve bio- and integrated-control of disease insect pest vector species under heterogeneous environments.

6.2. Materials and Methods

6.2.1. Animal collection and maintenance

Culex pipiens (2.9 ± 0.2 mm length; instar 2/3), hatched from egg rafts sampled from a water body (22° 35' 05.7S; 27° 06' 58.7E) situated on the Botswana International University of Science and Technology (BIUST) campus, Palapye, Botswana, were used as prey. This size class prey was employed as both focal predatory species readily consumed mosquito larvae at these instars, facilitating a comparative assessment between the two

predator species. Further, this size class has been shown to be preferred by notonectids in other studies (Dalal *et al.*, 2020). The larvae were transferred into 3 L plastic containers (Length: 150 mm × Width: 150 mm × Height: 200 mm) holding ~2 L matured tap water, fed crushed rabbit food pellets (Westerman's Premium, Durban, South Africa) and reared in climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany) at 20°C ±2; 65% ±10 RH under a 12:12 light:dark photocycle. The chambers were housed in a temperature-controlled laboratory (20°C ±1). Adult predators (*A. sardea* [hereafter, *Anisops*] 8.3 ± 0.2 mm and *E. chinai* [hereafter, *Enithares*] 10.6 ± 0.2 mm length) were sampled using a 1000 µm mesh net from a temporary pool (76 m length × 54 m width) in the BIUST campus (22° 35' 46.8S; 27° 07' 30.5E) between May and June 2019. The pond was predominantly stuffed with *Cyperus* species and diverse invertebrates. Although sampling was done in different habitats, these predators are capable of dispersing aerially, accessing unique and diverse aquatic ecosystems in search of resources (Papáček 2013). In the laboratory (see before), the predators were identified using gross morphology (Hutchinson 1929) and sorted into two aerated 5 L plastic containers (Length: 150 mm × Width: 150 mm × Height: 300 mm) holding pond and mature tap water (50:50 ratio). These were fed with *Cx. pipiens* larvae *ad libitum*. Predators were housed for five days prior to experimentation and fasted for 24 h before being exposed to any experimental treatments.

6.2.2. Experimental design

A factorial experimental design was used, fully randomised with respect to predators (4 levels: 1 *Anisops*, 1 *Enithares*, 2 *Anisops*, 2 *Enithares*), habitat complexity (3 levels: low, medium, high) and prey supply (6 levels: 2, 4, 8, 16, 32, 64). Treatments were

replicated at least four times per experiment group. In simulating habitat complexity, stalks of *Cyperus* sp. (Height: 120 ± 0.1 mm \times Width: 5.31 ± 0.2 mm), common in local temporary pools, were placed in 350 ml experimental glass arenas (Height: 123 mm \times Diameter: 74 mm) and arranged uniformly along the habitat gradient as in keeping with Cuthbert *et al.* (2019a). Low habitat complexity held no stalks, medium habitat complexity had two stalks and high habitat complexity contained four stalks. Predator heterospecific pairs were trialed, but ultimately not used due to high instances of intraguild predation thus, *Enithares* devoured the *Anisops*. Prey were carefully supplied into glass arenas holding 200 ml tap water (dechlorinated) from a continuously aerated source and allowed to settle for 2 h. Predator treatments were then added to arenas containing corresponding prey supplies and allowed to feed in light conditions for 3 h. After the feeding duration, predators were removed and the larvae (prey) that remained alive were counted to quantify numbers killed. Three predator-free control replicates of each prey density and habitat complexity were used to quantify background prey mortality levels.

6.2.3. Data analyses

All statistical analyses were performed in R v3.4.4 (R Development Core Team 2018). A Poisson-distributed generalised linear model with log link was used to analyse differences in prey mortality as a function of predators (4 levels), habitat complexity (3 levels) and prey supply (6 levels) without corrections for overdispersion since there was no significant residual deviance. The predator \times habitat interaction effects were also included in the model. Analysis of deviance with Type III sums of squares was used to infer the significance levels of main effects, with estimated marginal means used for *post-*

hoc pairwise comparisons, where appropriate. Significance was considered at the 95 % confidence level for all analyses.

Logistic regression was used to infer FR Types among each predator and habitat complexity treatment phenomenologically. Here, a Type II FR is characterised by a significantly negative linear coefficient (Juliano 2001). As mosquito prey were not replaced as they were consumed, I fit Rogers' random predator equation:

$$N_e = N_0(1 - \exp(a(N_e h - T)))$$

(1)

where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack rate, h is the handling time and T is the total experimental period. The Lambert W function was integrated to solve the random predator equation (Bolker 2008).

To quantify emergent multiple predator effects, a population dynamic approach was followed wherein multiple predator consumption was simulated from individual predator FR parameters (McCoy *et al.*, 2012; Sentis and Boukal 2018). These inferences were made separately according to each predator and habitat level using a and h estimates from the random predator equation (see before):

$$\frac{dN}{dt} = - \sum_{i=1}^n f_i(N)P_i$$

(2)

where N is the prey population density, P_i ($i = 1, 2, \dots, n$) the population densities of predators i and $f_i(N)$ the FR of predator i (i.e. Equation 1). These simulations were subsequently compared to observed FR curves to test for the presence of multiple predator

effects based on 95 % confidence intervals across prey densities. Confidence intervals were generated using global sensitivity analyses considering FR parameter estimates (i.e. a , h), standard errors and covariance-variance matrices. A Latin hypercube sampling algorithm was used to produce several parameter sets (Soetaert and Petzoldt 2010), with confidence intervals produced from 2.5 % and 97.5 % survival quantiles.

6.3. Results

All 378 mosquito larvae (prey) survived in controls, so all treatment/prey mortality were associated with predation by notonectids, which was also observed. Therefore, it was not necessary to adjust for background natural prey mortality in the analyses. The effects of habitat manifested differently according to predator treatment, owing to a significant predator \times habitat interaction term (GLM: $\chi^2 = 90.88$, $df = 6$, $p < 0.001$) (Figure 6.1). *Enithares* always consumed significantly more than *Anisops*, across all habitat and matched predator densities (all $p < 0.05$). Furthermore, conspecific pairs of *Enithares* always consumed significantly more than individual *Enithares* irrespective of habitat complexity (all $p < 0.05$). Contrastingly, conspecific pairs of *Anisops* did not consume significantly different numbers of prey than individuals under medium or high habitat complexity (both $p > 0.05$), yet conspecific consumption was significantly higher than that of individuals under low complexity ($p < 0.001$). Accordingly, the presence of habitat complexity dampened predatory impacts of multiple *Anisops*, but not *Enithares*. Larval mosquito consumption was also significantly positively related to the experimental prey supply (GLM: $\chi^2 = 2207.86$, $df = 5$, $p < 0.001$).

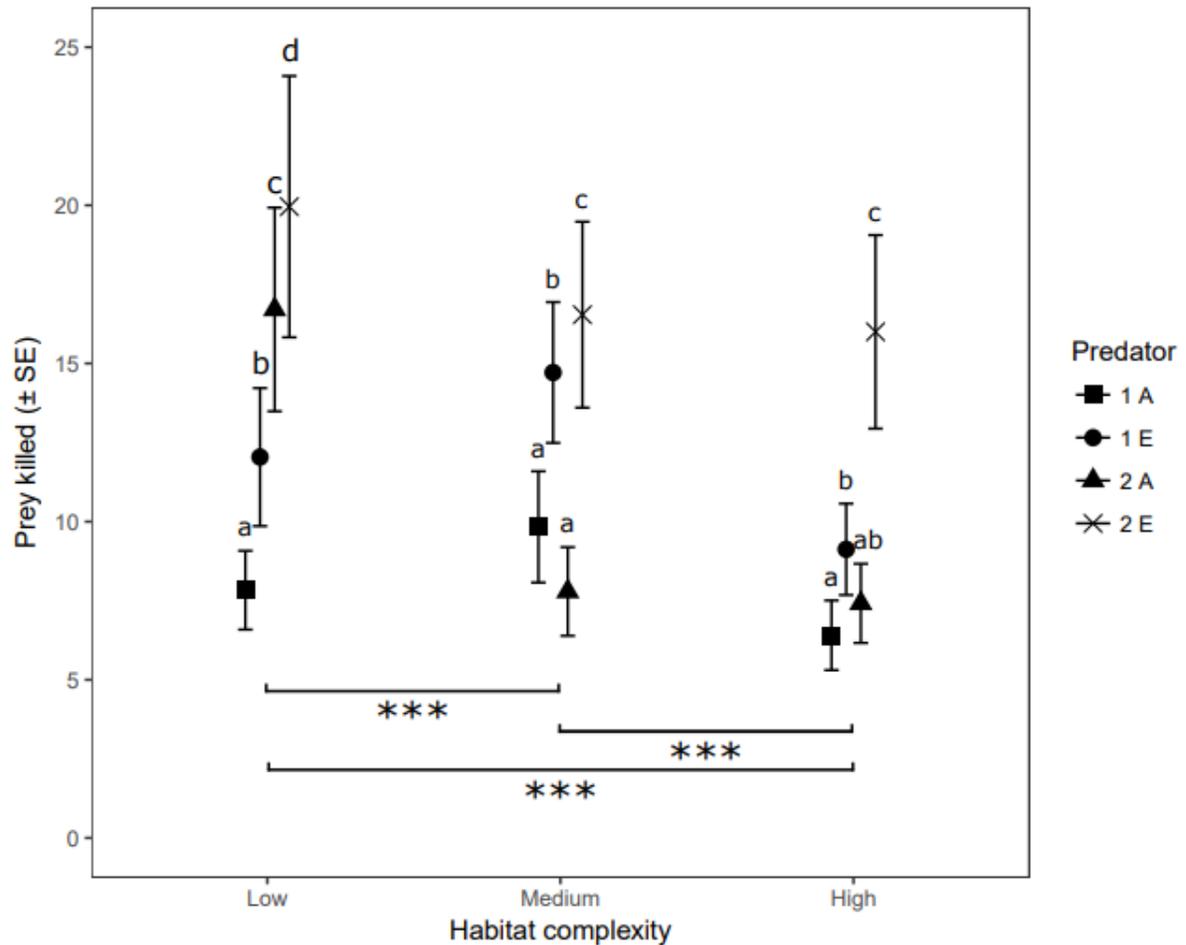


Figure 6. 1. Mean \pm SE larval mosquito prey mortality across different predator treatments (*Anisops*, A; *Enithares*, E) and habitat complexities, irrespective of prey supply. Letters and asterisks indicate significant differences.

Prey mortality was always significantly negatively related to prey supply, so all FRs were categorised as Type II (Table 6.1). Significant attack rate and handling time parameter estimates were returned across all predator and habitat treatment groups (Table 6.1). *Enithares* attack rates tended to peak unimodally under medium habitat complexities, whilst *Anisops* attack rates were generally reduced with increasing habitat. *Enithares* attack rates were always higher than *Anisops* (Table 6.1). Individual *Anisops* handling times also related negatively to increasing habitat complexity, whilst conspecific handling times

lengthened. Individual *Enithares* handling times were shortest at intermediate habitat complexities, whilst the inverse was true for conspecific pairs. *Enithares* handling times were also mostly shorter than those of *Anisops* under matched treatment groups (Table 6.1), driving higher maximum feeding rates (Figure 6.2).

Table 6. 1. First order terms resulting from logistic regression of the proportion of prey killed as a function of prey density across different predator (*Anisops*, A; *Enithares*, E) and habitat complexity treatments. Attack rate and handling time parameter estimates result from Rogers’ random predator equation.

Predator	Habitat	Linear coefficient, p	Attack rate, p	Handling time, p
1 A	Low	-0.04, < 0.001	1.93, < 0.001	0.06, < 0.001
1 E	Low	-0.03, < 0.001	5.37, < 0.001	0.04, < 0.001
2 A	Low	-0.03, < 0.001	3.36, < 0.001	0.02, < 0.001
2 E	Low	-0.07, 0.001	6.93, < 0.001	0.01, < 0.001
1 A	Medium	-0.03, < 0.001	1.85, < 0.001	0.04, < 0.001
1 E	Medium	-0.07, < 0.001	10.61, < 0.001	0.04, < 0.001
2 A	Medium	-0.02, < 0.001	0.97, < 0.001	0.04, < 0.001
2 E	Medium	-0.08, < 0.001	9.46, < 0.001	0.02, < 0.001
1 A	High	-0.01, 0.01	0.50, < 0.001	0.03, 0.004
1 E	High	-0.04, < 0.001	2.08, < 0.001	0.05, < 0.001
2 A	High	-0.02, < 0.001	1.04, < 0.001	0.04, < 0.001
2 E	High	-0.03, < 0.001	3.04, < 0.001	0.02, < 0.001

Under low habitat complexity, single *Enithares* exhibited a significantly higher FR than *Anisops* (Figure 6.2a), characterised by higher attack rates and shorter handling times (Table 6.1). Both *Anisops* (Figure 6.2b) and *Enithares* (Figure 6.2c) tended to display synergistic multiple predator effects where habitat complexity was absent. For *Anisops*, the FR was significantly greater than that simulated under high prey densities, whilst the

Enithares FR consistently displayed some overlap in confidence intervals. Under medium habitat complexities, individual *Enithares* FR magnitudes were again significantly greater than individual *Anisops* under low-intermediate prey densities (Figure 6.2d), aligning with greater attack rates but more similar handling times (Table 6.1). Conspecific *Anisops* pairs displayed significantly reduced FRs compared to simulations (Figure 6.2e), indicating antagonistic multiple predator effects that reduced prey risk with higher habitat complexity. Antagonistic multiple predator effects were also evident between *Enithares* conspecific pairs (Figure 6.2f), albeit differences were less marked and only divergent at high densities. Under high habitat complexities, individual *Enithares*, again, exhibited a significantly greater FR magnitude than individual *Anisops* at low-intermediate prey densities (Figure 6.2g), characterised by greater attack rates (Table 6.1). Whilst confidence intervals always overlapped, *Anisops* conspecifics tended to interact antagonistically and reduce prey risk as compared to simulations (Figure 6.2h), whilst observed FRs of *Enithares* pairs were more similar (Figure 6.2i).

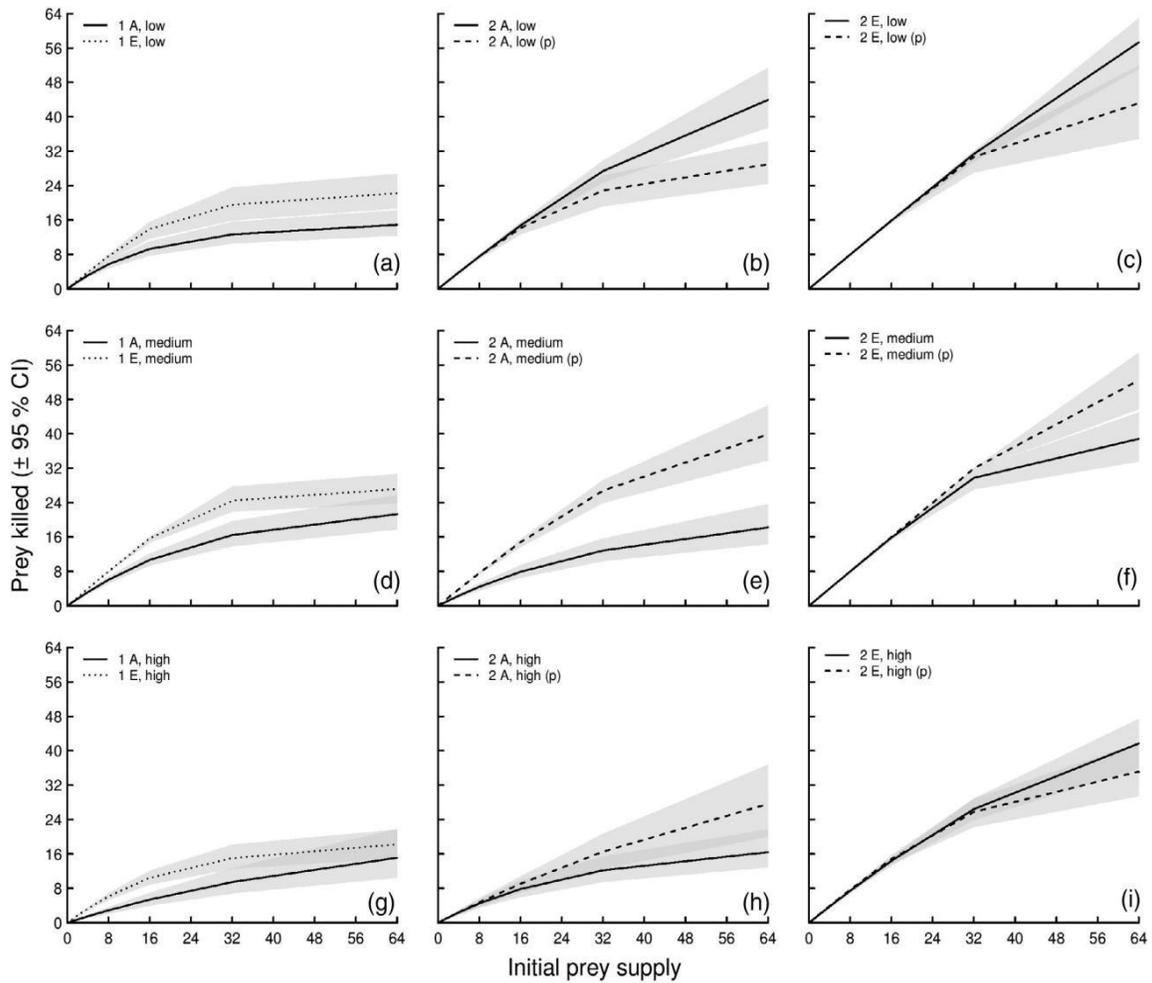


Figure 6. 2. Functional responses towards larval mosquito prey under low (a, b, c), intermediate (d, e, f) and high (g, h, i) habitat complexities by different combinations of notonectid predators (*Anisops*, A; *Enithares*, E). Multiple predator predictions (p) result from simulations based on individual predator functional responses (a, d, g). Shaded areas are 95% confidence intervals.

