Aquatic Biodiversity and Mosquito Ecology in Urban Wetlands

Jayne K. Hanford

April 2020

School of Life and Environmental Sciences

The University of Sydney

A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

Declaration

This is to certify that the content of this thesis is my own work. This thesis has not been submitted for any other degree or diploma at any other university or institution. I consent to this thesis being made available for photocopying and loan under the appropriate Australian copyright laws.

Funding sources

This research was financially funded in part through grants from the Holsworth Wildlife Research Endowment (grant numbers HOLSW2016-R1-F113, 2017-R1, 2019-R2) and the Society of Wetlands Scientists Student Research Award (2016).

Jayne K. Hanford

Title page photos L-R: mosquito trap, J. Hanford; juvenile corixid, J. Hanford; macroinvertebrate sampling, C. Tyler.

Table of Contents

Author attributions

Chapter 2 of this thesis is "Hanford, J. K., Webb, C. E. & Hochuli, D. F. (2020) Integrating urban wetlands research across scales and study systems to improve environmental and human health outcomes."

All authors conceived the project. J. K. Hanford collected the data. All authors contributed to manuscript preparation.

Chapter 3 of this thesis is published as "Hanford, J. K., Webb, C.E. & Hochuli, D. F. (2019) Habitat traits associated with mosquito risk and aquatic diversity in urban wetlands. Wetlands, 39(4), 743-758."

All authors conceived the project. J. K Hanford and C. E. Webb collected and identified the specimens. All authors contributed to data analyses and manuscript preparation.

Chapter 4 of this thesis is "Hanford, J. K., Webb, C. E. & Hochuli, D. F. (2020) Human population density is linked to abundance of container-inhabiting mosquitoes at urban wetlands."

All authors conceived the project. J.K Hanford and C. E. Webb collected and identified the specimens. All authors contributed to data analyses and manuscript preparation.

Chapter 5 of this thesis has been accepted for publication as "Hanford, J. K., Hochuli, D. F. & Webb, C. E. (2020) Observations of an unexpected abundance of estuarine mosquitoes associated with an urban freshwater wetland. Australian Zoologist, In press https://doi.org/10.7882/AZ.2020.014."

J.K Hanford and C. E. Webb conceived the project. All authors contributed to manuscript preparation.

Chapter 6 of this thesis has been published as "Hanford, J. K., Webb, C. E. & Hochuli, D. F. (2020) Management of urban wetlands for conservation can reduce aquatic biodiversity and increase mosquito risk. Journal of Applied Ecology, 57(4), 794-805."

All authors conceived the project and collected the data. J. K Hanford and C. E. Webb identified the specimens. All authors contributed to data analyses and manuscript preparation.

Chapter 7 of this thesis has been published as "Hanford, J. K., Hochuli, D. F. & Webb, C. E. (2019) Oviposition behavior of *Culex annulirostris* (Diptera: Culicidae) is affected by the recent presence of invasive *Gambusia holbrooki* (Cyprinodontiformes: Poeciliidae). Journal of Medical Entomology, 56(4), 1165-1169."

J.K Hanford and C. E. Webb conceived the project and collected the data. All authors contributed to data analyses and manuscript preparation.

Acknowledgements

I feel very lucky to be at the end of my PhD journey and still love the prospect of visiting a wetland and checking out its smallest inhabitants. I'm sure maintaining your love of your study system over 5 years of PhD research is only possible with an amazing support network, and I am certainly very grateful to have had one of those.

Both of my supervisors – Dieter Hochuli and Cameron Webb – have been nothing but supportive, not only of my research, but also in maintaining a life outside study and looking towards the post-PhD future. Both have been willing to explore new areas of research and have continued to challenge me to extend myself and further my skill set, which no doubt made my PhD journey all the more worthwhile and rewarding. Asking questions I didn't want to hear (e.g. "Do you think you can fit in another round of fieldwork before the birth of your first child in 2 months?") was especially helpful and ultimately improved the quality of my research while always maintaining the fine balance between boundless research possibilities and avoiding unrealistic expectations. I'm also very grateful for your positivity and pep talks – no matter how well things are going on the research front, sometimes you just need some reassurance and I always felt I could turn to you when I needed to.

The Hochuli Lab Write Club have also been a great support. Manuel, Ryan K, Casey, Daniel, Caitlyn and Lucy have all read multiple draft manuscripts and their feedback has helped me improve my writing and publish many of the chapters in this thesis. Just as important have been coffee discussions with Ryan L, Jo and Lily to keep things in perspective, and Caragh and Lizzy to work out how to balance research and family life, which was a steep learning curve indeed!

Lastly, Christian went above and beyond in helping me complete my PhD. From field volunteer, to lunchbox packer, to sounding board, you always had my back, didn't begrudge a hangry outburst and took on more than half the childcare during fieldwork and intensive writing periods. Children might slow you down, but they don't hold you back – especially when you have an amazing significant other. And while it's inconclusive which was more challenging to produce over the past 5 years – child #1, child #2, or my PhD thesis – what is certain is all three would look very different without you.

Abstract

Global wetland loss means urban wetlands are an increasingly valuable conservation resource. Concerns around mosquito-borne diseases will restrict how we manage these wetlands for conservation, yet the impacts of common wetland management practices on aquatic biodiversity and mosquitoes are often untested, and our understanding of interactions between biodiversity, mosquitoes and wetland and landscape traits is severely limited. I used a combination of observational and landscape scale manipulative field experiments and laboratory experiments to characterise relationships between aquatic biodiversity, mosquitoes, wetland traits, surrounding human population density, invasive species and wetland management regimes. I found that aquatic biodiversity and abundance of mosquito species of pest and public health importance respond to wetland design traits and human population density, though biodiversity and mosquitoes are not necessarily responding to each other. Wetland management regimes and the presence of invasive species can significantly affect aquatic biodiversity and mosquito abundance, as well as mosquito behaviour. Direct and indirect relationships between mosquitoes and other aquatic biota appear to influence subsequent community recovery following such disturbances. Relationships altered due to wetland management regimes and the presence of invasive species in turn have the potential to shape mosquito risks associated with freshwater urban wetlands and influence the value of these wetlands to urban biodiversity. To maximise the ecological benefits of the growing number of freshwater urban wetlands without creating perverse impacts on public health and wellbeing associated with mosquito exposure requires not only improved integration between the traditionally separate fields of wetland ecology and medical entomology, but also consideration of species-specific responses and design traits to pre-emptively minimise potential risks.

Chapter 1:

General introduction

As the global population continues to grow and become increasingly urbanised, we see ongoing degradation of natural habitats, but also innovative conservation solutions and methods for integrating nature into our cities. Wetlands have been recognised as one of the most valuable ecosystems on the planet due to the many essential ecosystem services they provide, such as provision of food, water and habitat, waste treatment and moderation of disturbances (de Groot et al. 2012, Costanza et al. 2014). Yet wetlands are also among our most threatened ecosystems; available data conservatively suggests more than 70% of the world's wetlands have been lost since 1900 (Davidson 2014).

Wetlands in urban areas are often simultaneously a degraded version of their former selves and a refuge for wildlife struggling to survive in challenging urban ecosystems. Human-made wetlands are the only group of wetlands increasing in number globally (Davidson 2014). Used for wastewater treatment for centuries (Kadlec and Wallace 2009), the value of wetlands for cost-effective stormwater management and potential co-benefits has contributed to the increasing abundance of multi-purpose constructed wetlands in urban areas. Urban green and blue spaces, including wetlands, are recognised as essential to maintaining human emotional wellbeing and the liveability of cities (Carrus et al. 2015, Mekala et al. 2015, Taylor et al. 2018). Urban wetlands can also provide habitat to support the persistence of urban wildlife, including many threatened species around the world (Spinks et al. 2003, Darcovich and O'Meara 2008, Faller and McCleery 2017).

While urban wetlands provide habitat for a wide range of wildlife, mosquitoes associated with these wetlands can present challenges for local authorities. Being embedded within urban areas means that human exposure to mosquitoes, an intrinsic inhabitant of wetland ecosystems, is inevitable. Concerns regarding disease-causing pathogens vectored by mosquitoes are global and well-recognised (Russell 1999, Walton 2012, Medlock and Vaux 2015). Perhaps less widely recognised are the nuisance biting impacts of mosquitoes, which can also severely effect human wellbeing and outdoor quality of life (Dickinson and Paskewitz 2012, Darbro et al. 2017). This in turn can impact public support for urban wetlands, reducing the potential ecological benefits they provide.

Australia has a diverse range of mosquitoes, with more than 300 species known from the region (Webb et al. 2016). However, relatively few species are of significant pest or public health concern. The key mosquitoes, particularly in temperate regions of the country, that are known to be associated with urban freshwater wetlands, readily bite humans, and are implicated in the transmission of arboviruses, are *Coquillettidia linealis*, *Culex annulirostris*, *Culex quinquefasciatus*, and *Mansonia uniformis*. These four species are routinely reported as being present and occasionally abundant in urban wetlands and have either been the focus of vector competence experiments demonstrating the ability to transmit arbovirus of human health concern, or have had such pathogens identified from field collected specimens (Ryan and Kay 1999).

Mosquito-borne disease is a concern for health authorities across Australia. While potentially fatal illnesses caused by pathogens such as Murray Valley encephalitis virus are not a major threat to metropolitan regions (Knox et al. 2012), other arboviruses associated with potentially severe or debilitating illness, such as Ross River virus, Barmah Forest virus, and West Nile (Kunjin) virus have been reported from urban areas across the country (Doggett et al. 2009, Claflin and Webb 2015, Maute et al. 2019). As these arboviruses all rely on a vertebrate reservoir host, including domestic and introduced species, to drive disease epidemics (Flies et al. 2018, Stephenson et al. 2019), understanding the interaction between mosquitoes, wetlands, and wildlife is critical to assessing public health risks and the development of appropriate responses.

While management goals for urban wetlands frequently include water quality targets (Metzger 2005), habitat for wildlife and public amenity (Zedler and Leach 1998, O'Meara and

Darcovich 2015), mosquito management is rarely considered until problems arise. Reactive mosquito management generally involves the use of pesticides which are expensive and can be detrimental to aquatic food webs and primary productivity (Duguma et al. 2015, Allgeier et al. 2019). While there are ecologically sustainable mosquito control agents (Russell and Kay 2008) that can be integrated into overall wetland management strategies, understanding links between modified urban environments, such as wetlands, and mosquitoes of pest and public health significance will facilitate proactive management to avoid mosquito problems and enhance wetland conservation in urban areas.

Aquatic macroinvertebrates are an excellent indicator of aquatic health and capacity for aquatic systems, including urban systems, to support other wildlife such as birds, amphibians and fish (Batzer and Wissinger 1996, Chessman et al. 2002, Chessman and McEvoy 2012). Aquatic macroinvertebrates are central to the functioning of aquatic ecosystems due to their position as the primary trophic link between plant productivity and higher animals, and typically constitute a large component of aquatic biodiversity (Faith and Norris 1989, Rosenberg and Resh 1993, Wellborn et al. 1996). Their abundance, diversity and ability to reflect longer-term ecological condition, providing an integrated perspective of aquatic health, underpins their widespread use as a measure of aquatic biodiversity, including in several research chapters in this thesis.

Thesis scope and aims

In this thesis I explore relationships between the aquatic biodiversity of freshwater urban wetlands, and the abundance and behaviour of mosquitoes of pest and public health significance. I use a combination of field surveys, landscape scale manipulative field experiments and laboratory experiments to examine these relationships at multiple spatial scales. My research outputs are intended to provide information relevant to practitioners and researchers in the fields of wetland ecology and management, urban planning, and medical entomology.

Cross-disciplinary wetland and mosquito research

It is widely recognised that collaborations across traditional research fields are essential if we are to solve the large scale environmental and social challenges associated with global urbanisation (Bugnot et al. 2019). In Chapter 2, through an extensive review of current literature on mosquitoes and wetland ecology, I show that even though mosquitoes are intrinsic inhabitants of urban wetland ecosystems, research on mosquitoes and urban wetland ecology rarely intersects. This lack of research integration between one of the world's most valuable ecosystems and most significant threats to human health has substantial economic, biodiversity and epidemiological implications. I identify barriers and opportunities for improved integration across the wetland and mosquito research communities. Chapter 2 is placed in this thesis in lieu of a traditional literature review, given that the introductory sections of each research chapter present relevant key literature and recent research underpinning the individual research projects.

Common assumptions that wetlands are sources of pest mosquitoes (Russell 1999) mean public health and conservation priorities are frequently deemed incompatible (Dale and Knight 2008). In Chapter 3 I demonstrate that conservation and public health priorities are not necessarily incompatible. My large-scale survey of wetlands across the greater Sydney region suggested wetlands with higher aquatic biodiversity did not necessarily have increased abundances of mosquitoes of pest and public health significance. While aquatic macroinvertebrates and some mosquito species showed relationships with particular wetland habitat traits, it appeared there were no direct relationships between aquatic biodiversity and mosquito abundance, and that it may be possible to design wetlands such that traits associated with geographically relevant problematic mosquito risks are reduced without negatively affecting other wetland values. Furthermore, landscape scale traits, such as land use and the human population density surrounding a wetland, may have a relatively more important role in shaping urban mosquito risks compared to site scale variables. In Chapter 4 I show that the abundance of container-inhabiting mosquitoes captured at freshwater urban wetlands is strongly linked to surrounding human population density, demonstrating the need to consider habitat availability at the landscape scale when mitigating site scale mosquito problems.

Managing native and invasive species at landscape scales in urban mosaics

At the landscape scale, natural environments can be impacted through species 'spillover' from surrounding managed environments (Blitzer et al. 2012). A similar effect may occur between different landscapes within the urban mosaic (Claflin and Webb 2017), presenting a challenge to urban planners and environmental managers operating at the site scale. In Chapter 5 I present the first published evidence of an estuarine mosquito of pest and public health significance, *Aedes vigilax*, successfully completing development in urban freshwater wetlands. Use of freshwater wetlands by this estuarine species has implications for our understanding of wetland and mosquito ecology and wetland management.

Invasive species can also present management conundrums for urban wetlands. The removal of invasive species with the goal of benefiting native species has been shown to occasionally have unintended, indirect and undesirable consequences in terrestrial ecosystems (Bergstrom et al. 2009). In Chapter 6 I show how management of urban wetlands for conservation can also have unintended outcomes. I conducted a landscape scale manipulative field experiment to investigate the impacts of short-term wetland draining on aquatic biodiversity and mosquito communities (Hanford et al. 2020). A group of freshwater wetlands are drained each year to reduce or temporarily eradicate *Gambusia holbrooki* and provide more favourable breeding conditions for the endangered green and golden bell frog, *Litoria aurea*, though such a management regime is also applicable in routine urban wetland maintenance activities. I found that this short-term wetland draining regime had a significant impact on both mosquito abundance and aquatic biodiversity, and some of these changes were not mitigated over time. This has implications for temporal and spatial plans of management for urban wetlands, both for conservation and other purposes.

Furthermore, invasive species can significantly alter innate behaviours of native species, including mosquitoes of pest and public health significance. In Chapter 7 I show that the ubiquitous invasive fish *G. holbrooki*, known locally as the plague minnow, significantly affects oviposition behaviour of a mosquito species of pest and public health concern, *Culex annulirostris*. The presence of *G. holbrooki* can have devastating consequences for native amphibians (Hamer et al. 2002) and fish (Pyke 2008), and though it is widespread globally, in certain situations it may therefore be preferable to undertake control actions to ensure the persistence of native species. However, I show that given the interactions between *G. holbrooki* and *Cx. annulirostris*, management may have unintended consequences for potential mosquito risks, which is an especially important consideration in our expanding urban areas.

Thesis conclusion

In Chapter 8 I provide a general discussion integrating the results across my data chapters and identify potential directions for future research that would further the development of best practice management for urban wetlands and mosquitoes. The outcomes of my research have identified some key pathways of further research that will be critical for developing strategic management of urban wetlands in a future influenced by climate change and ever-increasing urbanisation.

A note on redundancy and authorship

This thesis is written as a series of manuscripts for publication. These manuscripts have either been published or have been submitted to a scholarly journal and are currently under review. Consequently, there is some redundancy in the introductions and discussions of individual chapters given the common objectives of this research. There is also some variation in use of Australian and US spelling and use of species authorities, reflecting the requirements of the relevant scholarly journal. At the beginning of each chapter I have provided details of the publication where appropriate.

References

Allgeier, S., A. Friedrich, and C. A. Brühl. 2019. Mosquito control based on *Bacillus thuringiensis israelensis* (Bti) interrupts artificial wetland food chains. Science of the Total Environment **686**:1173-1184.

Batzer, D. P., and S. A. Wissinger. 1996. Ecology of insect communities in nontidal wetlands. Annual Review of Entomology **41**:75-100.

Bergstrom, D. M., A. Lucieer, K. Kiefer, J. Wasley, L. Belbin, T. K. Pedersen, and S. L. Chown. 2009. Indirect effects of invasive species removal devastate World Heritage Island. Journal of Applied Ecology **46**:73-81.

Blitzer, E. J., C. F. Dormann, A. Holzschuh, A.-M. Klein, T. A. Rand, and T. Tscharntke. 2012. Spillover of functionally important organisms between managed and natural habitats. Agriculture, Ecosystems & Environment **146**:34-43.

Bugnot, A. B., G. C. Hose, C. J. Walsh, O. Floerl, K. French, K. A. Dafforn, J. Hanford, E. C. Lowe, and A. K. Hahs. 2019. Urban impacts across realms: Making the case for inter-realm monitoring and management. Science of the Total Environment **648**:711-719.

Carrus, G., M. Scopelliti, R. Lafortezza, G. Colangelo, F. Ferrini, F. Salbitano, M. Agrimi, L. Portoghesi, P. Semenzato, and G. Sanesi. 2015. Go greener, feel better? The positive effects of biodiversity on the well-being of individuals visiting urban and peri-urban green areas. Landscape and Urban Planning **134**:221-228.

Chessman, B. C., and P. K. McEvoy. 2012. Insights into human impacts on streams from tolerance profiles of macroinvertebrate assemblages. Water Air and Soil Pollution **223**:1343-1352.

Chessman, B. C., K. M. Trayler, and J. A. Davis. 2002. Family- and species-level biotic indices for macroinvertebrates of wetlands on the Swan Coastal Plain, Western Australia. Marine and Freshwater Research **53**:919-930.

Claflin, S. B., and C. E. Webb. 2015. Ross River virus: Many vectors and unusual hosts make for an unpredictable pathogen. PLoS Pathogens **11**:e1005070.

Claflin, S. B., and C. E. Webb. 2017. Surrounding land use significantly influences adult mosquito abundance and species richness in urban mangroves. Wetlands Ecology and Management **25**:331-344.

Costanza, R., R. de Groot, P. Sutton, S. van der Ploeg, S. J. Anderson, I. Kubiszewski, S. Farber, and R. K. Turner. 2014. Changes in the global value of ecosystem services. Global Environmental Change **26**:152-158.

Dale, P. E. R., and J. M. Knight. 2008. Wetlands and mosquitoes: A review. Wetlands Ecology and Management **16**:255-276.

Darbro, J., Y. Halasa, B. Montgomery, M. Muller, D. Shepard, G. Devine, and P. Mwebaze. 2017. An economic analysis of the threats posed by the establishment of *Aedes albopictus* in Brisbane, Queensland. Ecological Economics **142**:203-213.

Darcovich, K., and J. O'Meara. 2008. An olympic legacy: Green and Golden Bell Frog conservation at Sydney Olympic Park 1993-2006. Australian Zoologist **34**:236-248.

Davidson, N. C. 2014. How much wetland has the world lost? Long-term and recent trends in global wetland area. Marine and Freshwater Research **65**:934-941.

de Groot, R., L. Brander, S. van der Ploeg, R. Costanza, F. Bernard, L. Braat, M. Christie, N. Crossman, A. Ghermandi, L. Hein, S. Hussain, P. Kumar, A. McVittie, R. Portela, L. C. Rodriguez, P. ten Brink, and P. van Beukeringh. 2012. Global estimates of the value of ecosystems and their services in monetary units. Ecosystem Services **1**:50-61.

Dickinson, K., and S. Paskewitz. 2012. Willingness to pay for mosquito control: How important is West Nile virus risk compared to the nuisance of mosquitoes? Vector-Borne and Zoonotic Diseases **12**:886-892.

Doggett, S. L., J. Clancy, J. Haniotis, C. E. Webb, L. Hueston, M. Marchetti, E. Blyth, S. Howard, D. E. Dwyer, and R. C. Russell. 2009. Arbovirus and vector surveillance in NSW, 2004/05-2007-08. Arbovirus Research in Australia **10**:28-37.

Duguma, D., M. W. Hall, P. Rugman-Jones, R. Stouthamer, J. D. Neufeld, and W. E. Walton. 2015. Microbial communities and nutrient dynamics in experimental microcosms are altered after the application of a high dose of Bti. Journal of Applied Ecology **52**:763-773.

Faith, D. P., and R. H. Norris. 1989. Correlation of environmental variables with patterns of distribution and abundance of common and rare freshwater macroinvertebrates. Biological Conservation **50**:77-98.

Faller, C. R., and R. A. McCleery. 2017. Urban land cover decreases the occurrence of a wetland endemic mammal and its associated vegetation. Urban Ecosystems **20**:573-580.

Flies, E. J., C. L. Lau, S. Carver, and P. Weinstein. 2018. Another emerging mosquito-borne disease? Endemic Ross River virus transmission in the absence of marsupial reservoirs. Bioscience **68**:288-293.

Hamer, A. J., S. J. Lane, and M. J. Mahony. 2002. The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia. Oecologia **132**:445-452.

Hanford, J. K., C. E. Webb, and D. F. Hochuli. 2020. Management of urban wetlands for conservation can reduce aquatic biodiversity and increase mosquito risk. Journal of Applied Ecology **57**:794-805.

Kadlec, R. H., and S. D. Wallace. 2009. Treatment Wetlands. Second edition edition. CRC Press, Taylor & Francis Group, Boca Raton, Florida.

Knox, J., R. Cowan, J. Doyle, M. Ligtermoet, J. Archer, J. Burrow, S. Tong, B. Currie, J. Mackenzie, D. Smith, and M. Catton. 2012. Murray Valley encephalitis: A review of clinical features, diagnosis and treatment. Medical Journal of Australia **196**:322-326.

Maute, K., C. Webb, D. Phalen, J. Martin, J. Hobson-Peters, R. E. Major, and K. French. 2019. Clean bill of health? Towards an understanding of health risks posed by urban ibis. Journal of Urban Ecology **5**:p.juz006.

Medlock, J. M., and A. G. Vaux. 2015. Impacts of the creation, expansion and management of English wetlands on mosquito presence and abundance - Developing strategies for future disease mitigation. Parasites & vectors **8**.

Mekala, G., R. Jones, and D. MacDonald. 2015. Valuing the Benefits of Creek Rehabilitation: Building a Business Case for Public Investments in Urban Green Infrastructure. Environmental Management:1-12.

Metzger, M. E. 2005. Managing mosquitoes in stormwater treatment devices. Pages 1-13 *in* American Society of Civil Engineers, editor. World Water and Environmental Resources Congress 2005. Impacts of Global Climate Change, Anchorage, Alaska, United States.

O'Meara, J., and K. Darcovich. 2015. Twelve years on: Ecological restoration and rehabilitation at Sydney Olympic Park. Ecological Management & Restoration **16**:14-28.

Pyke, G. H. 2008. Plague minnow or mosquito fish? A review of the biology and impacts of introduced *Gambusia* species. Annual Review of Ecology, Evolution, and Systematics **39**:171-191.

Rosenberg, D. M., and V. H. Resh. 1993. Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall, New York.

Russell, R. C. 1999. Constructed wetlands and mosquitoes: Health hazards and management options—an Australian perspective. Ecological Engineering **12**:107-124.

Russell, T. L., and B. H. Kay. 2008. Biologically based insecticides for the control of immature Australian mosquitoes: A review. Australian Journal of Entomology **47**:232-242.

Ryan, P. A., and B. H. Kay. 1999. Vector competence of mosquitoes (Diptera: Culicidae) from Maroochy Shire, Australia, for Barmah Forest virus. Journal of Medical Entomology **36**:856-860.

Spinks, P. Q., G. B. Pauly, J. J. Crayon, and H. Bradley Shaffer. 2003. Survival of the western pond turtle (*Emys marmorata*) in an urban California environment. Biological Conservation **113**:257-267.

Stephenson, E. B., A. K. Murphy, C. C. Jansen, A. J. Peel, and H. McCallum. 2019. Interpreting mosquito feeding patterns in Australia through an ecological lens: An analysis of blood meal studies. Parasites & Vectors **12**:156.

Taylor, L., A. K. Hahs, and D. F. Hochuli. 2018. Wellbeing and urban living: Nurtured by nature. Urban Ecosystems **21**:197-208.

Walton, W. E. 2012. Design and management of free water surface constructed wetlands to minimize mosquito production. Wetlands Ecology and Management **20**:22.

Webb, C., S. Doggett, and R. Russell. 2016. A Guide to Mosquitoes of Australia. CSIRO Publishing, Clayton South, Victoria.

Wellborn, G. A., D. K. Skelly, and E. E. Werner. 1996. Mechanisms creating community structure across a freshwater habitat gradient. Annual Review of Ecology and Systematics **27**:337-363.

Zedler, J. B., and M. K. Leach. 1998. Managing urban wetlands for multiple use: Research, restoration, and recreation. Urban Ecosystems **2**:189-204.

Chapter 2:

Integrating urban wetland and mosquito research to link environmental and human health

Abstract

Solving large-scale environmental and social challenges associated with urbanization requires collaborations across traditional research fields. Mosquitoes and urban wetlands, though often studied by different research communities, regularly share physical space, yet these areas of research rarely intersect. Wetlands constructed in urban areas continue to increase in number. Given concerns about mosquito-borne diseases, understanding interactions between these valuable habitats and significant threats to human health is essential for both conservation of urban wildlife and maintenance of human wellbeing. We reviewed the urban wetland, urban mosquito and interdisciplinary literature to identify barriers and opportunities for integrating across these fields. There is a critical gap in interdisciplinary research, exacerbated by the inaccessibility of discipline-specific journals. We identified numerous economic, biodiversity and epidemiological benefits to be gained by integrating these sections of the scientific community, allowing each to broaden their research impact and improve environmental and public health outcomes.

In a nutshell

- Wetlands are among our most valuable ecosystems, while mosquitoes, a natural component of wetlands, are among our most significant threats to human health and wellbeing globally.
- Wetland and mosquito research, despite frequently representing different scales of the same physical habitat, rarely intersects. Separation of these fields can result in poorly designed and managed urban wetlands that either suppress mosquitoes but do not support urban biodiversity, or provide habitat for urban wildlife while also proliferating mosquitoes. This lack of integration limits our understanding and management of mosquito-borne pathogens and associated diseases, and results in substantial economic and biodiversity costs.
- We reviewed available literature on urban wetlands and urban mosquitoes and identified more than 7000 published articles in the ten years from 2009-2018, yet only 76 of these considered both wetlands and mosquitoes.
- There are many benefits to be gained scientifically, economically and ecologically through improved integration between wetland and mosquito research. We highlight why interdisciplinary wetland and mosquito research is necessary and suggest actions to facilitate improved integration across these different research fields based within a common habitat.

Understanding the ecology of urban wetlands requires an integrated and interdisciplinary approach to reconciling the utility and biodiversity values of wetlands, such as their contribution to improved water quality and conservation, with human needs and perceptions. One theme frequently missing from urban wetland research is mosquito ecology and associated risks to human health and wellbeing, making it difficult to identify potential trade-offs and synergies.

Wetlands provide some of our most valuable ecosystem services (Costanza et al. 2014). Urban wetlands can simultaneously manage stormwater, which is critical to maintaining the quality of urban ecosystems, while also reducing flooding, increasing groundwater recharge, stabilizing soil, and providing recreational and biodiversity services. But mosquitoes can also provide ecosystem disservices. While they represent a small component of wetland ecosystems (Figure 2.1), they can pose costly threats to human health and wellbeing due to their transmission of disease-causing pathogens and nuisance-biting impacts (Walton 2000).

Despite common assumptions that wetlands are sources of pest mosquitoes (Russell 1999), mosquito and wetland research rarely intersects, and public health and conservation priorities are frequently deemed incompatible (Dale and Knight 2008). Our lack of understanding of interactions between one of our most valuable ecosystems and one of our most significant vectors of human disease-causing pathogens is a major challenge to the development of best practice wetland design and management for urban areas. Consequently, these gaps in our understanding create significant opportunity costs for urban biodiversity.

While there is little doubt that urban wetlands can provide habitat for mosquito species of pest and public health concern, the risks can vary greatly between wetlands. The abundance and diversity of mosquitoes can drive increased risk of mosquito-borne disease, especially in areas where vertebrate wildlife provide suitable reservoirs for zoonotic pathogens (Walton 2000). For example, mosquito species associated with constructed wetlands are known vectors of Ross River virus (Claflin and Webb 2015). Yet there is also evidence that the

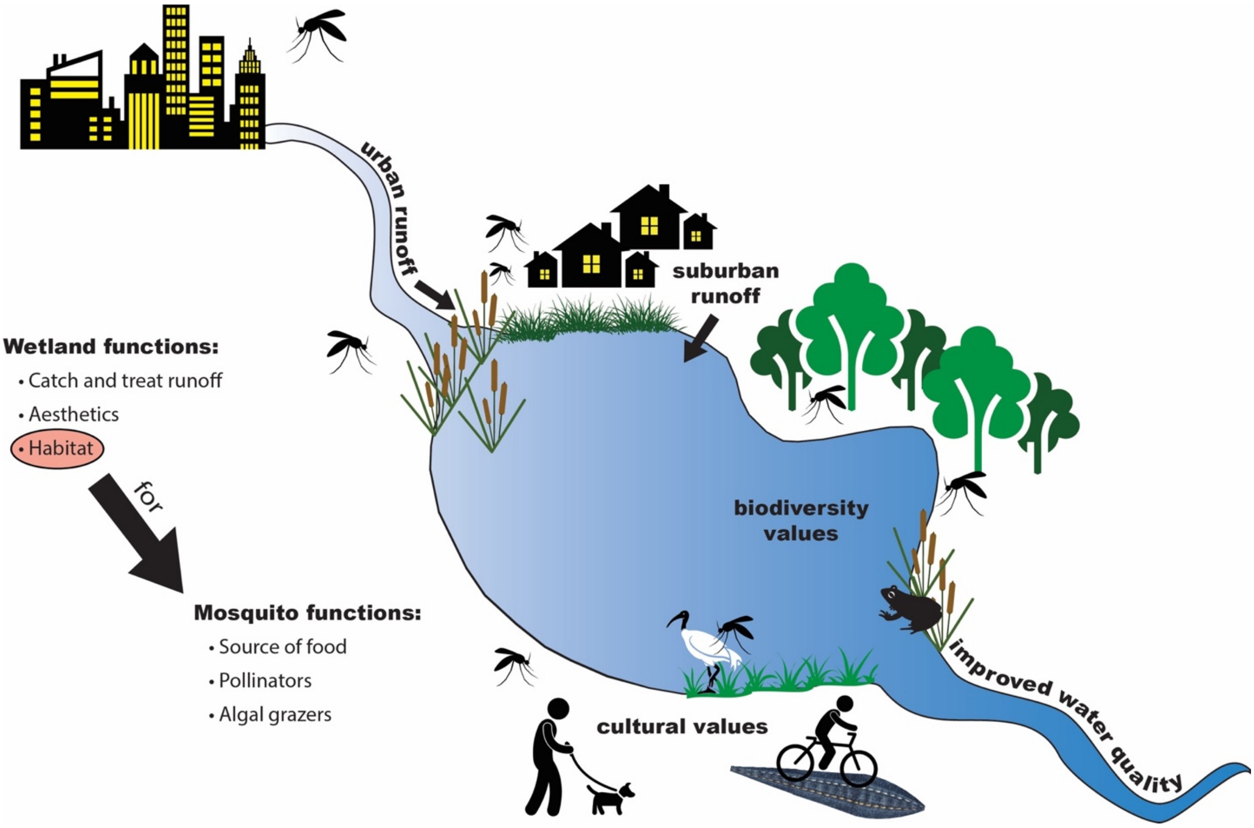


Figure 2.1. Even though functions provided by mosquitoes sit within urban wetland functions, there is very little overlap in research from these two fields, but great potential for each to inform the other.

presence of wetlands may reduce the risks of some mosquito-borne diseases. The prevalence of mosquito-borne West Nile virus can be lower around urban wetlands compared to adjacent urban residential areas (Johnson et al. 2012), and dengue occurrence can be lower in neighbourhoods with greater wetland cover (de Jesús Crespo et al. 2018). Public fear and dislike of mosquitoes often overshadows these findings and leads to design and management of urban wetlands that not only reduces mosquito risks, but also minimizes their biodiversity value.

Epidemiology of mosquito-borne pathogens also suffers from this ill-considered view of wetlands, with increased reliance on reactive rather than proactive mosquito management. A lack of understanding or consideration of interactions between key pest and vector species and environmental drivers of abundance is a barrier to proactive mosquito management. In

Australia, pre-emptive control across a range of wetland habitats is required to reduce the risks of mosquito-borne disease (Tomerini et al. 2011) but often control can be *ad hoc*, driven by issues such as the community's perception of nuisance. This approach necessitates expensive applications of pesticides which may have direct or indirect detrimental impacts to aquatic ecosystems (Duguma et al. 2015, Allgeier et al. 2019).

Why are urban wetlands growing in number?

Human survival is fundamentally linked to nature through our dependence on ecosystem services for our health and livelihoods (Costanza et al. 2014) and intrinsic need for contact with natural environments for our mental wellbeing (Taylor et al. 2018). Urban wetlands are also a cost-effective alternative to conventional wastewater treatment (Kadlec and Wallace 2009), making them an attractive, multi-benefit investment. There is a clear recognition that water management in urban environments is critical in the response to environmental and human health threats posed by a changing climate and continued urbanization. Wetlands have been used as wastewater discharge sites for as long as sewage has been collected (Kadlec and Wallace 2009). Once considered to be of minor value, wetlands are now accepted as being critically important, and in many countries, constructed wetlands are considered best management practice for urban stormwater and have become prominent features of new developments in cities (Kadlec and Wallace 2009).

Stormwater management priorities focus on public health and safety via flood mitigation and reduction of waterborne pathogens (Metzger 2005). However, stormwater best management practice frequently overlooks mosquito risks that may increase as an unintended consequence of wetland construction (Metzger 2005, National Research Council 2009). Furthermore, although the public generally 'like' wetlands in urban areas, perceptions of what is a 'good' wetland vary and are often not ecologically sound (Dobbie and Green 2013, Scholte et al. 2016), though public perceptions continue to influence design of, and public support for, these habitats in communities. While mosquitoes may be of concern, they are rarely considered in urban wetland design, and there is limited information available on mosquitoes associated with constructed urban wetlands. This leads to wetlands being designed to reduce ‘general’ mosquito risks, for example by removing aquatic vegetation and including features such as hard vertical edges (Russell and Kuginis 1998) that are also unfavorable to animals such as birds, frogs and fish, reducing the wetland’s potential to benefit local biodiversity while also potentially reducing the capacity for water treatment.

Addressing the large scale environmental and social challenges presented by urbanization requires problem-solving across traditional research systems (Bugnot et al. 2019). We show that integrating our knowledge of mosquitoes with our understanding of urban wetland design and management offers substantial potential benefits. These opportunities operate at multiple scales, from individuals to societies and ecosystems. We offer suggestions to build bridges between these linked fields and facilitate cross-disciplinary collaboration, with the objective of improving translation of research into practice.

Intersections between urban wetlands and mosquitoes

To identify areas where urban wetland and mosquito research currently intersects, and future opportunities and co-benefits of collaboration between these fields, we searched all peer-reviewed literature available in Scopus that was published in the 10 years between 2009 and 2018. We searched titles, abstracts and key words using the search strings (wetland* OR swamp* OR pond*) AND (*urban OR urban*) for urban wetland research; (mosquito*) AND (*urban OR urban*) for urban mosquito research; and (mosquito*) AND (wetland* OR swamp* OR pond*) AND (*urban OR urban*) for interdisciplinary mosquitoes and urban wetlands research. This search strategy was intended to target research undertaken in urban areas.

We reviewed each abstract returned in the cross-discipline ‘mosquitoes and urban wetlands’ search to determine the main research themes, and if research outputs inform our understanding of links between mosquitoes and urban wetlands. During screening we recorded the primary study question, disease focus (if any), study landscape, habitat, and the continent of any field work. We also noted publications returned that were focused on non-wetland inhabiting mosquito species, modelling or laboratory-only studies without field applicability, reported routine surveillance results with no ecological interpretation or analyses, taxonomic papers and those that were not about mosquitoes (mosquitoes were used in the study as a food source for example). If it was not possible to determine the main themes and research question from the abstract, the paper in full was reviewed. One person (JKH) screened all papers to ensure consistency. Abstracts that were returned from the broad urban mosquitoes and urban wetlands searches were not screened in detail, instead data on the journal of publication and country of research were used to identify general trends.

Our searches returned 5,117 publications on urban wetlands, 1,952 publications on urban mosquitoes, and 76 publications on the interdisciplinary study of mosquitoes and urban wetlands.

Urban wetland research identified in our searches was predominantly published in systems-focused journals (eg *Ecological Engineering*, *Science of the Total Environment*, *Wetlands*), while research on urban mosquitoes was overwhelmingly published in discipline-specific journals (eg *Parasites and Vectors*, *Malaria Journal*, *Plos Neglected Tropical Diseases*).

Research on mosquitoes in urban wetlands was also typically published in discipline-specific mosquito journals (*Parasites and Vectors*, *Journal of the American Mosquito Control Association*, *Malaria Journal*). More than half (53%) of research on mosquitoes in urban wetlands was focused on mosquito-borne pathogens and the diseases they cause, such as West Nile virus (13% of references) dengue virus (7% of references), and ongoing research into malaria (18% of references). The majority of cross-disciplinary papers were describing seasonal mosquito dynamics as they related to wetlands compared to other nearby habitats (38% of references), but only 11% linked mosquitoes to habitat traits (Figure 2.2).

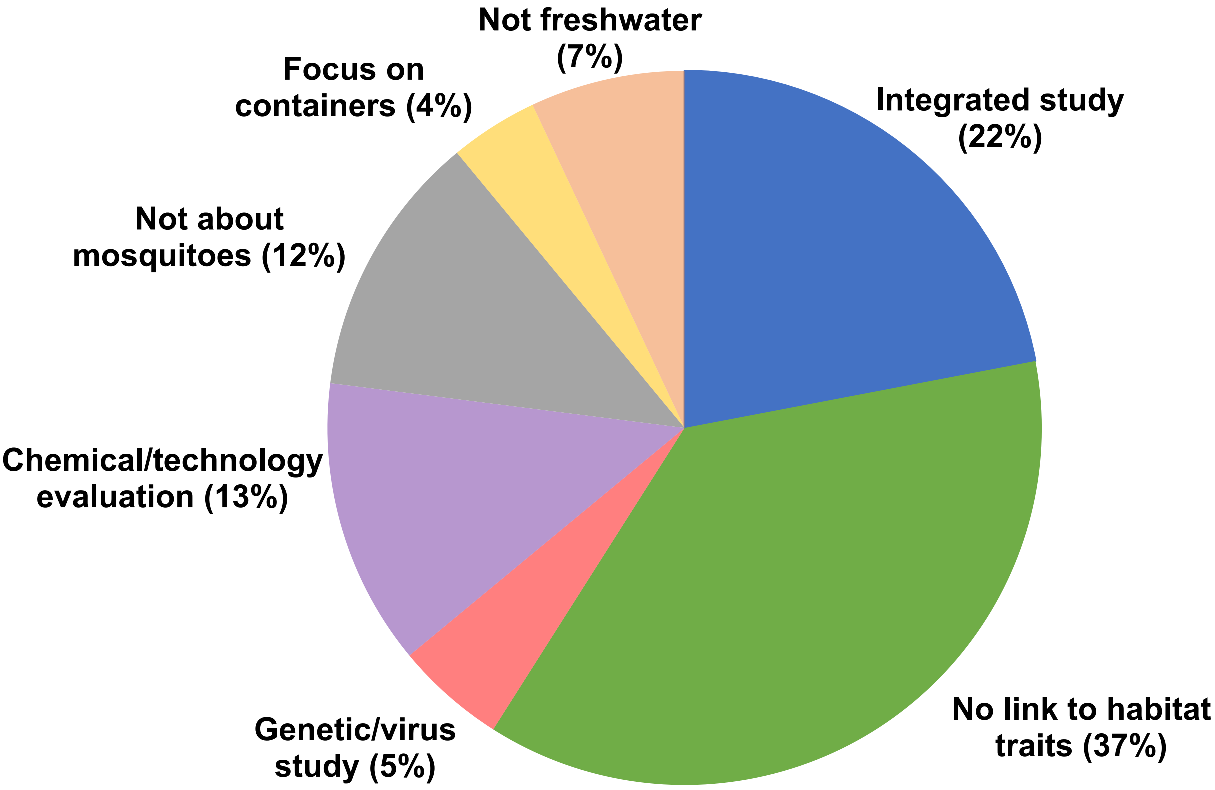


Figure 2.2. Of the 76 publications returned in our cross-disciplinary mosquitoes and urban wetlands literature search, many had little relevance to researchers and practitioners seeking to balance multiple competing priorities of designing and managing urban wetlands. Research was often returned due to mosquitoes being used as a food source (not about mosquitoes category) or being focused on container-inhabiting and saltmarsh species and habitats (focus on containers and not freshwater categories), or evaluating new pesticides and mapping technology.

There were strong geographical differences in the countries producing wetland research, and countries looking at relationships between mosquitoes and wetlands based on location of first authors (Figure 2.3). China was a strong publisher of general wetland research (19% of urban wetland research was undertaken in China, second only to the US with 27% of urban wetland research), but was not among the top 10 countries for number of publications on mosquitoes and wetlands research. This could potentially indicate future mosquito risks emerging in China, where severe diseases such as Japanese encephalitis are endemic, if they do not consider this important aspect of wetland design and management. The geographical imbalance of mosquitoes and wetlands research, which was concentrated in the global north and poorly represented in Africa, South America and Asia (Figure 2.3) is representative of research more generally (Di Marco et al. 2017). This presents another challenge to both conservation and epidemiology due to the mismatch between the location of research and locations of urban expansion, which is growing at a comparably faster rate in these underrepresented regions (Seto et al. 2012).

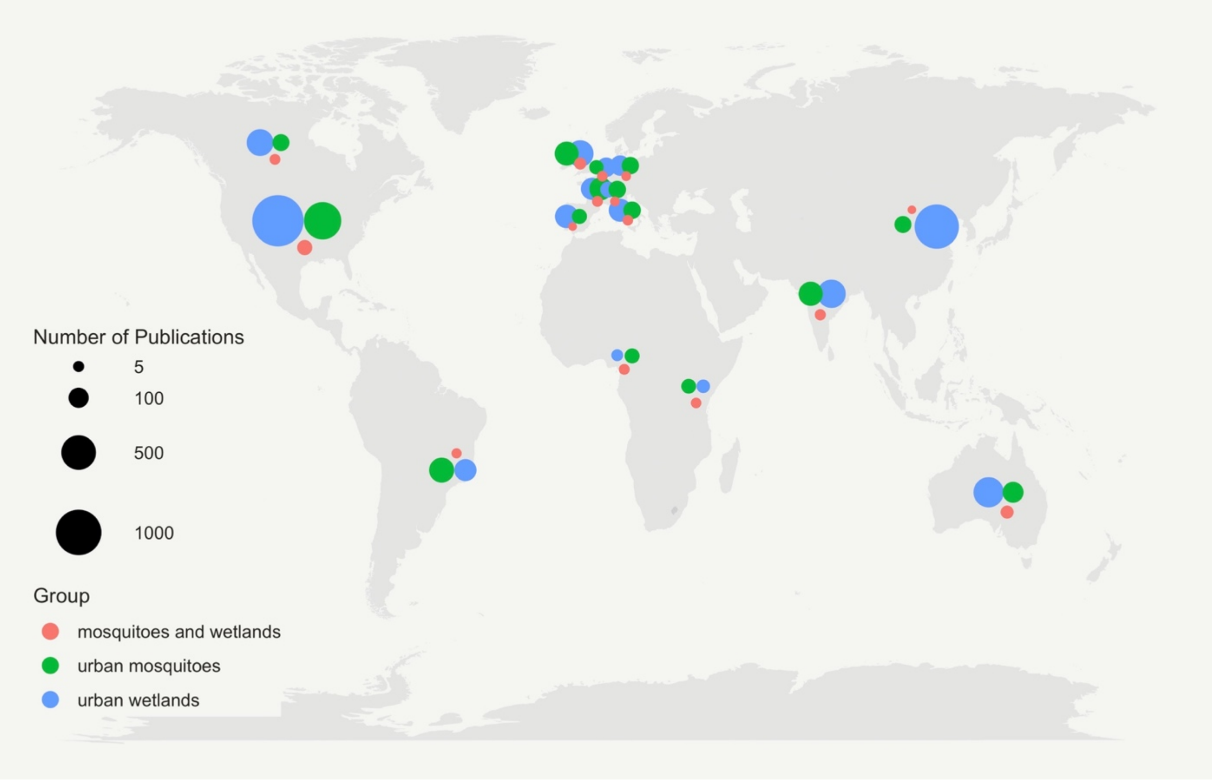


Figure 2.3. The countries that were the most prolific publishers of scientific articles for each search group, based on location of first authors of scientific articles published between 2009 and 2018 and available through Scopus.

It has long been recognized that management of any animal, mosquitoes included, requires information at a scale relevant to the organism in question – in the case of mosquitoes this is often deemed to be identification of larval habitats of specific vectors (Dale and Knight 2008). While place-based knowledge of seasonal mosquito dynamics and species distributions are undoubtedly important in assessing potential risks and informing wetland design and management, this information is largely established for the most common mosquito species. There is a critical gap in the current literature linking mosquitoes to habitat traits in a way that supports the development of design and management guidelines for urban wetlands. Future mosquito research effort and investment may achieve a broader impact by linking mosquitoes to habitat traits that can be managed, and identifying the mechanisms that drive abundance of different mosquito species. This systems- and landscape-based understanding can then be applied to both wetland design and management and epidemiology of mosquito-borne pathogens, which is essential for integrating multi-purpose wetlands into urban areas while pre-emptively, rather than reactively, responding to potential mosquito threats.

One key barrier to increasing knowledge exchange between mosquito and wetland researchers, and achieving co-benefits of research integration, is the lack of access to or awareness of discipline-specific journals where relevant knowledge is held. For example, key references on mosquito management in urban wetlands that would have relevance to mosquito and wetland managers (eg Thullen et al. 2002, Knight et al. 2003) were not returned in our search results, as some ‘industry standard’ journals are not indexed by major databases. This situation poses challenges for identifying and exploiting synergies and inhibits identification of broader research applications from each field.

Opportunities for multiple benefits through building bridges

Our review of the literature clearly demonstrates there remains a significant separation between mosquito research and wetland research. This has substantial opportunity costs to researchers, practitioners, biodiversity and the public.

Economic and health benefits

Cost-benefit analyses for wetlands as water treatment systems typically do not include costs associated with mosquito abatement (Walton 2000), which can be upwards of US$5000 ha^{-1} in poorly designed treatment wetlands, especially in arid areas where constructed wetlands can more significantly alter mosquito habitat availability and seasonal ecology. The separation demonstrated in the results of our literature search between where wetland research is published and where mosquito research is published, and likely lack of overlap in readership, would contribute to this oversight of what is ultimately a costly vector of disease. The lack of integration of mosquito risk management into wetland planning can significantly reduce the economic value of wetlands in urban areas by necessitating *ad-hoc* mosquito abatement activities, and may even increase public health risks in poorly designed and managed systems.

While some local-level guidelines discuss the potential pest and public health risks associated with urban wetlands (eg Russell and Kuginis 1998, Minnesota Stormwater Manual 2019) these texts are often not widely available and can be difficult or unsuitable to apply in other contexts. Even the seminal textbook on treatment wetland design and management, Kadlec and Wallace (2009), only mentions mosquitoes in wetland management, with no discussion of wetland design techniques to proactively reduce potential mosquito issues. The public, however, places significant importance on control of nuisance-biting and pathogen-transmitting mosquitoes, being willing to pay for control of nuisance-biting mosquitoes even when disease risk is low (Dickinson and Paskewitz 2012), and highly valuing eradication of invasive container-breeding species to preserve outdoor amenity (Darbro et al. 2017), and that attitude is likely to extend to those mosquitoes typically associated with wetlands.

Improving integration between wetland and mosquito research has the potential to not only reduce the economic and wellbeing burdens of mosquito-borne illnesses, but also further our understanding of the epidemiology of mosquito-borne diseases by linking landscapes perspectives with fine-scale mosquito research (Panel 2.1).

Panel 2.1. Co-benefits through improved integration.

Including wetlands in mosquito research	**Including mosquitoes in wetland research**
Aim: Characterize abundance and distribution of mosquito species in urban San Juan, Puerto Rico, targeting vectors of dengue and West Nile virus.	*Aim*: Inform plant selection for optimum nutrient removal in constructed treatment wetlands in subtropical Australia.
Method: Array of adult mosquito traps across urban wetland areas, urban forested areas, and high- and low-density residential areas.	*Method*: Compared growing conditions, phosphorus and nitrogen content in leaf/stem and root/rhizome of 12 aquatic plants across 15 constructed and treatment wetlands.
Areas for integration: Record details of wetland and other landscape traits at trap locations rather than coarse land use categories to provide specific traits to target through design and management.	*Areas for integration*: Consideration of potential pest and public health risks associated with each of the 12 plants due to associations with specific mosquito species, using available literature if not possible to include in field component of research.
Co-benefits: Lessons from this place-based research could be applied to geographically similar contexts for broader research and human health benefits. Direct targeted mosquito abatement in areas with comparable habitat traits before outbreaks of disease are recorded in that area. Potential to inform epidemiology by linking landscape- and place-based perspectives.	*Co-benefits*: Inform pre-emptive mosquito mitigation leading to potential public health benefits and improved community support, especially as treatment wetlands are commonly located close to or within urban developments. Reduce reliance on insecticides with associated economic and biodiversity benefits.
Smith, J., Amador, M. & Barrera, R. (2009). Seasonal and Habitat Effects on Dengue and West Nile Virus Vectors in San Juan, Puerto Rico. Journal of the American Mosquito Control Association, 25(1):38-46. DOI: http://dx.doi.org/10.2987/08-5782.1	*Greenway, M. (2003) Suitability of macrophytes for nutrient removal from surface flow constructed wetlands receiving secondary treated sewage effluent in Queensland, Australia. Water Science and Technology, 48(2):121-128. DOI: http://doi.org/10.2166/wst.2003.0101*

Biodiversity benefits

An incidental advantage of greater consideration of risks during the planning phase would be reduced reliance on insecticides (Table 2.1). Constructed urban wetlands can offer valuable food and habitat resources to birds (McKinney et al. 2011), amphibians (Hamer et al. 2012) , reptiles and insects (Goertzen and Suhling 2013) in what are generally challenging and resource-poor environments. Reactionary use of pesticides to control mosquitoes can negatively affect wetland food webs and primary productivity, with impacts persisting longer than control of the target larval mosquitoes (Duguma et al. 2015, Allgeier et al. 2019). Use of pesticides in this way can be minimized or even avoided through careful consideration of wetland habitat traits to reduce larval populations of potentially problematic mosquito species, and ongoing and proactive wetland management (Thullen et al. 2002, Knight et al. 2003, Popko and Walton 2013).

Incorporating knowledge of links between mosquitoes and constructed wetland traits into wetland design guidelines would reduce designing for ‘general’ mosquito risks and missing opportunities to maximise and diversify the ecological benefits of essential stormwater infrastructure (Figure 2.4). The small proportion of cross-disciplinary research we found that linked mosquitoes to wetland habitat traits currently makes this a challenging undertaking, though indicates a key area for future research. The goals of high aquatic biodiversity and reduced mosquito abundance are not necessarily incompatible if habitat preferences of geographically-relevant mosquito species are taken into account and wetlands designed and managed to facilitate access by mosquito predators (Walton et al. 2016, Hanford et al. 2019) and ensure maintenance activities occur during low-risk periods(Hanford et al. 2020). Importantly, reducing mosquito issues in urban wetlands would also improve public confidence and support of these habitats, leading to authorities more readily embracing this approach to increase urban biodiversity resources.

Figure 2.4. Constructed wetlands can provide many positive services to urban areas, but their proximity to housing (a) means mosquitoes should be a key consideration in design and management. Designs such as sprinklers (b) and moderate vegetation density (c) are key features to disrupt mosquito lifecycles and ensure access by mosquito predators. Local communities and urban wildlife (d) have much to gain from improved integration between mosquito and wetland research.

Connecting wetlands and mosquito research

Urban wetland and mosquito research are both dynamic, growing, and productive fields, but there is much to gain from increased integration across their different scales and approaches. Integrating mosquito and wetland research would facilitate wetland design that co-benefits multiple species and serves multiple purposes, and broaden each field’s research impact, reducing disease, improving ecological services and leading to more informed management decisions for both fields.

Taxon-focused research is central to understanding the types of mosquito risk (nuisance vs various health risks), and geographically-specific species lists are key to interpreting these risks for a given area. To connect wetland and mosquito practitioners and researchers an online database linking mosquito species to habitat traits and landscape variables, with geographically- and urban-specific risk profiles, may assist managers to assess risk in very broad terms without the need for additional training.

Finally, the small-scale, spatially-explicit nature of much mosquito research can make it difficult for systems-focused fields to recognize its relevance and value. We encourage mosquito researchers to look beyond discipline-specific journals to ensure their outputs reach fields where diverse applications of their knowledge can have significant positive impact on environmental and human health outcomes.

References

Allgeier, S., A. Friedrich, and C. A. Brühl. 2019. Mosquito control based on *Bacillus thuringiensis israelensis* (Bti) interrupts artificial wetland food chains. Science of the Total Environment **686**:1173-1184.

Bugnot, A. B., G. C. Hose, C. J. Walsh, O. Floerl, K. French, K. A. Dafforn, J. Hanford, E. C. Lowe, and A. K. Hahs. 2019. Urban impacts across realms: Making the case for inter-realm monitoring and management. Science of the Total Environment **648**:711-719.

Claflin, S. B., and C. E. Webb. 2015. Ross River virus: Many vectors and unusual hosts make for an unpredictable pathogen. PLoS Pathogens **11**:e1005070.

Costanza, R., R. de Groot, P. Sutton, S. van der Ploeg, S. J. Anderson, I. Kubiszewski, S. Farber, and R. K. Turner. 2014. Changes in the global value of ecosystem services. Global Environmental Change **26**:152-158.

Dale, P. E. R., and J. M. Knight. 2008. Wetlands and mosquitoes: A review. Wetlands Ecology and Management **16**:255-276.

Darbro, J., Y. Halasa, B. Montgomery, M. Muller, D. Shepard, G. Devine, and P. Mwebaze. 2017. An economic analysis of the threats posed by the establishment of *Aedes albopictus* in Brisbane, Queensland. Ecological Economics **142**:203-213.

de Jesús Crespo, R., P. Méndez Lázaro, and S. H. Yee. 2018. Linking wetland ecosystem services to vector-borne disease: Dengue fever in the San Juan Bay Estuary, Puerto Rico. Wetlands **39**:1281-1293.

Di Marco, M., S. Chapman, G. Althor, S. Kearney, C. Besancon, N. Butt, J. M. Maina, H. P. Possingham, K. Rogalla von Bieberstein, O. Venter, and J. E. M. Watson. 2017. Changing trends and persisting biases in three decades of conservation science. Global Ecology and Conservation **10**:32-42.

Dickinson, K., and S. Paskewitz. 2012. Willingness to pay for mosquito control: How important is West Nile virus risk compared to the nuisance of mosquitoes? Vector-Borne and Zoonotic Diseases **12**:886-892.

Dobbie, M., and R. Green. 2013. Public perceptions of freshwater wetlands in Victoria, Australia. Landscape and Urban Planning **110**:143-154.

Duguma, D., M. W. Hall, P. Rugman-Jones, R. Stouthamer, J. D. Neufeld, and W. E. Walton. 2015. Microbial communities and nutrient dynamics in experimental microcosms are altered after the application of a high dose of Bti. Journal of Applied Ecology **52**:763-773.

Goertzen, D., and F. Suhling. 2013. Promoting dragonfly diversity in cities: Major determinants and implications for urban pond design. Journal of Insect Conservation **17**:399-409.

Hamer, A. J., P. J. Smith, and M. J. McDonnell. 2012. The importance of habitat design and aquatic connectivity in amphibian use of urban stormwater retention ponds. Urban Ecosystems **15**:451-471.

Hanford, J. K., C. E. Webb, and D. F. Hochuli. 2019. Habitat traits associated with mosquito risk and aquatic diversity in urban wetlands. Wetlands **39**:743-758.

Hanford, J. K., C. E. Webb, and D. F. Hochuli. 2020. Management of urban wetlands for conservation can reduce aquatic biodiversity and increase mosquito risk. Journal of Applied Ecology **57**:794-805.

Johnson, B. J., K. Munafo, L. Shappell, N. Tsipoura, M. Robson, J. Ehrenfeld, and M. V. K. Sukhdeo. 2012. The roles of mosquito and bird communities on the prevalence of West Nile virus in urban wetland and residential habitats. Urban Ecosystems **15**:513-531.

Kadlec, R. H., and S. D. Wallace. 2009. Treatment Wetlands. Second edition edition. CRC Press, Taylor & Francis Group, Boca Raton, Florida.

Knight, R. L., W. E. Walton, G. F. O'Meara, W. K. Reisen, and R. Wass. 2003. Strategies for effective mosquito control in constructed treatment wetlands. Ecological Engineering **21**:211-232.

McKinney, R. A., K. B. Raposa, and R. M. Cournoyer. 2011. Wetlands as habitat in urbanizing landscapes: Patterns of bird abundance and occupancy. Landscape and Urban Planning **100**:144-152.

Metzger, M. E. 2005. Managing mosquitoes in stormwater treatment devices. Pages 1-13 *in* American Society of Civil Engineers, editor. World Water and Environmental Resources Congress 2005. Impacts of Global Climate Change, Anchorage, Alaska, United States.

Minnesota Stormwater Manual. 2019. Mosquito control and stormwater management. https://stormwater.pca.state.mn.us/index.php?title=Mosquito_control_and_stormwater_management&oldid=43702. Accessed 14/01/2020.

National Research Council. 2009. Urban Stormwater Management in the United States. The National Academies Press, Washington, DC.

Popko, D. A., and W. E. Walton. 2013. Small-stature emergent macrophytes and crepuscular sprinkler disturbance reduce mosquito abundance in wetland mesocosms. Journal of Vector Ecology **38**:379-389.

Russell, R., and L. Kuginis. 1998. Mosquito risk assessment and management.*in* D. o. L. a. W. Conservation, editor. The Constructed Wetlands Manual. Department of Land and Water Conservation New South Wales, Sydney, Australia.

Russell, R. C. 1999. Constructed wetlands and mosquitoes: Health hazards and management options—an Australian perspective. Ecological Engineering **12**:107-124.

Scholte, S. S. K., M. Todorova, A. J. A. van Teeffelen, and P. H. Verburg. 2016. Public support for wetland restoration: What is the link with ecosystem service values? Wetlands **36**:467-481.

Seto, K. C., B. Güneralp, and L. R. Hutyra. 2012. Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools. Proceedings of the National Academy of Sciences **109**:16083.

Taylor, L., A. K. Hahs, and D. F. Hochuli. 2018. Wellbeing and urban living: Nurtured by nature. Urban Ecosystems **21**:197-208.

Thullen, J. S., J. J. Sartoris, and W. E. Walton. 2002. Effects of vegetation management in constructed wetland treatment cells on water quality and mosquito production. Ecological Engineering **18**:441-457.

Tomerini, D. M., P. E. Dale, and N. Sipe. 2011. Does mosquito control have an effect on mosquito-borne disease? The case of Ross River virus disease and mosquito management in Queensland, Australia. Journal of the American Mosquito Control Association **27**:39-44.

Walton, W. E. 2000. Multipurpose constructed treatment wetlands in the arid southwestern United States: Are the benefits worth the risks? INTECOL International Wetlands Conference, Quebec City, Canada.

Walton, W. E., D. Duguma, T. Min, D. A. Popko, and S. Nygren. 2016. Integrated mosquito management in experimental constructed wetlands: Efficacy of small-stature macrophytes and fluctuating hydroperiod. Water **8**:421.

Chapter 3:

Habitat traits associated with mosquito risk and aquatic diversity in urban wetlands

This chapter has been published:

Hanford, J. K., Webb, C. E. & Hochuli, D. F. (2019) Habitat traits associated with mosquito risk and aquatic diversity in urban wetlands. Wetlands, 39(4), 743-758.

The paper is available online at:

https://link.springer.com/article/10.1007/s13157-019-01133-2

The supplementary material for this paper is available in Appendix 1:

Abstract

Urban wetlands are increasingly being used to manage stormwater, provide valuable resources for biodiversity, and to improve public amenity and wellbeing. However, community concern regarding an increase in the abundance of mosquitoes that pose risks to public health can severely undermine support for these habitats. Our limited understanding of relationships between wetlands, aquatic biodiversity, and mosquitoes in urban areas exacerbates this fear and inhibits our ability to assess potential risks and manage existing conflicts. We sampled adult mosquitoes and aquatic macroinvertebrates at 23 wetlands across the greater Sydney region, Australia. We analysed relationships between macroinvertebrates and mosquito abundance, and examined links with habitat, wetland design and surrounding land use. The mosquito assemblage as a whole showed no links with macroinvertebrate diversity or wetland design, but there were relationships between these variables and individual mosquito species. The diversity of aquatic macroinvertebrates was correlated with surrounding land use and wetland design rather than available aquatic habitat. When assessing mosquito risk, mosquitoes should not be considered collectively as that does not reveal links with urban wetland habitats or measures of aquatic diversity. Instead, strategic species-specific responses should be implemented to create urban wetlands that maximise multiple values without posing threats to public health.

Introduction

As the global population becomes increasingly urban (United Nations 2018), destruction of wetlands continues apace. As much as 87% of global wetland area has been lost since the beginning of the 18th century, driven primarily by economic and human population growth (Davidson 2014), and despite wetlands providing disproportionately more ecosystem services compared to other habitats and being vital to species persistence (Costanza et al. 1997, An et al. 2007, Ramsar Convention on Wetlands 2018).

In this context of destruction and degradation, wetlands in urban areas are becoming increasingly valuable as pockets of refuge and food resources, especially for threatened species of water birds and amphibians (Brand and Snodgrass 2010, Ma et al. 2010, McKinney et al. 2011, Scheffers and Paszkowski 2013). In fact, constructed wetlands are the only group of wetlands that are increasing in global extent, rather than decreasing (Davidson 2014). While many constructed wetlands are primarily built for stormwater management purposes, wetlands are also increasingly associated with the growing popularity of ‘green infrastructure’ and water recycling programs, which are linked to benefits for human health and wellbeing, as well as improving water quality (Soulsbury and White 2015, Eaton 2018). These habitats can simultaneously provide many secondary services such as biodiversity conservation, carbon sequestration, flood control, sediment retention, recreation, and education (Knight et al. 2003, Pankratz et al. 2007, Moore and Hunt 2012).

Enthusiasm within the local community for wetlands in urban areas is severely undermined, however, if they also provide ecosystem ‘disservices’, such as mosquito-friendly habitat and unpleasant odours (Knight et al. 2003). Public fear and dislike of mosquitoes and potential mosquito-borne diseases presents a significant barrier to widespread adoption of wetlands as dual-purpose biodiversity resources.

There is concern that increasing emphasis on both the benefits of green infrastructure for health and wellbeing, and for improving water quality, may have consequences for the spread and prevalence of vector-borne diseases in urban areas in the future (Russell 1999, Soulsbury and White 2015). The emergence and re-emergence of vector-borne diseases pose considerable risk to public health, the environment and the economy across the globe (Russell 1999, Bengis et al. 2004, Cunningham et al. 2017) especially in the face of rapid urbanisation and a changing climate (Vora 2008, World Health Organisation 2014). The economic costs of vector-borne diseases are substantial, and globally amount to tens of billions of US dollars each year due to lost productivity, healthcare costs, vector surveillance and vector control (Suaya et al. 2009, World Health Organisation 2013). For this reason many stormwater wetlands are designed with hard, vertical edges and little, if any, valuable habitat to support urban biodiversity (Department of Land and Water Conservation 1998, Mosquito Control Association of Australia 2008, Walton 2012).

Given the popularity and potential benefits associated with wetlands in urban areas, it is vital to consider both the services and disservices provided by these systems in an urban context to successfully maximise their multiple benefits. The process of assessing potential risks and benefits is hampered by our severely limited understanding of variation in and relationships between urban wetlands and mosquito risks, and between urban wetlands and wetland-inhabiting mosquitoes, which vary regionally as well as between mosquito species. Furthermore, we have little empirical evidence to show that creating wetlands that benefit urban biodiversity will or will not increase mosquito-related public health risks. Despite the obvious links between mosquitoes and aquatic habitats, work on mosquito ecology is rarely integrated with work on aquatic biodiversity. To effectively maximise the multiple values of urban wetlands, while assessing and managing mosquito risks, we must understand these relationships between mosquito risks and biodiversity, and in order to plan at the landscape scale, we must first examine small-scale mosquito responses at habitat level.

The conservation and management of freshwater systems remains a significantly understudied topic despite the potential for far-reaching impacts on global biodiversity conservation (Jucker et al. 2018), and until recently there has been little recognition of the need to manage both terrestrial and aquatic urban environments to achieve desired conservation outcomes (Bugnot et al. 2019). Ponds can contribute disproportionately to regional diversity compared to rivers, streams, and ditches, especially for unique and scarce species of macrophytes and macroinvertebrates (Williams et al. 2003, Le Viol et al. 2009). Few studies have applied the use of macroinvertebrates in wetland assessments in urban areas, though they are the most commonly used biotic indicator of stream health, including urban stream health (e.g. Dunn 2000, Spieles and Mitsch 2000, Chessman et al. 2002, Freeman and Schorr 2004, Hassall et al. 2011, Chessman and McEvoy 2012). Aquatic macroinvertebrates constitute a large component of biodiversity and are typically the primary trophic link between plant productivity and higher animals. This means they are central to the functioning of aquatic ecosystems and a good indicator of capacity for supporting other biodiversity such as birds, amphibians and fish, providing an integrated perspective of aquatic health (Faith and Norris 1989, Rosenberg and Resh 1993, Marchant et al. 1995, Batzer and Wissinger 1996, Wellborn et al. 1996, Chessman et al. 2002).

Using aquatic macroinvertebrates as a measure of wetland aquatic diversity, we investigated associations between mosquitoes and wetlands ranging from highly urban through to peri-urban to determine if mosquito abundance is related to aquatic diversity in urban wetland environments. We also identified the wetland habitat and landscape traits driving aquatic diversity and mosquito abundance to identify potential conflicts between the habitat requirements for high aquatic diversity and low mosquito risk.

Methods

Study area and study sites

We used satellite imagery (Land and Property Information 2015) with a scale of 1:282 at 96 DPI and ArcGIS (v10.0 Service Pack 3) to identify more than 120 waterbodies that represented potential study wetlands across the Greater Sydney region, Australia, an area comprising a complex urban matrix, expanding urban fringe, and providing a representative situation of urban expansion globally. Field surveys allowed us to narrow this selection to 23 wetlands (Supp. Table 1.1) that were a similar shape and size (average size of 0.33ha ±0.19, median size 0.29ha) and represented the gradient of urbanisation i.e. spanned highly urban areas out to peri-urban and suburban areas (Figure 3.1). Selected wetlands held permanent, open fresh water (permanence was established through multiple field visits across seasons), and represented a range of primary purposes including stormwater treatment and natural wetlands. Although some mosquito species also breed in fresh ephemeral waters, we selected permanent waters for this study to allow sampling of macroinvertebrates, and to represent the permanent standing waters often associated with in urban amenity and stormwater wetlands.

Measuring aquatic biodiversity

At each wetland we used a D-frame hand-held sweep net with 0.25mm mesh (e.g. Marchant et al. 1995) to collect aquatic macroinvertebrates from 10m of representative edge habitat (different edge habitats were sampled in proportion to their availability within the wetland) once in summer (December 2015-February 2016) and once again in autumn (March 2016).

Sweep net samples were sub-sampled in the field by transferring the collection to a white 45x60cm tray and live-picking specimens with forceps and pipettes for 30-60 minutes to achieve a relatively constant processing effort (Marchant et al. 1995, Turak et al. 2004). Specimens were returned to the laboratory in 70% ethanol and identified to the lowest recognisable taxonomic unit (RTU) practicable using keys from Theischinger (2002), Watts (2002), Dean (2004) and Hawking et al. (2013). The functional feeding group (predators, gatherers/filterers, shredders/scrapers, macrophyte piercers) of each taxon was also recorded for analyses.

Assessing the mosquito assemblage

We actively targeted host-seeking female mosquitoes using carbon dioxide-baited Encephalitis Virus Surveillance (EVS) traps (Supp. Figure 1.1) (Rohe and Fall 1979). Though this sampling method targets nocturnal- and crepuscular-feeding mosquito species, it is an effective technique for detection of adult mosquitoes and has been used successfully to measure the abundance and diversity of mosquitoes of pest and public health concern (Williams et al. 2012, Claflin and Webb 2016, Crocker et al. 2017), and comparable trapping methodology was followed. In summary, traps contained approximately 500 g of dry ice pellets, which release carbon dioxide as they sublimate and attracts host-seeking females. To comprehensively sample mosquitoes at each wetland, four traps were simultaneously

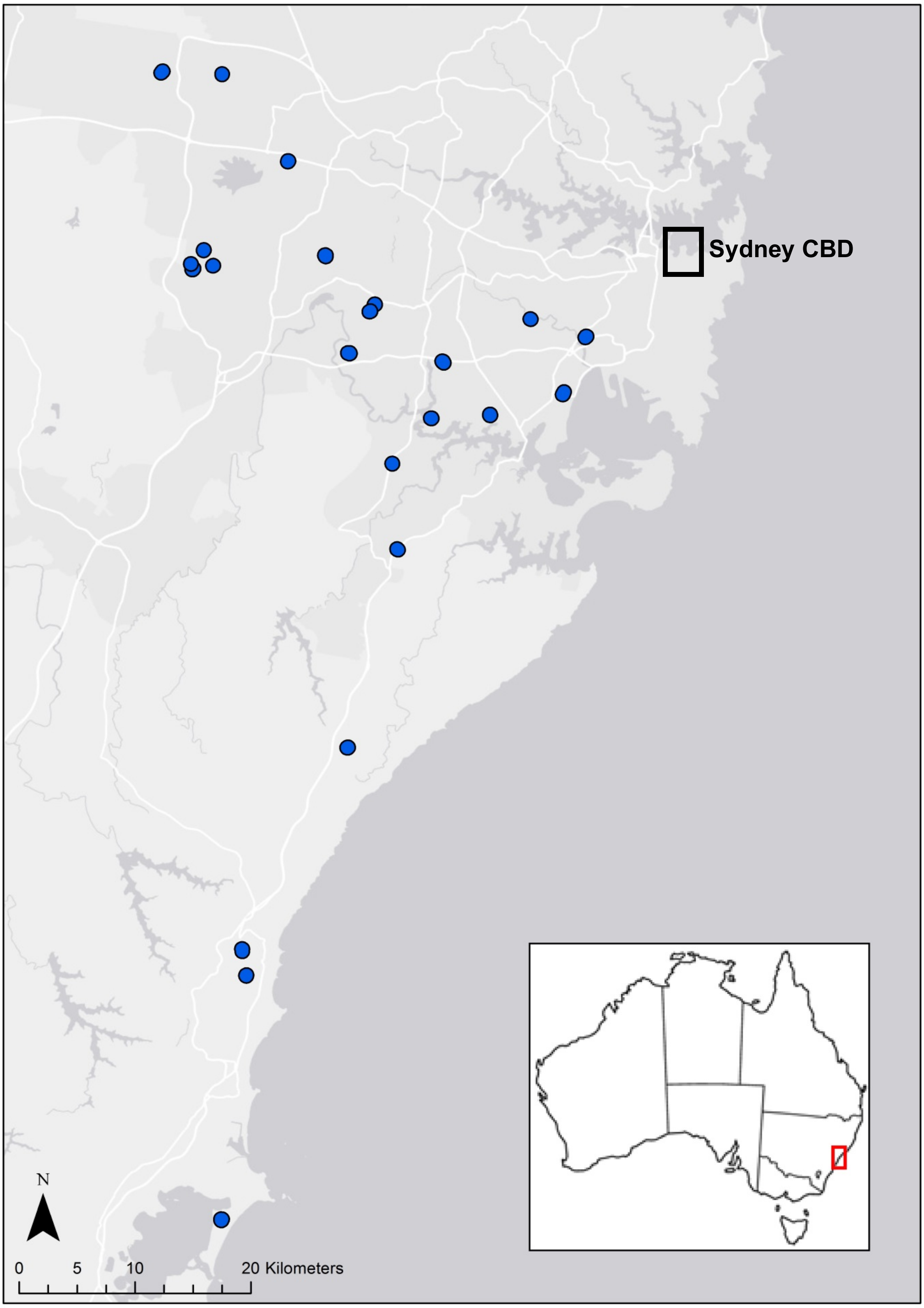


Figure 3.1 Location of study wetlands (circles) in relation to Sydney Central Business District

placed around the perimeter of the wetland and these four samples were pooled to give a single sample capturing the potential variation around the wetland due to habitat availability or other factors; where possible traps were separated by at least 50 m. Traps were placed away from areas of high pedestrian traffic to minimise potential disturbance or vandalism, and targeted shaded, vegetated areas where adult mosquitoes may have been seeking refuge.