6.4. Discussion

Natural predator communities within temporary aquatic ecosystems may play a key role in the population suppression of vector mosquito populations, thus providing an important ecosystem service (Saha *et al.*, 2012; Wesner *et al.*, 2012; Culler *et al.*, 2014; Faithpraise *et al.*, 2014). However, many natural enemies within these systems have remained unexamined quantitatively in terms of their contributions to vector population

regulation (Cuthbert *et al.*, 2018b). The present study quantifies predator efficiencies of two locally-abundant notonectids towards larvae of the vector mosquito *Cx. pipiens*. Whilst both notonectids displayed high predatory effects on larval mosquitoes, interspecific differences were present, with *Enithares* significantly more voracious than *Anisops*, irrespective of habitat context. Predator-predator interactions within both species were positive in the absence of habitat complexity, driving greater prey risk compared to predictions from population dynamic simulations. However, the effects of increasing habitat complexity drove differential emergent multiple predator effects between the two predator species. Specifically, antagonistic interactions were driven in *Anisops*, and particularly under intermediate habitat levels. Conversely, *Enithares* multiple predator effects were less impacted by habitat complexity.

Predator-prey interaction outcomes are the result of both predator and prey performance. Although prey behaviour was not explicitly assessed in the present study, *Cx. pipiens* larvae prey mostly rest and filter-feed at the surface and their escape response involves swimming downwards from the surface, whereby predation risk could be increased. *Anisops* and *Enithares* belong to two distinct notonectid subfamilies, Anisopinae and Notonectinae, respectively. Whilst members of the former group are able to manipulate bouyancy within water columns through haemoglobin regulation in tracheated cells in the abdomen (Matthews and Seymour 2008), the latter group must cling to habitat structures to remain submerged. Accordingly, *Anisops* is presumably better-adapted to the pelagic zone of waterbodies in the absence of vegetative structure, while *Enithares* with are better-adapted to structured habitats. This study therefore comprises two predatory species with

differing behaviours exposed to one species of prey, under varying habitat complexity scenarios. The results of the study indicate emergent effects between predator density and habitat complexity, which differ among predator species, and such differences may reflect habitat occupancy and prey capture characteristics interspecifically.

Both notonectid predators exhibited Type II FRs, characterised by high predation levels at low densities of *Cx. pipiens*. High magnitude Type II FRs, with high attack rates and low handling times, are desirable in a natural enemy context as predation pressures are high even when target prey are rare (Cuthbert *et al.*, 2019c). This observed FR Type was also robust to variations in habitat complexity and predator density, suggesting that notonectids may drive destabilising effects on target mosquito populations in structurally dynamic aquatic environments, such as temporary ponds. This result is in keeping with Cuthbert *et al.* (2018c), wherein a congeneric notonectid FR was relatively unaffected by changes in vegetative habitat complexity. However, FR magnitudes of notonectids towards mosquitoes are known to differ depending on prey size class, with optimal relative predator-prey body sizes found under intermediate instar stages in certain species (Dalal *et al.*, 2019). Although factors such as the presence of alternative prey may also alter the shape and magnitude of FRs (Blaustein and Chase 2007; Sahoo 2012).

Higher conspecific densities caused increased consumption levels for both predators in the absence of habitat complexity. Indeed, compared to simulations, both predator species exhibited positive multiple predator effects which increased prey risk. Since notonectids are visual predators and are attracted to movement, swimming by prey

likely elicited similar attention from both predatory species under low habitat complexity scenarios, with increasing predator numbers increasing encounter rates and potentially prey activity. It thus appears that neither of the focal predators interfere with conspecifics in simple environments, and may act in synergy to exacerbate prey risk. Contrastingly, as aquatic environments increased in structural complexity, predatory impacts of multiple *Anisops* were reduced, whilst multiple *Enithares* predatory impacts remained significantly higher than individuals. Functional responses of individual *Anisops* were always similar to those of pairs in the presence of vegetative habitat complexity. These trends are also reflected in predator attack rates, which were reduced under structured habitats in *Anisops*, yet tended to peak unimodally at interediate habitat complexity levels in *Enithares*. For both predator species, increases in predator abundance might increase prey activity through increased encounter rates and escape responses, with implications for prey detection. However, under increasing habitat complexities, the differences between how predators navigate around structure seems to become important. The larger *Enithares* can likely detect prey as they move between structure and may even have faster attack speeds with implications for capture of prey as they move through habitat. *Enithares* also utilise the structure to maintain position at depth and so likely receive some benefit from structure. Their persistence in both open water and close to structure may increase their encounter rates with prey should the latter utilise structure for hiding. For *Anisops*, however, increasingly complex habitats presumably hindered line-of-sight and thus the efficacy of straight-line attacks. Accordingly, their preference for open habitats may reduce prey encounter rates under scenarios where prey utilise structure for cover. However, for *Anisops*, the effects of habitat complexity on multiple predator effects were most

pronounced within intermediate habitat levels, and therefore the contribution of predator-predator interference relative to habitat appears to be reduced as environments become highly complex. Furthermore, niche models may also play a role in determining the outcomes of the MPEs presented here. Future studies may also look in the intraguild predation presented by *Enithares* and *Anisops* both in laboratory conditions and in the field.

While lab-based predator-prey simulations do not necessarily translate to community-level dynamics, such studies are important for phenomological outcome determination and are often necessary to gain insight into specific components of food web ecology. However, the limitations of such investigations need to be considered. The artificial nature of the experimental arenas and the comparative FR approach precluded the investigation of more than two conspecifics. As such, observed conspecific MPEs may be an underestimation of MPE dynamics in the wild. Furthermore, given high levels of consumption of *Anisops* by *Enithares* in preliminary trials, I could not address inter-specific MPE dynamics with this particular predator system and experimental design. It is suggested that future studies consider alternate designs to cater for the shortcomings of the present study, with particular emphasis on incorporating field data or *in situ* experimental approaches. However, despite these shortcomings, the importance of such lab-based interaction studies should not be overlooked. The predator-prey system investigated here is highly relevant, and closes major existing knowledge gaps. Notonectids are able to colonise ephemeral habitat patches across landscape through aerial dispersal during their adult stage (Bogan and Boersma 2012). This includes polluted aquatic habitats within urban and agro-rural landscapes (Domingos and Arcifa 2017), where mosquitoes may

proliferate (Muturi *et al.*, 2007). Given limited tolerances to pollution in wholly-aquatic invertebrates (e.g. copepods) (Drira *et al.*, 2018), this suggests an important predatory role of notonectids in suppressing mosquito populations within patchy aquatic habitats at the landscape scale. These results enhance understanding of environmental factors which alter the predatory effects of these important natural enemies of mosquitoes, and indicate interspecific differences within taxonomic groups.

It was demonstrated that high predatory impacts of two notonectid species within two sub-families towards larvae of the vector mosquito complex *Cx. pipiens*. Whilst both predator species are capable of handling mosquito prey in complex habitats, predator density effects are emergent between species across habitat gradients. Accordingly, promoting and conserving diverse predator populations which are adapted to different environmental conditions may assist in population suppression of vector pests and their associated ecological disservices in dynamic habitats. Further research is recommended into the roles of biotic and abiotic parameters which impact predator-pest prey interactions in aquatic environments, including environmental tolerances. Moreover, assessment of natural enemy efficacies towards emerging invasive vector species (Brady and Hay 2020), and how best to facilitate such predators in their natural habitats, requires urgent examination. With an increasing awareness for detrimental environmental impacts associated with synthetic pesticides (Deneer 2000), the promotion and conservation of natural bio-control agents in aquatic environments is paramount to integrated vector control for improved public health and maintenance of ecological integrity.

6.5. References

- Alexander, M. E., Dick, J. T., and O'Connor, N. E. (2013). Trait-mediated indirect interactions in a marine intertidal system as quantified by functional responses. *Oikos*, 122: 1521-1531.
- Alexander, M. E., Dick, J. T., O'Connor, N. E., Haddaway, N. R., and Farnsworth, K. D. (2012). Functional responses of the intertidal amphipod *Echinogammarus marinus*: effects of prey supply, model selection and habitat complexity. *Marine Ecology Progress Series*, 468: 191-202.
- Anholt, B. R., and Werner, E. E. (1998). Predictable changes in predation mortality as a consequence of changes in food availability and predation risk. *Evolutionary Ecology*, 12: 729-738.
- Baber, M. J., Fleishman, E. J., Babbitt, K. L., and Tarr, T. (2004). The relationship between wetland hydroperiod and nestedness patterns in assemblages of larval amphibians and predatory macroinvertebrates. *Oikos*, 107: 16-27.
- Barrios-O'Neill, D., Dick, J. T., Emmerson, M. C., Ricciardi, A., and MacIsaac, H. J. (2015). Predator-free space, functional responses and biological invasions. *Functional Ecology*, 29: 377-384.
- Barrios-O'Neill, D., Dick, J. T., Ricciardi, A., MacIsaac, H. J., and Emmerson, M. C. (2014). Deep impact: in situ functional responses reveal context-dependent interactions between vertically migrating invasive and native mesopredators and shared prey. *Freshwater Biology*, 59: 2194-2203.
- Blaustein, L., and Chase, J. M. (2007). Interactions between mosquito larvae and species that share the same trophic level. *Annual Review of Entomology*, 52: 489-507.
- Blaustein, L. (1998). Influence of the predatory backswimmer, *Notonecta maculata*, on invertebrate community structure. *Ecological Entomology*, 23: 246-252.
- Bogan, M. T., and Boersma, K. S. (2012). Aerial dispersal of aquatic invertebrates along and away from arid-land streams. *Freshwater Science*, 31: 1131-1144.

- Bolker, B. M. (2008). *Ecological models and data in R*. Princeton University Press, New Jersey, USA.
- Brady, O. J., and Hay, S. I. (2020). The global expansion of dengue: How *Aedes aegypti* mosquitoes enabled the first pandemic arbovirus. *Annual Review of Entomology*, 65: 191-208.
- Cardinale, B. J., Harvey, C. T., Gross, K., and Ives, A. R. (2003). Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. *Ecology Letters*, 6: 857-865.
- Chamberlain, S. A., Bronstein, J. L., and Rudgers, J. A. (2014). How context dependent are species interactions?. *Ecology Letters*, 17: 881-890.
- Culler, L. E., Ohba, S. Y., and Crumrine, P. (2014). Predator-prey interactions of dytiscids. In *Ecology, systematics, and the natural history of predaceous diving beetles (Coleoptera: Dytiscidae)* (pp. 363-386). Springer, Dordrecht.
- Cuthbert, R. N., Dalu, T., Wasserman, R. J., Callaghan, A., Weyl, O. L., and Dick, J. T. (2019a). Using functional responses to quantify notonectid predatory impacts across increasingly complex environments. *Acta Oecologica*, 95: 116-119.
- Cuthbert, R. N., Weyl, O. L., Wasserman, R. J., Dick, J. T., Froneman, P. W., Callaghan, A., and Dalu, T. (2019b). Combined impacts of warming and salinisation on trophic interactions and mortality of a specialist ephemeral wetland predator. *Freshwater Biology*, 64: 1584-1592.
- Cuthbert, R. N., Callaghan, A., Sentis, A., Dalal, A., and Dick, J. T. (2019c). Additive multiple predator effects can reduce mosquito populations. *Ecological Entomology*, 45: 243-250.
- Cuthbert, R. N., Dalu, T., Wasserman, R. J., Callaghan, A., Weyl, O. L., and Dick, J. T. (2018a). Calanoid copepods: an overlooked tool in the control of disease vector mosquitoes. *Journal of Medical Entomology*, 55: 1656-1658.

- Cuthbert, R. N., Dick, J. T., and Callaghan, A. (2018b). Interspecific variation, habitat complexity and ovipositional responses modulate the efficacy of cyclopoid copepods in disease vector control. *Biological Control*, 121: 80-87.
- Cuthbert, R. N., Dick, J. T., Callaghan, A., and Dickey, J. W. (2018c). Biological control agent selection under environmental change using functional responses, abundances and fecundities; the Relative Control Potential (RCP) metric. *Biological Control*, 121: 50-57.
- Dalal, A., Cuthbert, R. N., Dick, J. T., and Gupta, S. (2020). Prey preferences of notonectids towards larval mosquitoes across prey ontogeny and search area. *Pest Management Science*, 76: 609-616.
- Dalal, A., Cuthbert, R. N., Dick, J. T. and Gupta, S. (2019). Water depth-dependent notonectid predatory impacts across larval mosquito ontogeny. *Pest Management Science*, 75: 2610-2617.
- Daldorph, P. W., and Thomas, J. D. (1991). The effect of nutrient enrichment on a freshwater community dominated by macrophytes and molluscs and its relevance to snail control. *Journal of Applied Ecology*, 1: 685-702.
- Deneer, J. W. (2000). Toxicity of mixtures of pesticides in aquatic systems. *Pest Management Science*, 56: 516-520.
- Dick, J. T., Alexander, M. E., Jeschke, J. M., Ricciardi, A., MacIsaac, H. J., Robinson, T. B., and Paterson, R. A. (2014). Advancing impact prediction and hypothesis testing in invasion ecology using a comparative functional response approach. *Biological Invasions*, 16: 735-753.
- Dimentman, C., and Margalit, J. (1981). Rainpools as breeding and dispersal sites of mosquitos and other aquatic insects in the Central Negev Desert. *Journal of Arid Environments*, 4: 123-129.

- Domingos, A. R., and Arcifa, M. S. (2017). Distribution and fluctuations of backswimmers (Notonectidae) in a tropical shallow lake and predation on microcrustaceans. *Brazilian Journal of Biology*, 77: 108-117.
- Drira, Z., Kmiha-Megdiche, S., Sahnoun, H., Pagano, M., Tedetti, M., and Ayadi, H. (2018). Water quality affects the structure of copepod assemblages along the Sfax southern coast (Tunisia, southern Mediterranean Sea). *Marine and Freshwater Research*, 69: 220-223.
- Ellis, R. A., and Borden, J. H. (1970). Predation by *Notonecta undulata* (Heteroptera: Notonectidae) on larvae of the yellow-fever mosquito. *Annual Entomological Society of America*, 63: 963-973.
- Faithpraise, F. O., Idung, J., Usibe, B., Chatwin, C. R., Young, R., and Birch, P. (2014). Natural control of the mosquito population via Odonata and Toxorhynchites. *International Journal of Innovative Research in Science, Engineering and Technology*, 3: 12898-12911.
- Ghosh, A., Mandal, S., Bhattacharjee, I., and Chandra, G. (2005). Biological control of vector mosquitoes by some common exotic fish predators. *Turkish Journal of Biology*, 29: 167-171.
- Grabowski, J. H. (2004). Habitat complexity disrupts predator–prey interactions but not the trophic cascade on oyster reefs. *Ecology*, 85: 995-1004.
- Griffin, J. N., De La Haye, K. L., Hawkins, S. J., Thompson, R. C., and Jenkins, S. R. (2008). Predator diversity and ecosystem functioning: density modifies the effect of resource partitioning. *Ecology*, 89: 298-305.
- Heithaus, M. R., Wirsing, A. J., and Dill, L. M. (2012). The ecological importance of intact top-predator populations: a synthesis of 15 years of research in a seagrass ecosystem. *Marine and Freshwater Research*, 63: 1039-1050.
- Holling, C. S. (1959). Some characteristics of simple types of predation and parasitism. *Canadian Entomologist*, 91: 385-398.

- Hulsmans, A., Vanschoenwinkel, B., Pyke, C., Riddoch, B. J., and Brendonck, L. (2008). Quantifying the hydroregime of a temporary pool habitat: a modelling approach for ephemeral rock pools in South Eastern Botswana. *Ecosystems* 11: 89-100.
- Hutchinson, G. E. (1929). A revision of the Notonectidae and Corixidae of South Africa. *Annals of the South African Museum*, 25: 359– 474.
- Jackman, J. A., and Olson, J. K. (2002). Mosquitoes and the Diseases they Transmit. Texas Farmer Collection. Texas, USA.
- Juliano, S. A. (2001). Non-linear curve fitting: predation and functional response curve. Design and Analysis of Ecological Experiment. Oxford University Press, Oxford, UK.
- Kaiser, E. W. (1940). Zur Biologie und Morphologie von *Anisops persica* Lindberg im Vergleich mit *Buena Kirk* und *Notonecta L.* from *Danish Set Invettig in Iran. Part III*, pp. 139-158.
- Leeuwen, E. V., Jansen, V. A. A., and Bright, P. W. (2007). How population dynamics shape the functional response in a one-predator–two-prey system. *Ecology*, 88: 1571-1581.
- Mandal, S. K., Ghosh, A., Bhattacharjee, I., and Chandra, G. (2008). Biocontrol efficiency of odonate nymphs against larvae of the mosquito, *Culex quinquefasciatus* Say, 1823. *Acta Tropica*, 106: 109-114.
- Matthews, P. G., and Seymour, R. S. (2008). Haemoglobin as a buoyancy regulator and oxygen supply in the backswimmer (Notonectidae, *Anisops*). *Journal of Experimental Biology*, 211: 3790-3799.
- Matthews, P. G., and Seymour, R. S. (2006). Diving insects boost their buoyancy bubbles. *Nature*, 441: 171.
- McCoy, M. W., Stier, A. C., and Osenberg, C. W. (2012). Emergent effects of multiple predators on prey survival: the importance of depletion and the functional response. *Ecology Letters*, 15: 1449-1456.