Traps were set at least one hour before sunset and collected the following morning. Mosquitoes were transported to the laboratory where traps were placed in a freezer for approximately 10 minutes to ‘knock-down’ the specimens, which were then transferred to plastic petri dishes and stored at -20°C until counting and identification using Russell (1993) and Webb et al. (2016). We did not set traps on nights when more than 10mm of rain was predicted or strong winds forecast as anecdotal evidence indicates fewer individuals are trapped during such weather events. Mosquitoes were sampled once in summer and again in autumn to account for seasonal variations in the mosquito assemblage and were sampled within a 5-day period where consistent weather was forecast and observed, (8-12 January 2016; 19-23 March 2016).

Aquatic habitat, wetland structure and surrounding landscape

To assess aquatic habitat type and availability we used a modified Braun-Blanquet cover-abundance scale (Van Der Maarel 1979) for different types of aquatic vegetation (submerged, floating, emergent, trailing) and other available aquatic habitats (leaf packs, rocks, bare edge, woody debris). Aquatic habitats were scored according to the coverage of the habitat within a 2m band around the wetland perimeter. Habitats were scored from 0 to 5, where 0≦1% coverage within the 2m perimeter band, 1=1-10% coverage, 2=10-35% coverage, 3=35-65% coverage, 4=64-90% coverage, and 5=>90% coverage. Rapid assessment techniques such as this (i.e. visual assessments and overall estimation) have been shown to provide robust assessments of physical and biotic stream condition, linking the physical environment to ecological condition and the types of macroinvertebrate communities found there (Parsons et al. 2004). In addition, it gives an indication of the larval habitat available to wetland-utilising mosquito species, which are generally found within vegetated regions as opposed to open water areas (Walton and Workman 1998). Surveys of Australian wetlands have recorded low larval densities in open water areas of wetlands where predation is an important factor reducing larval survivorship (Greenway et al. 2003).

To characterise wetland shape, we measured wetland area and perimeter length using ArcGIS (v10.0 Service Pack 3) and satellite imagery corroborated with hand-held GPS points (Garmin eTrex 20X). We calculated perimeter complexity as the ratio of wetland perimeter to the perimeter of a circle with the same area as the wetland (Murray et al. 2013). We also recorded the land use immediately surrounding the wetland i.e. if the wetland was located within a landscaped parkland (park), bush area (bushland) or residential area where houses were within 50m of the wetland (residential), width of surrounding vegetation (riparian zone) if any, and water inlet and outlet type (pipe, channel, creek, overflow).

Data analyses

We analysed macroinvertebrate and mosquito data against wetland habitat and landscape traits using PRIMER v7 with PERMANOVA+ package for multivariate analyses and SPSS (version 22.0.0.0) for bivariate analyses. We also analysed mosquito data against macroinvertebrate data to determine if the macroinvertebrate assemblage contributes to shaping mosquito abundance and therefore potential risk.

For each wetland, we calculated the total number of individual macroinvertebrates, taxa richness (based on RTUs), Simpson diversity index, Shannon diversity and the relative percent abundance of macroinvertebrate functional feeding groups. We analysed relationships between these macroinvertebrate measures and habitat traits, between mosquito abundance and habitat traits and between macroinvertebrate measures and mosquito abundance, using Spearman’s correlations. To determine if the type of water inlet, water outlet, or immediate surrounding land use affected aquatic diversity or mosquito abundance we used Kruskal-Wallis tests (Anderson et al. 2008).

We tested if macroinvertebrate and mosquito assemblages differed between summer and autumn using one-factor (season) permutational multivariate analysis of variance (PERMANOVA) based on Bray Curtis similarity matrices of square-root transformed data. We explored associations between macroinvertebrates and habitat attributes and mosquitoes and habitat attributes using Canonical Analysis of Principle Components (CAP).

Similarity matrices of biotic assemblages and aquatic habitat variables were compared across sites using RELATE, a Mantel-type test, with 999 permutations. To determine if habitat traits can be used to explain macroinvertebrate and mosquito assemblages, we used distance-based linear models (DISTLM). We used AICc selection criterion with 9999 permutations (Anderson et al. 2008). Prior to DISTLM analyses some sites were removed from the data to construct identical matrices, as due to algal blooms two sites (BAN07 and FAI05) could not be sampled for macroinvertebrates in autumn, and due to vandalism one site (BLA02) could not be surveyed for mosquitoes in either summer or autumn.

Results

Aquatic biodiversity

Aquatic macroinvertebrates were significantly more abundant in summer (116.9±11.00) than in autumn (86.90±13.62) (t(20)=2.174, p=0.042), and the composition of assemblages was also significantly different between seasons (pseudo $F_{(1,42)}$=2.41, p=0.008, perm=998). Seasonal assemblage differences were driven by non-biting midges (Diptera: Chironomidae: Chironominae) and flatworms (Tricladida: Dugesiidae), both more abundant in summer, and water mites (Trombidiformes: Hydrachnidae), which were more abundant in autumn (Table 3.1). Despite these seasonal differences, we found that macroinvertebrate richness, Simpson and Shannon diversity and relative abundance of functional feeding groups were not different between summer and autumn. This suggests the seasonal assemblage differences were driven by changes in abundance rather than species composition.

Table 3.1. SIMPER results for aquatic macroinvertebrate assemblages; 11 taxa explained 53% of the difference between summer and autumn assemblages. The average abundance of each taxon (summer, autumn), their contribution to between group dissimilarities (Av.Diss), the consistency of these contributions (Diss/SD), and the individual and cumulative percentage of difference explain are shown. Taxa are listed in descending order for individual contribution to between-group dissimilarity, up to >50% of dissimilarity explained.

Taxon	Summer	Autumn	Av.Diss	Diss/SD	Contrib%	Cum.%
Chironominae	5.04	4.59	5.78	1.23	8.68	8.68
Hydrachna	1.81	1.86	3.73	0.98	5.60	14.28
Dugesiidae	2.42	1.17	3.54	1.18	5.32	19.60
Oligochaeta	2.43	2.21	3.24	1.16	4.87	24.47
Glossiphoniidae	1.93	0.73	3.16	1.02	4.75	29.22
Corixidae(juv)	1.61	0.89	2.85	1.04	4.28	33.50
Micronecta	1.57	0.59	2.85	0.80	4.28	37.78
Ischnura	1.83	1.75	2.67	1.32	4.01	41.79
Physa	1.56	0.84	2.61	1.07	3.91	45.71
Triplectides	1.28	0.80	2.45	0.88	3.67	49.38
Anisops	1.02	0.77	2.10	0.78	3.15	52.53

We collected and identified 79 aquatic macroinvertebrate taxa (2,770 specimens) in summer (Supp. Table 1.2) and 76 aquatic macroinvertebrate taxa (1,825 specimens) in autumn (Supp. Table 1.3). Together these samples represented 59 families. Two wetlands were not sampled in autumn due to algal blooms, which clogged collection nets and prevented accurate sampling. The vast majority (84% in both summer and autumn) of taxa collected were classified as tolerant or very tolerant of pollution (Gooderham and Tsyrlin 2003, Hawking et al. 2013). In summer only one site, WSP01, recorded any 'very sensitive' aquatic macroinvertebrates (a single trichopteran of genus *Cal. B*), and in autumn no sites recorded any very sensitive taxa. Sensitive taxa were dominated by trichopterans (mostly Leptoceridae) and water mites, almost 80% of tolerant taxa comprised the Chironominae subfamily of chironomids, and the taxa considered to be very tolerant of pollution were mostly comprised of segmented worms (Oligochaeta), water boatmen (Corixidae), damselflies of the family Coenagrionidae, and flatworms (Turbellaria). Chironominae were the most abundant taxa collected overall, accounting for 28% of individuals collected in summer and 32% of those collected in autumn, though were not the dominant taxa at every wetland.

Mosquitoes

Summer and autumn mosquito assemblages were significantly different ($F_{(1,42)}$=5.39, p=0.001, perm=998), driven by the abundance of *Culex annulirostris, Aedes notoscriptus* and *Cx. quinquefasciatus* (Table 3.2). This is not unexpected as a multitude of studies (e.g. Schafer et al. 2004, Williams et al. 2009, Claflin and Webb 2016) have demonstrated the highly seasonal nature of mosquito assemblages in temperate regions.

Table 3.2. SIMPER results for mosquito assemblages; 5 taxa explained 72% of the difference between summer and autumn assemblages. The average abundance of each taxon (summer, autumn), their contribution to between group dissimilarities (Av.Diss), the consistency of these contributions (Diss/SD), and the individual and cumulative percentage of difference explain are shown. Taxa are listed in descending order for individual contribution to between-group dissimilarity, up to >70% of dissimilarity explained.

Species	Summer	Autumn	Av.Diss	Diss/SD	Contrib%	Cum.%
Culex annulirostris	1.09	5.42	11.60	1.50	21.04	21.04
Aedes notoscriptus	6.37	4.65	9.62	1.30	17.44	38.48
Culex quinquefasciatus	4.01	3.55	8.45	1.22	15.32	53.81
Mansonia uniformis	0.79	1.93	5.21	0.97	9.45	63.26
Coquillettidia linealis	1.67	1.23	4.62	0.90	8.37	71.63

We collected and identified 22 mosquito species (2,093 specimens) in summer (Supp. Table 1.4) and 16 mosquito species (2,569 specimens) in autumn (Supp. Table 1.5). One site was excluded from mosquito sampling due to vandalism of traps during summer sampling. The most common species captured were *Cx. annulirostris* (summer n=58, autumn n=1,006), *Cx. quinquefasciatus* (summer n=551, autumn n=432), *Mansonia uniformis* (summer n=61, autumn n=175), *Coquillettidia linealis* (summer n=160, autumn n=89), and *Ae. notoscriptus* (summer n=1,058, autumn n=659), accounting for 92.4% of mosquitoes identified.

However, *Ae. notoscriptus* is predominantly a container-inhabiting species and rarely utilises wetland habitats, but rather disperses into wetland areas from surrounding urban environments (Watson et al. 2000). Further consideration of the ecology of individual mosquito species reveals that whole-assemblage analyses are unlikely to reflect wetland-specific features or conditions. For example, *Cx. molestus* is a known domestic pest and was detected at 4 of our 23 wetlands, however *Cx. molestus* larvae are generally associated with subterranean habitats such as sewers and septic tanks (Kassim et al. 2013, Webb et al. 2016); their presence is hence unlikely influenced by the wetland condition or aquatic biota, but may indicate factors other than wetland that should be addressed in the local area. Similarly, many collected mosquito species, such as *Cx. sitiens* and *Ae. vigilax* are associated with saline and brackish water habitats (Webb et al. 2016) and are also unlikely to indicate potential threats associated with freshwater wetlands.

Considering the whole mosquito assemblage therefore prevents robust evaluation of the potential mosquito risks associated with a wetland habitat, particularly in urban areas where many other container breeding habitats are abundantly available such as roof gutters, abandoned tyres, pot plants and rubbish (Webb et al. 2016). Consequently, further analyses pertain only to the four most abundant mosquito species known to utilise wetland habitats: *Cx. annulirostris, Cx. quinquefasciatus, Ma. uniformis,* and *Cq. linealis.* In addition, these four mosquito species are of known pest and public health significance in our study region (Doggett et al. 2009), and are highly relevant to determining if the goals of high aquatic biodiversity and low mosquito risk are compatible in urban wetland habitats.

While total mosquito abundance did not differ significantly between summer and autumn, when we focused on our four species of interest we found *Cx. annulirostris* abundance was significantly lower in summer (2.636±0.933) compared to autumn (45.727±14.563) (t(22)=-2.987, p=0.007), as was *Ma. uniformis* abundance between summer (2.773±1.581) and autumn (7.955±2.733) (t(22)=-3.344, p=0.003) (Figure 3.2).

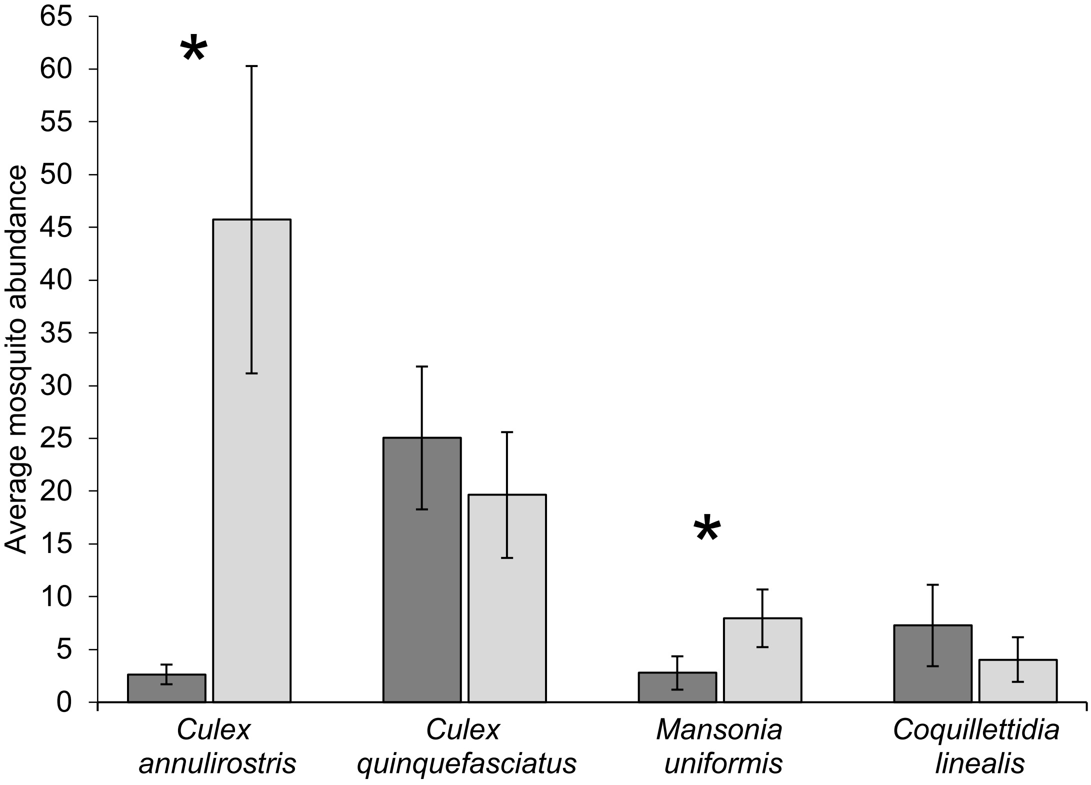


Figure 3.2. Average abundance (±SE) of four key mosquito species across n = 23 wetlands in summer (dark grey) and autumn (light grey). * denotes significant difference in abundance between summer and autumn

Relationships with aquatic habitat

Differences in the macroinvertebrate assemblage between sites did not reflect differences in overall habitat availability between sites (RELATE; summer Rho=0.077, p=0.256, perm=999; autumn Rho=0.062, p=0.749, perm=999). Modelling suggested that habitat traits alone have poor explanatory power for macroinvertebrate assemblages. The three best models for explaining summer macroinvertebrate assemblages were single-variable models using floating vegetation, immediate land use, and filamentous algae respectively, with each explaining less than 10% of the variation in macroinvertebrate assemblages observed (Table 3.3). This was also the case for autumn macroinvertebrate assemblages, with best models including rocks, which explained 10% of variation, and inlet and immediate surrounding land use, which each explained less than 10% of the variation (Table 3.3).

Table 3.3. The three best fit habitat variables models for macroinvertebrate and mosquito assemblages for summer and autumn.

		AICc	R^2	RSS	Variables Included
Macroinvertebrate assemblages	Summer	176.67	0.0955	40817	Floating vegetation
		176.94	0.0849	41291	Immediate land use
		176.97	0.0836	41350	Filamentous algae
	Autumn	163.43	0.1026	40321	Rocks
		163.59	0.0955	40640	Inlet type
		163.91	0.0819	41253	Immediate land use
Mosquito assemblages	Summer	158.03	0.1152	23478	Filamentous algae
		158.10	0.1126	23546	Immediate land use
		158.43	0.0992	23902	Riparian width
	Autumn	160.59	0.1192	26374	Immediate land use
		160.89	0.1084	26698	Inlet type
		160.87	0.2109	23627	Immediate land use, emergent vegetation

Differences in the mosquito assemblage were also not related to habitat differences (summer Rho=0.093, p=0.228, perm=999; autumn Rho =0.159, p=0.127, perm=999). The three best models for explaining summer mosquito assemblages were single-variable models using filamentous algae, immediate surrounding land use and riparian width respectively (Table 3.3), rather than a combination of variables. This was also observed for the autumn mosquito assemblage, with best models including immediate land use, inlet, and both variables together (Table 3.3).

Summer macroinvertebrate abundance was positively related to the availability of rocky habitat (r = 0.456, p = 0.038), while autumn abundance was negatively related to artificial habitats (r = -0.485, p = 0.026). In summer, we found positive relationships between floating vegetation and macroinvertebrate richness (r = 0.467, p = 0.033), Simpson diversity (r = 0.519, p = 0.016), and Shannon diversity (r = 0.538, p = 0.012). In autumn, we found negative relationships between filamentous algae and Simpson diversity (r = -0.498, p = 0.021) and Shannon diversity (r = -0.454, p = 0.039). Relative abundance of the shredder/scraper functional feeding group showed a negative relationship with artificial aquatic habitat in summer (r = -0.422, p = 0.045). No other functional feeding groups were related to aquatic habitat variables in summer or autumn.

Observations indicated that filamentous algae was more abundant in autumn compared to summer, but this difference was not significant. Furthermore, *Anopheles annulipes,* a mosquito species found preferentially in association with algal mats and generally in greater abundance in autumn (Webb et al. 2016), did not display increased abundance at our wetland sites, perhaps reflecting the absence of a significant difference in algal abundance.

Summer abundance of *Cx. quinquefasciatus* was positively related to filamentous algae (r=0.542, p=0.009). Conversely, *Cq. linealis* displayed similar relationships with aquatic habitat as macroinvertebrate variables. Summer *Cq. linealis* abundance was negatively related to filamentous algae (r=-0.544, p=0.009), while autumn abundance was positively related to leaf packs (r=0.513, p=0.015) and floating vegetation (r=0.527, p=0.012). These relationships were inconsistent across seasons, though that may be due to the mosquito assemblage being significantly different in autumn, when *Cx. annulirostris* was dominant and significantly more abundant.

Wetland configuration

Macroinvertebrate abundance, richness and diversity indices were not related to wetland area, perimeter length, perimeter complexity, or riparian width. As with macroinvertebrate variables, summer and autumn abundances of *Cx. annulirostris*, *Cx. quinquefasciatus*, *Ma. uniformis*, and *Cq. linealis* were not correlated with wetland area, perimeter length or perimeter complexity. Autumn abundance of *Cx. quinquefasciatus* was negatively related to riparian width (r = -0.457, p = 0.032, n = 22).

Landscape traits

We found that macroinvertebrate assemblages were predominantly linked to variables larger than wetland scale, including type of water inlet and the immediate surrounding land use (Figure 3.3, 3.4).

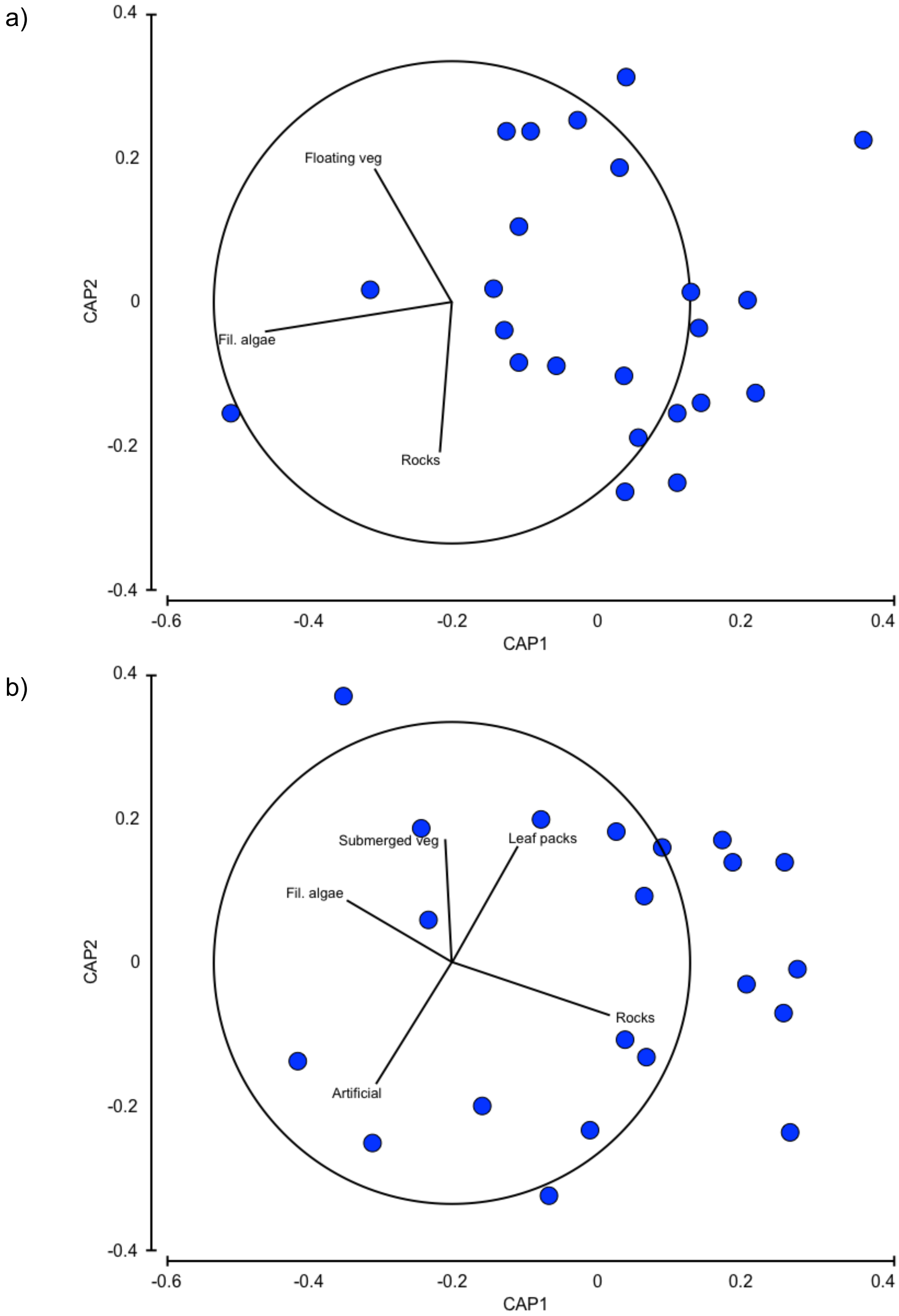


Figure 3.3 Canonical Analysis of Principle Coordinates (CAP) of (a) summer aquatic macroinvertebrate assemblage composition and (b) autumn aquatic macroinvertebrate assemblage composition at each wetland, based on Bray-Curtis similarities calculated from square-root transformed data. Vector overlays are Spearman Rank correlations >0.5 for wetland aquatic habitat attributes, indicating the drivers in differences in aquatic macroinvertebrate assemblages between wetlands. The circle overlay is scaled to the maximum correlation value indicating magnitude of effect

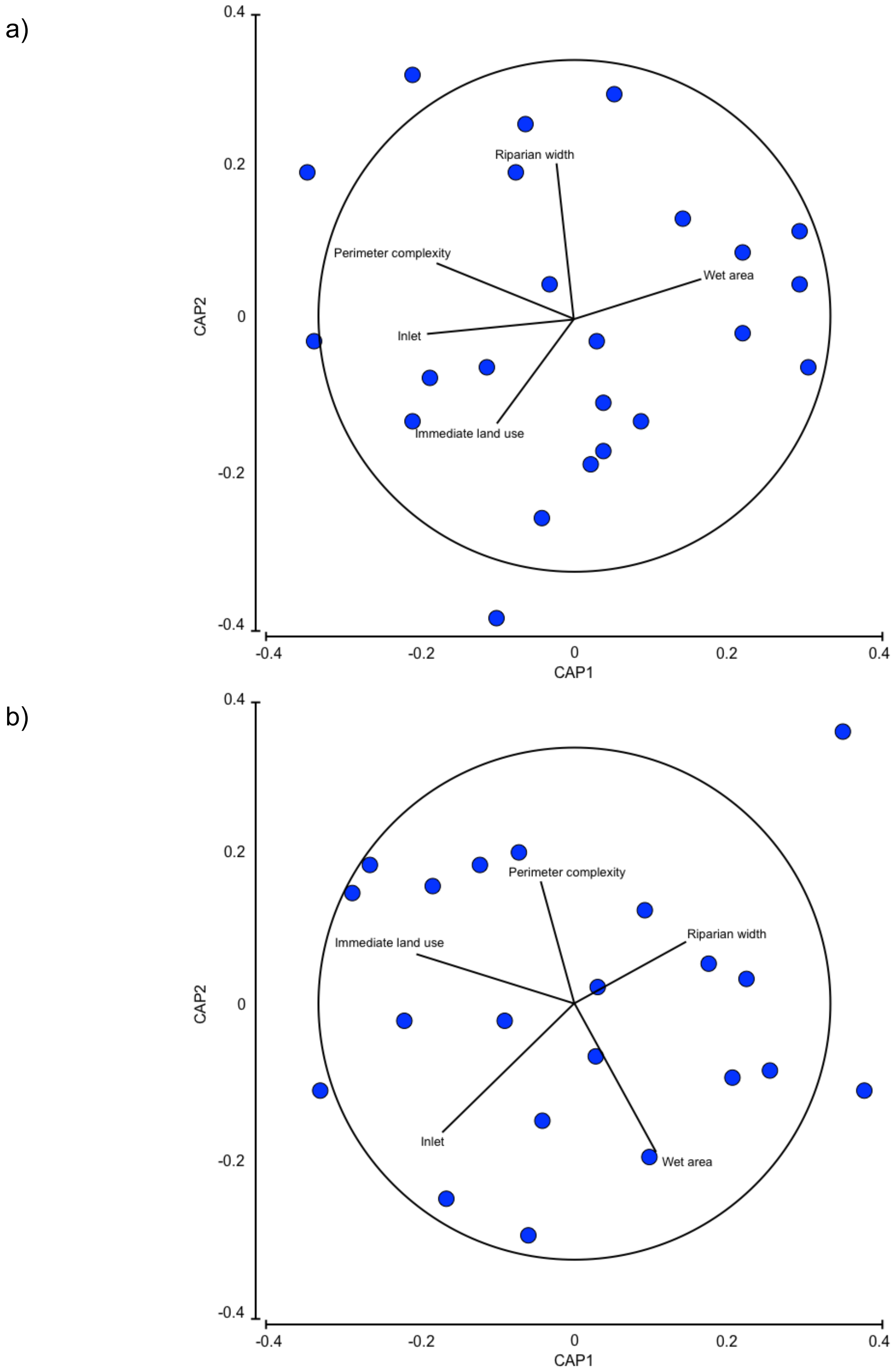


Figure 3.4 Canonical Analysis of Principle Coordinates (CAP) of (a) summer aquatic macroinvertebrate assemblage composition and (b) autumn aquatic macroinvertebrate assemblage composition at each wetland, based on Bray-Curtis similarities calculated from square-root transformed data. Vector overlays are Spearman Rank correlations >0.5 for wetland structure and surrounding land use attributes, indicating the drivers in differences in aquatic macroinvertebrate assemblages between wetlands. The circle overlay is scaled to the maximum correlation value indicating magnitude of effect.

Larger-scale structural variables had a greater effect on aquatic diversity measures compared to aquatic habitat or wetland configuration. In summer macroinvertebrate richness was associated with inlet type (*H*(2) = 10.58, *p* = 0.005); pairwise comparisons revealed wetlands with creek inlets had significantly higher richness than wetlands with Gross Pollutant Trap (GPT) inlets (p = 0.003, r = 0.902). Summer Simpson diversity was also related to inlet type (*H*(2) = 6.706, *p* = 0.035) as was Shannon diversity (*H*(2) = 7.789, *p* = 0.020), where again pairwise comparisons revealed wetlands with creek inlets had higher diversity for both indices compared to wetlands with GPT inlets (p = 0.042, r = 0.683; p = 0.026, r = 0.730). We found no relationships between inlet type and autumn macroinvertebrate variables or summer or autumn relative abundance of functional feeding groups.

Outlet type was also related to macroinvertebrate richness in summer (*H*(2) = 6.119, *p* = 0.047), however pairwise comparisons showed there were no significant differences between the individual outlet types. Relative abundance of shredders/scrapers was related to the type of water outlet (*H*(2) = 8.043, *p* = 0.018); pairwise comparisons revealed wetlands with creek outlets had significantly higher abundance of shredders/scrapers compared to wetlands with pipe outlets (p = 0.014, r = 0.634). Autumn relative abundance of macrophyte piercers was also significantly affected by outlet type (*H*(2) = 9.500, *p* = 0.009). Wetlands with a creek outlet had significantly lower relative abundance of macrophyte piercers compared to wetlands with a spillway outlet (p = 0.010, r = 0.813), while wetlands with a pipe outlet also had significantly lower relative abundance compared to the wetlands with a spillway outlet (p = 0.009, r = 0.820).

In both summer and autumn we found no relationships between inlet types and the abundance of the four dominant mosquito species. We also found no relationships with summer mosquito abundance and the type of water outlet. In autumn, *Ma. uniformis* abundance was related to outlet type (*H*(2) = 8.723, *p* = 0.013), with wetlands with creek outlets having significantly greater *Ma. uniformis* abundance than wetlands with pipe outlets (p = 0.010, r = 0.659).

The type of land use immediately surrounding a wetland (bushland, park, residential) had no effect on summer or autumn macroinvertebrate variables, relative abundances of macroinvertebrate functional feeding groups, or abundance of different mosquito species. Land use also did not affect aquatic habitat traits, and the type of inlet, outlet or surrounding land use was unrelated to wetland structural traits (perimeter complexity, perimeter length, wetland area or riparian width).

Inlet type had no overall effect on wetland aquatic habitat traits, aside from floating vegetation, H(2) = 8.698, p = 0.013. Pairwise comparisons showed that floating vegetation scores at sites with GPT inlets (M = 5.50) were lower than sites with pipe inlets (M = 14.55) (p = 0.016, r = 0.696) and sites with creek inlets (M = 13.93) (p = 0.048, r = 0.668). Outlet type significantly affected leaf pack scores (H(2) =7.443, p = 0.024). Pairwise comparisons revealed wetlands with pipe outlets had significantly lower leaf pack scores (M = 9.30) compared to wetlands with spillway outlets (M = 20.83) (p = 0.019, r = 0.757).

Interactions between mosquitoes and aquatic biodiversity

While adult mosquitoes do not interact with aquatic macroinvertebrates directly, interactions while both the mosquitoes and the macroinvertebrates were in the aquatic stage would undoubtedly have flow-through effects on the adult mosquito assemblage. Associations between macroinvertebrates and adult mosquitoes varied greatly between mosquito species. There were no relationships between our measures of aquatic biodiversity and *Cx. annulirostris* or *Cx. quinquefasciatus,* however there were several relationships with *Ma. uniformis* and *Cq. linealis*. Summer macroinvertebrate richness was positively related to *Cq. linealis* abundance (r=0.449, p=0.036, n=22) and *Ma. uniformis* abundance (r=0.497, p=0.019, n=22). There was also a positive relationship between macroinvertebrate Shannon diversity and *Ma. uniformis* abundance (r=0.468, p=0.028, n=22), and between *Ma. uniformis* abundance and the proportion of macroinvertebrates that belong to the shedder/scraper FFG (r=0.497, p=0.019, n=22). In autumn, *Cq. linealis* abundance was again positively associated with macroinvertebrate richness (r=0.680, p=0.001, n=20) and also macroinvertebrate abundance (r=0.525, p=0.017, n=20).

Discussion

Managing the diverse ecological benefits of urban wetlands while considering potential pest and public health threats associated with mosquito populations is a complex issue. Our results suggest that relationships between aquatic diversity and abundance of pest mosquito species are inconsistent and vary greatly between mosquito species. To create wetlands of high value to urban biodiversity that do not increase general mosquito risks, emphasis must be placed on consideration of the specific mosquito species of concern within the local region, and how the habitat requirements and environmental drivers of these species interact with those of local biodiversity. The simple presence of a certain mosquito species does present unequivocal risks to public health. When assessing the public health risks associated with wetlands, notwithstanding the propensity of mosquitoes to bite people and capacity of mosquitoes to transmit pathogens of concern, other factors such as the presence and abundance of reservoir hosts must be understood (Stephenson et al. 2018, Walsh and Webb 2018). Key mosquito species collected in this study are known nuisance biting pests (Webb et al. 2016) and have been demonstrated as potentially effective vectors of Ross River virus (Kay et al. 1982, Harley et al. 2001, Jeffery et al. 2002). Furthermore, consideration must be given to the relative ecological role of mosquitoes where strategies to reduce the abundance of key species may indirectly impact insectivorous wildlife, such as bats (Gonsalves et al. 2013a, Gonsalves et al. 2013c).

Relationships between mosquitoes and aquatic macroinvertebrates

A large proportion of aquatic macroinvertebrates collected from our urban wetlands were taxa considered to be tolerant or very tolerant of pollution. We found that non-biting midges, worms and water boatmen were numerically dominant across most wetlands, a pattern that is regularly observed in urban streams (Walsh et al. 2001, Freeman and Schorr 2004, Davies et al. 2010) and stormwater wetlands (Mackintosh et al. 2015). These pollution-tolerant taxa form a major component of food webs in urban wetlands (Mackintosh et al. 2017).

Our mosquito sampling confirmed the presence of diverse and locally abundant mosquitoes that represent potential pest and vector species of concern. While we detected 23 species, it is known that over 50 species have previously been recorded from the Greater Sydney region (Webb et al. 2001), though not all are pest species or pose potential public health risks. The difference between the number of species we detected and known species is likely due to mosquito habitat preferences not represented in our study sites (Webb et al. 2001, Webb et al. 2016), the variable abundance of less common species, and trap efficiency for different mosquito species in different habitats (Lühken et al. 2014).

While in some instances our measures of aquatic biodiversity showed positive relationships with mosquito abundance, as we found in summer with *Cq. linealis* abundance and *Ma. uniformis* abundance, and in autumn with *Cq. linealis* abundance, it appears that these taxa are responding to similar wetland traits, such as the presence of filamentous algae, leaf packs and floating vegetation, rather than to the presence or abundance of each other. The relative absence of relationships between abundance of mosquito species of interest and aquatic biodiversity measures, in particular predatory macroinvertebrates, was surprising, as many studies based on mesocosms and natural wetlands have suggested taxa such as dragonfly larvae, water boatmen, backswimmers, fish, tadpoles, and copepods are successful biological control agents for mosquito larvae, though some are more effective than others (Willems et al. 2005a, b, Kumar and Hwang 2006, Dale et al. 2007, Culler and Lamp 2009).

Other studies have similarly found inconsistent relationships between abundance of mosquito larvae and abundance of macroinvertebrate predators (Walton and Workman

1998), indicating that additional factors must be responsible for controlling populations of mosquitoes. In the presence of alternative prey, mosquito larvae may become less attractive to predators, especially as they grow in size and become more difficult to capture (Kumar et al. 2008). Furthermore, urban waterways are often nutrient-rich habitats which increases primary productivity (Mackintosh et al. 2017), and this abundance of resources may result in a decoupling of top-down interactions between mosquito larvae and predatory macroinvertebrates (Walton 2012). Another possible explanation may be the influence of abundant introduced predators, such as the invasive fish *Gambusia holbrooki,* which are pervasive in urban aquatic environments, and while originally introduced to Australia to control mosquito populations, have now been shown to adversely impact other aquatic wildlife, particularly frogs (Webb and Joss 1997, Hamer et al. 2002). Recent research has suggested that non-native predators may indirectly increase mosquito abundance through altering competition or predation relationships (Bucciarelli et al. 2019).