- McCoy, M. W., and Bolker, B. M. (2008). Trait-mediated interactions: influence of prey size, density and experience. *Journal of Animal Ecology*, 1: 478-486.
- Michel, M. J., and Adams, M. M. (2009). Differential effects of structural complexity on predator foraging behavior. *Behavioral Ecology*, 20: 313-317.
- Miller, P. L. (1966). The function of haemoglobin in relation to the maintenance of neutral buoyancy in *Anisops pellucens* (Notonectidae, Hemiptera). *Journal of Experimental Biology*, 44: 529-543.
- Muturi, E. J., Shililu, J. I., Gu, W., Jacob, B. G., Githure, J. I., and Novak, R. J. (2007). Larval habitat dynamics and diversity of *Culex* mosquitoes in rice agro-ecosystem in Mwea, Kenya. *American Journal of Tropical Medicine and Hygiene*, 76: 95-102.
- Nicolle, A., Hansson, L. A., Brodersen, J., Nilsson, P. A., and Brönmark, C. (2011). Interactions between predation and resources shape zooplankton population dynamics. *PloS ONE*, 6: e16534.
- Oaten, A., and Murdoch, W. W. (1975). Functional response and stability in predator-prey systems. *The American Naturalist*, 109: 289-298.
- Ohba, S. Y., Ohtsuka, M., Sunahara, T., Sonoda, Y., Kawashima, E., and Takagi, M. (2012). Differential responses to predator cues between two mosquito species breeding in different habitats. *Ecological Entomology*, 37: 410-418.
- O'Neill, B. J., and Thorp, J. H. (2014). Untangling food-web structure in an ephemeral ecosystem. *Freshwater Biology*, 59: 1462-1473.
- Papáček, M. (2013). Small aquatic and ripicolous bugs (Heteroptera: Nepomorpha) as predators and prey: The question of economic importance. *European Journal of Entomology*, 98: 1-12.
- Paterson, R. A., Dick, J. T., Pritchard, D. W., Ennis, M., Hatcher, M. J., and Dunn, A. M. (2015). Predicting invasive species impacts: a community module functional

- response approach reveals context dependencies. *Journal of Animal Ecology*, 84: 453-463.
- Raffel, T. R., Hoverman, J. T., Halstead, N. T., Michel, P. J., and Rohr, J. R. (2010). Parasitism in a community context: trait-mediated interactions with competition and predation. *Ecology*, 91: 1900-1907.
- Robson, B. J. (1996). Habitat architecture and trophic interaction strength in a river: riffle-scale effects. *Oecologia*, 107: 411-420.
- Ross, C. T., and Winterhalder, B. (2015). Sit-and-wait versus active-search hunting: A behavioral ecological model of optimal search mode. *Journal of Theoretical Biology*, 387: 76-87.
- Saha, N., Aditya, G., Banerjee, S., and Saha, G. K. (2012). Predation potential of odonates on mosquito larvae: Implications for biological control. *Biological Control*, 63: 1-8.
- Sahoo, B. (2012). Effects of additional food on predator-prey systems with monotonic and non-monotonic functional response: a comparative study. *International Journal of Business and Applied Sciences*, 1: 124-136.
- Scott, M. A., and Murdoch, W. W. (1983). Selective predation by the backswimmer, *Notonecta* 1. *Limnology and Oceanography*, 28: 352-366.
- Sentis, A., and Boukal, D. S. (2018). On the use of functional responses to quantify emergent multiple predator effects. *Scientific Reports*, 8: 1-12.
- Shears, N. T., Babcock, R. C., and Salomon, A. K. (2008). Context-dependent effects of fishing: variation in trophic cascades across environmental gradients. *Ecological Applications*, 18: 1860-1873.
- Skalski, G. T., and Gilliam, J. F. (2001). Functional responses with predator interference: viable alternatives to the Holling type II model. *Ecology*, 82: 3083-3092.

- Soetaert, K., and Petzoldt, T. (2010). Inverse modelling, sensitivity and monte carlo analysis in R using package FME. *Journal of Statistical Software* 33: 1-28.
- Solomon, M. E. (1949). The natural control of animal populations. *The Journal of Animal Ecology*, 18: 1-35.
- Streams, F. A. (1992). Intrageneric predation by *Notonecta* (Hemiptera: Notonectidae) in the laboratory and in nature. *Annual Entomological Society of America*, 85: 265-273.
- Svensson, B. G., Tallmark, B., and Petersson, E. (2000). Habitat Heterogeneity, Coexistence and Habitat Utilization in Five Backswimmer Species (*Notonecta* spp.; Hemiptera, Notonectidae). *Aquatic Insects*, 22: 81-98.
- Thorp, C. J., Alexander, M. E., Vonesh, J. R., and Measey, J. (2018). Size-dependent functional response of *Xenopus laevis* feeding on mosquito larvae. *PeerJ*, 6: e5813.
- Van Driesche, R., and Hoddle, M. (2009). Control of pests and weeds by natural enemies: an introduction to biological control. Blackwell publishing, Oxford, UK.
- Vanschoenwinkel, B., Hulsmans, A. N., De Roeck, E., De Vries, C., Seaman, M., and Brendonck, L. (2009). Community structure in temporary freshwater pools: disentangling the effects of habitat size and hydroregime. *Freshwater Biology*. 54: 1487-1500.
- Vonesh, J. R., and Blaustein, L. (2010). Predator-induced shifts in mosquito oviposition site selection: a meta-analysis and implications for vector control. *Israel Journal of Ecology and Evolution*, 56: 263-279.
- Vonesh, J. R., Kraus, J. M., Rosenberg, J. S., and Chase, J. M. (2009). Predator effects on aquatic community assembly: disentangling the roles of habitat selection and post-colonization processes. *Oikos*, 118: 1219-1229.
- Wesner, J. S., Billman, E. J., and Belk, M. C. (2012). Multiple predators indirectly alter community assembly across ecological boundaries. *Ecology*, 93: 1674-1682.

- Wasserman, R. J., Weston, M., Weyl, O. L., Froneman, P. W., Welch, R. J., Vink, T. J., and Dalu, T. (2018). Sacrificial males: the potential role of copulation and predation in contributing to copepod sex-skewed ratios. *Oikos* 127: 970-980.
- Wasserman, R. J., Alexander, M. E., Dalu, T., Ellender, B. R., Kaiser, H., and Weyl, O. L. (2016a). Using functional responses to quantify interaction effects among predators. *Functional Ecology*, 30: 1988-1998.
- Wasserman, R. J., Alexander, M. E., Weyl, O. L., Barrios-O'Neill, D., Froneman, P. W., and Dalu, T. (2016b). Emergent effects of structural complexity and temperature on predator–prey interactions. *Ecosphere*, 7: e01239.
- Wasserman, R. J., Noyon, M., Avery, T. S., and Froneman, P. W. (2013). Trophic level stability-inducing effects of predaceous early juvenile fish in an estuarine mesocosm study. *PloS ONE*, 8: e61019.
- Weis, J. S. (2011). Invasion and predation in aquatic ecosystems. *Current Zoology*, 57: 613-624.
- Zuharah, W. F., and Lester, P. J. (2011). Are exotic invaders less susceptible to native predators? A test using native and exotic mosquito species in New Zealand. *Population Ecology*, 53: 307-317.

CHAPTER 7

Implications of increasing temperature stress for predatory biocontrol of mosquito vectors

Published as: **Buxton, M.**, Nyamukondiwa, C., Dalu, T., Cuthbert, R. N., and Wasserman, R. J. (2020). Implications of increasing temperature stress for predatory biocontrol of vector mosquitoes. *Parasites and Vectors*, 13: 1-11.

7.1. Introduction

Population size in aquatic ecosystems is known to be largely dependent on ecological interactions such as competition and predation pressure (Holomuzki *et al.*, 2010; Arribas *et al.*, 2018). Predation plays a pivotal role in regulating problematic species (e.g., proliferating mosquitoes), through density-mediated effects, whereby population numbers are directly controlled through predatory removal, or through indirect, trait-mediated effects such as compromised fecundity, growth rate and longevity of prey (Zuharah and Lester 2010; Roux and Robert 2019). Key mosquito genera (e.g., *Aedes*, *Anopheles*, *Culex*) are of public concern globally, transmitting pathogens that cause common debilitating diseases to humans (e.g., chikungunya, dengue, multiple kinds of encephalitis, elephantiasis, malaria, yellow fever, Zika), livestock (Rift Valley fever) and wildlife (avian malaria, West Nile) Lebl *et al.*, 2015; Tandina *et al.*, 2019). Moreover, mosquitoes are semi-aquatic insects that colonise and develop (egg, larva, pupae) in aquatic habitats across natural, urban and peri-urban environments (Foster and Walker 2019).

Naturally, mosquitoes coexist in aquatic microhabitats with a large faunal community (Kocher *et al.*, 2014), including aquatic predators that curb mosquito populations (Chandra *et al.*, 2008; Ohba *et al.*, 2011; Acquah-Lampsey and Brandl 2018; Cuthbert *et al.*, 2018a). In these environments, both the predator and mosquito prey experience stressful thermal extremes, with variable effects on performance given differential thermal physiological responses (Abrahams *et al.*, 2007; Broitman *et al.*, 2009). Mosquitoes breed in diverse, often cryptic, aquatic habitats such as rock crevices, phytotelmata (e.g., tree holes), animal hoof prints and artificial containers, across small-to-large scale temporary and permanent water bodies (Karuitha *et al.*, 2019; Wilke *et al.*,

2019). These environments are prone to extreme environmental fluctuations, which are expected to become the new norm in a warming world, typically becoming more intense, prolonged and more frequent (Meehl and Tebaldi 2004; Thuiller 2007; Stillman 2019). Mosquitoes have also adapted to colonise clean to highly compromised water quality sources, sunlit or shaded and of varying nutrient levels (Dom *et al.*, 2016). Predators of mosquitoes can persist in these environments as wholly aquatic organisms, access through aerial dispersal as semi-aquatic predators, or are purposefully introduced as agents for desired ecosystem services (Dida *et al.*, 2015). The variable utilisation of different water bodies by mosquito larvae, and their aquatic predators, has implications for performance of these organisms and ultimately, predator-prey interaction outcomes. Understanding these dynamics could thus prove useful in determining the sustainability of natural and augmentative mosquito biological control in aquatic habitats, particularly within the context of changing environments (Scholes 2016).

Predator-prey interaction strengths can be mediated by abiotic environmental factors (Johansson and Brodin 2003). Temperature is particularly important in this regard, affecting organismal physiology, ecology, metabolism and overall fitness (Gillooly *et al.*, 2001; Chown and Nicolson 2004). Temperature in water bodies is essential as a regulatory mechanism that drives biochemical and physiological processes (Teoh *et al.*, 2004; Burton and Einum 2020), with implications for behaviour, performance, and predator-prey interaction outcomes (Domenici *et al.*, 2007; Vucic-Pestic *et al.*, 2011; Wasserman *et al.*, 2015). Indeed, empirical studies have shown environmental variability likely affects higher trophic levels, e.g., predators, more significantly than prey (Hance *et al.*, 2007; Machekano

et al., 2018a; Mutamiswa *et al.*, 2018a). Furthermore, thermal performance is highly enigmatic and varies among species, ontogeny, age (Bowler and Terblanche 2008) and size (Burton and Einum 2020). Moreover, natural enemy efficacy also depends on the fate of bottom-up and top-down effects, which have been reported to favour pest and vector species (Chidawanyika *et al.*, 2019). As such, even slight alterations to temperature can compromise or heighten species fitness, community interactions and structure (Jiang and Morin 2004; Deutsch *et al.*, 2008). This makes predicting the fate of natural enemy effects in the face of climate change highly complex. Additionally, at the autecological level, understanding how organismal critical thermal limits (CTLs) of varied species are affected by oscillating temperatures can be useful for broader ecological inferences, such as interaction dynamics between species (Terblanche *et al.*, 2011; Andersen *et al.*, 2015). Definitively, CTLs represent temperatures at which an organism stops activity. Ecologically, activity here represents key fitness traits e.g., swimming, foraging ability (Chown and Nicolson 2004; Mutamiswa *et al.*, 2018b).

The effects of temperature are critical in determining the fate of trophic interactions under changing environments (Thomas *et al.*, 2004). However, whilst few studies have focused on terrestrial environments (Agosta *et al.*, 2018; Machekano *et al.*, 2018a; Mutamiswa *et al.*, 2018a), to our knowledge, few have concerned aquatic habitats in the context of thermal tolerance, particularly for vector mosquitoes and their predators. Here, the study aimed to assess thermal tolerance (lower and upper) of three regionally-abundant aquatic mosquito predators (*Enithares chinai* and *Anisops sardea* [Insecta: Hemiptera], *Lovenula falcifera* [Copepoda: Calanoida]) and their vectorially-important larval mosquito

prey (*Aedes aegypti*, *Anopheles quadriannulatus*, *Culex pipiens* [Diptera: Culicidae]), in a semi-arid subtropical southern African landscape. All three mosquito species are commonly encountered in peri- and urban landscapes of the study region (Cornel *et al.*, 2018; Coetzee *et al.*, 2020), with *Ae. aegypti* and *Cx. pipiens* well-known vectors of various pathogens that cause disease (Tolle 2009; Braack *et al.*, 2018; Papa 2019). *Anopheles quadriannulatus* is not currently a known vector to human pathogens (Habtewold *et al.*, 2008), however, is susceptible to *Plasmodium* infection (Takken *et al.*, 2009; Lobo *et al.*, 2015). Physiological limits of this species may nevertheless serve as reasonable proxies of congeneric malaria vector species. While increases in extreme temperature events are predicted to be the future norm (Meehl and Tebaldi 2004; Ravelo *et al.*, 2004; Thuiller 2007; Seidel *et al.*, 2008; Wu *et al.*, 2012; Stillman 2019), semi-arid southern Africa is projected to be particularly impacted by shifting climatic conditions (Engelbrecht *et al.*, 2015). It was hypothesised that: (i) larval thermal tolerance would vary across mosquito species, with container-breeding specialists (principally *Ae. aegypti*) having the widest thermal window; (ii) for predators, the wholly aquatic copepod would have the narrowest thermal window, and; (iii) all predators would have narrower and, therefore, asynchronised thermal windows compared to the mosquito species. The results of the study have far reaching implications for the efficacy of natural biological control and fate of these ecosystem services under global change scenarios.

7.2. Materials and Methods

7.2.1 Animal collection and maintenance

Adult aquatic predators, *Lovenula falcifera* and *Anisops sardea* were collected from a clay-lined temporary pond, Central district, Botswana (Figure 8.2a; 022° 52' 16.0S; 027° 47' 42.7E) while *Enithares chinai* were collected from a concrete-lined water pool on the Botswana International University of Science and Technology (BIUST) campus (022° 35' 46.8S; 027° 07' 30.5E). The predators were housed in separate aerated 3 L plastic containers, covered with a net to prevent winged predator escape, comprising ~2 L of a 50:50 ratio of habitat water and matured tap water, and placed in climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany) set at 28 °C ± 2 and 65 % ± 10 relative humidity under a 12:12 light:dark photocycle to stabilise animals. All predator species were fed *Cx. pipiens* larvae *ad libitum*. *Culex pipiens* larvae (Accession Number: MT741514) originated from egg rafts sampled from a concrete-lined water body situated in BIUST campus (022° 35' 05.7S; 027° 06' 58.7E). *Aedes aegypti* larvae (Accession Number: MK571449) were collected using a 1000 µm mesh net from a 20 L container holding ~10 L rain water in a homestead in Palapye village (022° 32' 97.6S; 027° 11' 50.4E) while *An. quadriannulatus* larvae (Accession Number: MT741513) were sourced from a stagnant river water near Hogs Creek (022° 34' 79.3S; 028° 19' 96.1E). The larvae were reared, separately according to species, to different instar levels in 3 L plastic containers holding ~2 L matured tap water housed in climate chambers (as above) and fed with crushed rabbit food pellets *ad libitum* (Westerman's Premium, Durban, South Africa). Both predators and their prey were kept at similar densities (10 individuals/ L in a 3L container holding ~2 L of a 50:50 ratio of habitat water and matured tap water) to avoid overcrowding effects on thermal fitness (Sørensen and Loeschcke 2001). Both the predators and prey were collected between

January and February 2020, and experienced similar thermal environments. Prior to all experiments, predators were kept for at least 7 days in laboratory rearing conditions while prey developmental stages were monitored until appropriate instars sizes were reached.