Understanding responses of different taxa to wetland traits that are specific to urban wetlands is vital to manage these habitats for their multiple values. The ecology of individual mosquito species should be considered in conjunction with wetland habitat traits to assess potential mosquito risks associated with an urban wetland. For example, *Ma. uniformis* has a unique ecology that means they are closely associated with floating vegetation (Webb et al. 2016). Filamentous algae has been shown to enhance conditions for *Anopheles* spp. whose larvae exploit small pockets of water above the floating algal mats (Webb et al. 2016). Similarly, some *Coquillettidia* and *Mansonia* spp. exhibit a preference for wetlands which support specific macrophyte types, either emergent or floating vegetation, that may be related to the biological requirements of these mosquitoes; their larvae have specialised siphons that pierce submerged parts of aquatic plants. From this, it appears that some mosquito species are more abundant in higher quality wetlands, such as *Cq. linealis* and *Ma. uniformis*, and others in poor quality wetlands, such as *Cx. annulirostris* and *Cx. quinquefasciatus*. These species may be actively selecting poor quality wetlands as desirable ovipositing habitat, or their ecology may make these species less vulnerable to predation by hiding in vegetation mats. It is widely acknowledged that the interactions between aquatic vegetation and production of mosquitoes in constructed treatment wetlands are complicated, and difficult to determine if it is plant species or plant density (and therefore vulnerability to predation) that is responsible for observed patterns of abundance (Jiannino and Walton 2004). In addition, some mosquito species, such as *Cx. quinquefasciatus*, may be utilising nearby habitats within urban environments (e.g. water-filled containers in residential dwellings) while others, such as *Cx. annulirostris*, may be exploiting nearby ephemeral habitats.

The mosquito species of greatest concern for freshwater urban wetlands is *Cx. annulirostris* as it is a nuisance-biting pest and vector of pathogens including Ross River virus, Barmah Forest virus, Kunjin virus and Japanese encephalitis (Webb et al. 2016). While *Cx. annulirostris* abundance varied between sites and seasons, it was detected at 21 of our 23 sites, confirming its status as a ubiquitous potential threat and pest species. Interestingly, the two sites where we did not detect *Cx. annulirostris* were also the sites surrounded by the most bushland (WOL02 and SUT01). Surrounding vegetation may have inhibited *Cx. annulirostris* dispersal and therefore plays a relatively more important role in abundance compared to wetland quality in urban and peri-urban areas. Alternatively, perhaps abundance of terrestrial predators of mosquitoes was higher at these sites. Predators such as insectivorous bats have been shown to be more active in urban forested areas compared to open areas, even though prey was more abundant in open areas and proportionally more bat feeding activity occurred in open areas (Gonsalves et al. 2013b).

Spatial and temporal assemblage variations

In temperate regions globally, mosquito assemblages show strong seasonal variations in identity and abundance (e.g. Schafer et al. 2004, Williams et al. 2009, Claflin and Webb 2016). However, while much of the world also experiences seasonal variations in macroinvertebrate assemblages (Sporka et al. 2006, Leung et al. 2012), our study confirms a small number of others (Tippler et al. 2014) that demonstrate this pattern does not apply to temperate Australian macroinvertebrate assemblages. Macroinvertebrate assemblages, diversity and richness did not vary significantly between seasons, suggesting that assessments of potential mosquito risks associated with urban wetlands might use macroinvertebrates as a measure of aquatic health and habitat quality and availability throughout the year, and these data on aquatic health and habitat could then be cross-referenced with requirements of the mosquito species of interest (based on geographical location and season), rather than looking at macroinvertebrates as direct biocontrol agents of mosquitoes. Aquatic macroinvertebrates are widely considered to provide a rapid and robust assessment of aquatic health (Faith and Norris 1989, Rosenberg and Resh 1993, Marchant et al. 1995). However, it seems that in the context of urban wetlands, which are frequently degraded, homogenised systems, mosquito assemblages should not be directly associated with macroinvertebrates themselves.

Links between urban wetland biota and surrounding landscape

Aquatic diversity measures and mosquito abundance both displayed strong relationships with larger-scale physical traits considered to be part of the wetland design, especially the type of water inlet and water outlet, despite these variables being unrelated to aquatic habitat availability within the wetland, or to the type of immediate surrounding land use. The lower water level and possible ephemeral water supply in creek inlets and outlets may contribute to fewer predators of mosquito larvae, or increased refuge and food availability for macroinvertebrates. Differences between types of inlets and outlets, and between the inlets and outlets themselves, should therefore be considered alongside mosquito ecology to determine appropriate designs and management for specific geographical areas to identify conflicts and synergies with improving biodiversity resources. Evaluating the specific contribution of these inlet and outlet areas to the services and disservices associated with urban wetlands would provide further contributions to explain interactions between biodiversity and mosquito abundance.

Balancing multiple values in the urban environment

Evidence is growing that homogenisation of the built environment in urban areas is reflected in, and causes, homogenisation of ecological communities and ecosystem functions around the world (McKinney 2006, Groffman et al. 2014). Previous research has demonstrated that macroinvertebrate communities in urban wetlands are relatively homogeneous, independently of the degree of surrounding urbanisation (Mackintosh et al. 2015), however studies have not looked at how this may be related to wetland design. Walsh et al. (2001) suggested that to survive in urban waterways, aquatic macroinvertebrates must not only be tolerant of pollutants, but also possess life history traits that allow re-establishment of populations following frequent irregular disturbances, such as increased frequency and intensity of floods due to more efficient stormwater drainage.

While aquatic habitat availability and quality is commonly considered to directly influence biotic communities (Parsons et al. 2002), the overall disconnect between aquatic habitat and macroinvertebrate assemblages found in our study may be due to widespread homogenisation of aquatic habitats by the pervasive pressures associated with urbanisation (Roy et al. 2003, Mackintosh et al. 2017), such as increased sediment and nutrient inputs, limited fluvial connectivity and pulse stress associated with rainfall onto impervious surfaces. These urbanisation pressures are magnified in areas where drainage systems are dominated by pipes as opposed to open, pervious channels which increase infiltration and thereby somewhat mitigate the effects of pollution in a highly impervious catchment (Walsh et al. 2001). These place constraints on aquatic macroinvertebrate communities irrespective of created aquatic habitat. That our samples were mostly characterized by high abundances of a few hardy taxa is typical of degraded urban streams around the world so perhaps not a surprising find in urban wetlands fed by the same streams.

When considering how best to design and manage urban wetlands for multiple, sometimes conflicting values, managers must balance features that are intended to address the poor water quality that inhibits biodiversity, as well as features intended to reduce mosquito risks.

Our finding that potential mosquito risks remained unchanged by wetland size, despite a perceived increased in available oviposition area, contrasts with that of Schafer et al. (2004) who found mosquito abundance across constructed and natural wetlands in southern Sweden was significantly correlated with wetland size, though mosquito diversity showed no correlation with wetland size. This discrepancy between findings might be due to homogenisation of urban wetlands compared to non-urban wetlands, or perhaps geographical differences in mosquito species.

Also important to note is that abundances of mosquito species of concern were unrelated to the edge complexity score, despite a common recommendation for reducing mosquito risks being to construct wetlands with a low edge to area ratio (Mosquito Control Association of Australia 2008). This contrasts with evidence for increasing habitat suitability for birds (Murray et al. 2013), highlighting the difficulty in managing wetlands for multiple values, and also the lost opportunities for creating synergistic benefits when evidence of mosquito use and requirements is lacking. To further complicate managing urban wetlands for multiple values, potential threats to public health are shaped not only by the identity of mosquito species present at the wetland, but the potential for these species to encounter reservoir hosts for pathogens, which may also be related to wetland characteristics (Knight et al. 2003).

Conclusions

We found no coherent signal between aquatic macroinvertebrate assemblages and mosquitoes collectively, although some mosquito species exhibit strong associations. It is vitally important that mosquitoes be considered as individual species, not as a single entity, when discussing potential risks and benefits of urban wetlands, as mosquito species have vastly different habitat requirements and will not respond to the same, or even to similar, cues.

While there may be little interaction between aquatic macroinvertebrates and abundance of dominant mosquito species in our urban wetland systems, we have shown that both macroinvertebrates and mosquitoes have complex responses to aquatic habitat traits at a range of temporal and spatial scales. These complex responses can be used to better design and manage urban wetlands to maximise their multiple values without increasing mosquito risks.

Acknowledgements

This work was conducted under NSW National Parks and Wildlife Service Scientific Licence SL101581. We thank the various local councils and Western Sydney Parklands for granting access to the study wetlands. This work was conducted with support from a Holsworth Wildlife Research Endowment and a Society of Wetlands Scientists Student Research Grant both awarded to J. Hanford.

References

An, S., H. Li, B. Guan, C. Zhou, Z. Wang, Z. Deng, Y. Zhi, Y. Liu, C. Xu, S. Fang, J. Jiang, and H. Li. 2007. China's natural wetlands: Past problems, current status, and future challenges. Ambio **36**:335-342.

Anderson, M. J., R. N. Gorley, and K. R. Clarke. 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E Ltd., Plymouth, U.K.

Batzer, D. P., and S. A. Wissinger. 1996. Ecology of insect communities in nontidal wetlands. Annual Review of Entomology **41**:75-100.

Bengis, R. G., F. A. Leighton, J. R. Fischer, M. Artois, T. Morner, and C. M. Tate. 2004. The role of wildlife in emerging and re-emerging zoonoses. Revue Scientifique et Technique Office International des Epizooties **23**:497-511.

Brand, A. B., and J. W. Snodgrass. 2010. Value of artificial habitats for amphibian reproduction in altered landscapes. Conservation Biology **24**:295-301.

Bucciarelli, G. M., D. Suh, A. Davis, D. Roberts, D. Sharpton, H. B. Shaffer, R. N. Fisher, and L. B. Kats. 2019. Assessing effects of non-native crayfish on mosquito survival. Conservation Biology **33**:122-131.

Bugnot, A. B., G. C. Hose, C. J. Walsh, O. Floerl, K. French, K. A. Dafforn, J. Hanford, E. C. Lowe, and A. K. Hahs. 2019. Urban impacts across realms: Making the case for inter-realm monitoring and management. Science of the Total Environment **648**:711-719.

Chessman, B. C., and P. K. McEvoy. 2012. Insights into human impacts on streams from tolerance profiles of macroinvertebrate assemblages. Water Air and Soil Pollution **223**:1343-1352.

Chessman, B. C., K. M. Trayler, and J. A. Davis. 2002. Family- and species-level biotic indices for macroinvertebrates of wetlands on the Swan Coastal Plain, Western Australia. Marine and Freshwater Research **53**:919-930.

Claflin, S. B., and C. E. Webb. 2016. Surrounding land use significantly influences adult mosquito abundance and species richness in urban mangroves. Wetlands Ecology and Management **25**:331-344.

Costanza, R., R. d'Arge, R. deGroot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. O'Neill, J. Paruelo, R. Raskin, P. Sutton, and M. van den Belt. 1997. The value of the world's ecosystem services and natural capital. Nature **387**:253-260.

Crocker, W., K. Maute, C. Webb, and K. French. 2017. Mosquito assemblages associated with urban water bodies: Implications for pest and public health threats. Landscape and Urban Planning **162**:115-125.

Culler, L. E., and W. O. Lamp. 2009. Selective predation by larval *Agabus* (Coleoptera: Dytiscidae) on mosquitoes: Support for conservation-based mosquito suppression in constructed wetlands. Freshwater Biology **54**:2003-2014.

Cunningham, A. A., P. Daszak, and J. L. N. Wood. 2017. One Health, emerging infectious diseases and wildlife: Two decades of progress? Philosophical Transactions of the Royal Society of London B Biological Sciences **372**:20160167.

Dale, P. E. R., M. Greenway, H. Chapman, and M. J. Breitfuss. 2007. Constructed wetlands for sewage effluent treatment and mosquito larvae at two sites in subtropical Australia. Journal of the American Mosquito Control Association **23**:109-116.

Davidson, N. C. 2014. How much wetland has the world lost? Long-term and recent trends in global wetland area. Marine and Freshwater Research **65**:934-941.

Davies, P. J., I. A. Wright, S. J. Findlay, O. J. Jonasson, and S. Burgin. 2010. Impact of urban development on aquatic macroinvertebrates in south eastern Australia: Degradation of in-stream habitats and comparison with non-urban streams. Aquatic Ecology **44**:685-700.

Dean, J. C. 2004. Identification keys to Australian families and genera of caddis-fly larvae (Trichoptera). Co-operative Research Centre for Freshwater Ecology, Albury, Australia.

Department of Land and Water Conservation. 1998. The Constructed Wetlands Manual.*in* N. Department of Land and Water Conservation, editor. Department of Land and Water Conservation, NSW, Sydney, Australia.

Doggett, S. L., J. Clancy, J. Haniotis, C. E. Webb, L. Hueston, M. Marchetti, E. Blyth, S. Howard, D. E. Dwyer, and R. C. Russell. 2009. Arbovirus and vector surveillance in NSW, 2004/05-2007-08. Arbovirus Research in Australia **10**:28-37.

Dunn, H. 2000. Identifying and Protecting Rivers of High Ecological Value.*in* Land and Water Resources Research and Development Corporation, editor. Land and Water Resources Research and Development Corporation,, Canberra ACT.

Eaton, T. T. 2018. Approach and case-study of green infrastructure screening analysis for urban stormwater control. Journal of Environmental Management **209**:495-504.

Faith, D. P., and R. H. Norris. 1989. Correlation of environmental variables with patterns of distribution and abundance of common and rare freshwater macroinvertebrates. Biological Conservation **50**:77-98.

Freeman, P. L., and M. S. Schorr. 2004. Influence of watershed urbanization on fine sediment and macroinvertebrate assemblage characteristics in Tennessee Ridge and Valley Streams. Journal of Freshwater Ecology **19**:353-362.

Gonsalves, L., B. Bicknell, B. Law, C. Webb, and V. Monamy. 2013a. Mosquito consumption by insectivorous bats: Does size matter? Plos One **8**:e77183.

Gonsalves, L., S. Lamb, C. Webb, B. Law, and V. Monamy. 2013b. Do mosquitoes influence bat activity in coastal habitats? Wildlife Research **40**:10-24.

Gonsalves, L., B. Law, C. Webb, and V. Monamy. 2013c. Foraging ranges of insectivorous bats shift relative to changes in mosquito abundance. Plos One **8**:e64081.

Gooderham, J., and E. Tsyrlin. 2003. The waterbug book: A guide to the freshwater macroinvertebrates of temperate Australia. CSIRO Publishing, Collingwood Victoria.

Greenway, M., P. Dale, and H. Chapman. 2003. An assessment of mosquito breeding and control in four surface flow wetlands in tropical-subtropical Australia. Water Science and Technology **48**:249-256.

Groffman, P. M., J. Cavender-Bares, N. D. Bettez, J. M. Grove, S. J. Hall, J. B. Heffernan, S. E. Hobbie, K. L. Larson, J. L. Morse, C. Neill, K. Nelson, J. O'Neil-Dunne, L. Ogden, D. E. Pataki, C. Polsky, R. R. Chowdhury, and M. K. Steele. 2014. Ecological homogenization of urban USA. Frontiers in Ecology and the Environment **12**:74-81.

Hamer, A. J., S. J. Lane, and M. J. Mahony. 2002. The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia. Oecologia **132**:445-452.

Harley, D., A. Sleigh, and S. A. Ritchie. 2001. Ross River virus transmission, infection, and disease: A cross-disciplinary review. Clinical Microbiology Reviews **14**:909-932.

Hassall, C., J. Hollinshead, and A. Hull. 2011. Environmental correlates of plant and invertebrate species richness in ponds. Biodiversity and Conservation **20**:3189-3222.

Hawking, J. H., L. M. Smith, K. LeBusque, and C. Davey. 2013. Identification and Ecology of Australian Freshwater Invertebrates. http://www.mdfrc.org.au/bugguide. Accessed 09/08/2016.

Jeffery, J. A., P. A. Ryan, S. A. Lyons, and B. H. Kay. 2002. Vector competence of *Coquillettidia linealis* (Skuse)(Diptera: Culicidae) for Ross River and Barmah Forest viruses. Australian Journal of Entomology **41**:339-344.

Jiannino, J. A., and W. E. Walton. 2004. Evaluation of vegetation management strategies for controlling mosquitoes in a southern California constructed wetland. Journal of the American Mosquito Control Association **20**:18-26.

Jucker, T., B. Wintle, G. Shackelford, P. Bocquillon, J. L. Geffert, T. Kasoar, E. Kovacs, H. S. Mumby, C. Orland, J. Schleicher, E. R. Tew, A. Zabala, T. Amano, A. Bell, B. Bongalov, J. M. Chambers, C. Corrigan, A. P. Durán, L.-A. Duvic-Paoli, C. Emilson, E. J. S. Emilson, J. F. da Silva, E. E. Garnett, E. J. Green, M. K. Guth, A. Hacket-Pain, A. Hinsley, J. Igea, M. Kunz, S. H. Luke, W. Lynam, P. A. Martin, M. H. Nunes, N. Ockendon, A. Pavitt, C. L. R. Payne, V. Plutshack, T. T. Rademacher, R. J. Robertson, D. C. Rose, A. Serban, B. I. Simmons, C. Tayleur, C. F. R. Wordley, and N. Mukherjee. 2018. Ten-year assessment of the 100 priority questions for global biodiversity conservation. Conservation Biology **32**:1457-1463.

Kassim, N. F., C. E. Webb, Q. Wang, and R. C. Russell. 2013. Australian distribution, genetic status and seasonal abundance of the exotic mosquito *Culex molestus* (Forskal)(Diptera: Culicidae). Australian Journal of Entomology **52**:185-198.

Kay, B. H., I. D. Fanning, and J. G. Carley. 1982. Vector competence of *Culex pipiens quinquefasciatus* for Murray Valley encephalitis, Kunjin, and Ross River viruses from Australia. The American Journal of Tropical Medicine and Hygiene **31**:844-848.

Knight, R. L., W. E. Walton, G. F. O'Meara, W. K. Reisen, and R. Wass. 2003. Strategies for effective mosquito control in constructed treatment wetlands. Ecological Engineering **21**:211-232.

Kumar, R., and J. S. Hwang. 2006. Larvicidal efficiency of aquatic predators: A perspective for mosquito biocontrol. Zoological Studies **45**:447-466.

Kumar, R., P. Muhid, H. U. Dahms, L. C. Tseng, and J. S. Hwang. 2008. Potential of three aquatic predators to control mosquitoes in the presence of alternative prey: A comparative experimental assessment. Marine and Freshwater Research **59**:817-835.

Land and Property Information. 2015. NSW Imagery Web Services.*in* Land and Property Information, editor.

Le Viol, I., J. Mocq, R. Julliard, and C. Kerbiriou. 2009. The contribution of motorway stormwater retention ponds to the biodiversity of aquatic macroinvertebrates. Biological Conservation **142**:3163-3171.

Leung, A. S. L., A. O. Y. Li, and D. Dudgeon. 2012. Scales of spatiotemporal variation in macroinvertebrate assemblage structure in monsoonal streams: The importance of season. Freshwater Biology **57**:218-231.

Lühken, R., W. P. Pfitzner, J. Börstler, R. Garms, K. Huber, N. Schork, S. Steinke, E. Kiel, N. Becker, E. Tannich, and A. Krüger. 2014. Field evaluation of four widely used mosquito traps in Central Europe. Parasites & vectors **7**:268-268.

Ma, Z., Y. Cai, B. Li, and J. Chen. 2010. Managing wetland habitats for waterbirds: An international perspective. Wetlands **30**:15-27.

Mackintosh, T. J., J. A. Davis, and R. M. Thompson. 2015. The influence of urbanisation on macroinvertebrate biodiversity in constructed stormwater wetlands. Science of the Total Environment **536**:527-537.

Mackintosh, T. J., J. A. Davis, and R. M. Thompson. 2017. The effects of urbanization on trophic relationships in constructed wetlands. Freshwater Science **36**:138-150.

Marchant, R., L. A. Barmuta, and B. C. Chessman. 1995. Influence of sample quantification and taxonomic resolution on the ordination of macroinvertebrate communities from running waters in Victoria, Australia. Marine and Freshwater Research **46**:501-506.

McKinney, M. L. 2006. Urbanization as a major cause of biotic homogenization. Biological Conservation **127**:247-260.

McKinney, R. A., K. B. Raposa, and R. M. Cournoyer. 2011. Wetlands as habitat in urbanizing landscapes: Patterns of bird abundance and occupancy. Landscape and Urban Planning **100**:144-152.

Moore, T. L. C., and W. F. Hunt. 2012. Ecosystem service provision by stormwater wetlands and ponds - A means for evaluation? Water Research **46**:6811-6823.

Mosquito Control Association of Australia. 2008. Mosquito Management in Engineered Wetlands.*in* Mosquito Control Association of Australia Inc., editor. Australian Mosquito Control Manual, Sydney, Australia.

Murray, C. G., S. Kasel, R. H. Loyn, G. Hepworth, and A. J. Hamilton. 2013. Waterbird use of artificial wetlands in an Australian urban landscape. Hydrobiologia **716**:131-146.

Pankratz, S., T. Young, H. Cuevas-Arellano, R. Kumar, R. F. Ambrose, and I. H. Suffet. 2007. The ecological value of constructed wetlands for treating urban runoff. Water Science and Technology **55**:63-69.

Parsons, M., M. C. Thoms, and R. H. Norris. 2002. Austrlian River Assessment System: Review of Physical River Assessment Methods - A Biological Perspective. Commonwealth of Australia and University of Canberra, Canberra.

Parsons, M., M. C. Thoms, and R. H. Norris. 2004. Development of a standardised approach to river habitat assessment in Australia. Environmental Monitoring and Assessment **98**:109-130.

Ramsar Convention on Wetlands. 2018. Global Wetland Outlook: State of the World's Wetlands and their Services to People. Gland, Switzerland.

Rohe, D. L., and R. P. Fall. 1979. A miniature battery powered CO2 baited light trap for mosquito borne encephalitis surveillance. Bulletin of the Society of Vector Ecology **4**:21-27.

Rosenberg, D. M., and V. H. Resh. 1993. Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall, New York.

Roy, A. H., A. D. Rosemond, M. J. Paul, D. S. Leigh, and J. B. Wallace. 2003. Stream macroinvertebrate response to catchment urbanisation (Georgia, U.S.A.). Freshwater Biology **48**:329-346.

Russell, R. C. 1993. Mosquitoes and mosquito-borne disease in southeastern Australia: A guide to the biology, relation to disease, surveillance, control and the identification of mosquitoes in southeastern Australia. Westmead Hospital, Department of Medical Entomology.

Russell, R. C. 1999. Constructed wetlands and mosquitoes: Health hazards and management options—An Australian perspective. Ecological Engineering **12**:107-124.

Schafer, M. L., J. O. Lundstrom, M. Pfeffer, E. Lundkvist, and J. Landin. 2004. Biological diversity versus risk for mosquito nuisance and disease transmission in constructed wetlands in southern Sweden. Medical and Veterinary Entomology **18**:256-267.

Scheffers, B. R., and C. A. Paszkowski. 2013. Amphibian use of urban stormwater wetlands: The role of natural habitat features. Landscape and Urban Planning **113**:139-149.

Soulsbury, C. D., and P. C. L. White. 2015. Human-wildlife interactions in urban areas: a review of conflicts, benefits and opportunities. Wildlife Research **42**:541-553.

Spieles, D. J., and W. J. Mitsch. 2000. Macroinvertebrate community structure in high- and low-nutrient constructed wetlands. Wetlands **20**:716-729.

Sporka, F., H. E. Vlek, E. Bulankova, and I. j. Krno. 2006. Influence of seasonal variation on bioassessment of streams using macroinvertebrates. Hydrobiologia **566**:543-555.

Stephenson, E. B., A. J. Peel, S. A. Reid, C. C. Jansen, and H. McCallum. 2018. The non-human reservoirs of Ross River virus: A systematic review of the evidence. Parasites & vectors **11**.

Suaya, J. A., D. S. Shepard, J. B. Siqueira, C. T. Martelli, L. C. S. Lum, L. H. Tan, S. Kongsin, S. Jiamton, F. Garrido, R. Montoya, B. Armien, R. Huy, L. Castillo, M. Caram, B. K. Sah, R. Sughayyar, K. R. Tyo, and S. B. Halstead. 2009. Cost of Dengue cases in eight countries in the Americas and Asia: A prospective study. The American Journal of Tropical Medicine and Hygiene **80**:846-855.

Theischinger, G. 2002. Preliminary keys for the identification of larvae of the Australian Petaluridae, Archipetaliidae, Austropetaliidae, Telephlebiidae and Aeshnidae (Odonata). CRC for Freshwater Ecology, Albury, N.S.W.

Tippler, C., S. Findlay, I. A. Wright, P. J. Davies, C. Evans, and M. Ahmed. 2014. Does seasonality influence freshwater macroinvertebrate communities in the temperate paradise of Sydney? Pages 292-299 *in* G. Vietz, I. D. Rutherfurd, and R. Hughes, editors. 7th Australian Stream Management Conference, Townsville, Queensland.

Turak, E., N. Waddell, and G. Johnston. 2004. New South Wales Australian River Assessment Sampling and Processing Manual. Department of Environment and Conservation (NSW), Sydney.

United Nations. 2018. World Urbanization Prospects: The 2018 Revision.*in* Department of Economic and Social Affairs (Population Division), editor. United Nations, New York.

Van Der Maarel, E. 1979. Transformation of cover abundance values in phytosociology and its effects on community similarity. Vegetatio **39**:97-114.

Vora, N. 2008. Impact of anthropogenic environmental alterations on vector-borne diseases. The Medscape Journal of Medicine **10**:238-238.

Walsh, C. J., A. K. Sharpe, P. F. Breen, and J. A. Sonneman. 2001. Effects of urbanization on streams of the Melbourne region, Victoria, Australia. I. Benthic macroinvertebrate communities. Freshwater Biology **46**:535-551.

Walsh, M. G., and C. Webb. 2018. Hydrological features and the ecological niches of mammalian hosts delineate elevated risk for Ross River virus epidemics in anthropogenic landscapes in Australia. Parasites & vectors **11**:192.

Walton, W. E. 2012. Design and management of free water surface constructed wetlands to minimize mosquito production. Wetlands Ecology and Management **20**:22.

Walton, W. E., and P. D. Workman. 1998. Effect of marsh design on the abundance of mosquitoes in experimental constructed wetlands in southern California. Journal of the American Mosquito Control Association **14**:95-107.

Watson, T. M., A. Saul, and B. H. Kay. 2000. *Aedes notoscriptus* (Diptera: Culicidae) survival and dispersal estimated by mark-release-recapture in Brisbane, Queensland, Australia. Journal of Medical Entomology **37**:380-384.

Watts, C. H. S. 2002. Checklists and Guides to the Identification, to Genus, of Adult and Larval Australian Water Beetles of the Families Dytiscidae, Noteridae, Hygrobiidae, Halplidae, Gyrinidae, Hydranidae and the Superfamily Hydrophiloidea (Insecta: Coleoptera). CRC for Freshwater Ecology, Albury N.S.W.

Webb, C., S. Doggett, and R. Russell. 2016. A Guide to Mosquitoes of Australia. CSIRO Publishing, Clayton South, Victoria.

Webb, C., and J. Joss. 1997. Does predation by the fish *Gambusia holbrooki* (Atheriniformes: Poeciliidae) contribute to declining frog populations? Australian Zoologist **30**:316-324.

Webb, C. E., S. L. Doggett, K. J. Willems, R. C. Russell, and M. J. Geary. 2001. Mosquito species recorded from the Sydney basin region of NSW. Arbovirus Research in Australia **8**:391-394.

Wellborn, G. A., D. K. Skelly, and E. E. Werner. 1996. Mechanisms creating community structure across a freshwater habitat gradient. Annual Review of Ecology and Systematics **27**:337-363.

Willems, K. J., C. E. Webb, and R. C. Russell. 2005a. A comparison of mosquito predation by the fish *Pseudomugil signifier* Kner and *Gambusia holbrooki* (Girard) in laboratory trials. Journal of Vector Ecology **30**:87-90.

Willems, K. J., C. E. Webb, and R. C. Russell. 2005b. Tadpoles of four common Australian frogs are not effective predators of the common pest and vector mosquito *Culex annulirostris*. Journal of the American Mosquito Control Association **21**:492-494.

Williams, C. R., C. A. Bader, S. R. Williams, and P. I. Whelan. 2012. Adult mosquito trap sensitivity for detecting exotic mosquito incursions and eradication: A study using EVS traps and the Australian southern saltmarsh mosquito, *Aedes camptorhynchus*. Journal of Vector Ecology **37**:110-116.

Williams, C. R., S. R. Williams, J. Nicholson, S. M. Little, J. Riordan, S. R. Fricker, and M. J. Kokkinn. 2009. Diversity and seasonal succession of coastal mosquitoes (Diptera: Culicidae) in the northern Adelaide region of South Australia. Australian Journal of Entomology **48**:107-112.

Williams, P., M. Whitfield, J. Biggs, S. Bray, G. Fox, P. Nicolet, and D. Sear. 2003. Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. Biological Conservation **115**:329-341.

World Health Organisation. 2013. World Malaria Report 2013. Geneva.

World Health Organisation. 2014. A global brief on vector-borne diseases. Geneva.

Chapter 4:

Human population density is linked to abundance of container-inhabiting mosquitoes at urban wetlands

The supplementary material for this paper is available in Appendix 2:

Supplementary Table 2.1. Details of study wetlands locations. Page 172.

Abstract

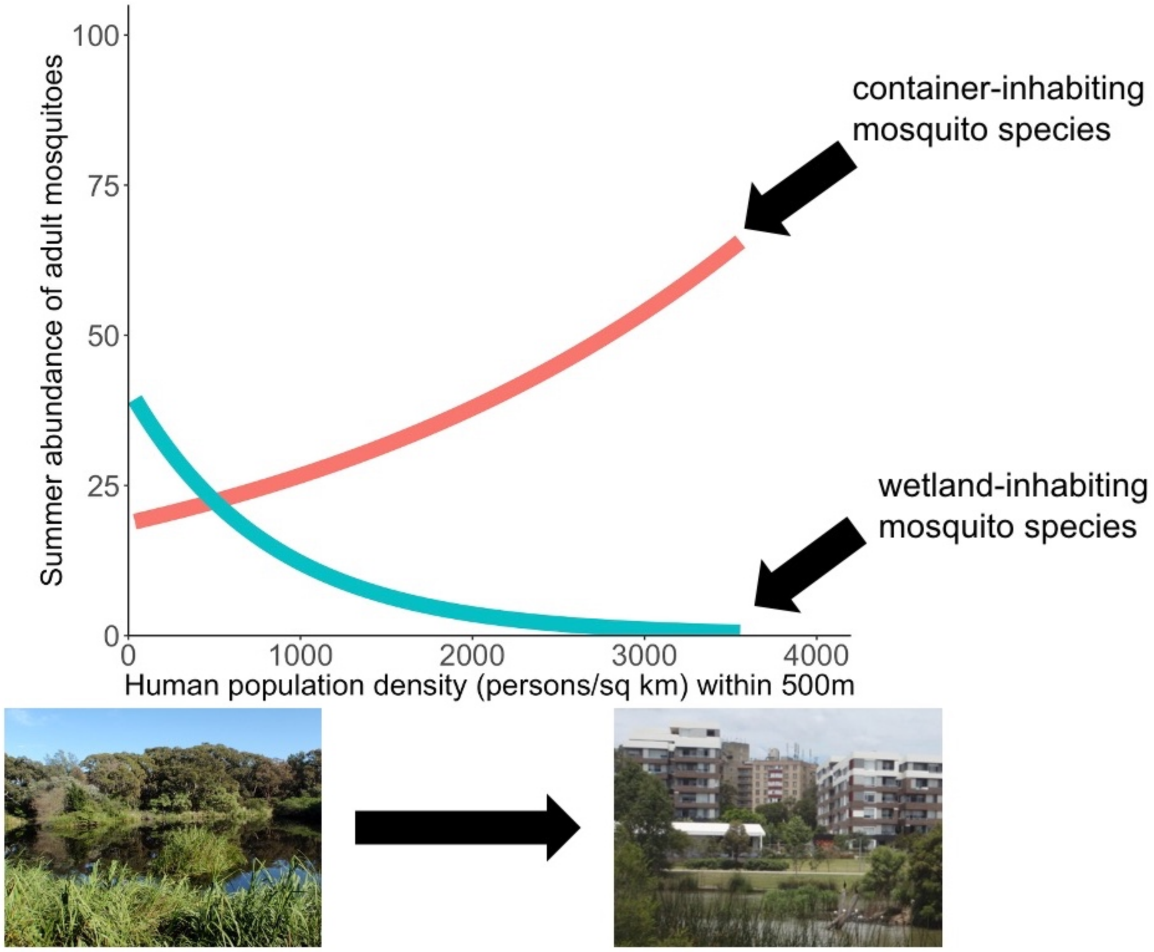
Conserving nature in cities and reducing human exposure to pest and disease risks are some of the many environmental and social challenges associated with urbanisation. Constructed wetlands are a popular solution for managing increased stormwater volumes associated with urbanisation due to their cost-effectiveness, ecological services, and human wellbeing benefits. Reducing the potential for these urban wetlands to create mosquito problems for adjacent communities requires a better understanding of the relationships between human landscape traits and the mosquitoes found around urban wetlands.

We sampled adult mosquitoes at freshwater wetlands across highly urban through to suburban and bushland sites. We analysed patterns of mosquito species abundance against human population density at the scale of 100m, 500m and 1000m surrounding the wetland to investigate relationships between potential mosquito problems and human population density at multiple landscape scales. The abundance of mosquito species known to inhabit container habitats was positively related to surrounding human population densities at the fine scales of 100m and 500m, but not 1000m. Conversely, the abundance of mosquito

species known to utilise wetland habitats was negatively related to human population density at 100m and 500m, and again not at 1000m.

The strong relationships between abundance of different mosquito species and fine scale human population densities reflect changes in mosquito habitat availability associated with human landscape traits. Identifying the sources of problematic mosquito species is critical to avoid adverse impacts on both wetland conservation and public health and wellbeing. Assumptions on the sources of pest mosquitoes, such as blaming wetlands for mosquito problems, are not always well placed. Sampling at multiple scales is therefore essential to inform landscape management in urban areas and maintain public support for natural habitats in cities.

Graphical Abstract



Introduction

As urban areas expand around the world, decentralised stormwater management is increasingly recognised as an environmentally, economically and socially preferable option for managing the associated increased volume of stormwater. Urban design with provision of ecosystem services in mind is known by many names, including Water Sensitive Urban Design in Australia, Nature-Based Solutions in Europe, Sponge Cities in China, and Low Impact Development in the USA (Kuller et al. 2019), the centrepiece of many of these solutions involves constructed permanent or ephemeral urban wetlands. These wetlands capture stormwater with the intention of improving water quality, reducing flood risk, decreasing erosion and facilitating groundwater recharge. Constructed urban wetlands can also simultaneously provide food and shelter resources for urban wildlife, including threatened birds, frogs, reptiles, insects and mammals (e.g. McKinney et al. 2011, Hamer et al. 2012, Goertzen and Suhling 2013, Murray et al. 2013, Faller and McCleery 2017). These areas can provide attractive recreation spaces for communities, increasing health and wellbeing (Carrus et al. 2015, Taylor et al. 2018). And economically, wetlands for stormwater management offer a more cost-effective solution compared to traditional water treatment (Kadlec and Wallace 2009, Lentz et al. 2014). Constructed urban wetlands will continue to be a critical contributor to maintaining liveable cities with adequate green and blue space.

Concerns that urban wetlands may provide ‘mosquito-friendly’ habitats have been raised for many years (Russell 1999), yet wetland and mosquito research rarely intersects, presenting significant challenges to urban managers and wetland designers looking to implement best practice without creating mosquito risks. When outbreaks of life-threatening and/or debilitating mosquito-borne diseases are reported, such as West Nile virus in the USA, Ross River virus in Australia, and Japanese encephalitis virus across south-east Asia, we see a resurgence in concern regarding wetland environments. Notwithstanding the human health risks posed by mosquitoes associated with wetlands, container-inhabiting mosquitoes in many urban areas of the world also pose a risk of transmitting pathogens such as dengue, chikungunya, and Zika viruses responsible for potentially serious illness. Even in the absence of disease, mosquitoes can present a major nuisance-biting issue which people are willing to pay to control in order to maintain their quality of outdoor and indoor life (Dickinson and Paskewitz 2012, Darbro et al. 2017).

Given public fear and dislike of mosquitoes, and the potential economic and wellbeing impacts of the pathogens they transmit, understanding the sources of mosquitoes in urban landscapes and how to mitigate potential risks before they become problematic is critically important in urban planning, environmental management and pest management.

Urban areas can support diverse and abundant mosquito populations that reflect the wide range of suitable habitats available. Many problematic mosquito species of pest and public health concern are associated with water-holding containers, such as water tanks, clogged roof gutters and planter pot saucers around suburban balconies and backyards (Webb et al. 2016). The assemblages of mosquitoes inhabiting these container habitats are vastly different to those inhabiting wetlands, and require not only different mitigation actions, but perhaps more importantly, different considerations in landscape and urban planning that take into account human behaviours and anthropogenic impacts on mosquito communities and their habitats (Ferraguti et al. 2016, Madewell et al. 2019).

Given the increasing abundance of freshwater wetlands in urban areas, it is important to understand relationships between mosquitoes found in these wetland areas and the surrounding human population. Wetlands are frequently incorrectly blamed as epicentres of mosquito problems in urban areas (Johnson et al. 2012, de Jesús Crespo et al. 2018), leading to habitat modifications that reduce a wetland’s potential value to the environment and community. Furthermore, there is evidence that urban wetlands do not necessarily increase local abundance of mosquitoes, and that anthropogenic landscape traits may be especially important when assessing urban mosquito risks and patterns of abundance (Claflin and Webb 2017, Hanford et al. 2019, Hanford et al. 2020). It is, therefore, essential we understand associations between urban wetlands, the human landscape they are sited in, and potential mosquito risks, to inform urban planning strategies and adaptive wetland design and management. This will allow site- and/or mosquito-specific assessment of potential disease risks, development of targeted public education, and indicates where mitigation may be required.

Human population density and associated activity may be directly and indirectly influencing the mosquito abundance and diversity around urban wetlands. Urban density around waterways is a key predictor of water quality degradation and loss of aquatic biodiversity (Walsh et al. 2001). The proximity and density of humans to coastal wetlands has also been identified as a risk factor for mosquito-borne disease (Vally et al. 2012). Notwithstanding the dispersal of mosquitoes from urban wetlands into surrounding urban areas, a suite of mosquito species associated with non-wetland habitats within urban areas may be moving into the environment surrounding urban wetlands. These mosquitoes may be contributing to pest and public health concerns but also confounding the response of local authorities to managing mosquito risk with ill-informed response decisions.

The aim of this study was to test relationships between human population density at multiple spatial scales and the abundance of mosquitoes of pest and public health concern at urban wetlands. We used surrounding human population density as a measurable landscape trait associated with urbanisation and examined relationships with mosquito communities to determine how anthropogenic landscape traits may shape potential mosquito risk.

Materials and Methods

Study area

The greater Sydney region has a temperate climate and stretches up to 100 km inland from the coast. The region's fast-growing urban population (approx. 5.1 million people, CoS 2016) and expanding urban fringe are typical of many large temperate cities around the world. Hundreds of freshwater wetlands exist in the region. In addition to remnant waterbodies and ornamental ponds, there are some naturally occurring and repurposed for stormwater management while others are specifically constructed to manage the increased volumes of stormwater associated with urbanisation. There are also a handful of freshwater wetlands constructed to provide habitat for urban wildlife, but more often this is a secondary design purpose. We surveyed a range of wetlands including some built for stormwater management, remnant natural wetlands and some built as wildlife habitat (Hanford et al. 2019). Wetland locations ranged from highly urbanized areas to native parklands and bushland areas representing a continuum from high population density to low population density (Supp. Table 2.1).

Mosquitoes

We collected adult mosquitoes using carbon dioxide-baited Encephalitis Virus Surveillance (EVS) traps (Rohe and Fall 1979) at 22 freshwater wetlands across the greater Sydney region in summer (January) and again in autumn (March) in 2016. Traps were set at least 2h before dusk in vegetation at the perimeter of the wetlands and likely to provide adult mosquito refuge from wind and direct sunlight. Traps were collected the following morning. Mosquitoes were transported to the laboratory where traps were placed in a freezer for approximately 10min to ‘knock down’ specimens, which were then transferred to plastic petri dishes and stored at -20°C until counting and identification using the keys of (Russell 1993) and (Webb et al. 2016). The abundance of each mosquito species was recorded according to trap date and individual wetland.

We also recorded wetland traits including the type of inlet and outlet (pipe, gross pollutant trap, channel/ditch) and immediate surrounding land use (residential, parkland, bushland) and related these to abundance of different mosquito species. This work with wetland traits formed a component of a related project examining links between wetland biodiversity and mosquitoes, and the results are reported elsewhere (Hanford et al. 2019).

Human population density

We used 2011 census data at the scale of mesh blocks (Australian Bureau of Statistics 2011) to calculate the population density at 100m, 500m, and 1000m surrounding each study wetland. Each wetland's perimeter was mapped using satellite imagery with a scale of 1:282 at 96 DPI (Land and Property Information 2015) and ground-truthed using a Garmin eTrex 20X. We used ArcGIS (v10.0 Service Pack 3) to create the respective buffers around each wetland and determine their areas, and calculated the population density within each area through intersections with the mesh block data.

Data analyses

To analyse relationships between population density and the abundance of the most common mosquito species captured as well as total mosquito abundance and species richness we used Spearman's correlations as most data were non-normal. Correlations were performed using the *cor.test* function from the stats package in R version 3.5.2 (R Core Team 2018).

Results

We collected a total of 2029 mosquitoes from 22 species in summer and 2569 mosquitoes from 16 species in autumn. In total this represented 23 unique mosquito species, including 13 that are thought to primarily utilise freshwater wetland habitats, 2 that are thought to primarily inhabit water-holding containers, and 3 that are thought to primarily use brackish and saline water habitats (Table 4.1). Thirteen of the recorded species were found at only one or two wetland sites in either summer, autumn, or both sampling seasons.

The most abundant mosquito species in summer were *Aedes notoscriptus* (52% of total), *Culex quinquefasciatus* (27% of total) and *Coquillettidia linealis* (8% of total). The most abundant mosquito species in autumn were *Cx. annulirostris* (39% of total), *Ae. notoscriptus* (26% of total), *Cx. quinquefasciatus* (17% of total) and *Mansonia uniformis* (7% of total) (Table 4.2). These species were highly cosmopolitan, being collected from most wetlands in each sampling season.

Coquillettidia linealis and *Ma. uniformis* are known to inhabit freshwater wetlands of varying quality, while *Ae. notoscriptus* is known to inhabit artificial and natural water-holding containers, making it highly adapted to urban environments. *Cx. annulirostris* and *Cx. quinquefasciatus* have been known to inhabit water-filled containers on occasion, however are more commonly found in larger waterbodies such as freshwater wetlands.

Of these 5 most abundant mosquito species, most showed no relationships with human population density at 100m, 500m or 1000m from the wetland. Notable exceptions were the abundance of *Ae. notoscriptus*, which was positively correlated with population density at 100m and 500m in summer (Figure 4.1), and *Cq. linealis*, which was negatively correlated with population density at 100m and 500m in both summer and autumn (Figure 4.2, Table 4.3). The abundance of *Cx. quinquefasciatus* was also positively correlated with population density at 500m for summer and autumn, and with 100m in autumn only.

Mosquito species richness tended to decrease with increasing population density at 100m (Figure 4.3), but this relationship was not significant. No mosquito species showed relationships with population density at 1000m.

Table 4.1. Summary of available details on the ecology of the 23 adult mosquito species collected across summer and autumn from urban wetlands in the Greater Sydney region and their human nuisance and health concern (Ryan et al. 2000, Russell 2002, Jardine et al. 2014, Webb et al. 2016, Stephenson et al. 2019). RRV = Ross River virus; KUNV = Kunjin virus; MVE = Murray Valley encephalitis; BFV = Barmah Forest virus.

Species	Pest status	Vector status	Flight distance	Larval habitats
Aedes alboannulatus	Bites humans; pest close to larval habitats	Unknown	Unknown	Freshwater; bushland ground pools
Aedes camptorhynchus	Bites humans and a major pest	RRV, BFV, KUNV, MVE	>2km	Brackish water and estuarine; marshlands, inundated pastures
Aedes mallochi	Uncertain	Unknown	Unknown	Freshwater; tree holes
Aedes multiplex	Bites humans; moderate pest close to larval habitats	BFV	Unknown	Freshwater; shaded ground pools
Aedes notoscriptus	Bites humans; major nuisance-biting pest	RRV, BFV, MVE	<200m	Freshwater; natural and artificial water-holding containers
Aedes procax	Bites humans; pest close to larval habitats	RRV, BFV	Unknown	Freshwater and mildly brackish water; permanent and ephemeral pools
Aedes rubrithorax	Bites humans; minor pest close to larval habitats	unknown	Unknown	Freshwater; bushland ground pools, occasionally large containers
Aedes vigilax	Major nuisance-biting pest	MVE, RRV, BFV	>5km	Estuarine; saltmarsh, sedgelands, and mangroves
Anopheles annulipes	Bites humans; moderate nuisance-biting pest when abundant	Malaria (historically), RRV,	<2km	Freshwater and brackish water; permanent and semi-permanent wetlands, esp. if filamentous algae present
Coquillettidia linealis	Bites humans; moderate nuisance-biting pest when abundant	RRV, BFV	<2km	Freshwater; permanent wetlands esp. with abundant emergent vegetation

Species	Pest status	Vector status	Flight distance	Larval habitats
Coquillettidia xanthogaster	Bites humans; moderate nuisance-biting pest when abundant	RRV	~4km	Freshwater; permanent wetlands esp. with abundant emergent vegetation
Culex Marks #*32*	Uncertain	Unknown	Unknown	Freshwater; heavily vegetated freshwater pools
Culex annulirostris	Major nuisance-biting pest	MVE, Kunjin, BFV, RRV, dog heartworm,	5-10kms	Freshwater; wide range of permanent and semi-permanent habitats
Culex australicus	Not a pest, prefers biting birds	Unknown	Unknown	Freshwater; permanent and semi-permanent habitats
Culex bitaeniorhynchus	Uncertain	Unknown	Unknown	Freshwater; permanent and semi-permanent habitats
Culex molestus	Moderate nuisance pest including entering buildings	MVE	<1km	Freshwater; subterranean water-holding structures
Culex orbostiensis	Bites humans; moderate pest close to larval habitats	Unknown	Unknown	Freshwater; permanent and semi-permanent habitats
Culex quinquefasciatus	Moderate nuisance pest including entering buildings	MVE, dog heartworm	<2km	Freshwater; permanent and semi-permanent habitats with high organic content
Culex sitiens	Not a pest, prefers biting birds	RRV	>3km	Estuarine and brackish water; coastal wetlands
Culiseta antipodea	Uncertain	Unknown	Unknown	Freshwater; permanent and semi-permanent pools in coastal swamp forests
Lutzia halifaxii	Uncertain	Unknown	Unknown	Freshwater; permanent and semi-permanent habitats

Species	**Pest status**	**Vector status**	**Flight distance**	**Larval habitats**
Mansonia uniformis	Bites humans; moderate nuisance-biting pest	RRV, MVE, KUNV	4km	Freshwater; permanent wetlands esp. with abundant emergent vegetation
Verrallina Marks *#52*	Bites humans; moderate pest close to larval habitats	Unknown	Unknown	Freshwater and brackish water; semi-permanent and ephemeral coastal swamp forest

Table 4.2. The most abundant adult mosquito species collected in summer (January) and autumn (March) from 22 urban wetlands across the Greater Sydney region. Including less abundant species, in summer we collected a total of 2029 mosquitoes, and in autumn we collected a total of 2569 mosquitoes.

Species	Summer	Autumn	% sites detected in summer	% sites detected in autumn
Aedes alboannulatus	1	1	5	5
Aedes camptorhynchus	1	0	5	0
Aedes mallochi	1	0	5	0
Aedes multiplex	12	2	5	5
Aedes notoscriptus	1058	659	100	95
Aedes procax	3	0	9	0
Aedes rubrithorax	2	1	9	5
Aedes vigilax	37	3	32	14
Anopheles annulipes	9	12	23	41
Coquillettidia linealis	160	89	68	59
Coquillettidia xanthogaster	1	0	5	0
Culex Marks *#32*	1	0	5	0
Culex annulirostris	58	1006	55	91
Culex australicus	20	45	36	50
Culex bitaeniorhynchus	8	47	5	27
Culex molestus	12	28	14	9
Culex orbostiensis	27	23	36	32
Culex quinquefasciatus	551	432	95	95
Culex sitiens	1	45	5	27
Culiseta antipodea	3	0	5	0
Lutzia halifaxii	0	1	0	5
Mansonia uniformis	61	175	36	68
Verrallina Marks *#52*	2	0	9	0
Total	2029	2569		

Table 4.3. Values for Spearman's correlations between abundance of common mosquito species at urban wetlands (n=22) and population density within varying distances from the wetland. Mosquitoes were sampled twice at each wetland, once in summer (January) and again in autumn (March). * Significant at *P* <0.05. ** Significant at *P* <0.01.

Species	Distance	Summer		Autumn	
		P	r_s	*P*	r_s
Aedes notoscriptus	100m	*0.019	0.495	0.089	0.371
	500m	*0.044	0.433	0.590	0.122
	1000m	0.126	0.337	0.709	0.084
Coquillettidia linealis	100m	**0.004	-0.585	**0.008	-0.547
	500m	**0.009	-0.545	**<0.001	-0.718
	1000m	0.161	-0.309	0.119	-0.343
Culex annulirostris	100m	0.990	-0.003	0.924	0.021
	500m	0.445	0.172	0.368	0.202
	1000m	0.282	0.240	0.079	0.382
Culex quinquefasciatus	100m	0.118	0.343	*0.046	0.430
	500m	*0.036	0.449	**0.008	0.549
	1000m	0.194	0.288	0.083	0.378
Mansonia uniformis	100m	0.296	-0.233	0.103	-0.356
	500m	0.079	-0.383	0.166	-0.306
	1000m	0.146	-0.320	0.828	-0.049
Species richness	100m	0.053	-0.418	0.056	-0.413
	500m	0.481	-0.158	0.469	-0.163
	1000m	0.458	-0.167	0.431	0.177
Total mosquito abundance	100m	0.129	0.334	0.813	-0.054
	500m	0.255	0.254	0.633	0.108
	1000m	0.171	0.303	0.617	0.113

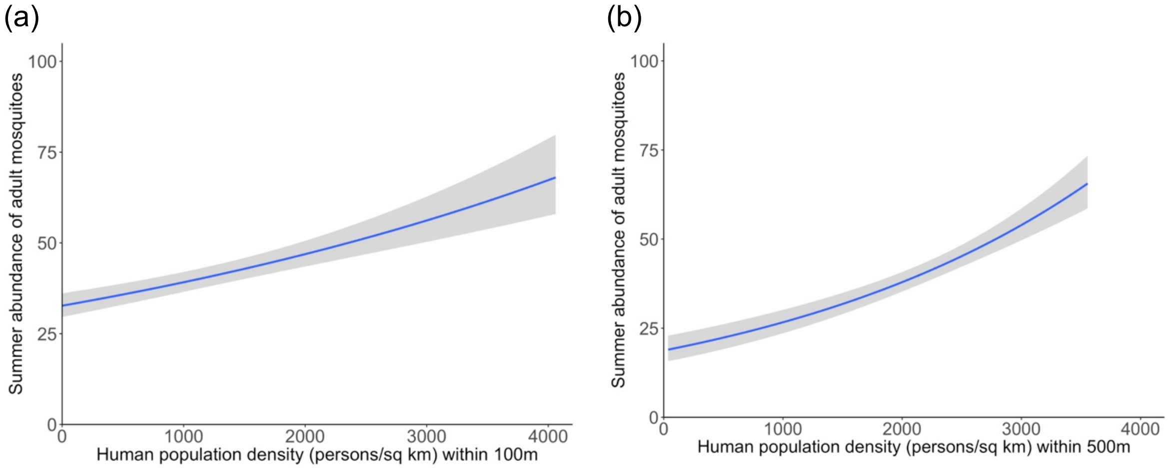


Figure 4.1. Relationships between summer abundance of *Aedes notoscriptus,* a container-inhabiting mosquito species, and human population density within (a) 100m (P = 0.019, r_s = 0.495) and (b) 500m (P = 0.044, r_s = 0.433) of the sampled wetlands. Shaded areas show 95% confidence interval.

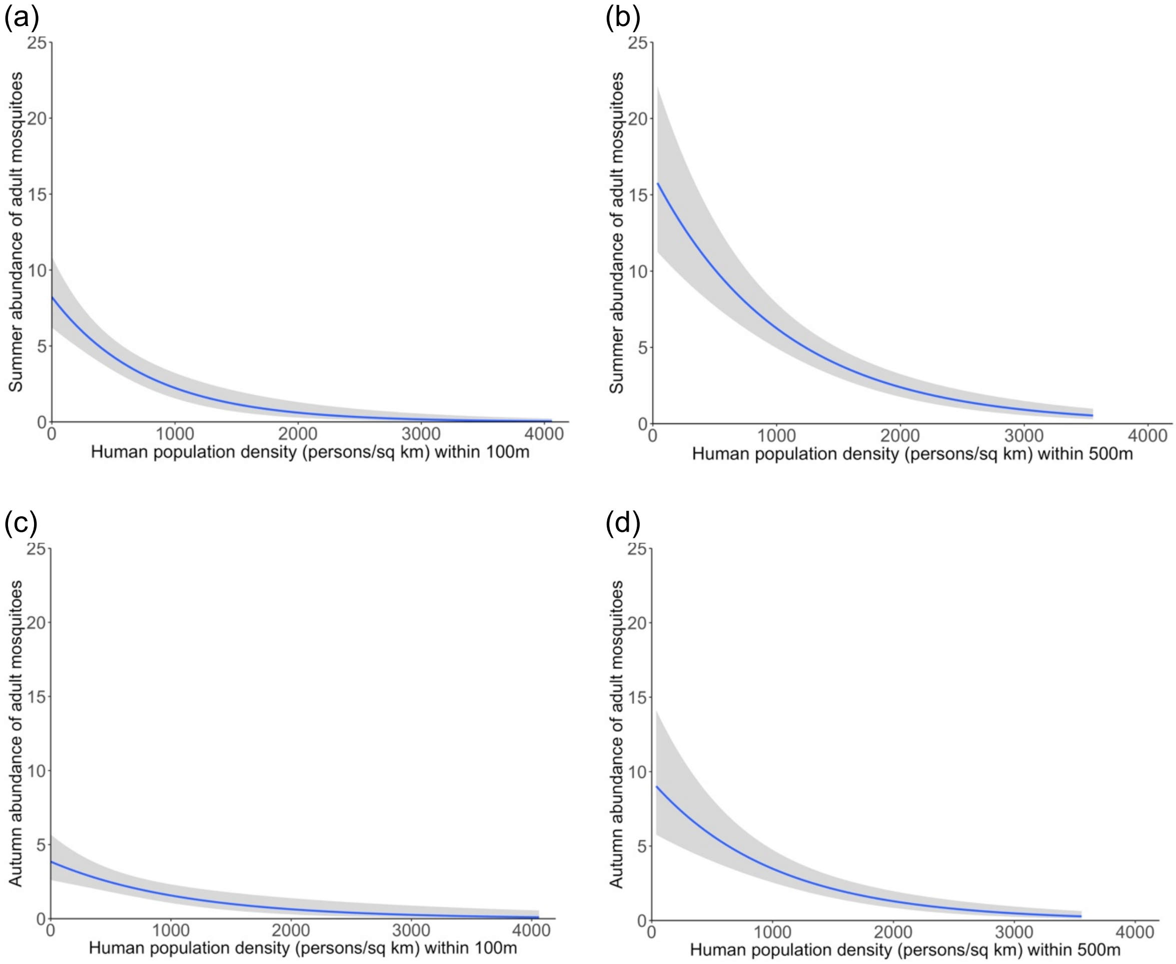


Figure 4.2. Relationships between abundance of *Coquillettidia linealis,* a wetland-inhabiting mosquito species, and human population density in summer within (a) 100m (P = 0.004, r_s = -0.585) and (b) 500m (P = 0.009, r_s = -0.545) of the sampled wetlands, and in autumn within (c) 100m (P = 0.008, r_s = -0.547) and (d) 500m (P < 0.001, r_s = -0.718) of the sampled wetlands. Shaded areas show 95% confidence interval.

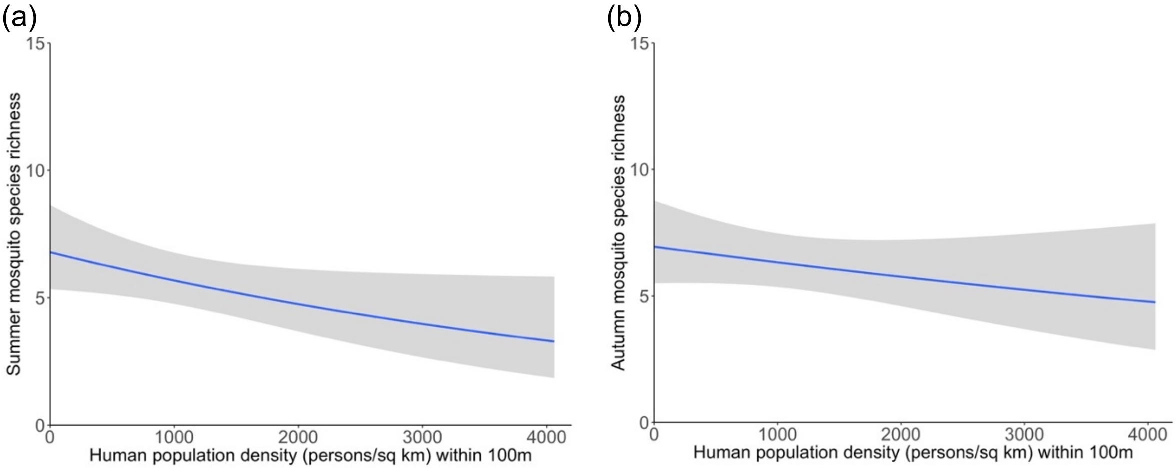


Figure 4.3. Relationships between mosquito species richness and human population density within 100m of the sampled wetlands in (a) summer (*P* = 0.053, r_s = -0.418) and (b) autumn (*P* = 0.056, r_s = -0.413). Shaded areas show 95% confidence interval.

Discussion

Our results demonstrate that mosquito species causing pest and public health concerns around urban freshwater wetlands may not be associated with the wetlands themselves, but with water-holding containers within neighbouring urban habitats. The relationships observed at the scales of 100m and 500m, but not at the coarse 1000m scale, reflect the fine scales at which mosquito species respond to habitat availability, and also the fine scales at which human populations influence urban landscape traits. It is therefore essential when initiating mosquito management strategies around these wetlands that fine scale sampling is integrated into landscape scale decision making and planning.

We found that mosquito species that generally inhabit water-holding containers can not only form a sizeable component of mosquitoes captured at urban freshwater wetlands, but are also strongly linked to fine scale human population density surrounding the wetland. This finding complements other work demonstrating landscape scale community patterns in mosquito occurrence, including links between mosquito communities found in urban mangrove ecosystems and surrounding land use (Claflin and Webb 2017), between container-inhabiting mosquito communities and distance from the city centre (Johnston et al. 2014, Zahouli et al. 2017), and between mosquitoes found in freshwater habitats and surrounding land use (Vanwambeke et al. 2007). The relationships between individual mosquito species and human population density, and lack of such relationships when considering the mosquito assemblage as a whole, also emphasises the need for species-specific sampling and monitoring (Hanford et al. 2019). Understanding these fine scale patterns of mosquito distribution and abundance and the mechanisms driving them is essential to inform landscape scale perspectives of potential mosquito nuisance and risk within urban mosaics, which in turn informs urban planning and management at the landscape scale.

Mosquito habitat associations and human population density

Differences in relationships between human population density and abundances of individual mosquito species are reflective of differences in the typical habitats these key mosquito species are associated with. While a diverse assemblage of mosquito species was collected, the abundance of key mosquito species that have specific habitat requirements can be used to investigate the role of human landscape factors in determining mosquito risk.

Mosquitoes such as *Cq. linealis* primarily inhabit wetland habitats and are known nuisance-biting pests (Williams 2005) and vectors of mosquito-borne pathogens such as Ross River virus (Jeffery et al. 2002). The immature stages of this mosquito rely on aquatic macrophytes for development (Johnson and Russell 2019a, Johnson and Russell 2019b) so are not associated with water-holding containers or ephemeral ground pools. As a consequence, this species may be a useful indicator species regarding possible impacts of human population density on wetland-inhabiting mosquito species. *Coquillettidia linealis* showed significant negative relationships with population density at 100m and 500m in both summer and autumn. The mechanism behind these negative relationships was not investigated, though is likely linked to concentrated detrimental impacts of urbanisation on local waterways including on aquatic plants (Walsh et al. 2005). There is little information available on the host feeding preferences of *Cq. linealis*, though available information indicates they feed readily on birds and other mammals (Jansen et al. 2009) which may have lower abundance and diversity in areas surrounding heavily urbanised wetlands.

Conversely, *Ae. notoscriptus,* the most abundant mosquito species recorded in summer, and to a lesser extent *Cx. quinquefasciatus*, a highly adaptable species that can be a significant nuisance, both showed significant positive relationships with population density at 100m and 500m from freshwater wetlands. *Aedes notoscriptus* is widespread in Australian cities, a known nuisance-biting pest (Webb et al. 2016) and vector of Ross River virus and Barmah Forest virus (Watson and Kay 1998, 1999). While this mosquito may be associated with natural occurring water-filled containers (e.g. tree holes, water-holding plants), it is more widely thought to be associated with artificial water-holding containers (Watson et al. 2000). Compared to more widely dispersing mosquitoes that can travel many kilometres from wetland habitats (Webb and Russell 2019), *Ae. notoscriptus* generally does not travel more than 200m from larval habitats (Watson et al. 2000, Trewin et al. 2020). Given the close proximity of abundant potential larval habitats in residential and industrial properties adjacent to wetlands, it is not surprising that this species would be dispersing into areas around wetlands that may offer greater refuge within terrestrial vegetation providing shaded and humid conditions away from wind disturbance. Comparable relationships have been observed for the container-inhabiting species *Ae. aegypti*, with distributions of this species being more accurately predicated based on human population data, such as human density and poverty, rather than climatic factors (Obenauer et al. 2017). The relationship between *Ae. aegypti* and human population density is also linked to increased dengue transmission (Padmanabha et al. 2012), highlighting the importance of understanding fine scale relationships and patterns of mosquito abundance to incorporate into planning for land-scape scale human population health and wellbeing. Beyond human population density, the support provided for increased biodiversity around urban wetlands may assist in reducing transmission of some pathogens where an abundance of "dead end" hosts reduces rates of mosquito feeding on humans and, subsequently, pathogen transmission (Carver et al. 2009).

Integrating fine scale and landscape scale to manage urban mosquitoes

That species with vastly different ecological requirements demonstrated relationships with population density at these fine 100m and 500m scales emphasises how the patchwork of green and blue spaces within the urban mosaic (Andersson 2018) requires a multi-pronged approach to managing mosquitoes due to the large variety of potential larval habitats. While *Ae. notoscriptus* is likely responding to the increased availability of container habitats in areas of high population density, the drivers of *Cq. linealis* abundance are likely to be more subtle, relating to degradation of water quality associated with urbanisation or the nature of constructed waterbodies in urban environments.

Mitigation of risks associated with container-inhabiting species such as *Ae. notoscriptus* may effectively include public education campaigns in addition to source reduction strategies within residential and industrial properties (e.g. screening on water tanks). Conversely, mitigation of risks associated with wetland-inhabiting species such as *Cq. linealis* which may be dispersing into residential areas or pose risks to users of wetland habitats (e.g. for recreation) might require long-term planning and improvement of wetland habitat quality, as higher quality wetland habitats are linked to reduced mosquito risks as well as a raft of incidental benefits (Carter 2015, Carver et al. 2015, Hanford et al. 2019).

Mosquito control can take various forms and each brings with it operational, financial, and ecological concerns and constraints. In Australia, wetland-inhabiting mosquitoes are often managed reactively, through techniques such as the application of mosquito control agents (e.g. larvicides, insect growth regulators, and adulticides) although these vary greatly between freshwater, saltmarsh and mangrove habitats (Dale and Knight 2008). Source reduction for intertidal (saltmarsh and mangrove) wetland-inhabiting species also occurs through habitat modification (Dale and Knight 2008). These techniques are not interchangeable, hence the importance of understanding which mosquito species is the cause of urban mosquito issues, and its relationship with surrounding fine and landscape scale environmental traits for effective control and maintenance of public health and wellbeing.

Conclusions

The dominance of container-inhabiting mosquitoes at urban wetland sites highlights the complexities of identifying and mitigating mosquito issues in urban areas. Our results clearly demonstrate that around urban wetlands in Sydney, high numbers of pest mosquitoes in routine monitoring may be caused by mosquitoes dispersing from nearby urban areas and not the wetland itself. Understanding the ecological requirements of adult mosquito species is an essential step in identifying larval sources of mosquitoes, which is critical for mosquito abatement (Dale and Knight 2008). While there may be pest and public health concerns associated with wetland mosquito species, there may also need to be active management of human perceptions and behaviour relating to nuisance-biting mosquitoes around wetlands.

In interconnected urban landscapes with mosaics of blue, green and built areas, we cannot make assumptions regarding the origin of mosquito problems. It is essential that urban planners understand fine and landscape scale drivers of distribution and abundance of mosquito species that may be of pest and public health concern in their region.

References

Andersson, E. 2018. Functional landscapes in cities: A systems approach. Landscape and Ecological Engineering **14**:193-199.

Australian Bureau of Statistics. 2011. Census Data. http://www.abs.gov.au/. Accessed April 2014.

Carrus, G., M. Scopelliti, R. Lafortezza, G. Colangelo, F. Ferrini, F. Salbitano, M. Agrimi, L. Portoghesi, P. Semenzato, and G. Sanesi. 2015. Go greener, feel better? The positive effects of biodiversity on the well-being of individuals visiting urban and peri-urban green areas. Landscape and Urban Planning **134**:221-228.

Carter, M. 2015. Wetlands and health: How do urban wetlands contribute to community well-being?*in* C. M. Finlayson, editor. Wetlands and Human Health. Springer, Dordrecht.

Carver, S., A. Bestall, A. Jardine, and R. S. Ostfeld. 2009. Influence of hosts on the ecology of arboviral transmission: Potential mechanisms influencing dengue, Murray Valley encephalitis, and Ross River virus in Australia. Vector Borne and Zoonotic Diseases **9**:51-64.

Carver, S., D. P. Slaney, P. T. Leisnham, and P. Weinstein. 2015. Healthy wetlands, healthy people: Mosquito borne disease.*in* C. M. Finlayson, editor. Wetlands and Human Health. Springer, Dordrecht.

City of Sydney. 2016. Research and Statistics. https://www.cityofsydney.nsw.gov.au/learn/research-and-statistics/the-city-at-a-glance/greater-sydney. Accessed 12/03/2020.

Claflin, S. B., and C. E. Webb. 2017. Surrounding land use significantly influences adult mosquito abundance and species richness in urban mangroves. Wetlands Ecology and Management **25**:331-344.

Dale, P. E. R., and J. M. Knight. 2008. Wetlands and mosquitoes: A review. Wetlands Ecology and Management **16**:255-276.

Darbro, J., Y. Halasa, B. Montgomery, M. Muller, D. Shepard, G. Devine, and P. Mwebaze. 2017. An economic analysis of the threats posed by the establishment of *Aedes albopictus* in Brisbane, Queensland. Ecological Economics **142**:203-213.

de Jesús Crespo, R., P. Méndez Lázaro, and S. H. Yee. 2018. Linking wetland ecosystem services to vector-borne disease: Dengue fever in the San Juan Bay Estuary, Puerto Rico. Wetlands **39**:1281-1293.

Dickinson, K., and S. Paskewitz. 2012. Willingness to pay for mosquito control: How important is West Nile virus risk compared to the nuisance of mosquitoes? Vector-Borne and Zoonotic Diseases **12**:886-892.

Faller, C. R., and R. A. McCleery. 2017. Urban land cover decreases the occurrence of a wetland endemic mammal and its associated vegetation. Urban Ecosystems **20**:573-580.

Ferraguti, M., J. Martínez-de la Puente, D. Roiz, S. Ruiz, R. Soriguer, and J. Figuerola. 2016. Effects of landscape anthropization on mosquito community composition and abundance. Scientific Reports **6**:29002.

Goertzen, D., and F. Suhling. 2013. Promoting dragonfly diversity in cities: Major determinants and implications for urban pond design. Journal of Insect Conservation **17**:399-409.

Hamer, A. J., P. J. Smith, and M. J. McDonnell. 2012. The importance of habitat design and aquatic connectivity in amphibian use of urban stormwater retention ponds. Urban Ecosystems **15**:451-471.

Hanford, J. K., D. F. Hochuli, and C. E. Webb. 2020. Observations of an unexpected abundance of estuarine mosquitoes associated with an urban freshwater wetland. Australian Zoologist doi: 10.7882/AZ.2020.014.

Hanford, J. K., C. E. Webb, and D. F. Hochuli. 2019. Habitat traits associated with mosquito risk and aquatic diversity in urban wetlands. Wetlands **39**:743-758.

Jansen, C. C., C. E. Webb, G. C. Graham, S. B. Craig, P. Zborowski, S. A. Ritchie, R. C. Russell, and A. F. van den Hurk. 2009. Blood sources of mosquitoes collected from urban and peri-urban environments in eastern Australia with species-specific molecular analysis of avian blood meals. American Journal of Tropical Medicine and Hygiene **81**:849-857.

Jardine, A., P. J. Neville, C. Dent, C. Webster, and M. D. A. Lindsay. 2014. Ross River virus risk associated with dispersal of *Aedes* (Ochlerotatus) *camptorhynchus* (Thomson) from Breeding Habitat into surrounding residential areas: Muddy Lakes, Western Australia. American Journal of Tropical Medicine and Hygiene **91**:101-108.

Jeffery, J. A., P. A. Ryan, S. A. Lyons, and B. H. Kay. 2002. Vector competence of *Coquillettidia linealis* (Skuse)(Diptera: Culicidae) for Ross River and Barmah Forest viruses. Australian Journal of Entomology **41**:339-344.

Johnson, B. J., K. Munafo, L. Shappell, N. Tsipoura, M. Robson, J. Ehrenfeld, and M. V. K. Sukhdeo. 2012. The roles of mosquito and bird communities on the prevalence of West Nile virus in urban wetland and residential habitats. Urban Ecosystems **15**:513-531.

Johnson, P. H., and R. C. Russell. 2019a. Colonization of *Coquillettidia linealis* (Skuse) with reference to other *Coquillettidia* and *Mansonia* species. Journal of Vector Ecology **44**:130-137.

Johnson, P. H., and R. C. Russell. 2019b. Effects of attachment substrate, larval diet, and temperature on development and survival of immature *Coquillettidia linealis* (Skuse) and *Coquillettidia xanthogaster* (Edwards). Journal of Vector Ecology **44**:138-148.

Johnston, E., P. Weinstein, D. Slaney, A. S. Flies, S. Fricker, and C. Williams. 2014. Mosquito communities with trap height and urban-rural gradient in Adelaide, South Australia: implications for disease vector surveillance. Journal of Vector Ecology **39**:48-55.

Kadlec, R. H., and S. D. Wallace. 2009. Treatment Wetlands. Second edition edition. CRC Press, Taylor & Francis Group, Boca Raton, Florida.

Kuller, M., P. M. Bach, S. Roberts, D. Browne, and A. Deletic. 2019. A planning-support tool for spatial suitability assessment of green urban stormwater infrastructure. Science of the Total Environment **686**:856-868.

Land and Property Information. 2015. NSW Imagery Web Services.*in* Land and Property Information, editor.

Lentz, A. H., A. W. Ando, and N. Brozović. 2014. Water quality trading with lumpy investments, credit stacking, and ancillary benefits. Journal of the American Water Resources Association **50**:83-100.

Madewell, Z. J., S. Sosa, K. C. Brouwer, J. G. Juárez, C. Romero, A. Lenhart, and C. Cordón-Rosales. 2019. Associations between household environmental factors and immature mosquito abundance in Quetzaltenango, Guatemala. BMC Public Health **19**:1729.

McKinney, R. A., K. B. Raposa, and R. M. Cournoyer. 2011. Wetlands as habitat in urbanizing landscapes: Patterns of bird abundance and occupancy. Landscape and Urban Planning **100**:144-152.

Murray, C. G., S. Kasel, R. H. Loyn, G. Hepworth, and A. J. Hamilton. 2013. Waterbird use of artificial wetlands in an Australian urban landscape. Hydrobiologia **716**:131-146.

Obenauer, J. F., T. Andrew Joyner, and J. B. Harris. 2017. The importance of human population characteristics in modeling *Aedes aegypti* distributions and assessing risk of mosquito-borne infectious diseases. Tropical Medicine and Health **45**:38.