7.2.2. Experimental design

The CTLs were assessed in an experimental design with respect to (i) predators (3 species types: *L. falcifera*, *A. sardea*, *E. chinai*) and (ii) mosquito larvae (3 species: *Cx. pipiens*, *Ae. aegypti*, *An. quadriannulatus*) across their instar stages (3 levels: mean length \pm SE, early [1st instar; 1.5 ± 0.2 mm], intermediate [2/3 instar; 3.0 ± 0.2 mm], late [4th instar; 5.1 ± 0.2 mm]). Critical thermal limits (CT_{\min} and CT_{\max}) were measured following modified protocols by Nyamukondiwa *et al.* (2013) and were done randomly across all treatments. A set of ten individual organisms at a time were each placed in a series of 10 insulated double-jacketed chambers, connected to a programmable water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany). The water bath contained a 1:1 water:propylene glycol ratio to sufficiently cater for sub-zero cooling temperatures. Each ‘organ pipe’ was filled with 50 mL matured tap water to house an individual animal, which was then given 10 minutes to stabilise at the 28°C temperature, i.e., equivalent to climate chamber rearing conditions. A thermocouple (type T 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Corporation, USA) was inserted into a central ‘organ pipe’ (the control pipe) to monitor the water temperature experienced by the test animals. Temperature was ramped up (CT_{\max}) or down (CT_{\min}) (from benign 28 °C) at $0.25 \text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ following described protocols (Stillman 2004; Nyamukondiwa *et al.*, 2013). The experiment was repeated twice (i.e., two runs per set of ten animals; $n = 20$) per treatment in keeping with Nyamukondiwa and Terblanche (2009). Here, I defined CTLs as the

temperature at which an animal lost coordinated muscle function or responses resulting from a slight prodding using a thermally-inert object congruous to Nyamukondiwa *et al.* (2013). For CT_{max} , this loss of coordinated muscle function always coincides with lethal temperatures and mortality such that recovery is not possible. However, for CT_{min} , recovery always occurs and thus the trait is not always lethal (Chown and Nicolson 2004; Terblanche *et al.*, 2011).

7.2.3. Microclimate data recordings

Microclimate temperature data was recorded from sunlit (i) temporary clay lined pond (123 m length \times 95 m width \times 1.5 m depth; 022° 52' 16.0S; 027° 47' 42.7E) and (ii) a temporary rock pool (2.4 m length \times 1.7 m width \times 13 cm depth; 22° 35' 46.07S; 27° 07' 16.46E), using programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy; 1 h sampling frequency) during the period between August 2019 and February 2020. This was to determine the thermal fluctuations in these temporary wetland aquatic habitats that represent the dominant available natural breeding habitats for predators and prey/ mosquitoes in the region (Chirebvu and Chimbari 2015). These temporary wetlands have been observed to host all larval species and the three predators tested. A data logger probe was placed on sediments at the bottom of the pond/ pool during the dry period and monitored throughout the wet phases to reflect temperature variations associated with these environments. The mean temperature for the wet phase, between both habitats, was used as the habitat temperature (T_{hab}).

7.2.4. Statistical analysis

Data analysis was performed using R, version 3.6.3. The residuals were first checked for normality and variance homogeneity using Shapiro-Wilk and Levene's tests, respectively, and were found to violate normality and variance homogeneity assumptions. Therefore, a one-way Kruskal-Wallis nonparametric test was employed. The CTLs (CT_{\max} and CT_{\min}) were considered separately as dependent variables, while the different prey species, instar stages and predator species were the independent factors. Statistically significant effects were examined pairwise *post hoc* using Dunn test. I thus fit two models to our data, whereby the two CTLs (CT_{\max} or CT_{\min}) of predators and prey (early, intermediate and late instars) were compared.

The thermal breadths (warming tolerance [WT] and cooling tolerance [CT]) for the predators and the larvae were calculated as described by Machekano *et al.* (2018b):

$$WT = CT_{\max} - T_{\text{hab}}$$

and,

$$CT = T_{\text{hab}} - CT_{\min}$$

where CT_{\max} and CT_{\min} were the CTLs for the predators and larval prey whereas T_{hab} was the mean daily habitat (clay lined pond and rock pool data combined means) temperature, reflective of natural conditions likely experienced by both predators and the larval prey in these dominant temporary wetland environments. The standard errors (SE) between CTLs and the T_{hab} for each species were also calculated.

7.3. Results

Critical thermal maxima (CT_{max}) differed significantly among predators and prey across instar stages ($\chi^2 = 229.43$, $df = 11$, $p < 0.001$) (Figure 7.1a). Among predators, the copepod *L. falcifera* exhibited lowest CT_{max} , which was highest for the notonectids *E. chinai* and *A. sardea*, yet only significantly for *E. chinai*. Among prey, *Ae. aegypti* generally exhibited consistently highest CT_{max} , whilst *An. quadriannulatus* was lowest and *Cx. pipiens* intermediate. Whilst 1st instar prey were all statistically similar, *Ae. aegypti* exhibited significantly higher CT_{max} than *An. quadriannulatus* at intermediate and late instar stages, but was statistically similar to *Cx. pipiens*. Within species, prey responses were in turn dependent on their instar stage, whereby 1st instar stages consistently exhibited significantly lowest CT_{max} , whilst the later instar stages were not statistically different (Figure 7.1a). As such, the extent of predator-prey CT_{max} mismatch was greatest when considering later instar stages.

Similarly, CT_{min} differed significantly across predator and prey types ($\chi^2 = 233.63$, $df = 11$, $p < 0.001$) (Figure 7.1b). Among predators, the notonectid *E. chinai* exhibited greatest cold tolerance (lowest CT_{min} temperature) compared to the other species, with CT_{min} significantly lower than *L. falcifera* or *A. sardea*. For prey, responses were inconsistent among species considering instar stage. For 1st instars, CT_{min} values were always statistically similar, whilst for 2nd-3rd and 4th instars *Cx. pipiens* CT_{min} was the highest, and significantly higher than *An. quadriannulatus*. Within prey species, 1st instar stages consistently exhibited significantly higher cold tolerance (lower CT_{min}), excepting between 1st and 2nd-3rd instars of *An. quadriannulatus* (Figure 7.1b). Accordingly, in

contrast to CT_{max} , cold tolerance mismatches between predators and prey tended to be greatest for early instars.

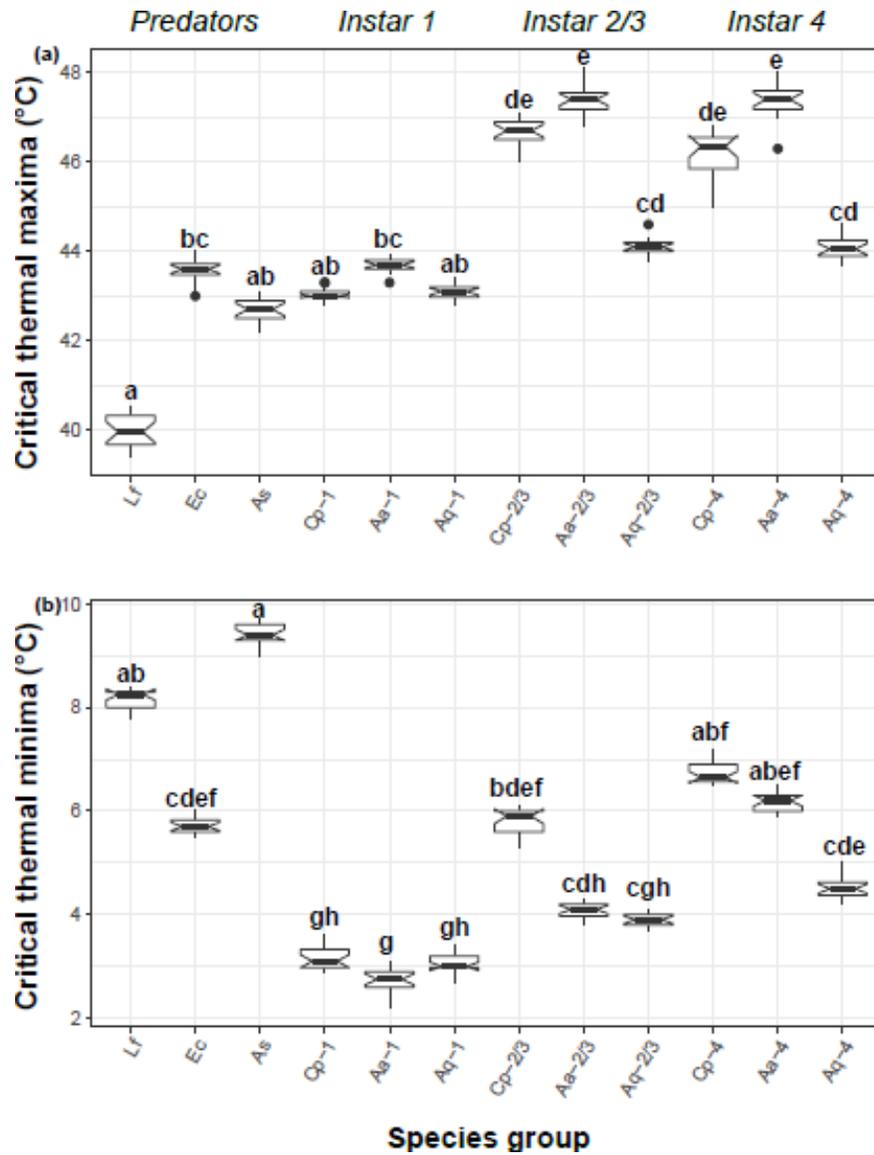


Figure 7. 1. Critical thermal limits ([a] CT_{max} and [b] CT_{min}) of three mosquito predators (*Lovenula falcifera* [*Lf*], *Enithares chinai* [*Ec*] and *Anisops sardea* [*As*]) and three larval prey (*Aedes aegypti* [*Aa*], *Anopheles quadriannulatus* [*Aq*] and *Culex pipiens* [*Cp*]) at 1st, 2/3rd and 4th instar stages. Group medians with different letters are statistically different from each other. ($n = 20$ per experimental group). In the boxplots, the box gives the interquartile ranges and the whiskers show the largest and smallest values up to $1.5 \times$ interquartile range. The points in each box show the median.

The mean daily T_{hab} obtained from the wet phases of the clay lined and rock pool temporary wetland was 25.2 °C (minimum: 21.9 °C; maximum: 29.7 °C), and this was further used to determine the thermal breadths of mosquito predators and the larval prey (Table 7.1; Figure 7.2). These results showed an overall trend of higher CTLs and wider thermal breadths for mosquito larval prey compared to their predators (Table 7.1). The notonectid *E. chinai* had greatest thermal breadths compared to the other predator species at both temperature extremes. In turn, the WT of *L. falcifera* was narrower than *A. sardea*, but the same species (*L. falcifera*) exhibited a wider CT (Table 7.1). Amongst larval mosquitoes, *Ae. aegypti* always had wider WT thermal breadths at matched instar stages. For CT, *Ae. aegypti* had the greatest breadths for the 1st instar stage alone; 2/3rd and 4th instar stages were greater than *Cx. pipiens*, but lower than *An. quadriannulatus*. For all species, 1st instar stages had narrower WT, but broader CT than later instars. Generally, aside from 1st instars, *An. quadriannulatus* had a narrower thermal breadth for WT and a wider CT breadth than *Cx. pipiens* (Table 7.1). *Enithares chinai* was an exception among predators, with wider WT and CT that was more similar to several larval mosquito stages (Table 7.1).

Table 7. 1. Summary of thermal breadths (warming and cooling tolerance) of predators and larval prey, calculated as temperatures between the critical thermal limits (CTLs) and the habitat temperature (T_{hab}). Means are shown alongside standard errors (SE).

Species	Warming tolerance (°C)	SE	Cooling tolerance (°C)	SE
Predators				
<i>Anisops sardea</i>	17.53	± 0.49	15.77	± 0.48
<i>Lovenula falcifera</i>	14.83	± 0.59	17	± 0.43
<i>Enithares chinai</i>	18.41	± 0.54	19.43	± 0.39
Larval prey				
<i>Aedes aegypti</i> 1	18.5	± 0.39	22.41	± 0.48
<i>Aedes aegypti</i> 2/3	22.23	± 0.62	21.1	± 0.39
<i>Aedes aegypti</i> 4	22.21	± 0.62	19.01	± 0.42
<i>Anopheles quadriannulatus</i> 1	17.91	± 0.41	22.10	± 0.44
<i>Anopheles quadriannulatus</i> 2/3	18.94	± 0.42	21.26	± 0.34
<i>Anopheles quadriannulatus</i> 4	18.95	± 0.51	20.66	± 0.48
<i>Culex pipiens</i> 1	17.88	± 0.37	22.01	± 0.48
<i>Culex pipiens</i> 2/3	21.49	± 0.57	19.37	± 0.52
<i>Culex pipiens</i> 4	21.02	± 0.73	18.44	± 0.48

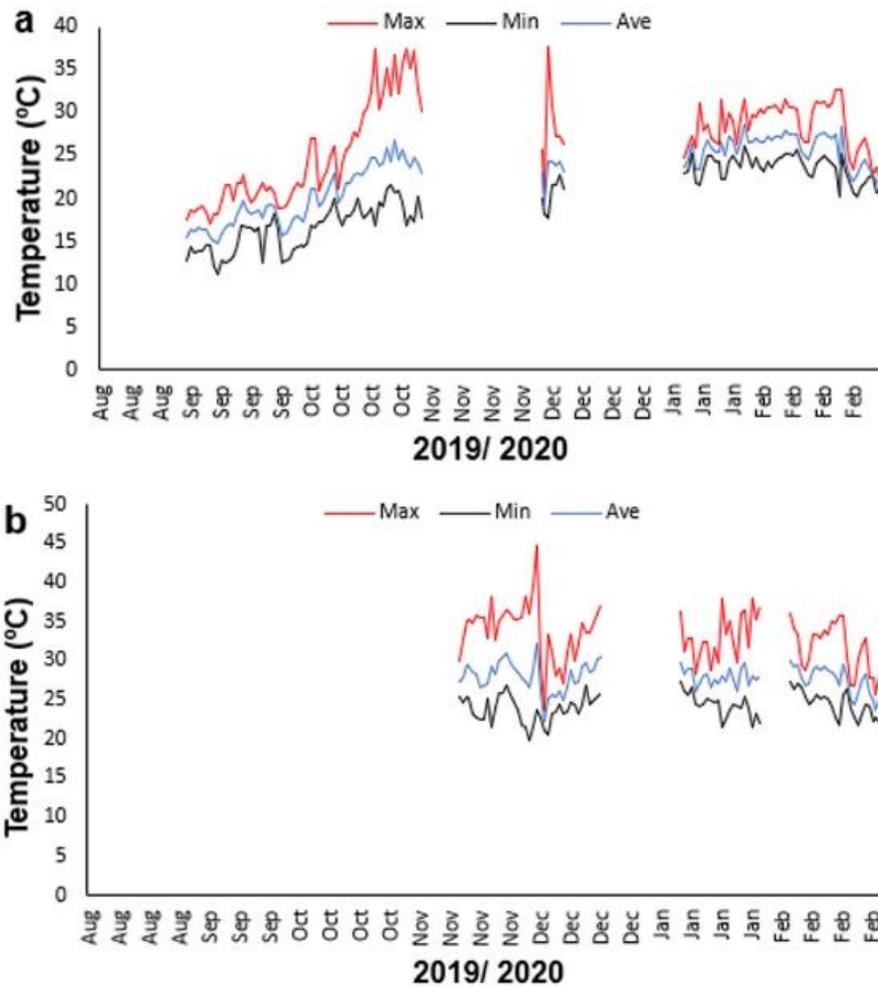


Figure 7. 2. Microclimatic data showing mean daily maximum (Max), minimum (Min) and average (Ave) temperature (°C) of (a) a clay lined pond and (b) a rock pool during their wet phase between August 2019 and February 2020.

7.4. Discussion

Biological control of mosquito larvae using aquatic predators is a sustainable and environmentally friendly approach in reducing disease vector populations (Benelli *et al.*, 2016; Sapari *et al.*, 2019). Dissociations in thermal tolerances between predators and prey may, however, adversely affect predator-prey interactions leading to compromised foraging impacts. The results showed varied thermal tolerance amongst mosquito larval prey, with early instars exhibiting compromised heat tolerance but having higher cold

tolerance. Further, the container-breeding specialist (*Ae. aegypti*) always had a wider WT (thermal breadth), indicating greater tolerance of heat stress. The wholly aquatic copepods had narrower thermal breadth, which was more compromised in terms of WT compared to other semi-aquatic notonectids. Overall, there was a mismatch between predators and the mosquito larval prey at both low and high temperature extremes. Predators showed lower thermal fitness and activity windows, suggesting that they may be impacted by temperature extremes earlier and more negatively than their mosquito prey. In keeping with Hunsicker *et al.* (2013), these results potentially represent a loss of predator optimal ecosystem services provision and a mosquito larval prey proliferation cost in aquatic ecosystems with shifting environments. This has subsequent negative implications on increased vector population and associated disease risks amid rapidly shifting climate environments (Xu *et al.*, 2020).