Padmanabha, H., D. Durham, F. Correa, M. Diuk-Wasser, and A. Galvani. 2012. The interactive roles of *Aedes aegypti* super-production and human density in dengue transmission. PLoS Neglected Tropical Diseases **6**:e1799.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rohe, D. L., and R. P. Fall. 1979. A miniature battery powered CO2 baited light trap for mosquito borne encephalitis surveillance. Bulletin of the Society of Vector Ecology **4**:21-27.

Russell, R. C. 1993. Mosquitoes and mosquito-borne disease in southeastern Australia: A guide to the biology, relation to disease, surveillance, control and the identification of mosquitoes in southeastern Australia. Westmead Hospital, Department of Medical Entomology.

Russell, R. C. 1999. Constructed wetlands and mosquitoes: Health hazards and management options—an Australian perspective. Ecological Engineering **12**:107-124.

Russell, R. C. 2002. Ross River virus: Ecology and distribution. Annual Review of Entomology **47**:1-31.

Ryan, P. A., K. A. Do, and B. H. Kay. 2000. Definition of Ross River virus vectors at Maroochy shire, Australia. Journal of Medical Entomology **37**:146-152.

Stephenson, E. B., A. K. Murphy, C. C. Jansen, A. J. Peel, and H. McCallum. 2019. Interpreting mosquito feeding patterns in Australia through an ecological lens: An analysis of blood meal studies. Parasites & vectors **12**:156.

Taylor, L., A. K. Hahs, and D. F. Hochuli. 2018. Wellbeing and urban living: Nurtured by nature. Urban Ecosystems **21**:197-208.

Trewin, B. J., D. E. Pagendam, M. P. Zaluck, J. M. Darbro, G. J. Devine, C. C. Jansen, and N. A. Schellhorn. 2020. Urban landscape features influence the movement and distribution of the Australian container-inhabiting mosquito vectors *Aedes aegypti* (Diptera: Culicidae) and *Aedes notoscriptus* (Diptera: Culicidae). Journal of Medical Entomology **57**:443-453.

Vally, H., M. Peel, G. K. Dowse, S. Cameron, J. P. Codde, I. Hanigan, and M. D. A. Lindsay. 2012. Geographic Information Systems used to describe the link between the risk of Ross River virus infection and proximity to the Leschenault estuary, WA. Australian and New Zealand Journal of Public Health **36**:229-235.

Vanwambeke, S. O., P. Somboon, R. E. Harbach, M. Isenstadt, E. F. Lambin, C. Walton, and R. K. Butlin. 2007. Landscape and land cover factors influence the presence of Aedes and Anopheles larvae. Journal of Medical Entomology **44**:133-144.

Walsh, C. J., A. H. Roy, J. W. Feminella, P. D. Cottingham, P. M. Groffman, and R. P. Morgan. 2005. The urban stream syndrome: Current knowledge and the search for a cure. Journal of the North American Benthological Society **24**:706-723.

Walsh, C. J., A. K. Sharpe, P. F. Breen, and J. A. Sonneman. 2001. Effects of urbanization on streams of the Melbourne region, Victoria, Australia. I. Benthic macroinvertebrate communities. Freshwater Biology **46**:535-551.

Watson, T. M., and B. H. Kay. 1998. Vector competence of *Aedes notoscriptus* (Diptera: Culicidae) for Ross River virus in Queensland, Australia. Journal of Medical Entomology **35**:104-106.

Watson, T. M., and B. H. Kay. 1999. Vector competence of *Aedes notoscriptus* (Diptera: Culicidae) for Barmah Forest virus and of *Aedes aegypti* (Diptera: Culicidae) for dengue 1–4 viruses in Queensland, Australia. Journal of Medical Entomology **36**:508-514.

Watson, T. M., A. Saul, and B. H. Kay. 2000. *Aedes notoscriptus* (Diptera: Culicidae) survival and dispersal estimated by mark-release-recapture in Brisbane, Queensland, Australia. Journal of Medical Entomology **37**:380-384.

Webb, C., S. Doggett, and R. Russell. 2016. A Guide to Mosquitoes of Australia. CSIRO Publishing, Clayton South, Victoria.

Webb, C. E., and R. C. Russell. 2019. Dispersal of the mosquito *Aedes vigilax* (Diptera: Culicidae) from urban estuarine wetlands in Sydney, Australia. Journal of Medical Entomology **56**:1290-1295.

Williams, C. R. 2005. Timing of host-seeking behaviour of the mosquitoes *Anopheles annulipes* sensu lato Walker and *Coquillettidia linealis* (Skuse)(Diptera: Culicidae) in the Murray River Valley, South Australia. Australian Journal of Entomology **44**:110-112.

Zahouli, J. B. Z., B. G. Koudou, P. Müller, D. Malone, Y. Tano, and J. Utzinger. 2017. Urbanization is a main driver for the larval ecology of Aedes mosquitoes in arbovirus-endemic settings in south-eastern Côte d'Ivoire. PLoS Neglected Tropical Diseases **11**.

Chapter 5:

Observations of an unexpected abundance of estuarine mosquitoes associated with an urban freshwater wetland

This chapter has been accepted for publication:

Hanford, J. K., Hochuli, D. F. & Webb, C. E. (2020) Observations of an unexpected abundance of estuarine mosquitoes associated with an urban freshwater wetland. Australian Zoologist, In press https://doi.org/10.7882/AZ.2020.014.

The paper is available online at:

https://publications.rzsnsw.org.au/doi/abs/10.7882/AZ.2020.014

Abstract

Urban freshwater wetlands have been identified as a potential source of mosquitoes of pest and public health concern in Australia. Mosquitoes most commonly associated with these habitats include *Anopheles annulipes*, *Culex annulirostris*, *Culex quinquefasciatus*, *Coquillettidia linealis* and *Mansonia uniformis*. However, adaptive management of freshwater wetlands for wildlife conservation or vegetation management can alter the local environmental conditions and may provide opportunities for other mosquito species to exploit these habitats. A series of small wetlands at Sydney Olympic Park, NSW, were drained in spring 2016 and allowed to refill via rainfall to improve conditions for the endangered green and golden bell frog

Litoria aurea. Below-average rainfall was experienced that summer and the wetlands did not fully refill until early autumn. Surprisingly, immature stages of the estuarine mosquito, *Aedes vigilax*, were observed in the freshwater wetlands in exceptional abundances during this period. Adjacent to these freshwater wetlands there are extensive saltmarsh and mangrove habitats where highly abundant populations of *Ae. vigilax* are common. Observations of use of freshwater habitats by this estuarine mosquito have implications for urban freshwater wetland management and rehabilitation strategies, which currently do not consider the potential pest and public health issues associated with estuarine mosquitoes.

Introduction

Australia has a diverse mosquito fauna with pest and non-pest species associated with a wide range of habitats (Webb et al. 2016). However, a majority of mosquitoes are typically specialised and use a narrow range of suitable habitats for immature development. Mosquitoes lay eggs on or near sources of water, in habitats ranging from estuarine wetlands to snow melt alpine pools. A suite of habitat traits determine where adult mosquitoes oviposit including water quality, salinity, physical structure, and vegetation. These close associations between key mosquito species and habitat often drive mosquito control and surveillance programs by local authorities seeking to mitigate actual and potential pest and public health risks associated with mosquitoes (Russell and Kay 2008).

In Australian freshwater wetlands, especially those associated with urban landscapes, the key pest and vector mosquito species include *Anopheles annulipes*

(Diptera: Culicidae), *Coquillettidia linealis* (Diptera: Culicidae), *Culex annulirostris* (Diptera: Culicidae), and *Culex quinquefasciatus* (Diptera: Culicidae). The abundance and diversity of mosquitoes associated with these habitats have the potential to drive pest and public health risk. With mosquito-borne pathogens, such as Ross River virus (RRV), becoming increasingly important at the fringes of metropolitan regions, increasing consideration is being given to strategies to assess and mitigate health risks associated with urban wetlands (Claflin and Webb 2015, Crocker et al. 2017, Hanford et al. 2019b). These strategies can vary from adaptive vegetation and water level management, to the application of mosquito control agents. The appropriateness of each strategy will vary with site characteristics and existing mosquito populations.

While freshwater wetlands provide suitable conditions for a diverse range of mosquitoes, one of the most important pest and vector mosquitoes in coastal regions of Australia is *Aedes vigilax* (Diptera: Culicidae). This mosquito is closely associated with tidally influenced estuarine wetlands and can be exceptionally abundant during periods of favourable climatic conditions (Kay and Jorgensen 1986, Gíslason and Russell 1997, Knight et al. 2012, Claflin and Webb 2017). The mosquito disperses widely from larval habitats, especially saltmarsh and mangroves (Webb and Russell 2019) and is a severe nuisance-biting pest that can impact local communities (Webb et al. 2016). Notwithstanding its role as a nuisance-biting pest, *Ae. vigilax* is considered an important vector of RRV (Claflin and Webb 2015) and the abundance of this mosquito has been associated with local outbreaks of disease (Ritchie et al. 1997, Jacups et al. 2008, Jardine et al. 2015).

Here we describe an opportunistic observation of exceptionally high abundances of immature stages of *Ae. vigilax* in freshwater wetlands in Sydney, NSW, confirming that this species will lay eggs around freshwater habitats and successfully complete development. Because this species is generally considered to be exclusively associated with estuarine wetlands, the public health risks and required management interventions for *Ae. vigilax* in freshwater wetlands are usually unnecessary. With urban freshwater wetlands increasingly being incorporated into coastal urban developments, assessing the potential for this mosquito to utilise these habitats, which may be adjacent to estuarine wetlands, requires an understanding of the likelihood of *Ae. vigilax* exploiting these habitats.

Methods

The study site

The Narawang Wetlands is a network of small constructed wetlands covering approximately 22 ha at Sydney Olympic Park, NSW. The wetlands sit within the flood plain of the intertidal Haslams Creek, however engineered structures prevent direct tidal exchange with Narawang Wetlands and the small wetlands within the Narawang system were constructed using engineered soils (Tyrrell 2011). Surrounding these freshwater wetlands are extensive areas of saltmarsh and mangrove habitats influenced by tidal flows from Haslams Creek, Powells Creek, and the Parramatta River. The area is approximately 20 kms from the ocean. A diverse range of mosquitoes have been recorded from the local area, with the most important nuisance-biting pest species being *Ae. vigilax* (Webb and Russell 1999, Claflin and

Webb 2017). The local authorities actively manage the abundance of this mosquito produced from estuarine wetlands through a program of mosquito control agent applications, a common approach to managing this mosquito in coastal wetlands in Australia (Tomerini et al. 2011, Dale and Knight 2012).

The freshwater Narawang Wetlands serve multiple local purposes, primarily water conservation and wildlife habitat. The small wetlands within the Narawang system are actively managed to reduce the impact of an invasive predatory fish, the plague minnow *Gambusia holbrooki* (Atheriniformes: Poeciliidae) on the endangered green and golden bell frog *Litoria aurea* (Anura: Hylidae) (O'Meara and Darcovich 2008). The abundance and distribution of *G. holbrooki* has been identified as adversely impacting frog populations across Sydney and specifically *L. aurea* tadpoles in the local area (Hamer et al. 2002, O'Meara and Darcovich 2008). A group of small wetlands within the system are drained each year in early spring (August/September) and left to dry to significantly reduce, or temporarily eradicate, *G. holbrooki* populations with the expectation that it will enhance local conditions for *L. aurea* (O'Meara and Darcovich 2008). The wetlands are then either refilled using water from adjacent freshwater wetlands or allowed to passively refill with rainfall. In late 2016, wetlands were drained and left to refill with rainfall (Figure 5.1). However, throughout the post-draining period, Sydney experienced above average temperatures and below average rainfall until a substantial rainfall event occurred during March 2017 (Figure 5.2). Observed salinity values in March 2017 in the wetlands ranged between 0.18 and 0.68 psu, recorded using a YSI ProDSS multiparameter water quality probe (Xylem Inc.). Salinity in the small wetland where mosquito observations were made was 0.20 psu. Freshwater is defined as having salinity values of less than 1.0 psu.

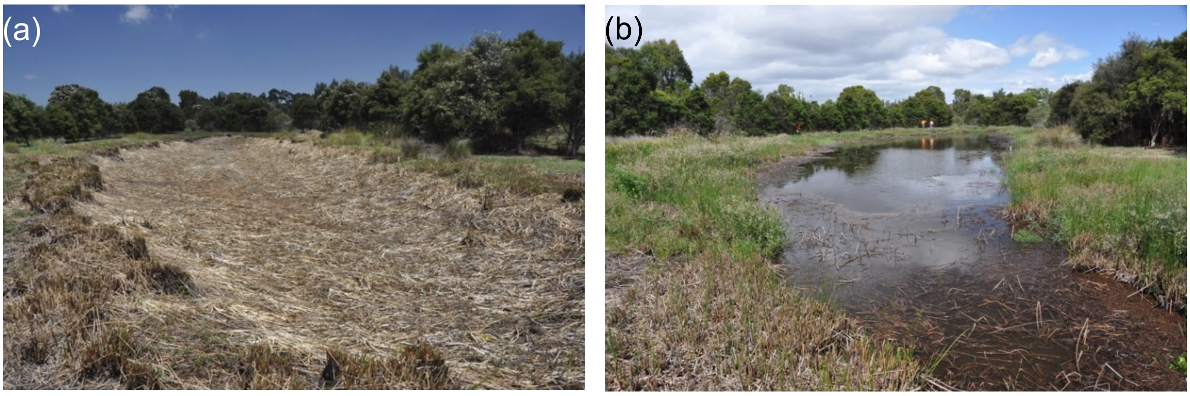


Figure 5.1. An example of a wetland in Narawang Wetlands in November 2016 after draining (a) and in March 2017 following refilling (b). Photos: J. Hanford.

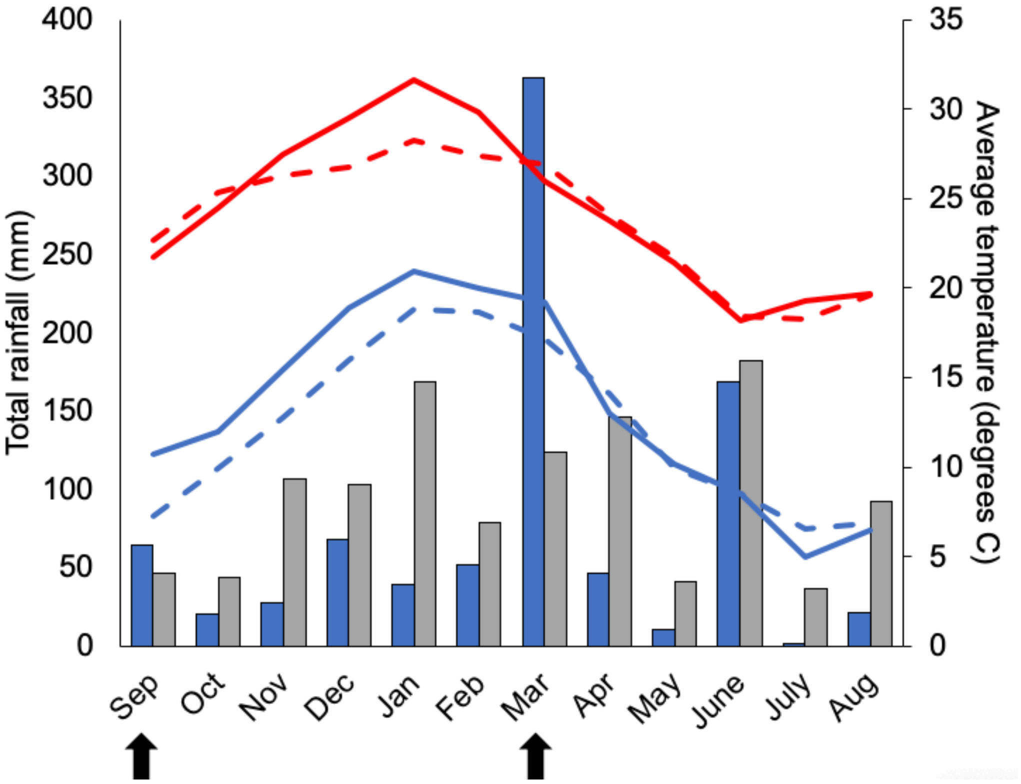


Figure 5.2. Rainfall and average minimum and maximum air temperature data for Sydney Olympic Park for study year (2016-17) and previous 5 years average (grey columns and broken lines). Wetlands were drained in September 2016 (indicated by black arrow) and above-average rainfall event occurred in March 2017 (again indicated by black arrow) following which high abundances of the saltmarsh mosquito *Ae. vigilax* were recorded from the freshwater wetlands. (Data: Bureau of Meteorology 2017, Sydney Olympic Park weather stations 066212 and 066195).

Mosquito observations

Local authorities had been receiving complaints of nuisance-biting mosquito impacts, despite the local mosquito control program in estuarine wetlands proving effective as measured by the associated surveillance program (S. Paul pers. comm. 2017, SOPA). During monitoring of the freshwater Narawang Wetlands for an investigation of the responses of aquatic macroinvertebrates to the management program (Hanford et al. 2019a, Hanford et al. 2020), observations of large numbers of immature mosquitoes were reported from one of the partially refilled wetlands in March 2017 (Figure 5.3). A sample of immature populations was collected using a 300ml dipper and returned to the laboratory for identification using the taxonomic keys of Russell (1993).

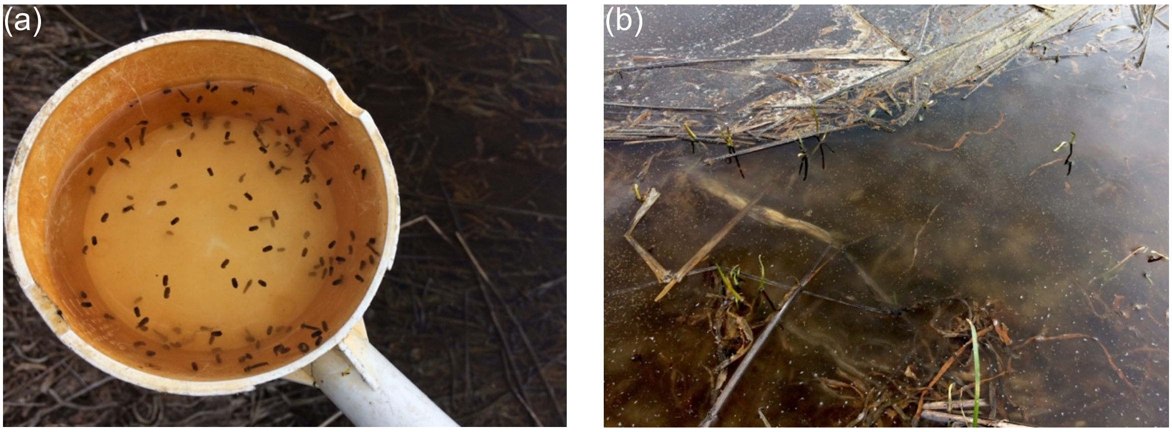


Figure 5.3. Large numbers of immature mosquitoes were collected from a wetland (a) at the freshwater Narawang Wetlands and identified in the lab as *Aedes vigilax*, a species commonly associated with estuarine habitats. The exceptional abundance of mosquito larvae and pupae could be seen on the surface of a wetland (b) as small specks covering the water surface. Photos: C. Webb.

Results and discussion

On inspection, the drained wetlands were approximately 25% refilled, with an abundance of aquatic macrophytes. Exceptional abundances of immature mosquitoes were observed, with late instar larvae and pupae collected with dip densities exceeding 100 per dip on multiple dips (Figure 5.3). This density was considered remarkable in comparison to densities of *Ae. vigilax* in nearby estuarine wetlands that trigger the need for control when exceeding 1 larva per dip across more than ten different sample sites (Webb and Russell 2001). All immature mosquito specimens collected were identified as *Ae. vigilax*. The relatively consistent development stages of mosquitoes corresponded to a single wetland-filling event resulting from rainfall of approximately 363mm in March 2017 (Bureau of Meteorology 2017).

The observations reported here confirm that *Ae. vigilax* will lay eggs around freshwater habitats and successfully complete development in fresh to mildly brackish conditions. There are few specific published accounts, but many anecdotal reports, of immature stages of *Ae. vigilax* occurring in freshwater habitats but their presence in these habitats has been considered occasional or uncommon (Lee et al. 1984). Marks (1982) reports that in Queensland *Ae. vigilax* can “breed successfully in fresh water but it is seldom taken from it” while Dobrotworsky (1965) reports that in Victoria it is found in “fresh water ground pools”.

The paucity of information on *Ae. vigilax* associated with freshwater habitats it not surprising given that the primary focus of previous investigations has been on the intertidal zone and associated habitat characteristics. It is well established through studies of eggs and immature stages that *Ae. vigilax* is most typically associated with tidally influenced estuarine wetlands, especially saltmarsh communities dominated by *Sarcocornia quinqueflora* (Bunge ex Ungen-Sternberg) A.J. Scott and *Sporobolus virginicus* (Linnaeus) Kunth as well as mangrove communities, in NSW dominated by *Avicennia marina* (Forssk.) Vierh. (Kay and Jorgensen 1986, Ritchie 1994, Gíslason and Russell 1997, Knight et al. 2012). It has been noted that, irrespective of vegetation, suitable habitats are typically those ephemeral in nature. The intermittent inundation is likely to be important, not only in allowing gravid mosquitoes to lay eggs on sediment or vegetation within the zone of inundation (Turner and Streever 1999) but also that the drying of their desiccation resistant eggs may be important to maximise hatching success. It is interesting to note that immature stages of *Ae. vigilax* have been reported in ephemeral habitats but that following rainfall and persistent inundation of habitats, a shift to *Cx. annulirostris* as the dominant species occurs (Lee et al. 1984). Dale et al. (2008) surveyed substrates within a she-oak woodland (dominated by *Casuarina* spp) for eggshells of *Ae. vigilax* but found that they were absent in 75% of the samples. These habitats are not necessarily freshwater systems and likely to retain some salinity due to proximity to tidally inundated wetlands.

There are several factors that may contribute to explaining the observation of estuarine mosquitoes in these freshwater wetlands. Although the Narawang Wetlands are freshwater, they are in close proximity to estuarine habitats (i.e. saltmarsh and mangrove communities) and *Ae. vigilax* are abundant in the local area (Claflin and Webb 2017). Given that *Ae. vigilax* can disperse up to 3km from local estuarine wetlands (Webb and Russell 2019), the species have ready access to freshwater habitats. This area is also likely to be a source of potential blood meals given the abundance of wildlife, especially birds and mammals common in the area. A study by (Jansen et al. 2009) found that *Ae. vigilax*, as well as other mosquitoes, were readily feeding on a range of animals in the local area. Therefore *Ae. vigilax* adults, once acquiring a blood meal, may lay eggs nearby rather than dispersing to new areas. With the exposure of wetland banks following draining, mosquitoes may respond to potentially favourable conditions. It is worth noting that while the wetlands may be drained, the sediment does not completely dry and retains some moisture. Consistent with other observations, in the wetlands where abundant immature stages of *Ae. vigilax* were detected, there was also substantial coverage of macrophytes, which may have further provided suitable conditions for oviposition.

There are important practical implications for mosquito and urban wetland management stemming from these observations. In areas where freshwater habitats are present adjacent to estuarine wetlands, it should be considered that ecological “spill over” in habitat use by *Ae. vigilax* may occur. This is noteworthy in two types of settings. Firstly, where water levels in wetlands are actively managed to enhance conditions for wildlife, they may become suitable for *Ae. vigilax* and other species that lay eggs on potentially inundated substrates (e.g. *Aedes* or *Verrallina* spp.). Secondly, where rehabilitation is proposed through the reintroduction of tidal exchange to a currently freshwater setting, and adjacent estuarine wetlands are present, it should be considered that *Ae. vigilax* eggs may be abundant in the environment and may pose a threat once habitats receive tidal exchange.

While the observations reported here relate directly to *Ae. vigilax*, it should be expected that under similar circumstances, other mosquitoes of pest and public health concern (e.g. *Aedes multiplex*, *Aedes procax*, *Verrallina funerea*) may exploit these changes in environmental conditions. Wetlands managed in this way in proximity to estuarine wetlands present the possibility that *Ae. vigilax* will lay eggs in freshwater habitats and may pose a localised nuisance-biting concern. Assumptions regarding the types of mosquitoes produced from these habitats based solely on salinity or vegetation communities should be considered more critically.

Acknowledgments

We thank Jenny O’Meara and Dr Swapan Paul of Sydney Olympic Park Authority for their assistance gaining access to field sites and acknowledge the Wann-gal people, the traditional custodians of the land on which this work was conducted.

References

Bureau of Meteorology. 2017. Climate Data Online. http://www.bom.gov.au/climate/data/. Accessed 10 March 2017.

Claflin, S. B., and C. E. Webb. 2015. Ross River virus: Many vectors and unusual hosts make for an unpredictable pathogen. PLoS Pathogens **11**:e1005070.

Claflin, S. B., and C. E. Webb. 2017. Surrounding land use significantly influences adult mosquito abundance and species richness in urban mangroves. Wetlands Ecology and Management **25**:331-344.

Crocker, W., K. Maute, C. Webb, and K. French. 2017. Mosquito assemblages associated with urban water bodies: Implications for pest and public health threats. Landscape and Urban Planning **162**:115-125.

Dale, P. E., J. Knight, B. H. Kay, H. Chapman, S. A. Ritchie, and M. D. Brown. 2008. Habitat characteristics and eggshell distribution of the salt marsh mosquito, *Aedes vigilax*, in marshes in subtropical eastern Australia. Journal of Insect Science **8**:25.

Dale, P. E. R., and J. M. Knight. 2012. Managing mosquitoes without destroying wetlands: An eastern Australian approach. Wetlands Ecology and Management **20**:233-242.

Dobrotworsky, N. V. 1965. The Mosquitoes of Victoria. Melbourne University Press, Victoria.

Gíslason, G. M., and R. C. Russell. 1997. Oviposition sites of the saltmarsh mosquito, *Aedes vigilax* (Skuse) (Diptera: Culicidae), at Homebush Bay, Sydney, NSW — a preliminary investigation. Australian Journal of Entomology **36**:97-100.

Hamer, A. J., S. J. Lane, and M. J. Mahony. 2002. The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia. Oecologia **132**:445-452.

Hanford, J. K., D. F. Hochuli, and C. E. Webb. 2019a. Oviposition behavior of *Culex annulirostris* (Diptera: Culicidae) is affected by the recent presence of invasive *Gambusia holbrooki* (Cyprinodontiformes: Poeciliidae). Journal of Medical Entomology **56**:1165-1169.

Hanford, J. K., C. E. Webb, and D. F. Hochuli. 2019b. Habitat traits associated with mosquito risk and aquatic diversity in urban wetlands. Wetlands **39**:743-758.

Hanford, J. K., C. E. Webb, and D. F. Hochuli. 2020. Management of urban wetlands for conservation can reduce aquatic biodiversity and increase mosquito risk. Journal of Applied Ecology **57**:794-805.

Jacups, S. P., P. I. Whelan, and B. J. Currie. 2008. Ross River virus and Barmah Forest virus infections: A review of history, ecology, and predictive models, with implications for tropical northern Australia. Vector Borne and Zoonotic Diseases **8**:283-298.

Jansen, C. C., N. A. Prow, C. E. Webb, R. A. Hall, A. T. Pyke, B. J. Harrower, I. L. Pritchard, P. Zborowski, S. A. Ritchie, R. C. Russell, and A. F. Van Den Hurk. 2009. Arboviruses isolated from mosquitoes collected from urban and peri-urban areas of Eastern Australia. Journal of the American Mosquito Control Association **25**:272-278.

Jardine, A., P. Neville, and M. D. Lindsay. 2015. Proximity to mosquito breeding habitat and Ross River virus risk in the peel region of Western Australia. Vector Borne and Zoonotic Diseases **15**:141-146.

Kay, B. H., and W. K. Jorgensen. 1986. Eggs of *Aedes vigilax* (Skuse) and their distribution on plants and soil in south east Queensland saltmarsh. Australian Journal of Entomology **25**:267-272.

Knight, J., L. Griffin, P. Dale, and S. Phinn. 2012. Oviposition and larval habitat preferences of the saltwater mosquito, *Aedes vigilax*, in a subtropical mangrove forest in Queensland, Australia. Journal of Insect Science **12**:6.

Lee, D. J., M. M. Hicks, M. L. Debenham, M. Griffiths, E. N. Marks, J. H. Bryan, and R. C. Russell. 1984. The Culicidae of the Australasian Region. Australian Government Publishing Service Press, Canberra.

Marks, E. N. 1982. An atlas of common Queensland mosquitoes with a guide to common Queensland biting midges. Queensland Institute of Medical Research, Herston, Queensland.

O'Meara, J., and K. Darcovich. 2008. *Gambusia* control through manipulation of water levels in Narawang Wetland, Sydney Olympic Park 2003-2005. Australian Zoologist **34**:285-290.

Ritchie, S., I. D. Fanning, D. A. Phillips, H. A. Standfast, D. McGinn, and B. Kay. 1997. Ross River virus in mosquitoes (Diptera: Culicidae) during the 1994 epidemic around Brisbane, Australia. Journal of Medical Entomology **34**:156-159.

Ritchie, S. A. 1994. Spatial stability of *Aedes vigilax* (Diptera: Culicidae) eggshells in southeastern Queensland salt marshes. Journal of Medical Entomology **31**:920-922.

Russell, R. C. 1993. Mosquitoes and mosquito-borne disease in southeastern Australia: A guide to the biology, relation to disease, surveillance, control and the identification of mosquitoes in southeastern Australia. Westmead Hospital, Department of Medical Entomology.

Russell, T. L., and B. H. Kay. 2008. Biologically based insecticides for the control of immature Australian mosquitoes: A review. Australian Journal of Entomology **47**:232-242.

Tomerini, D. M., P. E. Dale, and N. Sipe. 2011. Does mosquito control have an effect on mosquito-borne disease? The case of Ross River virus disease and mosquito management in Queensland, Australia. Journal of the American Mosquito Control Association **27**:39-44.

Turner, P. A., and W. J. Streever. 1999. Changes in productivity of the saltmarsh mosquito, *Aedes vigilax* (Diptera: Culicidae), and vegetation cover following culvert removal. Australian Journal of Ecology **24**:240-248.

Tyrrell, M. 2011. Millenium Parklands. Landscape Architecture Australia.

Webb, C., S. Doggett, and R. Russell. 2016. A Guide to Mosquitoes of Australia. CSIRO Publishing, Clayton South, Victoria.

Webb, C. E., and R. C. Russell. 1999. Towards management of mosquitoes at Homebush Bay, Sydney, Australia. I. Seasonal activity and relative abundance of adults of *Aedes vigilax*, *Culex sitiens*, and other salt-marsh species, 1993-94 through 1997-98. Journal of the American Mosquito Control Association **15**:242-249.

Webb, C. E., and R. C. Russell. 2001. Do we spray? Did it work? Indices for control of *Aedes vigilax* larvae in Homebush Bay. Arbovirus Research in Australia **8**:387-390.

Webb, C. E., and R. C. Russell. 2019. Dispersal of the mosquito *Aedes vigilax* (Diptera: Culicidae) from urban estuarine wetlands in Sydney, Australia. Journal of Medical Entomology **56**:1290-1295.

Chapter 6:

Management of urban wetlands for conservation can reduce aquatic biodiversity and increase mosquito risk

This chapter has been published:

Hanford, J. K., Webb, C. E. & Hochuli, D. F. (2020) Management of urban wetlands for conservation can reduce aquatic biodiversity and increase mosquito risk. *Journal of Applied Ecology*, 57(4), 794-805.

The paper is available online at:

https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2664.13576

The supplementary material for this paper is available in Appendix 3:

Supplementary Table 3.1. Number of adult mosquitoes captured adjacent to drained (n=6) and undrained (n=6) wetlands during each sampling month. Page 173.

Supplementary Table 3.2. Results of Welch two tailed t-tests for water quality parameters between drained and undrained wetlands for each month. Page 174.

Supplementary Table 3.3. Abundance of *Anopheles annulipes* and *Culex annulirostris* larvae were strongly linked to macroinvertebrate abundance and several water quality parameters. Several macroinvertebrate variables were also linked to water turbidity and dissolved oxygen. Page 175.

Abstract

Global wetland loss means constructed urban wetlands are an increasingly valuable resource for conservation. However, priorities for managing urban wetlands for conservation are often seen to conflict with management to reduce potential mosquito risks, such as nuisance biting and pathogen transmission. Understanding the ecological consequences of wetland management practices is vital to maximise the conservation value of urban wetlands

without negatively impacting public health, however management practices are often untested.

We conducted a landscape-scale experiment to test the ecological impacts of an existing urban wetland management regime, in which a group of urban wetlands are drained annually to reduce the abundance of an invasive fish, the plague minnow *Gambusia holbrooki*, and then refilled to provide breeding habitat for a threatened frog, the green and golden bell frog *Litoria aurea*. We collected and compared aquatic macroinvertebrates and mosquito larvae from these refilled wetlands, and adjacent undrained wetlands, as well as sampling adult mosquito populations on four occasions across summer and autumn.

Wetland draining had a significant effect on aquatic macroinvertebrates and larval mosquitoes. Twice as many macroinvertebrates were collected from drained wetlands compared to undrained wetlands, and almost all mosquito larvae were collected from drained wetlands. Differences in macroinvertebrate assemblages and larval mosquitoes at drained and undrained wetlands decreased over time, but total macroinvertebrate abundance and taxa richness did not.

Synthesis and applications. While conserving threatened habitats and species is vitally important, our results highlight how wetland management practices can unintentionally impact non-target species, and potentially public health. As constructed urban wetlands become more common, so too does the need for routine maintenance and management of threatened and invasive species. It is essential that future design and management of urban wetlands considers the impact mosquitoes might have on humans. Pre-emptive action to control geographically-relevant vectors of mosquito-borne pathogens and nuisance-biting species would reduce human exposure to mosquitoes and associated negative impacts, and increase positive conservation outcomes associated with urban wetlands.

Graphical Abstract

Introduction

Urban wetlands can serve many purposes including managing stormwater, providing refuge for threatened and migratory fauna, contributing to urban water conservation, and opportunities for community education and interaction with nature (Pankratz et al. 2007, Kadlec and Wallace 2009). While blame as epicentres for outbreaks of disease levelled at urban wetlands is often incorrect (Irwin et al. 2008, Johnson et al. 2012), nuisance biting and the transmission of disease-causing pathogens by mosquitoes associated with urban wetlands can impact human health and well-being and undermine community support for wetland conservation (Russell 1999, Knight et al. 2003).

Understanding the ecological interactions between potentially problematic mosquito species and their urban environments is critical to mitigate risks and balance the multiple goals and values of urban wetlands. However, gaps in our understanding of the ecological impacts of various urban wetland management strategies, including routine operational and maintenance actions as well as management for threatened and invasive species, presents a significant challenge for urban wetland managers attempting to balance conservation and public health goals.

The suitability of urban wetlands for different species of mosquitoes is influenced by numerous factors including vegetation, aquatic biodiversity, and the presence of predators (Bentley and Day 1989, Webb et al. 2012, Hanford et al. 2019a). Introductions of larvivorous species of fish for biological mosquito control have had catastrophic impacts on native species of fish, amphibians and invertebrates around the world (Hamer et al. 2002, Pyke 2008) and have frequently been ineffective in reducing mosquito problems (Kumar et al. 2008, Pyke 2008). *Gambusia holbrooki*, known commonly as the plague minnow or mosquitofish, is one of the world’s most significant invasive fish species (Pyke 2008, Ho et al. 2011). Since its introduction to provide mosquito control in Australia in the 1920s it has become widespread in waterways across the country, especially in degraded aquatic habitats in urban areas (Department of Primary Industries 2019). In Australia, *G. holbrooki* predation on eggs and tadpoles of the endangered green and golden bell frog *Litoria aurea* is a key threat to the survival of this iconic frog species (Hamer et al. 2002, O'Meara and Darcovich 2008).

To improve *L. aurea* breeding success, a series of small wetlands at Sydney Olympic Park, Sydney, Australia, are drained on a rotational basis each year to reduce or temporarily eradicate *G. holbrooki* (O'Meara and Darcovich 2008). Aquatic biodiversity, and subsequently ecosystem function, is critical for urban wetlands (Rosenberg and Resh 1993, Strayer and Dudgeon 2010, Lefcheck et al. 2015) but ephemeral waterbodies are also known to be significant sources of mosquito production (Carver et al. 2010). Drought can affect the abundance of mosquitoes and their predators (Chase and Knight 2003), and may also impact vector-borne disease risks (Brown et al. 2014). However, the gradual, prolonged nature of drought differs from the rapid, short-term periodic draining such as that seen in maintenance and management of small urban wetlands, and for which the impact on aquatic biodiversity and mosquitoes is unknown. In addition, drought conditions may influence populations of mosquitoes associated with non-wetland urban habitats (e.g. stormwater infrastructure and domestic water storage) differently to those associated with wetlands.