Aedes aegypti had a wider thermal window compared to the other mosquito species, having higher thermal limits to activity especially on the warming extreme. The species is highly invasive in tropical and subtropical regions of the world (Akiner *et al.*, 2016). This could be linked to its behavioural profile of associating with thermally heterogeneous transient or temporary microhabitats, although CTLs may vary across mosquito species, space and methodological context (Bayoh and Lindsay 2004; Ruybal *et al.*, 2016). The species is found in human dwellings and thrives in diverse artificial containers as breeding habitats (Ngugi *et al.*, 2017; Abilio *et al.*, 2018). Given that this species specialises in small water body utilisation for oviposition (e.g., tyres, tins, gutters, flowerpots), *Ae. aegypti* have likely adapted (e.g., through transgenerational plasticity; (Sgro *et al.*, 2016) and have

the ability to withstand extreme temperatures in small environments associated with less thermal inertia. Indeed, environmental history has consequences for thermal stress tolerance and reliable environmental stress cues experienced by parents can induce adaptive non-heritable phenotypic changes in their offspring owing to epigenetic gene expression (Sgro *et al.*, 2016). Related acclimation effects have been reported to improve fitness traits both within and across generations in *Ae. aegypti* and related dipterans (Stillman 2003; Nyamukondiwa and Terblanche 2010; Schneider *et al.*, 2011). With the highest thermal tolerance and breadth compared to its predators and other mosquito prey larvae tested, *Ae. aegypti* has potential to thrive in conditions where other vector species would otherwise be compromised. Moreover, during this time, mismatch between the *Ae. aegypti* and its predators could allow a rapid mosquito population growth owing to a lack of natural biotic suppression. This may further promote invasion success across the globe, with likely increased risks of associated pathogens and their diseases (Powell 2018). This has implications for emerging and reemerging diseases on vulnerable societies across rural-urban spheres with overall negative implications on public health and livelihoods. However, additional population regulatory effects of increased intraspecific competition should not be ignored.

Amongst the prey species assessed here, generally, *Cx. pipiens* was intermediate heat- (CT_{max}) and warming-tolerant. *Culex pipiens* can breed in highly compromised water quality habitats (e.g., water treatment plants, sewage ponds, septic tanks) (Weitzel *et al.*, 2015), and must oviposit directly onto water in contrast to *Ae. aegypti* which produce dormant eggs. These environments provide thermal variability cushion to the aquatic life-

stages as compared to relatively smaller microhabitats which are likely not as buffered. Conversely, the results showed that *An. quadriannulatus* was generally the most cold tolerant, and generally least heat tolerant, except at 1st instars. Activity at low temperatures means *An. quadriannulatus* can still maintain key life history traits (e.g. swimming, foraging and development) during winter periods. Although the largely zoophilic *An. quadriannulatus* is not presently considered a malaria vector to humans (Habtewold *et al.*, 2008), the species is susceptible to *Plasmodium* infection (Takken *et al.*, 1999; Lobo *et al.*, 2015). This may have future implications for spatial emerging-re-emerging infections to humans, given potential shifts in behavioural (Pates *et al.*, 2006; Seyoum *et al.*, 2012) and feeding preferences associated with such species (Dekker and Takken 1998; Chinula *et al.*, 2018). While *An. quadriannulatus* does not vector malaria, current thermal activity limit results are likely reasonable ecological proxies for more competent congeneric malaria vectors. To this end, further research considering thermal profiles of malaria implicated vectors regionally is recommended, and diverse mosquito predators, alongside actual predator-prey performance experiments, in changing climatic conditions owing to the risk of emerging and reemerging diseases (Sharma *et al.*, 2020).

The results of this study generally reported that mosquitoes had wider thermal windows than their predators. Owing to this temperature regime mismatch, mosquito larvae are likely to proliferate against their predators, suggesting reduced efficacy of predator-prey interaction under shifting aquatic environments (Grigaltchik *et al.*, 2016). *Enithares chinai* heat tolerance (CT_{max}) however synchronised with 1st instar stages of mosquitoes. However, Dalal *et al.* (2020) showed that, at this size, there are some prey refuge effects,

with the notonectid consuming 2/3 and 4th instar larvae more efficiently. This suggests that any observed thermal synchrony between *E. chinai* and 1st instar larvae may still offer limited biocontrol potential. Moreover, this study found that the wholly aquatic copepods are highly likely to be impacted in the warming temperature regimes as opposed to the semi-aquatic, air breathing predatory notonectids and the larval prey. Copepods' life-history traits are negatively affected by escalating temperatures as demonstrated by Lee *et al.* (2003). This has an overall implication for biocontrol in shifting environments, with the need to further identify diverse species, additively combine predators for sustainable mosquito regulation (Cuthbert *et al.*, 2019) and monitoring (physiological-mediated traits) in time and space given the varied habitat temperature exposure. Although species used here were a representation of a single location, the direction of the conclusions drawn are likely more broadly applicable for the studied taxa, all of which are widespread in southern Africa, and in certain instances Africa (e.g., *Lovenula* spp. and *Anopheles* spp.) or even globally (e.g., *Ae. aegypti*). It would, however, be useful for future studies to investigate temperature effects across space and closely related taxa in search of unifying patterns. Similarly, future studies should also investigate predator-prey thermal interaction effects using more controlled predator and prey developmental stages (juveniles), since thermal fitness might vary across insect ontogeny. The relationship between CTLs, and optimal temperatures also need to be explored, within the context of predator-prey behaviour and interaction outcomes.

Based on the predator-prey dissociations exhibited here, whether predator thermal fitness can co-evolve symmetrically with their prey in changing aquatic environments

remains a key question (Collins *et al.*, 2013). Field data has shown that, during the hydroperiod, performance of predators and mosquito larvae was within thermal breadths with no compromised activity on both extremes. Although pond temperature extremes could be seasonal and short-lived due to hydroperiods, more investigations on predator life-history traits are warranted, especially the dormant egg physiology and hatching phenology consequent to the extreme hot and dry phases evidenced from clay lined ponds and rock pools (Svensson *et al.*, 2000; Shadrin *et al.*, 2015). Furthermore, the current study only measured basal thermal traits with the need to further explore other physiological-mediated traits driving the fate of predator-prey interactions within aquatic heterogeneous systems. In particular, behavioral microclimate selection often drives invertebrate vulnerability to shifting climates (Pincebourde and Woods 2020). Thus, the role of behaviour in modulating thermal fitness and how this may reshape predator-prey interactions also ought to be investigated.

Critical thermal limits have widely been used in assessing insect responses to climate change (Chown and Nicolson 2004; Terblanche *et al.*, 2007; Hoffmann *et al.*, 2013), including aquatic invertebrates (Stillman 2003; Stillman 2004). As such, these assays have gained attention in explaining the fate of trophic interactions, e.g., coevolved predator prey and host-natural enemy association under high temperature stress (Machekano *et al.*, 2018a; Mutamiswa *et al.*, 2018a). It is nevertheless critical to directly investigate and establish predator-prey interaction dynamics under varied temperatures; (Mondal *et al.*, 2017; DeLong and Lyon 2020) and how optimal performance may relate to CTLs. This will allow for a more effective assessment of constraints of biological control

associated with thermal stress prior to organismal loss of physiological function e.g., through thermal performance curves (see discussions in Chidawanyika *et al.* [2019]). While CTLs are only a measure of the fate of an organism at extreme temperatures (Lyons *et al.*, 2012; Lyons *et al.*, 2016), they can still be useful in predictive models associated with population dynamics (Chown and Nicolson 2004; Hamblin *et al.*, 2017; Chidawanyika *et al.*, 2019). In addition, the outcomes have ecological implications not only in the long-term effects of global change (Hamblin *et al.*, 2017), but also in the short- to medium term whereby organisms may be exposed to unexpected extreme acute temperatures, such as cold snaps and heat waves (Stillman 2019; Xu *et al.*, 2020). Although some organisms can survive these harsh conditions, some may succumb to them, with implications for community dynamics. In this context, although feeding rates of mosquito natural enemies can relate positively to temperature (Cuthbert *et al.*, 2018b), as temperatures exceed thermal optimums a unimodal feeding relationship may arise (Englund *et al.*, 2011), which could alleviate prey from predation pressure and promote their proliferation.

Ectothermic organisms also often adaptively remodel their phenotypes to better survive stressful environments through plasticity (Stillman 2003; Whitman 2009), a near ubiquitous mechanism in insects. Thus, it is likely that the limited thermal tolerance and breadths for predators recorded here may be compensated for through higher phenotypic adjustments (Stillman 2003; Calosi *et al.*, 2008; Gunderson and Stillman 2015; van Heerwaarden *et al.*, 2016). Nevertheless, the presence, magnitude and extent to which phenotypic plasticity may cushion organisms to climate change remains debatable

(Gunderson and Stillman 2015; van Heerwaarden *et al.*, 2016). Thus, the exact extent to which plasticity may change the direction of interactions reported here warrants future investigation. Unraveling these physiological mechanisms will foster improved understanding of predator longevity and success, paramount to sustain mosquito biological control under climate change. This is significant in maintaining the integrity and efficacy of biocontrol agents in aquatic habitats given the increased extreme temperature means and fluctuations with high intensities under global change (Meehl and Tebaldi 2004; Thuiller 2007; Walther *et al.*, 2009; Xu *et al.*, 2020).

The results demonstrate a mismatch of thermal activity limits (CTLs) and thermal breadths (WT and CT) between key predators and their mosquito prey. Larval mosquitoes had significantly higher activity limits and wider thermal windows relative to their predator antagonists. This thermal mismatch may mean asynchrony in predator-prey phenologies in shifting habitats, consequently altering the aquatic ecosystem trophic community structures and functioning. Predators are thus projected to reduce interactive foraging strength towards an increasingly thermally-fit prey, giving vectors an advantage to proliferate in aquatic habitats. Implication for increasing temperature stress remains a challenge in predicting mosquito biocontrol using natural enemies, and more so under shifting aquatic habitats. In future research, the role of plastic thermal compensation for building resilience under climate change, should be explored. Conservation of mosquito predators, coupled with the use of other complementary biological control strategies, and used in an integrated approach, may help reduce disease vector populations and associated public health concerns.

7.5. References

- Abílio, A. P., Abudasse, G., Kampango, A., Candrinho, B., Sitoi, S., Luciano, J., and David, M. R. (2018). Distribution and breeding sites of *Aedes aegypti* and *Aedes albopictus* in 32 urban/peri-urban districts of Mozambique: implication for assessing the risk of arbovirus outbreaks. *PLoS Neglected Tropical Diseases*, 12: e0006692.
- Abrahams, M. V., Mangel, M., and Hedges, K. (2007). Predator–prey interactions and changing environments: who benefits? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 362: 2095-2104.
- Acquah-Lamptey, D., and Brandl, R. (2018). Effect of a dragonfly (*Bradinopyga strachani* Kirby, 1900) on the density of mosquito larvae in a field experiment using mesocosms. *Web Ecology*, 18: 81-89.
- Agosta, S. J., Joshi, K. A., and Kester, K. M. (2018). Upper thermal limits differ among and within component species in a tritrophic host-parasitoid-hyperparasitoid system. *PloS ONE*, 13: e0198803.
- Akiner, M. M., Demirci, B., Babuadze, G., Robert, V., and Schaffner, F. (2016). Spread of the invasive mosquitoes *Aedes aegypti* and *Aedes albopictus* in the Black Sea region increases risk of chikungunya, dengue, and Zika outbreaks in Europe. *PLoS Neglected Tropical Diseases*, 10: e0004664.
- Andersen, J. L., Manenti, T., Sørensen, J. G., MacMillan, H. A., Loeschcke, V., and Overgaard, J. (2015). How to assess *Drosophila* cold tolerance: chill coma temperature and lower lethal temperature are the best predictors of cold distribution limits. *Functional Ecology*, 29: 55-65.
- Arribas, R., Justin C. T., and Gomez-Mestre, I. (2018). "Predation and competition differentially affect the interactions and trophic niches of a Neotropical amphibian guild." *Frontiers in Ecology and Evolution* 6: 28.
- Bayoh, M. N., and Lindsay, S. W. (2004). Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae* in the laboratory. *Medical and Veterinary Entomology*, 18: 174-179.

- Benelli, G., Jeffries, C. L., and Walker, T. (2016). Biological control of mosquito vectors: past, present, and future. *Insects*, 7: 52.
- Bowler, K., and Terblanche, J. S. (2008). Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? *Biological Reviews*, 83: 339-355.
- Braack, L., de Almeida, A. P. G., Cornel, A. J., Swanepoel, R., and De Jager, C. (2018). Mosquito-borne arboviruses of African origin: review of key viruses and vectors. *Parasites and Vectors*, 11: 29.
- Broitman, B. R., Szathmary, P. L., Mislán, K. A. S., Blanchette, C. A., and Helmuth, B. (2009). Predator–prey interactions under climate change: the importance of habitat vs body temperature. *Oikos*, 118: 219-224.
- Burton, T., and Einum, S. (2020). The old and the large may suffer disproportionately during episodes of high temperature: evidence from a keystone zooplankton species. *Conservation Physiology*, 8: coaa038.
- Calosi, P., Bilton, D. T., and Spicer, J. I. (2008). Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biology Letters*, 4: 99-102.
- Chandra, G., Bhattacharjee, I., Chatterjee, S. N., and Ghosh, A. (2008). Mosquito control by larvivorous fish. *Indian Journal of Medical Research*, 127: 13.
- Chidawanyika, F., Mudavanhu, P., and Nyamukondiwa, C. (2019). Global climate change as a driver of bottom-up and top-down factors in agricultural landscapes and the fate of host-parasitoid interactions. *Frontiers in Ecology and Evolution*, 7: 80.
- Chinula, D., Hamainza, B., Chizema, E., Kavishe, D. R., Sikaala, C. H., and Killeen, G. F. (2018). Proportional decline of *Anopheles quadriannulatus* and increased contribution of *An. arabiensis* to the *An. gambiae* complex following introduction of indoor residual spraying with pirimiphos-methyl: an observational, retrospective secondary analysis of pre-existing data from south-east Zambia. *Parasites and Vectors*, 11: 1-7.
- Chirebvu, E., and Chimbari, M. J. (2015). Characteristics of *Anopheles arabiensis* larval habitats in Tubu village, Botswana. *Journal of Vector Ecology*, 40: 129-138.

- Coetzee, M., Kyalo, D., Irish, S., and Snow, R. (2020). Updated list of *Anopheles* species (Diptera: Culicidae) by country in the Afrotropical Region and associated islands. *Zootaxa*, 4743: 401–449.
- Cornel, A. J., Lee, Y., Almeida, A. P. G., Johnson, T., Mouatcho, J., Venter, M., and Braack, L. (2018). Mosquito community composition in South Africa and some neighboring countries. *Parasites and Vectors*, 11: 1-12.
- Chown, S. L., Chown, S., and Nicolson, S. (2004). *Insect physiological ecology: mechanisms and patterns*. Oxford University Press, New York, USA.
- Collins, M., Knutti, R., Arblaster, J., Dufresne, J. L., Fichefet, T., Friedlingstein, P., and Shongwe, M. (2013). Long-term climate change: projections, commitments and irreversibility. In *Climate Change 2013-The Physical Science Basis: Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 1029-1136). Cambridge University Press, New York, USA.
- Cuthbert, R. N., Dalu, T., Wasserman, R. J., Weyl, O. L., Froneman, P. W., Callaghan, A., and Dick, J. T. (2019). Additive multiple predator effects of two specialist paradiaptomid copepods towards larval mosquitoes. *Limnologica*, 79: 125727.
- Cuthbert, R. N., Dalu, T., Wasserman, R. J., Callaghan, A., Weyl, O. L., and Dick, J. T. (2018a). Calanoid copepods: an overlooked tool in the control of disease vector mosquitoes. *Journal of Medical Entomology*, 55: 1656-1658.
- Cuthbert, R. N., Dick, J. T., Callaghan, A., and Dickey, J. W. (2018b). Biological control agent selection under environmental change using functional responses, abundances and fecundities; the Relative Control Potential (RCP) metric. *Biological Control*, 121: 50-57.
- Dalal, A., Cuthbert, R. N., Dick, J. T., and Gupta, S. (2020). Prey preferences of notonectids towards larval mosquitoes across prey ontogeny and search area. *Pest Management Science*, 76: 609-616.
- Dekker, T., and Takken, W. (1998). Differential responses of mosquito sibling species *Anopheles arabiensis* and *An. quadriannulatus* to carbon dioxide, a man or a calf. *Medical and Veterinary Entomology*, 12: 136-140.

- DeLong, J. P., and Lyon, S. (2020). Temperature alters the shape of predator–prey cycles through effects on underlying mechanisms. *PeerJ*, 8: e9377.
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., and Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*, 105: 6668-6672.
- Dida, G. O., Gelder, F. B., Anyona, D. N., Abuom, P. O., Onyuka, J. O., Matano, A. S., and Ofulla, A. V. (2015). Presence and distribution of mosquito larvae predators and factors influencing their abundance along the Mara River, Kenya and Tanzania. *SpringerPlus*, 4: 136.
- Dom, N. C., Madzlan, M. F., Nur, S., Hasnan, A., and Misran, N. (2016). Water quality characteristics of dengue vectors breeding containers. *International Journal of Mosquito Research*, 3: 25-29.
- Domenici, P., Claireaux, G., and McKenzie, D. J. (2007). Environmental constraints upon locomotion and predator–prey interactions in aquatic organisms: an introduction. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 362: 1929–1936.
- Engelbrecht, F., Adegoke, J., Bopape, M. J., Naidoo, M., Garland, R., Thatcher, M., and Gatebe, C. (2015). Projections of rapidly rising surface temperatures over Africa under low mitigation. *Environmental Research Letters*, 10: 085004.
- Englund, G., Öhlund, G., Hein, C. L., and Diehl, S. (2011). Temperature dependence of the functional response. *Ecology Letters*, 14: 914-921.
- Foster, W. A., and Walker, E. D. (2019). Mosquitoes (Culicidae). In *Medical and veterinary entomology* (pp. 261-325). Academic press, San Diego, CA, USA.
- Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., and Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science*, 293: 2248-2251.
- Grigaltchik, V. S., Webb, C., and Seebacher, F. (2016). Temperature modulates the effects of predation and competition on mosquito larvae. *Ecological Entomology*, 41: 668-675.

- Gunderson, A. R., Dillon, M. E., and Stillman, J. H. (2017). Estimating the benefits of plasticity in ectotherm heat tolerance under natural thermal variability. *Functional Ecology*, 31: 1529-1539.
- Gunderson, A. R., and Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences*, 282: 20150401.
- Habtewold, T., Povelones, M., Blagborough, A. M., and Christophides, G. K. (2008). Transmission blocking immunity in the malaria non-vector mosquito *Anopheles quadriannulatus* species A. *PLoS Pathogens*, 4: e1000070.
- Hamblin, A. L., Youngsteadt, E., López-Urbe, M. M., and Frank, S. D. (2017). Physiological thermal limits predict differential responses of bees to urban heat-island effects. *Biology Letters*, 13: 20170125.
- Hance, T., van Baaren, J., Vernon, P., and Boivin, G. (2007). Impact of extreme temperatures on parasitoids in a climate change perspective. *Annual Reviews of Entomology*, 52: 107-126.
- Hoffmann, A. A., Chown, S. L., and Clusella-Trullas, S. (2013). Upper thermal limits in terrestrial ectotherms: how constrained are they? *Functional Ecology*, 27: 934-949.
- Holomuzki, J. R., Feminella, J. W., and Power, M. E. (2010). Biotic interactions in freshwater benthic habitats. *Journal of the North American Benthological Society*, 29: 220-244.
- Hunsicker, M. E., Ciannelli, L., Bailey, K. M., Zador, S., and Stige, L. C. (2013). Climate and demography dictate the strength of predator-prey overlap in a subarctic marine ecosystem. *PLoS ONE*, 8: e66025.
- Jiang, L., and Morin, P. J. (2004). Temperature-dependent interactions explain unexpected responses to environmental warming in communities of competitors. *Journal of Animal Ecology*, 73: 569-576.
- Johansson, F., and Brodin, T. (2003). Effects of fish predators and abiotic factors on dragonfly community structure. *Journal of Freshwater Ecology*, 18: 415-423.

- Karuitha, M., Bargul, J., Lutomiah, J., Muriu, S., Nzovu, J., Sang, R., and Mbogo, C. (2019). Larval habitat diversity and mosquito species distribution along the coast of Kenya. *Wellcome Open Research*, 4: 175.
- Kocher, D. K., Jamwal, S., Kansal, M., Bakshi, D. K., and Kocher, D. K. (2014). Coexistence of copepods and mosquito larvae in different standing water bodies of Punjab, India. *Scholars Academic Journal of Biosciences*, 2: 640-642.
- Lebl, K., Zित्रa, C., Silbermayr, K., Obwaller, A., Berer, D., Brugger, K., and Rubel, F. (2015). Mosquitoes (Diptera: Culicidae) and their relevance as disease vectors in the city of Vienna, Austria. *Parasitology Research*, 114: 707-713.
- Lee, H. W., Ban, S., Ikeda, T., and Matsuishi, T. (2003). Effect of temperature on development, growth and reproduction in the marine copepod *Pseudocalanus newmani* at satiating food condition. *Journal of Plankton Research*, 25: 261-271.
- Lobo, N. F., Laurent, B. S., Sikaala, C. H., Hamainza, B., Chanda, J., Chinula, D., and Boldt, H. L. (2015). Unexpected diversity of *Anopheles* species in Eastern Zambia: implications for evaluating vector behavior and interventions using molecular tools. *Scientific Reports*, 5: 17952.
- Lyons, C. L., Oliver, S. V., Hunt, R. H., and Coetzee, M. (2016). The Influence of Insecticide Resistance, Age, Sex, and Blood Feeding Frequency on Thermal Tolerance of Wild and Laboratory Phenotypes of *Anopheles funestus* (Diptera: Culicidae). *Journal of Medical Entomology*, 53: 394-400.
- Lyons, C. L., Coetzee, M., Terblanche, J. S., and Chown, S. L. (2012). Thermal limits of wild and laboratory strains of two African malaria vector species, *Anopheles arabiensis* and *Anopheles funestus*. *Malaria Journal*, 11: 226.
- Machekano, H., Mvumi, B. M., and Nyamukondiwa, C. (2018a). Loss of coevolved basal and plastic responses to temperature may underlie trophic level host-parasitoid interactions under global change. *Biological Control*, 118: 44-54.
- Machekano, H., Mutamiswa, R., and Nyamukondiwa, C. (2018b). Evidence of rapid spread and establishment of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in semi-arid Botswana. *Agriculture and Food Security*, 7: 48.

- Meehl, G. A., and Tebaldi, C. (2004). More intense, more frequent, and longer lasting heat waves in the 21st century. *Science*, 305: 994-997.
- Mondal, R. P., Chandra, G., Bandyopadhyay, S., and Ghosh, A. (2017). Effect of temperature and search area on the functional response of *Anisops sardea* (Hemiptera: Notonectidae) against *Anopheles stephensi* in laboratory bioassay. *Acta Tropica*, 166: 262-267.
- Mutamiswa, R., Chidawanyika, F., and Nyamukondiwa, C. (2018a). Thermal plasticity potentially mediates the interaction between host *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and endoparasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) in rapidly changing environments. *Pest Management Science*, 74: 1335-1345.
- Mutamiswa, R., Machezano, H., Chidawanyika, F., and Nyamukondiwa, C. (2018b). Thermal resilience may shape population abundance of two sympatric congeneric *Cotesia* species (Hymenoptera: Braconidae). *PloS ONE*, 13: e0191840.
- Ngugi, H. N., Mutuku, F. M., Ndenga, B. A., Musunzaji, P. S., Mbakaya, J. O., Aswani, P., and LaBeaud, A. D. (2017). Characterization and productivity profiles of *Aedes aegypti* (L.) breeding habitats across rural and urban landscapes in western and coastal Kenya. *Parasites and Vectors*, 10: 331.
- Nyamukondiwa, C., Weldon, C. W., Chown, S. L., le Roux, P. C., and Terblanche, J. S. (2013). Thermal biology, population fluctuations and implications of temperature extremes for the management of two globally significant insect pests. *Journal of Insect Physiology*, 59: 1199-1211.
- Nyamukondiwa, C., and Terblanche, J. S. (2010). Within-generation variation of critical thermal limits in adult Mediterranean and Natal fruit flies *Ceratitis capitata* and *Ceratitis rosa*: thermal history affects short-term responses to temperature. *Physiological Entomology*, 35: 255-264.
- Nyamukondiwa, C., and Terblanche, J. S. (2009). Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): effects of age, gender and feeding status. *Journal of Thermal Biology*, 34: 406-414.

- Ohba, S. Y., Huynh, T. T., Kawada, H., Le, L. L., Ngoc, H. T., Hoang, S. L., and Takagi, M. (2011). Heteropteran insects as mosquito predators in water jars in southern Vietnam. *Journal of Vector Ecology*, 36: 170-174.
- Papa, A. (2019). Emerging arboviruses of medical importance in the Mediterranean region. *Journal of Clinical Virology*, 115: 5-10.
- Pates, H. V., Takken, W., Curtis, C. F., and Jamet, H. (2006). Zoophilic *Anopheles quadriannulatus* species B found in a human habitation in Ethiopia. *Annals of Tropical Medicine and Parasitology*, 100: 177-179.
- Pincebourde, S., and Woods, H. A. (2020). There is plenty of room at the bottom: microclimates drive insect vulnerability to climate change. *Current Opinion in Insect Science*. 41: 63-70.
- Powell, J. R. (2018). Mosquito-borne human viral diseases: why *Aedes aegypti*? *The American Journal of Tropical Medicine and Hygiene*, 98: 1563-1565.
- Ravelo, A. C., Andreasen, D. H., Lyle, M., Lyle, A. O., and Wara, M. W. (2004). Regional climate shifts caused by gradual global cooling in the Pliocene epoch. *Nature*, 429: 263-267.
- Roux, O., and Robert, V. (2019). Larval predation in malaria vectors and its potential implication in malaria transmission: an overlooked ecosystem service? *Parasites and Vectors*, 12: 217.
- Ruybal, J. E., Kramer, L. D., and Kilpatrick, A. M. (2016). Geographic variation in the response of *Culex pipiens* life history traits to temperature. *Parasites and Vectors*, 9: 1-9.
- Sapari, N. B., Manan, T. S. B. A., and Yavari, S. (2019). Sustainable control of mosquito by larval predating *Micronecta polhemus* Niser for the prevention of mosquito breeding in water retaining structures. *International Journal of Mosquito Research*, 6: 31-37.
- Schneider, J. R., Chadee, D. D., Mori, A., Romero-Severson, J., and Severson, D. W. (2011). Heritability and adaptive phenotypic plasticity of adult body size in the

- mosquito *Aedes aegypti* with implications for dengue vector competence. *Infection, Genetics and Evolution*, 11: 11-16.
- Scholes, R. J. (2016). Climate change and ecosystem services. *Wiley Interdisciplinary Reviews: Climate Change*, 7: 537-550.
- Seidel, D. J., Fu, Q., Randel, W. J., and Reichler, T. J. (2008). Widening of the tropical belt in a changing climate. *Nature Geoscience*, 1: 21-24.
- Seyoum, A., Sikaala, C. H., Chanda, J., Chinula, D., Ntamatungiro, A. J., Hawela, M., and Killeen, G. F. (2012). Human exposure to anopheline mosquitoes occurs primarily indoors, even for users of insecticide-treated nets in Luangwa Valley, South-East Zambia. *Parasites and Vectors*, 5: 101.
- Sgro, C. M., Terblanche, J. S., and Hoffmann, A. A. (2016). What can plasticity contribute to insect responses to climate change? *Annual Review of Entomology*, 61: 433-451.
- Shadrin, N. V., Anufrieva, E. V., Amat, F., and Eremin, O. Y. (2015). Dormant stages of crustaceans as a mechanism of propagation in the extreme and unpredictable environment in the Crimean hypersaline lakes. *Chinese Journal of Oceanology and Limnology*, 33: 1362-1367.
- Sharma, V., Sharma, M., Dhull, D., Sharma, Y., Kaushik, S., and Kaushik, S. (2020). Zika virus: an emerging challenge to public health worldwide. *Canadian Journal of Microbiology*, 66: 87-98.
- Sørensen, J. G., and Loeschcke, V. (2001). Larval crowding in *Drosophila melanogaster* induces Hsp70 expression, and leads to increased adult longevity and adult thermal stress resistance. *Journal of Insect Physiology*, 47: 1301-1307.
- Stillman, J. H. (2019). Heat waves, the new normal: summertime temperature extremes will impact animals, ecosystems, and human communities. *Physiology*, 34: 86-100.
- Stillman, J. H. (2004). A comparative analysis of plasticity of thermal limits in porcelain crabs across latitudinal and intertidal zone clines. In *International Congress Series*, 1275: 267-274.
- Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science*, 301: 65.

- Svensson, B. G., Tallmark, B., and Petersson, E. (2000). Habitat heterogeneity, coexistence and habitat utilization in five backswimmer species (*Notonecta* spp.; Hemiptera, Notonectidae). *Aquatic Insects*, 22: 81-98.
- Takken, W., Eling, W., Hooghof, J., Dekker, T., Hunt, R., and Coetzee, M. (1999). Susceptibility of *Anopheles quadriannulatus* Theobald (Diptera: Culicidae) to *Plasmodium falciparum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 93: 578-580.
- Tandina, F., Doumbo, O., Traoré, S. F., Parola, P., and Robert, V. (2018). Mosquitoes (Diptera: Culicidae) and mosquito-borne diseases in Mali, West Africa. *Parasites and Vectors*, 11: 1-12.
- Teoh, M. L., Chu, W. L., and Phang, S. M. (2010). Effect of temperature change on physiology and biochemistry of algae: a review. *Malaysian Journal of Science*, 29: 82-97.
- Terblanche, J. S., Hoffmann, A. A., Mitchell, K. A., Rako, L., le Roux, P. C., and Chown, S. L. (2011). Ecologically relevant measures of tolerance to potentially lethal temperatures. *Journal of Experimental Biology*, 214: 3713-3725.
- Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C., and Chown, S. L. (2007). Critical thermal limits depend on methodological context. *Proceedings of the Royal Society B: Biological Sciences*, 274: 2935-2943.
- Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., and Hughes, L. (2004). Extinction risk from climate change. *Nature*, 427: 145-148.
- Thuiller, W. (2007). Climate change and the ecologist. *Nature*, 448: 550-552.
- Tolle, M. A. (2009). Mosquito-borne diseases. *Current Problems in Pediatric and Adolescent Health Care*, 39: 97-140.
- van Heerwaarden, B., Kellermann, V., and Sgrò, C. M. (2016). Limited scope for plasticity to increase upper thermal limits. *Functional Ecology*, 30: 1947-1956.

- Vucic-Pestic, O. L. I. V. E. R. A., Ehnes, R. B., Rall, B. C., and Brose, U. (2011). Warming up the system: higher predator feeding rates but lower energetic efficiencies. *Global Change Biology*, 17: 1301-1310.
- Walther, G. R., Roques, A., Hulme, P. E., Sykes, M. T., Pyšek, P., Kühn, I., and Czucz, B. (2009). Alien species in a warmer world: risks and opportunities. *Trends in Ecology and Evolution*, 24: 686-693.
- Wasserman, R. J., Vink, T. J., Dalu, T., and Froneman, P. W. (2015). Fish predation regimes modify benthic diatom community structures: Experimental evidence from an in situ mesocosm study. *Austral Ecology*, 40: 806-815.
- Weitzel, T., Jawień, P., Rydzanicz, K., Lonc, E., and Becker, N. (2015). *Culex pipiens* s.l. and *Culex torrentium* (Culicidae) in Wrocław area (Poland): occurrence and breeding site preferences of mosquito vectors. *Parasitology Research*, 114: 289-295.
- Whitman D. W. (2009). Acclimation. *Phenotypic Plasticity of Insects. Mechanisms and Consequences* (ed. By D.W. Whitman and T.N. Ananthkrishnan), (pp. 675-739). Enfield: Science Publishers, Boca Raton.
- Wilke, A. B., Chase, C., Vasquez, C., Carvajal, A., Medina, J., Petrie, W. D., and Beier, J. C. (2019). Urbanization creates diverse aquatic habitats for immature mosquitoes in urban areas. *Scientific Reports*, 9: 1-11.
- Wu, L., Cai, W., Zhang, L., Nakamura, H., Timmermann, A., Joyce, T., and Chang, P. (2012). Enhanced warming over the global subtropical western boundary currents. *Nature Climate Change*, 2: 161-166.
- Xu, C., Kohler, T. A., Lenton, T. M., Svenning, J. C., and Scheffer, M. (2020). Future of the human climate niche. *Proceedings of the National Academy of Sciences*, 117: 11350-11355.
- Zuharah, W. F., and Lester, P. J. (2010). The influence of aquatic predators on mosquito abundance in animal drinking troughs in New Zealand. *Journal of Vector Ecology*, 35: 347-353.

CHAPTER 8
General Discussion

Disease vector mosquitoes have been increasing in the past decades as have their associated parasites (Braack *et al.*, 2018; Franklinos *et al.*, 2019). This may be partly due to increased anthropogenic activities and the current shifts in climates (Brugueras *et al.*, 2020; Lee *et al.*, 2020; Schrama *et al.*, 2020). Despite the proliferation of vector species and the surge of disease, societal knowledge on general mosquito bio-ecology is often limited although malaria and its burden are well known (Govere *et al.*, 2000; Potter *et al.*, 2016). This study focused on the neglected region (Central district, Botswana) with vulnerable communities exposed to the infectious diseases such as malaria. The district has no baseline information to measure change in issues of mosquitoes and associated burden. Thus, the study aimed to bridge knowledge gaps in issues of community preparedness and education, vector prevalence and factors likely to exacerbate the impact of mosquito-borne infection. To this end, knowledge on mosquito bio-ecology is key for sustainable control of pest populations amidst apparent biotic and abiotic factors modulated by humans and climate.