In this landscape-scale experiment we investigated the impact of wetland draining on aquatic biodiversity and mosquitoes. We were interested in larval mosquitoes as they provide a measure of habitat-specific associations and existing literature on individual species allows assessment of potential risk to public health and wellbeing, and aquatic macroinvertebrates as a measure of aquatic biodiversity (Rosenberg and Resh 1993); we therefore sampled and analysed these groups separately. We compared biotic assemblages collected from drained and undrained wetlands, as well as individual species of interest and summary measures of abundance and diversity. Understanding the impact of this wetland management regime on aquatic macroinvertebrates and mosquitoes across time is important to inform wetland and wildlife management while strategically responding to potential mosquito threats.

Materials and Methods

Study area location and management

Narawang Wetlands were constructed in 2000 to provide habitat for *L. aurea* (O'Meara and Darcovich 2008). The wetlands complex consists of 22 small wetlands and 4 larger, deeper reservoir pools across a 1.6 km long, 20 ha habitat corridor at Sydney Olympic Park in the geographical centre of Sydney, Australia (Figure 6.1). The habitat corridor adjoins remnant Sydney turpentine ironbark forest, a critically endangered ecological community, and is constructed on a former industrial site using engineered soils (Tyrrell 2011). Following construction, *G. holbrooki* invaded the wetlands during seasonal flooding.

Since 2003 the Sydney Olympic Park Authority has been actively managing the wetlands to reduce the impact of *G. holbrooki* on *L. aurea*. The wetlands are drained on a rotational basis each year in early spring (August/September) and left to dry for approximately four weeks to significantly reduce, or temporarily eradicate, *G. holbrooki* populations before the onset of *L. aurea* breeding in summer (O'Meara and Darcovich 2008).

Six wetlands were drained in August 2016 and monitored for refilling via rainfall and runoff (Figure 6.2). To ensure adequate representation of drained and undrained wetlands we selected six similar and spatially independent undrained wetlands based on depth and surface area in consultation with the managing wetlands ecologists at Sydney Olympic Park Authority. For unknown reasons *G. holbrooki* re-invaded one of the drained wetlands in mid-January 2017. Following a major rainfall event in March 2017 the majority of wetlands flooded and *G. holbrooki* were then present throughout the drained and undrained wetlands.

Sampling aquatic biodiversity

Aquatic macroinvertebrates, larval and adult mosquitoes were sampled four times (January, March, April and May 2017), and water chemistry parameters were sampled on three occasions across summer and autumn 2016/2017 to compare characteristics between drained and undrained wetlands and determine whether differences, if any, are mitigated

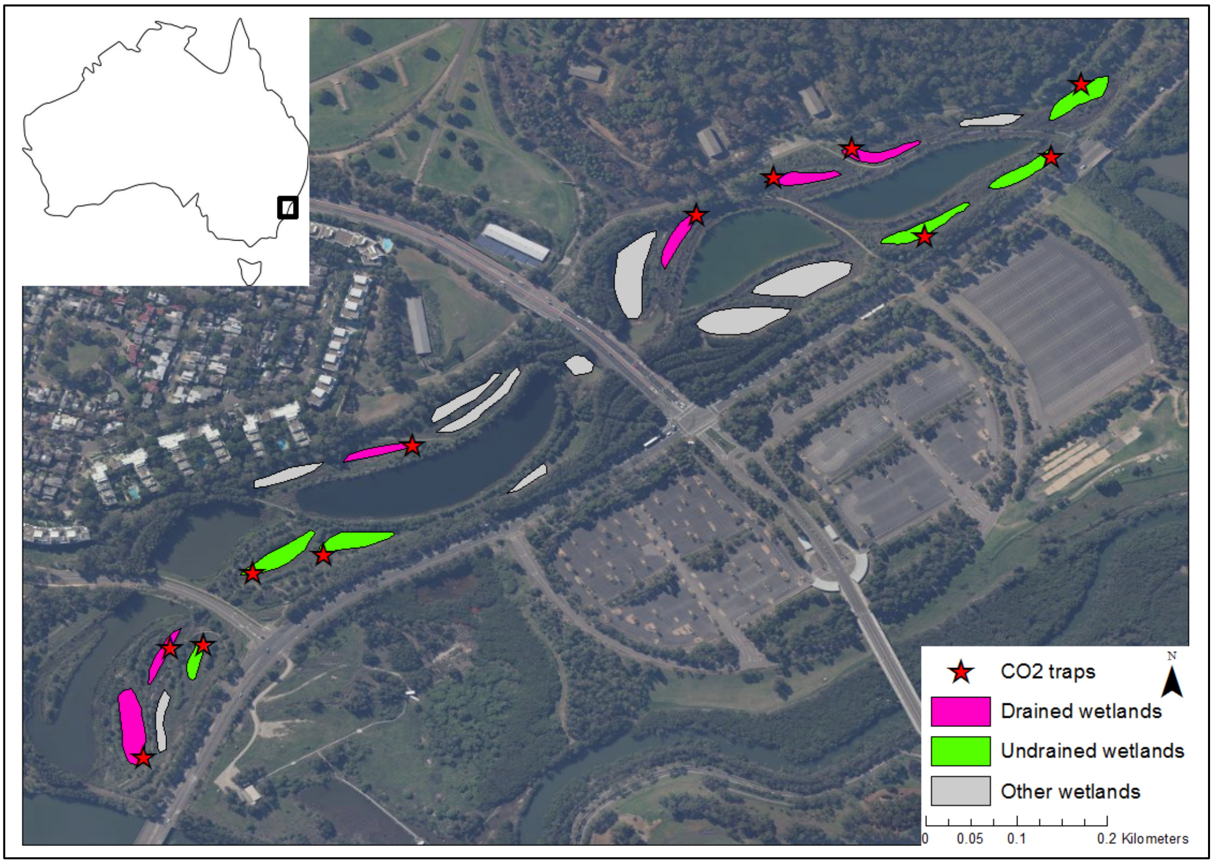


Figure 6.1. Narawang Wetlands at Sydney Olympic Park, in the geographical centre of Sydney, Australia, comprised of 22 freshwater small wetlands and 4 reservoir wetlands. The wetlands complex was constructed in 2000 to provide habitat for flora and fauna and water quality improvement for Haslams Creek.

with time since refilling (i.e. if drained and undrained wetlands become more similar across time).

We used 1 m sweeps of a 30 cm triangular-framed hand-held net with 0.25 mm mesh (Australian Entomological Supplies, Murwillumbah, Australia) to collect aquatic macroinvertebrates from approximately 10 m^2 of representative edge habitat at each wetland. Macroinvertebrates were collected once each in January, March, April and May 2017. Collections were sub-sampled in the field using timed live-picking based on new taxa discovery (Hanford et al. 2019b) and returned to the laboratory preserved in 70% ethanol for counting and identification using keys from Dean (2004) and Hawking et al. (2013). Macroinvertebrates were identified to the lowest practical taxonomic level, typically to genus. We included the zooplankton groups Cladocera, Copepoda and Podocopida in macroinvertebrate counts and analyses due to their important role as a food source, predators and competitors with mosquitoes (Carver et al. 2010, Rossi et al. 2011) and being readily identifiable in the macroinvertebrate sample.

To identify the relative abundance of mosquito species, we sampled drained and undrained wetlands for larval mosquitoes in January, March, April and May 2017. Larval mosquitoes were sampled using a telescopic plastic ladle of approximately 300 mL volume (Australian Entomological Supplies, Murwillumbah, Australia). Ten ‘dips’ were taken from each wetland in areas of aquatic vegetation or decaying plant matter likely to provide habitat for larvae (Hanford et al. 2019b).

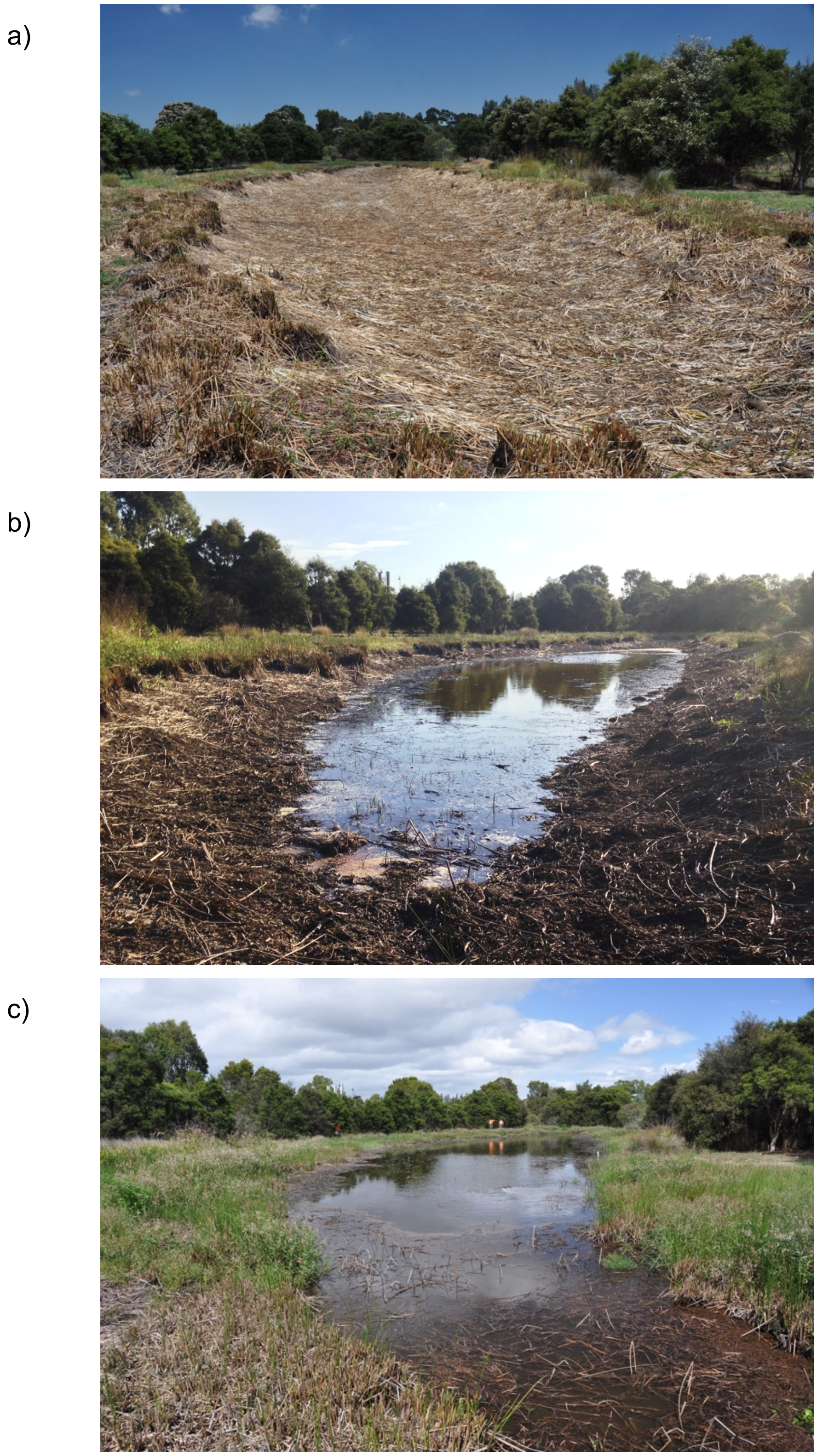


Figure 6.2. Wetland N5 was drained in August 2016 and by November (a) was completely dry. Following seasonal rainfall the wetland progressively refilled (b, January 2017), and by March 2017 (c) broke its banks allowing re-invasion of *Gambusia holbrooki* from adjacent undrained wetlands.

Adult mosquitoes were sampled using carbon dioxide-baited Encephalitis Virus Surveillance traps (Australian Entomological Supplies, Murwillumbah, Australia), which is an effective technique for detection of adult mosquitoes and provides information on mosquito community composition and relative abundance (Williams et al. 2012, Claflin and Webb 2017). One trap was deployed at each wetland at least 2 h before sunset, and placed at the wetland edge with the minimum distance between any two traps 50 m. Although the small distance between wetlands meant the attractive area of traps overlapped in some instances, we were interested to see if strong differences between wetlands might still be captured using this sampling technique, as attractiveness would decrease with distance from the trap. Traps were collected after sunrise and mosquitoes were stored in plastic petri dishes at -20°C until counting and identification using Russell (1993) and Webb et al. (2016).

Water quality parameters

Water quality measurements were collected from each wetland in March, April and May 2017. There was insufficient water depth to collect measurements from most wetlands in January 2017. We used a YSI ProDSS multiparameter water quality probe (Xylem Inc., Rye Brook, NY, USA) to measure salinity (psu), temperature, turbidity (FNU), dissolved oxygen (mg/L), and pH. Measurements were collected prior to sampling for aquatic macroinvertebrates. The probe was held at 30 cm depth where total water depth was approximately 1 m. We allowed readings to stabilise for at least one minute, then collected measurements every five seconds for approximately one minute and the average of these measurements was used for analyses.

Data analyses

We calculated total macroinvertebrate abundance, macroinvertebrate taxa richness, and total ostracod abundance for each wetland for each sample month. We were interested in ostracod abundance specifically because they have been identified as a widespread and abundant competitor with mosquito larvae in ephemeral water bodies (Carver et al. 2010).

To test for the impact of wetland status (drained or undrained, fixed factor) and sample (January, March, April, May, random factor) on macroinvertebrate, larval and adult mosquito, and water quality assemblages, we used four separate permutational multivariate analyses of variance (PERMANOVA+ package in PRIMER v7) (Anderson et al. 2008). We used Bray-Curtis similarity matrices for macroinvertebrate and adult mosquito assemblages, Euclidean distance matrices for larval mosquito assemblages as the large number of zeros in this dataset were biologically meaningful (Legendre and Legendre 2012), and Euclidean distance matrices for water quality.

We compared univariate measures between drained and undrained wetlands for each sample month using the *t.test* function for data with normal sampling distributions and the *wilcox.test* function for non-normal data, both from the stats package in R version 3.5.2 (R Core Team 2018). We analysed Kendall's correlations between univariate biota measures and individual water quality parameters using the *cor.test* function from the stats package in R (R Core Team 2018).

Results

We collected and identified a total of 6,078 aquatic macroinvertebrates across 69 taxa, 265 mosquito larvae across 6 species, and 18,914 adult mosquitoes across 18 species. Twice as many macroinvertebrates were collected from drained wetlands compared to undrained wetlands, almost all mosquito larvae were collected from drained wetlands, and slightly less than half of adult mosquitoes were collected adjacent to drained wetlands.

Aquatic biodiversity of drained and undrained wetlands

There was a strong interaction between wetland drainage status and sampling month in shaping aquatic macroinvertebrate assemblages (pseudo $F_{(3,40)} = 2.06$, $p < 0.01$, perm = 998). Pairwise tests revealed there was a strong early effect on assemblages (Table 6.1), as assemblages differed between drained and undrained wetlands for each sample month and these differences decreased over time. Aquatic macroinvertebrate assemblages overall were significantly different among sample months (pseudo $F_{(3,40)} = 3.94$, $p < 0.01$, perm = 998) (Figure 6.3) but not between drained and undrained wetlands (pseudo $F_{(1,40)} = 6.70$, $p = 0.06$, perm = 377).

The five main taxa driving differences between drained and undrained wetlands were immature Corixidae/Micronectidae, Podocopida, Cladocera, Copepoda and Culicinae (Table 6.2), though the relative importance of an individual taxa in discriminating between drained and undrained wetlands changed across time (Figure 6.4).

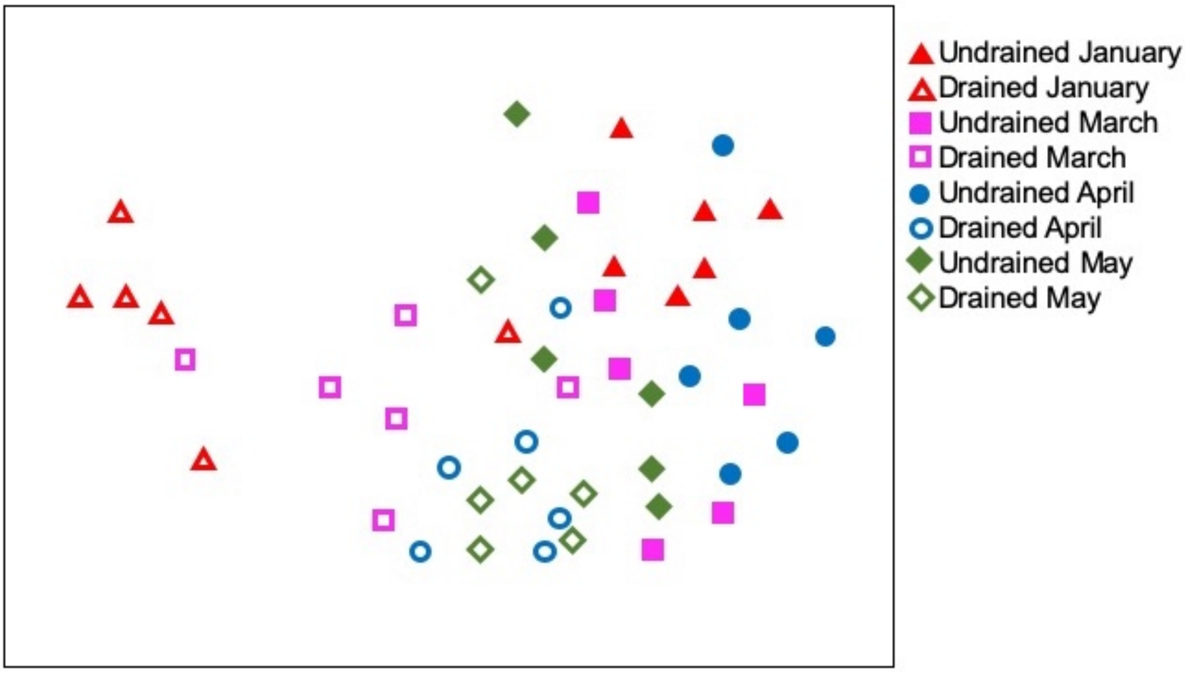


Figure 6.3. Macroinvertebrate assemblages differed between drained (n=6) and undrained (n=6) wetlands following wetland draining and refilling for each sample month, and these differences decreased over time, i.e. assemblages become more similar. Non-metric multi-dimensional scaling plot based on Bray-Curtis similarity matrices of fourth-root transformed data, 2D stress=0.2.

Table 6.1. Macroinvertebrate assemblages at drained (n=6) and undrained (n=6) wetlands become more similar over time. PERMANOVA pairwise tests for sample month within drainage status.

Month	Ave similarity between drained and undrained	t	p	perms
January	20	3.07	0.003	409
March	39	1.92	0.005	413
April	43	1.87	0.002	400
May	47	1.66	0.018	406

Table 6.2. The five macroinvertebrate taxa that contributed most to differences between drained and undrained wetlands varied between sample months. Average abundances given are drawn from fourth-root transformed data that were used for SIMPER analyses; as such, zeros may not represent true absences.

Sample	Species	Undrained ave. abundance	Drained ave. abundance	Ave. dissimilarity	Diss/SD	Contributing %	Cumulative %
January	Podocopida	2.16	0.79	5.71	2.43	7.15	7.15
	Culicinae	0.00	1.70	5.42	1.91	6.79	13.94
	Corixid/Micronectidae	0.00	1.60	5.06	1.98	6.33	20.27
	Hydrophilidae	0.20	1.46	3.80	1.97	4.76	25.04
	Chironominae	1.78	0.57	3.71	1.62	4.65	29.69
March	Corixid/Micronectidae	0.00	1.98	4.54	7.67	7.42	7.42
	Culicinae	0.00	1.18	2.76	1.29	4.51	11.93
	Anisops	0.00	1.18	2.73	1.95	4.46	16.39
	Copepoda	0.91	1.52	2.53	1.25	4.14	20.53
	Dytiscidae	0.57	1.44	2.47	1.56	4.04	24.57
April	Cladocera	0.33	1.82	3.40	1.73	5.95	5.95
	Ischnura	0.42	1.42	2.29	1.78	4.01	9.96
	Anisops	0.00	0.99	2.06	1.27	3.61	13.56
	Corixid/Micronectidae	0.00	0.96	2.04	1.30	3.57	17.13
	Tanypodinae	1.05	0.17	2.01	1.75	3.52	20.65
May	Copepoda	0.36	2.08	3.52	1.43	6.60	6.60
	Anax	0.00	1.37	2.65	2.09	4.97	11.57
	Cladocera	0.65	1.60	2.39	1.48	4.48	16.05
	Ischnura	0.70	1.79	2.28	1.46	4.27	20.31
	Hydrachnidae	0.92	1.23	1.90	1.44	3.56	23.88
All	Corixid/Micronectidae	0.13	1.39	3.17	1.56	4.87	4.87
	Podocopida	2.05	1.83	2.45	1.07	3.75	8.62
	Cladocera	0.40	1.04	2.27	1.08	3.47	12.09
	Copepoda	0.42	1.00	2.19	0.98	3.36	15.45
	Culicinae	0.00	0.81	2.18	0.80	3.34	18.79

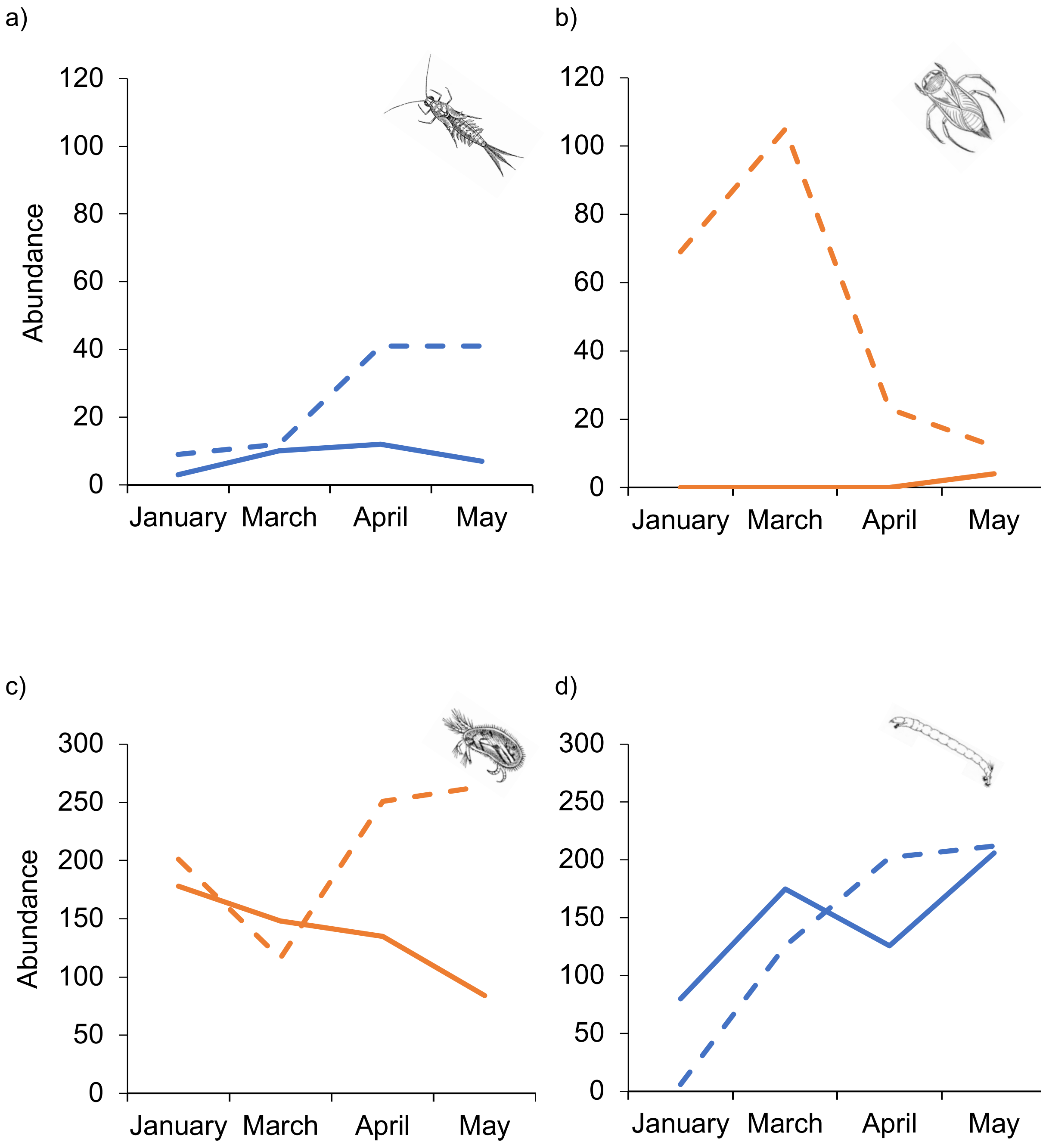


Figure 6.4. The abundance of individual macroinvertebrate taxa differed between drained (broken lines) and undrained (solid lines) wetlands and differences changed across time. Abundances of *Cloeon*, a mayfly (a), only increased in drained wetlands following several months of inundation, while remaining relatively constant at undrained wetlands. Conversely, immature Corixidae/Micronectidae (b) were early colonisers of ‘new; habitats presented by drained wetlands, and their abundance decreased and converged over time. While ostracod (Podocopida) abundance was initially similar between drained and undrained wetlands, as time went by their abundance increased dramatically in drained wetlands while decreasing in undrained wetlands (c). Abundance of chironomids was initially higher in undrained wetlands but levelled between drained and undrained wetlands by the end of sampling in May (d).

Macroinvertebrate summary measures at drained and undrained wetlands

There were multiple differences between drained and undrained wetlands for the macroinvertebrate summary measures total abundance and taxa richness (Table 6.3), though differences were sometimes inconsistent across sampling months (Table 6.4). Interestingly, while macroinvertebrate assemblage differences decreased over time, differences between summary macroinvertebrate measures actually increased. Differences in total macroinvertebrate abundance between drained and undrained wetlands increased over time, with drained wetlands having almost twice as many individuals compared to undrained wetlands by the end of the sampling season. This is attributable to the increase in a few hyper-abundant crustaceans: ostracods, copepods and cladocerans. Undrained wetlands generally had greater macroinvertebrate taxa richness than drained wetlands (Table 6.3).

Individual macroinvertebrate taxa patterns

Patterns of abundance of individual macroinvertebrate taxa varied substantially temporally and spatially. While some taxa appeared to quickly exploit the ‘new’ habitat presented by drained and refilled wetlands (e.g. Chironomidae, Cladocera, *Anax*, immature Corixidae/Micronectidae, Culicinae) (Figure 6.4) and were important in discriminating between drained and undrained wetlands (Table 6.2), others seemed more reliant on continuous water presence, only increasing in abundance in drained wetlands several months into the experiment while remaining relatively constant in undrained wetlands (Figure 6.4). Ostracods were initially present in comparable abundance at drained and undrained wetlands, and differences in abundance increased over time (Figure 6.4).

Undrained wetlands had a higher abundance of ‘rare’ taxa (< 10 individuals collected), 16 of which were only collected from undrained wetlands. In contrast, only two ‘rare’ taxa were found exclusively in drained wetlands (*Nannophlebia* and *Trianodes*). Four abundant taxa were only found in drained wetlands: *Anisops*, *Austrolestes*, Gerridae and Culicinae.

Table 6.3. Summary measures of aquatic macroinvertebrate assemblages at drained and refilled (n=6) and adjacent undrained (n=6) urban wetlands. Despite assemblages becoming more similar over time, abundance tended to become more different over time. Ostracods were considered individually due to their being controphic predators and competitors of mosquito larvae.

	Macroinvertebrate abundance		Macroinvertebrate taxa richness		Ostracod abundance	
	drained	undrained	drained	undrained	drained	undrained
January	615	520	32	35	201	178
March	895	534	34	38	116	148
April	949	512	42	36	251	135
May	1545	507	37	39	264	84
Total	**4005**	**2073**	**53**	**62**	**832**	**545**

Table 6.4. Differences in the abundance of macroinvertebrates between drained and undrained wetlands increased over time, while other macroinvertebrate measures were mostly similar between wetland types. Blank cells indicate where t-tests were not performed due to non-normal data.

	Sample	Welch two sample t-test				Wilcoxon rank-sum test	
		t	df	p	r	W	p
Total abundance of macroinvertebrates	Jan					17.000	0.937
	Mar					28.000	0.132
	Apr					30.500	0.054
	May					36.000	0.002
Macroinvertebrate taxa richness	Jan	-1.196	7.680	0.267	0.396		
	Mar	1.031	8.819	0.330	0.328		
	Apr	2.704	8.462	0.026	0.681		
	May	1.694	9.584	0.123	0.480		
Ostracod abundance	Jan					6.000	0.061
	Mar	-0.453	9.999	0.661	0.142		
	Apr					26	0.228
	May	2.262	5.872	0.065	0.682		

Larval mosquitoes at drained and undrained wetlands

Assemblages of larval mosquitoes were significantly different between drained and undrained wetlands (pseudo $F_{(1,40)} = 24.90$, $p = 0.027$, perm = 369), and also among sample months (pseudo $F_{(3,40)} = 3.75$, $p < 0.01$, perm = 995). Differences between wetlands were driven by the greater abundance of *Culex annulirostris* in drained wetlands (Figure 6.5). Differences among sampling months were also driven by *Cx. annulirostris* which was more abundant in January and March, and *Anopheles annulipes*, which was more abundant in May (Figure 6.5).

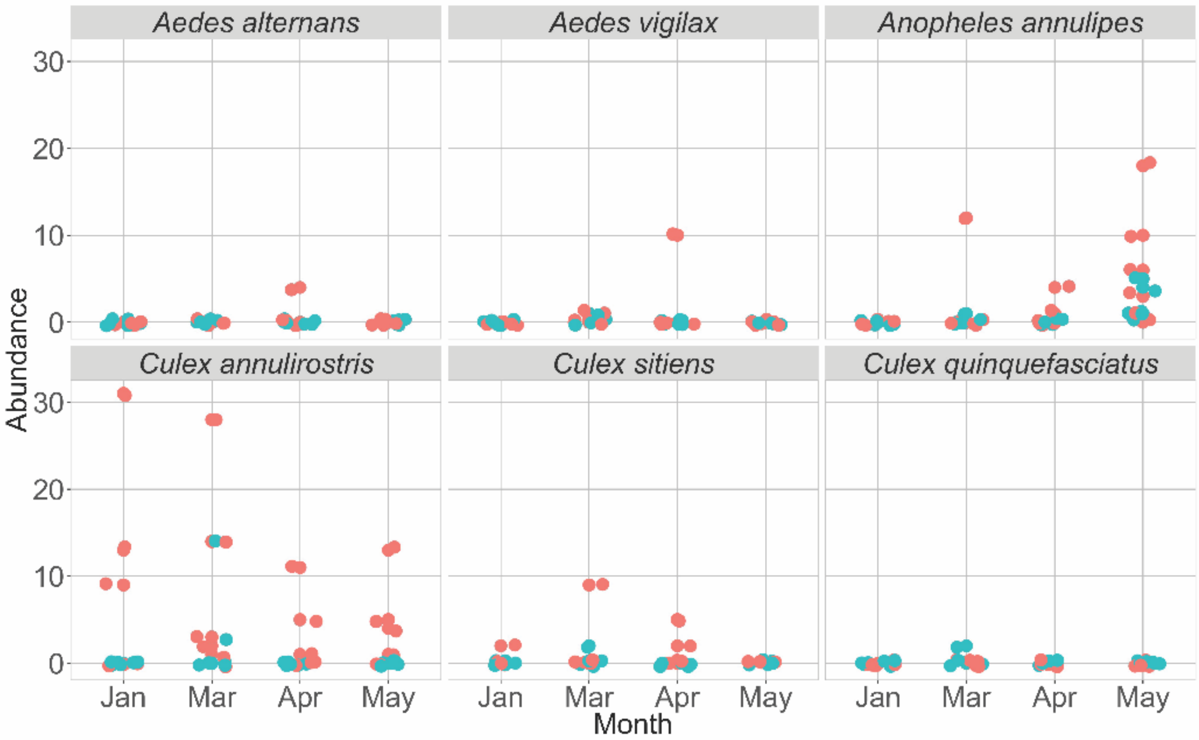


Figure 6.5. More larval mosquitoes were collected from drained (red) compared to undrained (blue) wetlands for all species across all sample months. *Culex annulirostris* and *Anopheles annulipes* were the two most abundant larvae captured, and had different peaks of abundance with *Cx. annulirostris* abundance higher early in the season and *An. annulipes* abundance higher later in the season.

All species of mosquito larvae were more abundant in drained compared to undrained wetlands, and 87% of mosquito larvae were collected from drained wetlands (Table 6.5). Only one drained wetland (N17) did not record any mosquito larvae. This wetland had early re-invasion of *G. holbrooki*, which may be related to the absence of mosquito larvae. *Culex annulirostris* and *An. annulipes*, the two most abundant species of mosquito larvae, had strong seasonal peaks: *Cx. annulirostris* in January/March and *An. annulipes* in May.

Table 6.5. Summary of larval and adult mosquito species collected from drained (n=6) and undrained (n=6) wetlands across the four sampling periods (January, March, April and May).

Mosquito species	Adults		Larvae	
	Drained	Undrained	Drained	Undrained
Aedes alternans	443	538	4	0
Aedes multiplex	11	3		
Aedes notoscriptus	16	12		
Aedes procax	10	3		
Aedes vigilax	5754	7063	12	1
Anopheles annulipes	386	283	55	13
Coquillettidia linealis	13	28		
Coquillettidia xanthogaster	9	20		
Culex annulirostris	1780	2016	141	17
Culex australicus	42	31		
Culex bitaeniorhynchus	3	6		
Culex molestus	24	19		
Culex orbostiensis	13	2		
Culex quinquefasciatus	102	153	0	2
Culex sitiens	436	221	18	2
Mansonia uniformis	39	101		
Mimomyia elegans	1	0		

Adult mosquitoes and wetland draining

Adult mosquito assemblages did not differ between drained and undrained wetlands (pseudo $F_{(1,40)} = 1.34$, $p = 0.306$, perm = 372), though assemblages did change over time as is routinely observed in temperate areas (pseudo $F_{(3,40)} = 12.0$, $p < 0.01$, perm = 999) (Figure 6.6) (e.g. Claflin and Webb 2017, Hanford et al. 2019b).

One-third of mosquito species collected as adults were also collected as larvae (*Aedes alternans*, *Ae. vigilax*, *An. annulipes*, *Cx. annulirostris*, *Cx. sitiens*, and *Cx. quinquefasciatus*) (Table 6.5). Collecting these six species as both larvae and adults confirms they used the study wetlands as larval habitat, and we therefore focussed on adults of these six species. There are other adult mosquito species collected whose larvae may also be associated with freshwater habitats but are likely to either be present in low abundance or may be associated with other unsampled freshwater habitats in the general area (e.g. ephemeral ground pools, drainage lines, stormwater infrastructure).

There were no differences in the adult abundances of our target mosquito species between drained and undrained wetlands for each sample month (all $p > 0.05$). Our six target mosquito species also had varied peaks in abundance of adult individuals, with *Ae. vigilax* in particular being collected in high abundances during January and very few individuals collected at other sample months (Supp. Table 3.1). *Culex annulirostris* was the second-most abundant species of adult mosquito sampled (Table 6.5), and the species of greatest concern in our study region associated with freshwater habitats. Abundance of *Cx. annulirostris* was highest in January and March.