To implement efficacious vector mosquito control, communities ought to be knowledgeable about the bio-ecology of the vectors and that of their associated parasites. Thus, it follows that the most severely affected communities are those likely having limited knowledge on the dynamics of mosquito bio-ecology and propagation (Wilke *et al.*, 2020). For example, mosquito species belonging to *Aedes* genus live in close association with humans, exploiting modified and artificial habitats (e.g., container style systems) for breeding (Brown *et al.*, 2014). Knowledge on how mosquitoes breed could bolster *Aedes* mosquito population reduction through simply ensuring that breeding potential habitats are

reduced or treated with larvicide. The survey of KAPs (Chapter 2) revealed major knowledge gaps on mosquitoes, their ecology and control aspects with no significant difference between malaria endemic and non-endemic sub-districts. In addition, all the communities exhibited concerns about the effects of vector mosquito burden, biased towards malaria but with limited knowledge on other vector-borne infections. The level of protection from vector mosquitos was also variable across their zones. The gaps unveiled by the KAPs are crucial in strengthening and mobilising the communities' knowledge on mosquito bio-ecology.

Chapter 3 scoped mosquito species and abundance across three sub-districts and reported *Ae. aegypti*, for the first time in the country. This vector species was recorded at all sites sampled, suggesting that it is widely distributed across Botswana. This is a milestone warranting further surveillance countrywide, and routine surveillance to update resident vector pest mosquito species' lists. The sub-districts were also populated by high abundance of *Cx. pipiens*, a biting nuisance, and a competent disease vector in humans (Brugman *et al.*, 2018), livestock (Brustolin *et al.*, 2017) and wildlife (Martínez-de la Puente *et al.*, 2016). Similarly, all sub-districts had a representation of *Anopheles* vector species with the risk of malaria spread given proximity of the sub-districts and the well-established *Plasmodium* parasite in the Central district. Although Serowe and Palapye are currently known to be non-endemic, evidence of sporadic cases reported by Chihanga *et al.* (2016) are worrisome. This calls for intensive vector and parasite surveillance even to the rest of the non-endemic zones in the country. As the current national vector control programme is biased towards *Anopheles* and malaria only, focus needs to extend to other

vector species. Knowledge of diverse species across the country, can help in modelling vector borne disease dynamics and assist in early warning systems for epidemics. Therefore, continuous monitoring of vector species and associated parasite incrimination is highly recommended, as it helps keep track of indigenous and exotic invasive vectors, key traits to managing vector-borne diseases. In addition, it is essential to taxonomically describe species identified to genus level (e.g., *Anopheles* sp.) in Chapter 3.

Determining factors that promote mosquito breeding is an essential component of any management protocol. Within the Botswana context, this is a major gap. Using a mesocosm approach, Chapter 4 investigated the effects of aquatic nutrification on mosquito breeding, using cattle dung inputs as a nutrient source. Botswana is a “cattle country” and when coupled with free range tenure system, as evident in most parts of the nation, it is likely to progressively account for aquatic systems degradation through dung inoculation. Chapter 4 demonstrated that aquatic water sources enriched with different concentrations of cattle dung nutrients, recruit different mosquito species (*An. rhodesiensis*, *Cx. pipiens*) for oviposition, consequently contributing to an increase in vector mosquito numbers. The potential risk factor here, is on the free-range cattle that are capable of accessing water sources even within human homesteads and/or settlements. The situation is much complex to deal with since the cattle industry is of valued socio-economic status in Botswana (Hillbom 2014; Temoso *et al.*, 2015). It is, therefore, recommend that cattle movement restriction be investigated in areas in and near human dwellings. This also calls for adoption and prioritisation of farm peripheral fences or paddocks establishment to monitor livestock movement as previously advocated by Selolwane (2001). The number of cattle reared may

also be important to review considering their potential as hosts for many zoophilic species (*An. quadriannulatus*, *An. arabiensis*, *An. gambiae*, *An. melas*, *An. merus*) some responsible for debilitating diseases (Sinka 2013; Athrey *et al.*, 2017). Moreover, *An. arabiensis*, is considered one of the vectors of malaria in Botswana (Tawe *et al.*, 2017), and also regarded zoophilic when compared to the highly anthropophilic *An. gambiae* (Sinka 2013). This further poses a risk of disease spread and transmission dynamics given the readily available free ranging cattle ‘as hosts’ within human habitations (Stone and Gross 2018). Based on the results of Chapter 4, designated and modified watering points for livestock need to be investigated for reduction of contamination by nutrifying inputs associated with cattle. Furthermore, sustainable uses of cattle dung such as energy generation, may be explored to minimise dung nitrification risks while increasing benefits (see Valela and Muzenda 2019).

Another major gap in the literature for the region are the ecosystem services of intact small aquatic environments. Given that these habitats can be used as mosquito breeding sites when compromised, the extent to which native predators may hinder mosquito numbers through larval control warranted investigation. Chapter 5 was aimed at identifying native mosquito predator species regulation mosquito juveniles (larvae) in local aquatic habitats. Since the national vector control programme focuses solely on adults, investigating larval biocontrol would allow synergism in vector control. Although trials have been piloted on larviciding in the Bobirwa sub-district (see Obopile *et al.*, 2018), these methods may be expensive, as treatment intervals need to be deployed every two weeks at > 80% larval mortality for efficacious results (Mpofu *et al.*, 2016). Chapter 5 and/or

Chapter 6 identified *Anisops sardea*, *Enithares chinai* and *Lovenula falcifera* as dominant native predators and quantified their predatory impacts towards mosquito larvae for potential exploration in natural aquatic systems. All the species exhibited the type II functional response toward mosquito larvae, suggesting that they efficiently contribute to the natural biological control of mosquitoes. It further highlights the role of predator loss (see discussion in Chapter 5), associated with habitat degradation, as a facilitator of mosquito proliferation. Future work should investigate mosquito egg predators, that may potentially provide complimentary synergistic control (Mukherjee and Blaustein 2019).

Chapter 6 investigated multiple predator effects of the native *Anisops sardea* and *Enithares chinai* under environmental context dependencies. Both predators demonstrated type II functional responses towards *Cx. pipiens* larval ontogeny (see Chapter 6). Synergistic multiple predator effects (i.e., prey risk enhancement) were observed under certain environmental contexts. Similarly, different notonectid predators performed optimally under different habitat complexity levels. These findings highlight that predator diversity and density is potentially important in mosquito larval regulation given environmental heterogeneity in breeding habitats. Future work should directly assess the role of habitat degradation in compromising aquatic predator diversity and abundance.

The most important abiotic factor that potentially mediates predator prey interaction is temperature (e.g., Wasserman *et al.*, 2016; Daugaard *et al.*, 2019). Given the current projections of warming average temperatures in Africa (Almazroui *et al.*, 2020), it is unknown how this may affect co-evolved predator-prey interactions. To investigate the

fate of predator prey interactions under climate change, thermal fitness of both the predator and prey should remain in synchronisation (e.g., Bestion *et al.*, 2019). Chapter 7 demonstrated thermal fitness trait mismatch between studied predators and the larval mosquito prey. The mosquito larvae better tolerated both cooling and warming regimes as compared to the predators. The ecological relevance of these findings suggest that mosquito larvae are likely to proliferate more than their predators, in the face of extreme temperature stress. Here, the invasive *Aedes aegypti* displayed higher thermal breadths than *An. quadriannulatus* and *Cx. pipiens* especially on the warming regimes. This has implications on increased *Ae. aegypti* invasion of novel habitats under changing climates (Iwamura *et al.*, 2020). Since all the sampled districts reported this species, intensive and regular surveillance is recommended across the country to assess the extent of invasiveness and where needed, establish control interventions congruent with Petrić *et al.* (2012). As climate change turns to favour mosquito development and survival (e.g., shortens generation time), early warning and monitoring systems remain essential in management of disease vector populations and associated risks.

In conclusion, this thesis reports substantial knowledge gaps on issues of mosquito bio-ecology and burden in the Central district, Botswana. First, this call for relevant stakeholders to strengthen public education across the country to combat impacts of mosquito burden. Given the first record of *Ae. aegypti* in the Central district and the country, there is need to extend the surveillance to other areas to inform vector borne disease epidemics. Second, it was showed that human activities may be influential in the proliferation of mosquito abundance. In particular, cattle rearing, and its potential for

aquatic degradation by dung nutrification is likely to enhance mosquito numbers. Therefore, informed communities and policy makers would put in place measures that mitigate against degradation of aquatic habitats thus reducing potential mosquito breeding hotspots. Third, a sustainable approach to vector mosquito management is worth investigating. The study has identified and quantified native predatory impacts towards the larval prey under artificial conditions. More work is required to determine if persistence and prevalence of these potential natural predators do, in fact, reduce the biting burden of mosquitoes. If the lab-based findings from this work are reflective of field conditions, there is a strong case for the incorporation of a conservation biocontrol approach in an integrated mosquito vector control strategy. This will complement the existing vector control initiatives targeting the adult vector mosquitoes in the country. Fourth, the increase in temperatures due to the current climate change are likely to negatively impact on the predators, giving the larval prey leverage to proliferate. Thus, insights from evolutionary physiology may be used to enhance predator fitness in increasingly warming aquatic habitats (see e.g., Sgrò *et al.*, 2010; Hoffmann and Sgrò 2011). Overall, the country is advised to effectively educate communities on mosquito ecology and parasite transmission dynamics while focusing on complementary and sustainable control of mosquitoes in aquatic ecosystems where breeding is mainly neglected. Fruitful areas of study would be (1) spatio-temporal species diversity and abundance of vector mosquitoes, (2) impact of livestock nutrification in aquatic ecosystems on a diversity of vector mosquito species and their native predators (3) effects of habitat temperature variation on predator-prey interaction outcomes and (4) investigating spatio-temporal patterns of insecticide susceptibility status of vector mosquitoes following the prolonged chemical intervention

strategy in the country. These results are essential in informing policy form the management of vector-borne infections. Furthermore, results may help in mechanistic models for mosquito-borne diseases' epidemiology (see e.g., Bartlow *et al.*, 2019; Tran *et al.*, 2020) and is significant for early warning systems on related vectors and infections.

References

- Almazroui, M., Saeed, F., Saeed, S., Islam, M. N., Ismail, M., Klutse, N. A. B., and Siddiqui, M. H. (2020). Projected change in temperature and precipitation over Africa from CMIP6. *Earth Systems and Environment*, 4: 455–475.
- Athrey, G., Cosme, L. V., Popkin-Hall, Z., Pathikonda, S., Takken, W., and Slotman, M. A. (2017). Chemosensory gene expression in olfactory organs of the anthropophilic *Anopheles coluzzii* and zoophilic *Anopheles quadriannulatus*. *BMC Genomics*, 18: 1-14.
- Bartlow, A. W., Manore, C., Xu, C., Kaufeld, K. A., Del Valle, S., Ziemann, A., and Fair, J. M. (2019). Forecasting zoonotic infectious disease response to climate change: mosquito vectors and a changing environment. *Veterinary Sciences*, 6: 40.
- Bestion, E., Soriano-Redondo, A., Cucherousset, J., Jacob, S., White, J., Zinger, L., and Cote, J. (2019). Altered trophic interactions in warming climates: consequences for predator diet breadth and fitness. *Proceedings of the Royal Society B*, 286: 20192227.
- Braack, L., de Almeida, A. P. G., Cornel, A. J., Swanepoel, R., and De Jager, C. (2018). Mosquito-borne arboviruses of African origin: review of key viruses and vectors. *Parasites and Vectors*, 11: 29.
- Brown, J. E., Evans, B. R., Zheng, W., Obas, V., Barrera-Martinez, L., Egizi, A., and Powell, J. R. (2014). Human impacts have shaped historical and recent evolution in *Aedes aegypti*, the dengue and yellow fever mosquito. *Evolution*, 68: 514-525.
- Brugman, V. A., Hernández-Triana, L. M., Medlock, J. M., Fooks, A. R., Carpenter, S., and Johnson, N. (2018). The role of *Culex pipiens* L. (Diptera: Culicidae) in virus

transmission in Europe. *International Journal of Environmental Research and Public Health*, 15: 389.

Brugueras, S., Martinez, B. F., de la Puente, J. M., Figuerola, J., Porro, T. M., Rius, C., and Gomez-Barroso, D. (2020). Environmental drivers, climate change and emergent diseases transmitted by mosquitoes and their vectors in southern Europe: a systematic review. *Environmental Research*, 191: 110038.

Brustolin, M., Talavera, S., Nuñez, A., Santamaría, C., Rivas, R., Pujol, N., and Busquets, N. (2017). Rift Valley fever virus and European mosquitoes: vector competence of *Culex pipiens* and *Stegomyia albopicta* (= *Aedes albopictus*). *Medical and Veterinary Entomology*, 31: 365-372.

Chihanga, S., Haque, U., Chanda, E., Mosweunyane, T., Moakofhi, K., Jibril, H. B., and Glass, G. E. (2016). Malaria elimination in Botswana, 2012–2014: achievements and challenges. *Parasites and Vectors*, 9: 99.

Daugaard, U., Petchey, O. L., and Pennekamp, F. (2019). Warming can destabilize predator–prey interactions by shifting the functional response from Type III to Type II. *Journal of Animal Ecology*, 88: 1575-1586.

Franklinos, L. H., Jones, K. E., Redding, D. W., and Abubakar, I. (2019). The effect of global change on mosquito-borne disease. *The Lancet Infectious Diseases*, 19: e302-e312.

Govere, J., Durrheim, D., la Grange, K., Mabuza, A., and Booman, M. (2000). Community knowledge and perceptions about malaria and practices influencing malaria control in Mpumalanga Province, South Africa. *South African Medical Journal*, 90: 611-618.

Hillbom, E. (2014). Cattle, diamonds and institutions: main drivers of Botswana's economic development, 1850 to present. *Journal of International Development*, 26: 155-176.

Hoffmann, A.A. and Sgrò, C.M. (2011). Climate change and evolutionary adaptation. *Nature*, 470: 479-485.

- Iwamura, T., Guzman-Holst, A., and Murray, K. A. (2020). Accelerating invasion potential of disease vector *Aedes aegypti* under climate change. *Nature Communications*, 11: 1-10.
- Lee, J. M., Wasserman, R. J., Gan, J. Y., Wilson, R. F., Rahman, S., and Yek, S. H. (2020). Human activities attract harmful mosquitoes in a tropical urban landscape. *EcoHealth*, 17: 52-63.
- Martínez-de la Puente, J., Ferraguti, M., Ruiz, S., Roiz, D., Soriguer, R. C., and Figuerola, J. (2016). *Culex pipiens* forms and urbanization: effects on blood feeding sources and transmission of avian Plasmodium. *Malaria Journal*, 15: 589.
- Mpofu, M., Becker, P., Mudambo, K., and Jager, C. (2016). Field effectiveness of microbial larvicides on mosquito larvae in malaria areas of Botswana and Zimbabwe. *Malaria Journal*, 15: 586.
- Mukherjee, S., and Blaustein, L. (2019). Effects of predator type and alternative prey on mosquito egg raft predation and destruction. *Hydrobiologia*, 846: 215-221.
- Obopile, M., Segoea, G., Waniwa, K., Ntebela, D. S., Moakofhi, K., Motlaleng, M., and Manzi, M. (2018). Did microbial larviciding contribute to a reduction in malaria cases in eastern Botswana in 2012–2013? *Public Health Action*, 8: S50-S54.
- Petrić, D., Zgomba, M., Bellini, R., and Becker, N. (2012). Surveillance of mosquito populations: a key element to understanding the spread of invasive vector species and vector-borne diseases in Europe. *Essays on Fundamental and Applied Environmental Topics*, 192: 224.
- Potter, A., Jardine, A., and Neville, P. J. (2016). A survey of knowledge, attitudes, and practices in relation to Mosquitoes and Mosquito-Borne disease in Western Australia. *Frontiers in Public Health*, 4: 32.
- Schrama, M., Hunting, E. R., Beechler, B. R., Guarido, M. M., Govender, D., Nijland, W., and Gorsich, E. E. (2020). Human practices promote presence and abundance of disease-transmitting mosquito species. *Scientific Reports*, 10: 1-6.

- Selolwane, O. (2001). The policy of fencing Botswana's communal rangelands. *Land Degradation and Sustainable Resource Management in sub-Saharan Africa*, 6: 94-108.
- Sgrò, C.M., Lowe, A.J., and Hoffmann, A.A. (2010). Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications*, 4: 326-337.
- Sinka, M. E. (2013). Global distribution of the dominant vector species of malaria. In *Anopheles mosquitoes-New insights into malaria vectors*. IntechOpen. p. 36. DOI: 10.5772/54163.
- Stone, C., and Gross, K. (2018). Evolution of host preference in anthropophilic mosquitoes. *Malaria Journal*, 17: 257.
- Tawe, L., Ramatho, P., Waniwa, K., Muthoga, C. W., Makate, N., Ntebela, D. S., and Paganotti, G. M. (2017). Preliminary survey on Anopheles species distribution in Botswana shows the presence of *Anopheles gambiae* and *Anopheles funestus* complexes. *Malaria Journal*, 16: 1-7.
- Temoso, O., Hadley, D., and Villano, R. (2015). Performance measurement of extensive beef cattle farms in Botswana. *Agrekon*, 54: 87-112.
- Tran, A., Mangeas, M., Demarchi, M., Roux, E., Degenne, P., Haramboure, M., and Dehecq, J. S. (2020). Complementarity of empirical and process-based approaches to modelling mosquito population dynamics with *Aedes albopictus* as an example—Application to the development of an operational mapping tool of vector populations. *PloS ONE*, 15: e0227407.
- Valela, I., and Muzenda, E. (2019). Design of a Biodigester to Treat Cow Dung in Botswana. In *2019 7th International Renewable and Sustainable Energy Conference*, IEEE, Agadir, Morocco, DOI: 10.1109/IRSEC48032.2019.9078244.
- Wasserman, R. J., Alexander, M. E., Weyl, O. L., Barrios-O'Neill, D., Froneman, P. W., and Dalu, T. (2016). Emergent effects of structural complexity and temperature on predator-prey interactions. *Ecosphere*, 7: e01239.

Wilke, A. B., Chase, C., Vasquez, C., Carvajal, A., Medina, J., Petrie, W. D., and Beier, J. C. (2019). Urbanization creates diverse aquatic habitats for immature mosquitoes in urban areas. *Scientific Reports*, 9: 1-11.

Appendix 1



S1: Mosquito Knowledge, Attitude and Practices by Communities in the Serowe, Palapye and Bobirwa Sub-districts, Botswana.

Household Questionnaire

GENERAL

Respondent name(s):	Surname: _____ First name: _____
Date :	_____ / _____ / _____
Interviewer Name:	_____ Interviewer ID _____
	Sub-district: _____
Location ID :	Village/ Town: _____
	GPS Coordinates _____

SECTION 1.0 DEMOGRAPHICS

1.1	What is your gender? 1= Male, 2= Female	
1.2	What is your age range? 1=18- 29 years, 2=30-39 years, 3=40- 49 years, 4= 50- 59 years, 5= ≥60 years	
1.3	What is your marital status? 1= Married, 2= Divorced, 3= Widowed, 4= single (never married), 5= Other (specify).....	
1.4	Are you disabled? 1= Yes, 2= No, 3= Prefer not to say	
1.5	Are you literate? 1= Yes, 2= No, 3= Prefer not to say	

1.6	What is your highest level of education? 1= None, 2= Primary, 3= JC, 4= Form 4-5 (senior), 4= vocational, 5= Tertiary, 6= Prefer not to say, 7= Other (Specify).....	
------------	---	--

1.7	What is your profession? 1= Farmer, 2= Self-employed, 3= Entrepreneur, 4= Employee, 5= Not working, 6= Other (Specify).....	
1.8	How do you access information about mosquitoes and mosquito-borne diseases? (May select more than one). 1= Audio/visual source (Radio/TV), 2=Health professionals, 3=Print media (newspapers and others), 4= Electronic sources (Cellphone, computers and others) 5= Friends/ family/ relatives, 6 = Own experience, 7 = Other (Specify).....	
1.9	How many of you are staying on the property? 1= 1-2, 2= 3-5, 3= 5-10, 4= >10	
1.10	Which of the following are found in your property? (May give more than one) 1= JoJo tank, 2= flowerpots, 3 = gutter, 4 = old tyres, 5= indoor containers, 6= None of the above, 7= Other artificial containers (Specify).....	
1.11	If your answer is 2 in 1.10, where are the flowerpots located? (If no, continue to 1.12) 1= In the house, 2= Outside in the veranda/ porch/ terrace/ balcony, 3= Outside around the house, 4= Outside distant from the house, Other (Specify).....	
1.12	Do you have a pit latrine in your yard? (If no, continue to 1.15) 1= Yes, 2= No	
1.13	If yes to 1.12, are there signs of mosquitoes in it? 1=Yes, 2=No, 3= Not sure, 4= Don't know	
1.14	Do you think it encourages mosquito breeding? 1=Yes, 2=No, 3= Not sure, 4= Don't know	
1.15	Is there a drainage system in your yard? (If no, continue to 1.18) 1= Yes, 2= No	
1.16	If yes to 1.15, do you think it has an effect on mosquito breeding? 1=Yes, 2=No, 3= Not sure, 4= Don't know	
1.17	Are there signs of mosquitoes in the drainage system? 1=Yes, 2=No, 3= Not sure, 4= Don't know, 5= Did not check	
1.18	Do you have stagnant waters in the yard, either 'natural'/artificial? (If your answer is 2,3 or 4, continue to 2.1) 1=Yes, 2=No, 3= Not sure, 4= Don't know	
1.19	If yes to 1.18, are there signs of mosquito activity? 1=Yes, 2=No, 3= Not sure, 4= Don't know	

SECTION 2.0 KNOWLEDGE

2.1	Do you know mosquitoes? 1= Yes, 2= No, 3= Not sure, 4= I don't know	
------------	---	--

2.2	How do you identify them? 1= Colour, 2= Size, 3= Sound, 4= body parts, 5= Taxonomy key, 6= Specify.....	
2.3	How many types of mosquitoes do you know? 1=0, 2=1, 3=2, 4=3, 5=4, 6= >5	
2.4	Can you identify the different types of adult mosquitoes? 1= Yes, 2= No, 3 =Not sure, 4= Don't know	
2.5	Have you seen an adult mosquito in the last 3 months? 1= Yes, 2= No, 3= Not sure	
2.6	If yes to 2.5, what is the colour of the mosquito(es) you have seen? 1= Light brownish, 2= black and white, 3= Not sure, 4= Other (Specify).....	
2.7	Do you know mosquitoes go through different developmental stages? 1= Yes, 2= No, 3= Not sure, 4= I don't know	
2.8	Have you ever seen the following developmental stages of mosquitoes apart from adults? (If no to all, continue to 2.10) 1. Eggs Yes.....No..... 2. Larvae Yes.....No..... 3. Pupae Yes.....No.....	
2.9	If yes to 2.8, where did you see them?	
2.10	Did you experience a mosquito bite in the last summer (October to March) in your village? 1= Yes, 2= No, 3= None, 4= Not sure, 5= Don't know	
2.11	If yes to 2.10, how many bites in a day? 1= Too many to count, 2= Many, 3= Moderate, 4= Few, 5= Very few	
2.12	Did you experience in your village, a mosquito bite in winter (April to July)? 1= Yes, 2= No, 3= None, 4= Not sure, 5= Don't know	
2.13	If yes to 2.12, how many bites in a day? 1= Too many to count, 2= Many, 3= Moderate, 4= Few, 5= Very few	
2.14	Where did you get bitten by the mosquito? 1= Indoors, 2= Outdoors, 3= Both indoors and outdoors, 4= I don't know	
2.15	What time of the day did you normally experience mosquito bites? 1= Morning, 2= Noon, 3= Afternoon, 4= Evening, 5= Night, 6= Other (Specify).....	
2.16	Which part of the body received lots of bites? 1= head, 2= neck, 3= chest, 4= back area, 5= arms, 6= legs, 7= Other (Specify)	
2.17	Do you think mosquitoes can transmit HIV during blood feed? 1= Yes, 2= No, 3= None, 4= Not sure, 5= Don't know	

2.18	What time of the year are mosquitoes abundant? (May choose more than one) 1= Winter, 2= Spring, 3= Summer, 4= Autumn, 5= All year round, 6= Not sure, 7= Other (Specify).....	
2.19	Do mosquitoes get plenty when it is hot or cold? 1= Hot, 2= Cold, 3= Both Hot and Cold, 4= Not sure, 5= Don't know	
2.20	In your opinion, what is the general trend of mosquito abundance over the past 10yrs? 1= increasing, 2= decreasing, 3= No change, 4= Don't know	
2.21	If your answer to 2.20 is 1, what do you think is the main contributing factor? 1= Temperature, 2= Precipitation, 3= hosts, 4= I don't know, 5= Other (Specify).....	
2.22	Which diseases do you think mosquitoes may spread?	
2.23	Do you know signs and symptoms of any diseases you mentioned? 1= Yes, 2= No, 3= None, 4= Not sure, 5= Don't know	
2.24	If yes in 2.23, state one disease and its signs and symptoms. Disease.....	Signs & symptoms
2.25	Do you know: a (i) Yellow fever? 1=Yes, 2=No, 3= Not sure, 4= Don't know..... a (ii) what vectors itand its parasite? b (i) Dengue fever? 1=Yes, 2= No, 3= Not sure, 4= Don't know..... b (ii) what vectors itand its parasite?..... c (i) human malaria? 1=Yes, 2= No, 3= Not sure, 4= Don't know..... c (ii) what vectors itand its parasite?..... c (iii) how is it diagnosed locally? d (i) Elephantiasis? 1=Yes, 2=No, 3= Not sure, 4= Don't know..... d (ii) what vectors itand its parasite? e (i) avian malaria? 1=Yes, 2= No, 3= Not sure, 4= Don't know..... e (ii) what vectors it?and its parasite?.....	
2.26	Have you ever suffered from a mosquito-borne disease? 1=Yes, 2=No, 3= Not sure, 4= Don't know	
2.27	Do you know anyone who suffered from a mosquito-borne disease? 1=Yes, 2=No, 3= Not sure, 4= Don't know	
2.28	If your answer to 2.26 and 2.27 is yes. Which diseases?	

2.29	Are there any new types of mosquito species in your area that were not here before? 1=Yes, 2=No, 3= Not sure, 4= Don't know	
2.30	Do you have cattle in your area/ village? 1=Yes, 2=No, 3= Not sure, 4= Don't know	
2.31	If yes to 2.30, are there reared in fenced farms or unfenced communal areas? 1= Fenced farms, 2=Unfenced communal areas, 3= Not sure, 4= Don't know	
2.32	As far as Cattle interact with the environment, do you think this may have an effect in mosquito numbers? 1=Yes, 2=No, 3= Not sure, 4= Don't know	
2.33	Do you think cattle dung (and other waste products) dropped in water bodies can be a good resource for breeding mosquitoes? 1=Yes, 2=No, 3= Not sure, 4= Don't know	
2.34	If water is contaminated with dung, what effect do you think it has on the numbers of breeding mosquitoes? 1= Increase, 2= Decrease, 3= Same, 4= Not sure, 5= I don't know	

SECTION 3.0 ATTITUDE

3.1	What do you think about the 'economic' significance of mosquitoes in your area? 1= No concern, 2= Pose a health risk, 3= Nuisance 4= Other (Specify)	
3.2	Could one mosquito bite be of any health risk? 1=Yes, 2=No, 3=Not sure, 4= Never	
3.3	After being bitten by a mosquito, what do you do? 1= Nothing, 2= consider protective measures, 3= wait for fever symptoms, 4= visit a clinic, 5= Other (Specify).....	
3.4	How can you rate your concern on a mosquito-borne infection? 1= Strongly concerned, 2= concerned, 3= Less concerned, 4= Not concerned	
3.5	What do you do to discourage mosquito breeding and resting habitats in your property? (May choose more than one) 1= Land clearing, 2=eliminating water holding containers, 3= Nothing, 4= Other (Specify).....	
3.6	What mosquito training do you need? 1= Biology, 2= Identification, 3= Ecology, 4= I do not want, 5= Other (Specify).....	

SECTION 4. PRACTICES

4.1	Which method do you use to protect against mosquitoes indoors? 1= Spray with insecticides, 2= Physical kill when spotted, 3= Use bed nets, 4= Electric fan, 5= Mosquito coil, 6= Burning cow dung, 7= Window gauze 8= Nothing, 9= Other (Specify).....	
4.2	If your answer to 4.1 is either 1, 2, 3, 4, 5, 6 or 7, which one(s) are most effective?	
4.3	How do you and your family protect yourselves from mosquito bites? 1= Stay indoors in the evening, 2= Wear long cloths/ sleeves, 3= Apply repellents, 4= Nothing, 5= Other (Specify).....	
4.4	If your answer to 4.3 is either 1, 2 or 3, which one (s) are most effective?	
4.5	Is the government spraying your houses to control mosquitoes? (If no, continue to 4.10) 1=Yes, 2= No, 3=Not sure, 4= I don't know	
4.6	If yes to 4.5, which chemicals are used for spraying?	
4.7	Do you think indoor residual spraying (IRS) is effective in controlling indoor resting mosquitoes? 1= Yes, 2=No, 3= Not sure, 4= I don't know	
4.8	After spraying, what happens to mosquito population densities indoors? 1= Increase, 2= Reduce, 3= Same, 4= Not sure, 5= I don't know.	
4.9	After spraying, what happens to mosquito biting rates? 1= Increase, 2= Reduce, 3= Same, 4= Not sure, 5= I don't know.	
4.10	Are there certain parts of the village where mosquitoes are more than others? 1=Yes, 2=No, 3=Not sure, 4= I don't know	
4.11	If yes to 4.10, where and why?	
4.12	Do you know of any animals/ organisms that eat adult mosquito? 1 = Yes, 2=No, 3 = Not sure	
4.13	If yes to 4.12, do you know which ones?.....	
4.14	Do you know any animals/ organisms that eat mosquito juveniles (egg-pupa)? 1= Yes, 2=No, 3 = Not sure	
4.15	If yes to 4.14, Which ones?	

4.16	Cross boarder tradings that traverse Serowe, Palapye and/or Bobirwa sub-districts have the risk of imported mosquito-borne infections. Do you agree with this notion? 1= Strongly agree, 2= Agree, 3= Disagree, 4= Strongly disagree, 5= Don't know	
4.17	Do you think cross-border screening of infectious diseases and foreign biological samples effectively carried out? 1=Yes, 2=No, 3= Sometimes, 4= Not sure, 5= Never, 6 Don't know	
4.18	Have you ever travelled to malaria endemic district/ sub-districts (Okavango, Ngamiland, Boteti, Chobe, Tutume, and Bobirwa? If no continue to 4.21. 1=Yes, 2=No, 3= I can't remember	
4.19	If yes to 4.18, how long was your stay? 1= less than one week, 2= up to two weeks, 3= more than two weeks	
4.20	Did you take any prescribed medication to these malaria endemic districts? 1=Yes, 2=No, 3= I can't remember	
4.21	Do you know any indigenous/ exotic plant that is used to repel mosquitoes? 1=Yes, 2=No, 3= Not sure, 4= Don't know	
4.22	If yes to 4.21, which one(s)?.....	
4.23	Do you know any indigenous/ exotic plant that treats mosquitoes-borne diseases? 1=Yes, 2=No, 3= Not sure, 4= Don't know	
4.24	If yes to 4.23, specify which.....	
4.25	Is there sufficient and available diagnosis and treatment for mosquito-borne diseases in your locality? 1=Yes, 2=No, 3= Not sure, 4= Don't know	
4.26	Is information sharing and awareness practices on mosquitoes and control measures sufficient in your area? 1=Yes, 2=No, 3= Not sure, 4= Don't know	
4.27	If no to 4.26, what can be done to better the situation? 1= Public education, 2= Community training, 3= Workshops, 4= Campaigns, 5= Not sure, 6= Other (Specify).....	

Appendix 2

Table S3. 1. Malaria cases and deaths between 2013 and 2018 transmission season for the Bobirwa, Palapye and Serowe sub-districts.

District	2013		2014		2015		2016		2017		2018		2019	
	Cases	Deaths												
Bobirwa	40	0	63	1	30	0	19	2	307	5	99	0	56	2
Palapye	10	1	56	3	14	0	15	0	85	2	61	1	32	0
Serowe	7	0	9	0	12	0	3	1	13	1	20	1	5	0

Table S3. 2. Mosquito sample collection site information for adult (homesteads) and the larvae (water sources) from Bobirwa, Palapye and Serowe sub-districts.

Adult collection			
District	Site Name	Site code	Coordinates
Bobirwa	Bobonong	1	S21°58'195, E28°25'426
Bobirwa	Gobojango	2	S21°49'717, E28°43'872
Bobirwa	Molalatau	3	S22°04'071, E28°35.907
Bobirwa	Mothabaneng	4	S22°10'712, E28°51.971
Bobirwa	Mabolwe	5	S21°49'081, E28°49'754
Bobirwa	Lepokole	6	S21°47'522, E28°20'705
Palapye	Palapye	7	S22°31'912, E27°08'310
Palapye	Topisi	8	S22°10'487, E27°15'021
Palapye	Radisele	9	S22°47'754, E27°00'262
Palapye	Ratholo	10	S22°43'246, E27°34'552
Palapye	Magapi	11	S22°19'458, E27°50'332
Palapye	Lerala	12	S22°47'199, E27°45'349
Serowe	Serowe	13	S22°22'945, E26°42'569
Serowe	Paje	14	S22°25'326, E26°79'029
Serowe	Mabeleapodi	15	S22°12'559, E26°50'038
Serowe	Tshimoyapula	16	S22°07'023, E26°55'419
Serowe	Mogorosi	17	S22°26'210, E26°33'464
Serowe	Motshegaletau	18	S22°34'752, E26°23'744

Larval collection		Habitat
-------------------	--	---------

Bobirwa	Motloutse river	1	Pool in drying stream	S21°57'457, E28°25'469
Bobirwa	Bobonong Bridge	2	Pool in drying stream	S21°58'288, E28°25'130
Bobirwa	Hogs Creek	3	Pool in drying stream	S22°34'793, E28°19'961
Palapye	Old Lecheng Road	4	Pool in drying stream	S22°32'596, E27°11'283
Palapye	Biust campus	5	Plastic containers	S22°35'467, E27°07'303
Palapye	Homestead	6	Plastic buckets	S22°32'976, E27°11'504
Serowe	Serowe river	7	Pool in drying stream	S22°25'117, E26°41'422
Serowe	Serowe river	8	Pool in drying stream	S22°24'435, E26°41'593
Serowe	Serowe river	9	Pool in drying stream	S22°24'543, E26°42'588