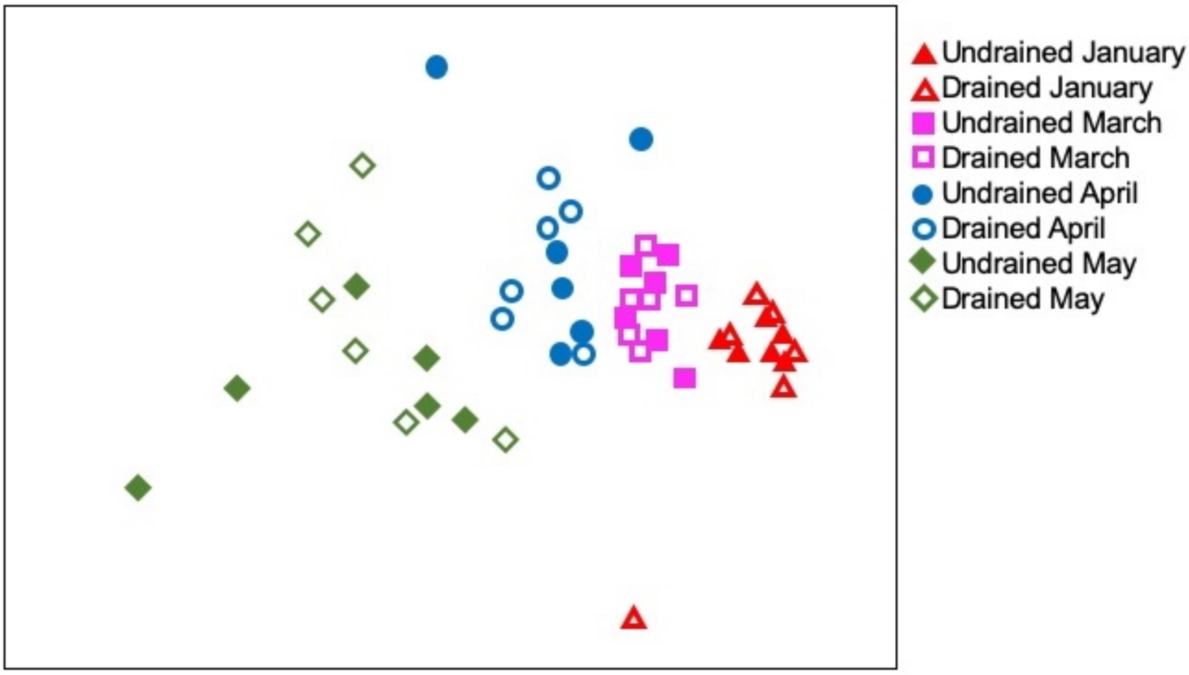


Figure 6.6. Adult mosquito assemblages showed strong seasonal shifts, but there was no difference between drained and undrained wetlands. Non-metric multi-dimensional scaling plot based on Bray-Curtis similarity matrices of fourth-root transformed data, 2D stress = 0.11.

Water quality parameters

Collective water quality (salinity, temperature, turbidity, dissolved oxygen and pH) did not differ between drained and undrained wetlands (pseudo $F_{(1,35)} = 1.12$, p(MC) = 0.373, perm = 38), but there were significant differences among sampling months (pseudo $F_{(2, 35)} = 11.0$, $p < 0.01$, perm = 997) due to differences in temperature. Overall, individual water quality parameters (temperature, salinity, pH, turbidity, dissolved oxygen) also did not differ between drained and undrained wetlands during each sample month (Supp. Table 3.2).

Links between water quality and biotic assemblages

The two most abundant species of mosquito larvae, *Cx. annulirostris* and *An. annulipes*, showed multiple correlations with individual water quality parameters (Supp. Table 3.3), most notably with salinity, temperature, turbidity, pH, and also with abundance of aquatic macroinvertebrates. Aquatic macroinvertebrate abundance was positively related to turbidity, while macroinvertebrate taxa richness was positively related to dissolved oxygen.

Discussion

While draining small urban wetlands may be a valuable tool for wetland maintenance and conservation of wildlife, it can have unexpected impacts on non-target aquatic biodiversity and potentially public health through increased exposure to mosquitoes. Differences in assemblages and individual taxa responses to short-term wetland draining and refilling may be due to water availability, presence of *G. holbrooki*, or a combination of these and other factors, highlighting the importance of considering ecological impacts of management strategies for urban wetlands.

Effects of draining on aquatic biodiversity

Whereas differences between macroinvertebrate assemblages at drained and undrained wetlands decreased over time, consistent with previous studies focused on wetland restoration (Spadafora et al. 2016), differences in summary measures such as taxa richness did not decrease, and differences in the total number of macroinvertebrates increased across time. Therefore, while macroinvertebrate assemblages appear to converge over time, consideration of summary indices suggests complex ecological consequences of wetland draining may not be entirely mitigated over the course of a single sampling season.

While we cannot say definitively if these differences were due to the temporary absence of water or the temporary absence of *G. holbrooki*, the presence of fish has been shown to affect aquatic macroinvertebrates in non-urban semipermanent wetlands (Zimmer et al. 2000). Some taxa, such as mayflies, have even been shown to actively move into fishless habitats such as floodplains despite few apparent benefits and many perceived disadvantages including unreliable water supply and high abundance of amphibian predators (Galatowitsch and Batzer 2011).

Contrary to general perceptions of ephemeral habitats being dominated by mosquitoes due to their ability to rapidly colonise newly created water bodies (Carver et al. 2010, Medlock and Vaux 2014), predators and competitors of mosquitoes are often found in ephemeral waterbodies (Carver et al. 2010, Rossi et al. 2011). We found that the increase in total macroinvertebrate abundance at drained wetlands was driven by several crustacean taxa – ostracods, cladocerans and copepods –typically associated with lesser water permanence in more isolated habitats such as ephemeral riverine wetlands (Chessman and Hardwick 2014). Although not typically considered to be macroinvertebrates, we were interested in these crustacean taxa as they are known controphic competitors and predators of mosquito larvae (Carver et al. 2010, Rossi et al. 2011), and our results demonstrate this can also be the case in urban wetlands. The general abundance of these crustacean taxa and their place in aquatic food webs makes them important sources of food for other invertebrates such as dragonfly nymphs and water mites (Rossi et al. 2011, Hawking et al. 2013). As algal grazers and filter feeders, they also exert a strong influence on algal dynamics, which is an essential consideration for urban wetlands receiving nutrient-enriched water (Lawrence et al. 2002, Kadlec and Wallace 2009).

Introductions of *G. holbrooki* are associated with increases in algal biomass due to their predation of algal grazers such as ostracods and cladocerans, although this top-down effect is reduced in systems previously dominated by notonectids (Ho et al. 2011), emphasising the important role of macroinvertebrates in algal dynamics. While *G. holbrooki* are known to reduce zooplankton abundance, in our experiment ostracod abundance increased following re-invasion of *G. holbrooki* (Figure 6.4c). This may be due to *G. holbrooki* preferentially preying on controphic competitors or predators of ostracods, or perhaps a time lag between wetland rewetting and responses in ostracod populations. An increased abundance of ostracods and other crustaceans was also observed under low stocking densities of the North American arroyo chub (Why et al. 2014), indicating competition release is an important consideration for both native and non-native species in small urban wetlands, and perhaps the initially lower density of *G. holbrooki* in re-invaded wetlands facilitated ostracod population increases.

Removal of invasive species such as *G. holbrooki* therefore may have mixed or counter-intuitive impacts for native fauna and wetland ecological character. In urban areas where temporary draining of wetlands might be considered a management option, it is highly likely that species such as *G. holbrooki* will re-invade at some point, and understanding the effects of their re-invasion should be considered as a component of understanding the effects of their removal.

Effects of draining on mosquitoes

We found no difference in adult mosquito assemblages or individual species of adults between drained and undrained wetlands; adults varied temporally but not at small spatial scales of hundreds of metres. Although the attractive area of our EVS traps overlapped other unsampled wetlands that would provide mosquito habitat, and in some cases two EVS trap areas overlapped, our results indicate strong larval differences cannot be detected using adult sampling, either because of the confounding effect of overlapping trap areas or the presence of multiple unsampled alternative mosquito habitats available in close proximity.

Mosquito larvae were primarily found in drained wetlands. Possible explanations for the low abundance of larval mosquitoes in undrained wetlands include: (1) mosquito eggs are deposited less frequently in undrained wetlands, potentially due to the deterrent effect of fish (Hanford et al. 2019a) or indirect effects associated with wetland draining, for example changes in aquatic habitat characteristics; and/or (2) mosquito eggs are deposited in undrained wetlands, and eggs and larvae consumed, for example by *G. holbrooki* or macroinvertebrate predators (Van Dam and Walton 2007). The relative importance of these explanations is likely to vary between mosquito species. Understanding fine-scale responses of different mosquito species to landscape-scale wetland management strategies is essential to successfully integrate wetlands as biodiversity resources into urban areas.

Responses to oviposition stimuli are highly species-specific and often reflect the evolutionary ecology of the species (Bentley and Day 1989, Van Dam and Walton 2008). The recent presence of fish, different types of organic infusions (manure, leaves, hay) and aquatic plants, and conspecific larvae have been found to deter or attract some ovipositing *Culex* spp. (Allan et al. 2005, Webb et al. 2012, Hanford et al. 2019a). *Culex annulirostris* was the most abundant and widespread species of mosquito larva collected in this study and it was found almost exclusively in drained wetlands (only two instances from undrained wetlands). It is also the species of greatest public health concern in our study region, being a known nuisance-biting pest and vector of mosquito-borne pathogens (e.g. Ross River virus) (Webb et al. 2016).

While most *An. annulipes* larvae in this study were collected from drained wetlands, their abundance was highest in April and May, when drained and undrained wetlands were becoming more similar in their macroinvertebrate assemblages, presence of *G. holbrooki*, water levels and aquatic vegetation. *Anopheles annulipes* is frequently associated with permanent freshwater habitats, especially where filamentous algae is present which offers protection from potential predators (Webb et al. 2016). Their abundance may therefore be linked to factors other than wetland draining, such as habitat traits or seasonal variation.

Surprisingly, *Ae. vigilax* larvae and adults were collected during initial sampling, however this species is usually associated with saline and brackish habitats and abundance decreased as the ponds refilled with freshwater. *Aedes vigilax* is one of the major nuisance biting pests and vector of Ross River virus in coastal Australia and is the target of mosquito control programs (Webb et al. 2016).

In interpreting the results of immature mosquito sampling, it is important to note potential sampling bias due to the ecology of certain species that may influence relative abundance and species richness in samples. In addition to effects of spatial variation in vegetation composition, some larvae will dive for long periods, while others have biological associations that make them difficult to sample when amongst dense vegetation. For example, immatures of *Coquillettidia* and *Mansonia* spp. attach to submerged parts of aquatic plants, making them less likely to be collected in traditional sampling using ladling (Johnson and Russell 2019). In this study we collected adults of two *Coquillettidia* spp. (70 individuals) and one species of *Mansonia* (140 individuals) (Table 6.5), however did not collect any larvae of these species. It is possible captured adults used habitats that were not sampled in this study due to the large attractive area of the EVS traps, and future studies may benefit from using emergence traps to confirm the presence of this difficult to sample species. Predation risk may also alter larval behaviour, and as a consequence, larvae may not be equally likely to be captured in drained and undrained wetlands. Though not all mosquito larvae will emerge (Greenway et al. 2003), our results provide an indication of potential mosquito risk and how this risk is influenced by the practice of wetland draining, and may be especially relevant in urban situations with fewer available habitats in such close proximity.

Management recommendations

Urban wetlands are challenging aquatic environments for taxa to persist in. Although urbanisation can decrease water quality and macroinvertebrate richness and abundance (Mackintosh et al. 2015), our undrained wetlands supported communities of macroinvertebrates that included 20 sensitive and rare taxa, despite having high abundances of *G. holbrooki*, suggesting that macroinvertebrates can persist in the face of such invasive species. Management actions to control invasive species must therefore be carefully considered, given the possibility that actions may be more disruptive than the invasive species themselves (Bergstrom et al. 2009).

Landscape-level *in situ* experiments in urban wetland management are rare, and our research highlights that, despite their impaired ecological character, management of urban wetlands can impact non-target species such as aquatic macroinvertebrates and larval mosquitoes. While these impacts are likely to vary geographically, and also between wetlands with different ecological characteristics (Hanford et al. 2019b), it is critical that abundance and diversity of mosquitoes is considered when evaluating potential management strategies, due to the potential nuisance-biting and pathogen-transmission impacts.

Substantial disruption to the aquatic ecosystem to control pest species must be compared to interventions, such as using crepuscular sprinkler systems to disrupt oviposition (Jiannino and Walton 2004, Popko and Walton 2013), or planting different vegetation species or vegetation designs that facilitate predator access to mosquito larvae within emergent vegetation (Thullen et al. 2002, Jiannino and Walton 2004) that may provide more beneficial and sustainable outcomes.

In urban environments where many small waterbodies are available within close proximity, draining only a subset of these to undertake maintenance works or manage endangered or invasive species would be preferable to draining all wetlands in the system, as this maintains habitat for water-reliant rare taxa, even though undesirable species may persist in the undrained wetlands. Furthermore, considering impacts on non-target species, especially those that may adversely affect human populations, would allow scheduling of management activities to occur when there is less opportunity for colonisation by mosquito species of concern to the local area.

While periodic draining may be considered as a management strategy for some urban wetlands, and indeed may be necessitated by maintenance and repair works, unintended consequences of such actions, including management for conservation, are non-trivial and must be considered.

Acknowledgements

We thank K. Darcovich and J. O’Meara for their assistance in gaining access to field sites. We acknowledge the Wann-gal people, the traditional custodians of the land on which this work was conducted. J.K.H. received support from a Holsworth Wildlife Research Endowment.

Data availability statement

Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.jm63xsj6k (Hanford, Webb & Hochuli, 2020).

References

Allan, S. A., U. R. Bernier, and D. L. Kline. 2005. Evaluation of oviposition substrates and organic infusions on collection of *Culex* in Florida. Journal of the American Mosquito Control Association **21**:268-273.

Anderson, M. J., R. N. Gorley, and K. R. Clarke. 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E Ltd., Plymouth, U.K.

Bentley, M. D., and J. F. Day. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. Annual Review of Entomology **34**:401-422.

Bergstrom, D. M., A. Lucieer, K. Kiefer, J. Wasley, L. Belbin, T. K. Pedersen, and S. L. Chown. 2009. Indirect effects of invasive species removal devastate World Heritage Island. Journal of Applied Ecology **46**:73-81.

Brown, L., J. Medlock, and V. Murray. 2014. Impact of drought on vector-borne diseases – how does one manage the risk? Public Health **128**:29-37.

Carver, S., H. Spafford, A. W. Storey, and P. Weinstein. 2010. The roles of predators, competitors, and secondary salinization in structuring mosquito (Diptera: Culicidae) assemblages in ephemeral water bodies of the wheatbelt of Western Australia. Environmental Entomology **39**:798-819.

Chase, J. M., and T. M. Knight. 2003. Drought-induced mosquito outbreaks in wetlands. Ecology Letters **6**:1017-1024.

Chessman, B. C., and L. Hardwick. 2014. Water regimes and macroinvertebrate assemblages in floodplain wetlands of the Murrumbidgee River, Australia. Wetlands **34**:661-672.

Claflin, S. B., and C. E. Webb. 2017. Surrounding land use significantly influences adult mosquito abundance and species richness in urban mangroves. Wetlands Ecology and Management **25**:331-344.

Dean, J. C. 2004. Identification keys to Australian families and genera of caddis-fly larvae (Trichoptera). Co-operative Research Centre for Freshwater Ecology, Albury, Australia.

Department of Primary Industries, N. S. W. 2019. Eastern gambusia. https://www.dpi.nsw.gov.au/fishing/pests-diseases/freshwater-pests/species/gambusia. Accessed 24/07/2019.

Galatowitsch, M. L., and D. P. Batzer. 2011. Benefits and costs of *Leptophlebia* (Ephemeroptera) mayfly movements between river channels and floodplain wetlands. Canadian Journal of Zoology-Revue Canadienne De Zoologie **89**:714-723.

Greenway, M., P. Dale, and H. Chapman. 2003. An assessment of mosquito breeding and control in four surface flow wetlands in tropical-subtropical Australia. Water Science and Technology **48**:249-256.

Hamer, A. J., S. J. Lane, and M. J. Mahony. 2002. The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia. Oecologia **132**:445-452.

Hanford, J. K., D. F. Hochuli, and C. E. Webb. 2019a. Oviposition behavior of *Culex annulirostris* (Diptera: Culicidae) is affected by the recent presence of invasive *Gambusia holbrooki* (Cyprinodontiformes: Poeciliidae). Journal of Medical Entomology **56**:1165-1169.

Hanford, J. K., C. E. Webb, and D. F. Hochuli. 2019b. Habitat traits associated with mosquito risk and aquatic diversity in urban wetlands. Wetlands **39**:743-758.

Hawking, J. H., L. M. Smith, K. LeBusque, and C. Davey. 2013. Identification and Ecology of Australian Freshwater Invertebrates. http://www.mdfrc.org.au/bugguide. Accessed 09/08/2016.

Ho, S. S., N. R. Bond, and P. S. Lake. 2011. Comparing food-web impacts of a native invertebrate and an invasive fish as predators in small floodplain wetlands. Marine and Freshwater Research **62**:372-382.

Irwin, P., C. Arcari, J. Hausbeck, and S. Paskewitz. 2008. Urban wet environment as mosquito habitat in the upper midwest. Ecohealth **5**:49-57.

Jiannino, J. A., and W. E. Walton. 2004. Evaluation of vegetation management strategies for controlling mosquitoes in a southern California constructed wetland. Journal of the American Mosquito Control Association **20**:18-26.

Johnson, B. J., K. Munafo, L. Shappell, N. Tsipoura, M. Robson, J. Ehrenfeld, and M. V. K. Sukhdeo. 2012. The roles of mosquito and bird communities on the prevalence of West Nile virus in urban wetland and residential habitats. Urban Ecosystems **15**:513-531.

Johnson, P. H., and R. C. Russell. 2019. Colonization of *Coquillettidia linealis* (Skuse) with reference to other *Coquillettidia* and *Mansonia* species. Journal of Vector Ecology **44**:130-137.

Kadlec, R. H., and S. D. Wallace. 2009. Treatment Wetlands. Second edition edition. CRC Press, Taylor & Francis Group, Boca Raton, Florida.

Knight, R. L., W. E. Walton, G. F. O'Meara, W. K. Reisen, and R. Wass. 2003. Strategies for effective mosquito control in constructed treatment wetlands. Ecological Engineering **21**:211-232.

Kumar, R., P. Muhid, H. U. Dahms, L. C. Tseng, and J. S. Hwang. 2008. Potential of three aquatic predators to control mosquitoes in the presence of alternative prey: A comparative experimental assessment. Marine and Freshwater Research **59**:817-835.

Lawrence, J. R., B. Scharf, G. Packroff, and T. R. Neu. 2002. Microscale evaluation of the effects of grazing by invertebrates with contrasting feeding modes on river biofilm architecture and composition. Microbial Ecology **44**:199-207.

Lefcheck, J. S., J. E. K. Byrnes, F. Isbell, L. Gamfeldt, J. N. Griffin, N. Eisenhauer, M. J. S. Hensel, A. Hector, B. J. Cardinale, and J. E. Duffy. 2015. Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. Nature Communications **6**:6936.

Legendre, P., and L. Legendre. 2012. Numerical Ecology 3rd Edition. Elsevier, Oxford, UK.

Mackintosh, T. J., J. A. Davis, and R. M. Thompson. 2015. The influence of urbanisation on macroinvertebrate biodiversity in constructed stormwater wetlands. Science of the Total Environment **536**:527-537.

Medlock, J. M., and A. G. C. Vaux. 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.

O'Meara, J., and K. Darcovich. 2008. *Gambusia* control through manipulation of water levels in Narawang Wetland, Sydney Olympic Park 2003-2005. Australian Zoologist **34**:285-290.

Pankratz, S., T. Young, H. Cuevas-Arellano, R. Kumar, R. F. Ambrose, and I. H. Suffet. 2007. The ecological value of constructed wetlands for treating urban runoff. Water Science and Technology **55**:63-69.

Popko, D. A., and W. E. Walton. 2013. Small-stature emergent macrophytes and crepuscular sprinkler disturbance reduce mosquito abundance in wetland mesocosms. Journal of Vector Ecology **38**:379-389.

Pyke, G. H. 2008. Plague minnow or mosquito fish? A review of the biology and impacts of introduced *Gambusia* species. Annual Review of Ecology, Evolution, and Systematics **39**:171-191.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rosenberg, D. M., and V. H. Resh. 1993. Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall, New York.

Rossi, V., G. Benassi, F. Belletti, and P. Menozzi. 2011. Colonization, population dynamics, predatory behaviour and cannibalism in *Heterocypris incongruens* (Crustacea: Ostracoda). Journal of Limnology **70**:102-108.

Russell, R. C. 1993. Mosquitoes and mosquito-borne disease in southeastern Australia: A guide to the biology, relation to disease, surveillance, control and the identification of mosquitoes in southeastern Australia. Westmead Hospital, Department of Medical Entomology.

Russell, R. C. 1999. Constructed wetlands and mosquitoes: Health hazards and management options—an Australian perspective. Ecological Engineering **12**:107-124.

Spadafora, E., A. W. Leslie, L. E. Culler, R. F. Smith, K. W. Staver, and W. O. Lamp. 2016. Macroinvertebrate community convergence between natural, rehabilitated, and created wetlands. Restoration Ecology **24**:463-470.

Strayer, D. L., and D. Dudgeon. 2010. Freshwater biodiversity conservation: Recent progress and future challenges. Journal of the North American Benthological Society **29**:344-358.

Thullen, J. S., J. J. Sartoris, and W. E. Walton. 2002. Effects of vegetation management in constructed wetland treatment cells on water quality and mosquito production. Ecological Engineering **18**:441-457.

Tyrrell, M. 2011. Millenium Parklands. Landscape Architecture Australia.

Van Dam, A. R., and W. E. Walton. 2007. Comparison of mosquito control provided by the Arroyo chub (*Gila orcutti*) and the mosquitofish (*Gambusia affinis*). Journal of the American Mosquito Control Association **23**:430-441.

Van Dam, A. R., and W. E. Walton. 2008. The effect of predatory fish exudates on the ovipostional behaviour of three mosquito species: *Culex quinquefasciatus*, *Aedes aegypti* and *Culex tarsalis*. Medical and Veterinary Entomology **22**:399-404.

Webb, C., S. Doggett, and R. Russell. 2016. A Guide to Mosquitoes of Australia. CSIRO Publishing, Clayton South, Victoria.

Webb, C. E., A. Ironside, and S. Mansfield. 2012. A comparison of oviposition preference in the presence of three aquatic plants by the mosquitoes *Culex annulirostris* Skuse and *Culex quinquefasciatus* Say (Culicidae: Diptera) in laboratory tests. General and Applied Entomology **41**:21-26.

Why, A. M., B. Johnson, S. Nygren, R. Zembal, J. Thieme, and W. E. Walton. 2014. Suitability of the Arroyo Chub (*Gila orcutti*) for the biological control of mosquitoes in a southern California treatment wetland. Proceedings and Papers of the Mosquito and Vector Control Association of California **82**:80-89.

Williams, C. R., C. A. Bader, S. R. Williams, and P. I. Whelan. 2012. Adult mosquito trap sensitivity for detecting exotic mosquito incursions and eradication: A study using EVS traps and the Australian southern saltmarsh mosquito, *Aedes camptorhynchus*. Journal of Vector Ecology **37**:110-116.

Zimmer, K. D., M. A. Hanson, and M. G. Butler. 2000. Factors influencing invertebrate communities in prairie wetlands: A multivariate approach. Canadian Journal of Fisheries and Aquatic Sciences **57**:76-85.

Chapter 7:

Oviposition behavior of *Culex annulirostris* (Diptera: Culicidae) is affected by the recent presence of invasive *Gambusia holbrooki* (Cyprinodontiformes: Poeciliidae)

This chapter has been published:

Hanford, J. K., Hochuli, D. F. & Webb, C. E. (2019) Oviposition behavior of *Culex annulirostris* (Diptera: Culicidae) is affected by the recent presence of invasive *Gambusia holbrooki* (Cyprinodontiformes: Poeciliidae). Journal of Medical Entomology, 56(4), 1165-1169.

The paper is available online at:

https://academic.oup.com/jme/article-abstract/56/4/1165/5420705

Abstract

Constructed wetlands are popular tools for managing threatened flora and fauna in urban settings, but there are concerns that these habitats may increase mosquito populations and mosquito-related public health risks. Understanding the interactions occurring between mosquitoes of public health concern and co-occurring organisms is critical to informing management of these habitats to mitigate potential health risks and balance the multiple values of urban wetlands. This study examined how oviposition behavior of *Culex annulirostris* Skuse (Diptera: Culicidae), the most important pest mosquito species associated with freshwater wetland habitats in Australia, is influenced by the presence of

Gambusia holbrooki Girard (Cyprinodontiformes: Poeciliidae), a widespread invasive fish. Water was collected from urban wetlands that are intensively managed to reduce *G. holbrooki* populations to assist conservation of locally threatened frogs, and adjacent unmanaged wetlands where *G. holbrooki* was abundant. Laboratory experiments were conducted to examine the oviposition response by *Cx. annulirostris* to water samples from these two habitats. Experiments were conducted on two occasions, once in February following draining and refilling of the urban wetlands, and repeated following a substantial rainfall event in March. The results clearly demonstrate that ovipositing mosquitoes were able to detect and avoid water derived from habitats containing fish, even in the absence of the fish themselves. Understanding how invasive species affect the behavior and spatial distribution of pest species such as *Cx. annulirostris* will enable future wetland design and management to maximise benefits of urban wetlands and minimize potential public health risks.

Introduction

Constructed wetlands are a common feature of urban landscapes as a tool for stormwater management and treatment (Mitsch et al. 2009) while also enhancing biodiversity and providing refuge for threatened flora and fauna (Mitsch et al. 2009). However, there are concerns about constructed wetlands potentially causing increases in mosquito populations and creating associated public health risks (Russell 1999). Notwithstanding the potential increase in mosquito populations associated with wetland construction itself, the way in which these habitats are managed may also impact the abundance of local mosquitoes and subsequent pest and public health threats. Balancing the multiple goals of urban wetlands therefore presents substantial challenges. The key to minimizing potential mosquito-related public health risks is understanding the modified ecological interactions occurring between species brought together by these created wetland habitats.

Predatory fish are often proposed to control mosquitoes in constructed wetlands (Angelon and Petranka 2002). However, introduction of fish outside their natural range, as has been the case with *Gambusia holbrooki* Girard (Cyprinodontiformes: Poeciliidae) in Australia, has had catastrophic impacts on multiple taxa including frogs and native fish (Webb and Joss 1997, Hamer et al. 2002). In many circumstances exotic fish have proven to be ineffective as biological control agents (Haas et al. 2003, Kumar et al. 2008). Given the potential impact on aquatic wildlife and less than effective mosquito control, local authorities are investigating strategies to reduce, remove or exclude fish such as *G. holbrooki* from urban wetlands. However, management of invasive fish in urban wetlands may have unintended impacts on mosquito populations and potentially on public health risks associated with mosquito-borne pathogens.

Culex annulirostris Skuse (Diptera: Culicidae) is a known nuisance-biting pest closely associated with freshwater habitats (Webb et al. 2016), is widespread across Australia, southeast Asia and Micronesia (Lee et al. 1984), and has been identified as a mosquito of concern in association with constructed wetlands in urban areas (Russell 1999). It has been implicated in the transmission of a range of arboviruses of public health concern, including Ross River, Barmah Forest, Murray Valley encephalitis, and West Nile (Kunjin subtype) viruses (Doggett et al. 2009).

When selecting oviposition sites, gravid mosquitoes detect and respond to a variety of chemical, physical and biological cues (Bentley and Day 1989), and several mosquito species have been shown to avoid ovipositing in water where the presence of potential predators can be detected (Angelon and Petranka 2002, Van Dam and Walton 2008, Hurst et al. 2010). While Australian native fish have been shown to prey upon *Cx. annulirostris* (Willems et al. 2005), there is little evidence to demonstrate the indirect impact of the presence of native or invasive fish on mosquito oviposition. There may be species-specific responses by mosquitoes to the presence of fish in water bodies, and invasive fish may actually play a relatively more important role in structuring mosquito populations due to their aggressive nature and exclusion of native fishes from urban wetlands (Haas et al. 2003).

The native fish *Melanotaenia duboulayi* (Castelnau) (Atheriniformes: Melanotaeniidae) has been shown to affect the oviposition behavior of *Cx. annulirostris* with the mosquito avoiding laying eggs in water that had recently contained *M. duboulayi*, while in the same study *Aedes notoscriptus* Skuse (Diptera: Culicidae), a container-inhabiting species with little natural likelihood of predation from *M. duboulayi* (Webb et al. 2016), actually preferred to oviposit in water that recently contained *M. duboulayi* (Hurst et al. 2010, Webb et al. 2016). As *Cx. annulirostris* has no shared evolutionary history with exotic fish such as *G. holbrooki*, *Cx. annulirostris* may not interpret the recent presence of *G. holbrooki* as a predatory threat (Bentley and Day 1989).

We examined oviposition behavior in a laboratory experiment using water samples collected from the field. We tested whether gravid *Cx. annulirostris* displayed an oviposition preference for water sourced from urban wetlands with abundant *G. holbrooki* populations, or water from wetlands comparatively free of *G. holbrooki*, in the absence of other visual or mechanical cues. As *Cx. annulirostris* has no shared evolutionary history with *G. holbrooki*, we predicted that *Cx. annulirostris* would not perceive *G. holbrooki* as a threat, so the recent presence of *G. holbrooki* would have no effect on where *Cx. annulirostris* chose to oviposit.

Materials and Methods

Wetland management

The Narawang Wetlands complex consists of 22 constructed freshwater habitat wetlands across 20 ha at Sydney Olympic Park in the geographical center of Sydney, Australia

(33°50’07”S, 151°03’51”E). The wetlands are actively managed to reduce the impact of *G. holbrooki* on the endangered green and golden bell frog, *Litoria aurea* (Lesson)(Anura: Hylidae) (O'Meara and Darcovich 2008). Predation by *G. holbrooki* on *L. aurea* tadpoles has been identified as a key threat to the breeding success of *L. aurea* (Hamer et al. 2002, O'Meara and Darcovich 2008). Three groups of wetlands in the Narawang complex are alternately drained each year in early spring (August/September) and left to dry for approximately four weeks to significantly reduce, or temporarily eradicate, *G. holbrooki* populations before the onset of *L. aurea* breeding in summer (O'Meara and Darcovich 2008).

Field methods

Wetlands were drained in late 2016 and monitored for refilling via rainfall and runoff. An above average rainfall event occurred in Sydney during March 2017 with over 300 mm being recorded (Bureau of Meteorology 2017). Water for oviposition preference experiments was collected from wetlands that had not been drained and where a visually abundant *G. holbrooki* population was present, and from previously drained and refilled wetlands that had not yet been reinvaded by *G. holbrooki* (referred to as ‘fish-free’). While it is possible that our ‘fish-free’ wetlands may have contained larval fish, monitoring has indicated that even during years that experience unseasonably heavy rain, the wetland draining program results in drained wetlands having no indication of *Gambusia* presence for an average of 2 months, and 4.5 months during average weather years (O'Meara and Darcovich 2008). The program for 2016-2017 experienced minimal early rain, and the first large rainfall event did not occur until March 2017. We have therefore used drained wetlands as ‘comparatively fish-free’ habitats.

Sample wetlands were within 50 m of each other, of equal age, similar size and depth, and with similar vegetation communities. We collected 1 liter of water from each wetland using a clean glass bottle with 0.5 mm mesh over the mouth to prevent collection of organic material and invertebrates. Each bottle was rinsed three times with wetland water, then the sample was collected from an undisturbed part of the wetland. Wetland water was transported to the laboratory and the experiment started within 1 h of collection. We repeated this experiment twice, in February prior to the major rainfall event, and in March following the rainfall event, on each occasion using different fish-free wetlands and the same fish-present wetland.

Laboratory methods

A laboratory colony of *Cx. annulirostris* was used for both experiments. The colony was derived from specimens collected in 1975 from northern Victoria, Australia, and has been blood fed primarily on sedated rats. The colony was not supplemented at any time with field collected specimens. Mosquitoes were provided a bloodmeal using a sedated rat for approximately 30 min. All male and non-blood fed female mosquitoes were removed from the cage and remaining specimens were maintained for 4 d in a climate controlled room at 25°C (±2°C), 75% (±10%) relative humidity and 12:12 h[L:D], and provided with a 10% sugar solution soaked cotton pad.

Oviposition preference experiments were conducted five days following blood feeding using (n=10) screened cages (approx. 30 x 30 x 30 cm) (Figure 7.1). Two disposable clear plastic cups (150 mL) containing 50 mL of water from either the *G. holbrooki*-inhabited wetland or the fish-free wetland were provided as oviposition sites in each cage, and a bamboo skewer was provided as a resting site within each cup. Ten blood-fed mosquitoes were introduced into each cage and could choose between the two cups, placed in opposite and random corners of the cage, for oviposition. This low stocking density of females was to minimise the potential for oviposition behavior to be affected by existing egg density, as studies have demonstrated that *Aedes aegypti* (L.) females prefer to oviposit in the presence of existing conspecific and heterospecific eggs (Allan and Kline 1998).

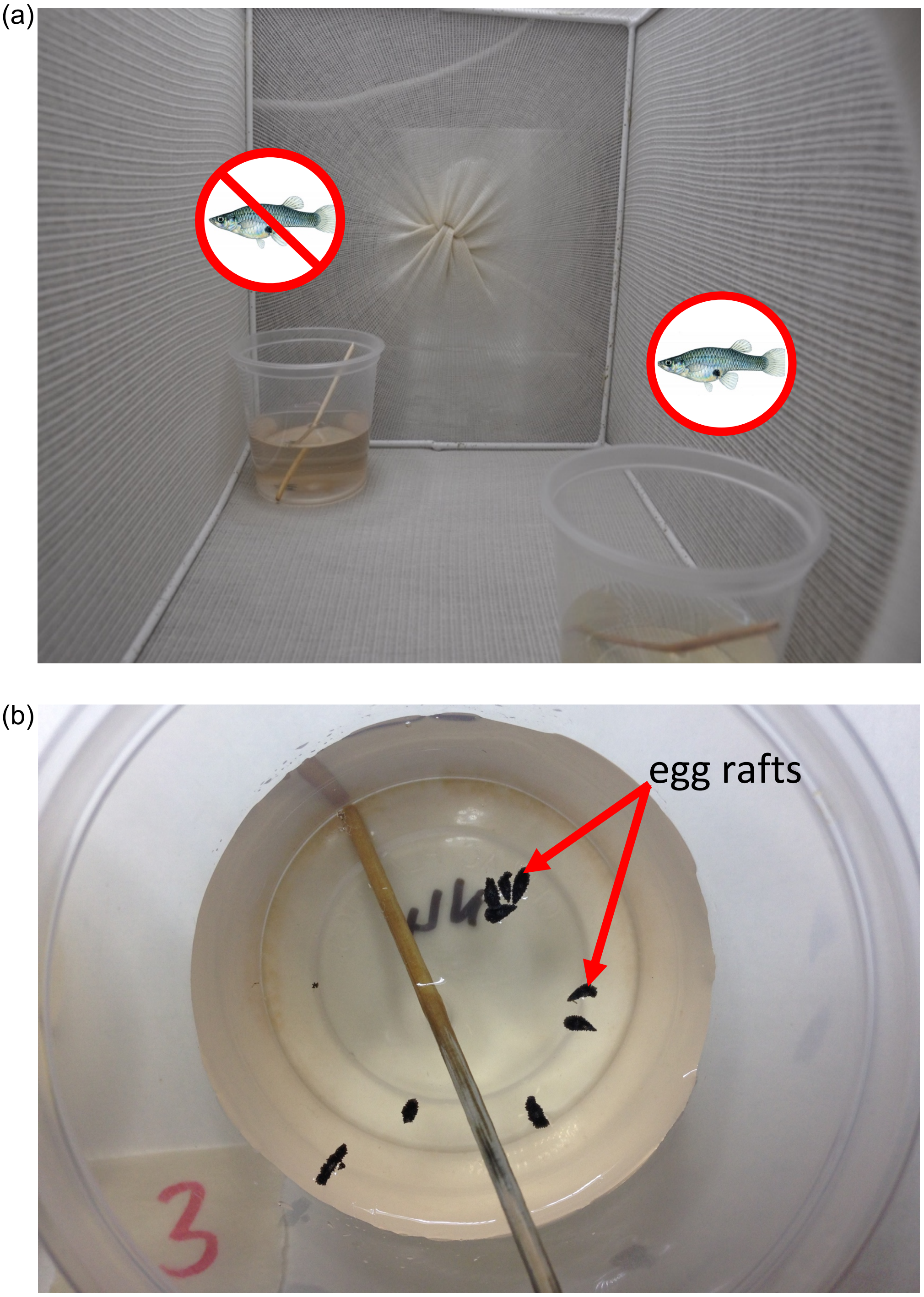


Figure 7.1. (a) Laboratory set-up for oviposition choice experiment. Ten screened cages approx. 30x30x30cm with two cups of water, one from the *G. holbrooki*-inhabited wetland and one from the fish-free wetland as oviposition sites in each cage. (b) Egg rafts deposited in a sample cup.

Mosquitoes were maintained in the 10 cages for 48 h. We then removed mosquitoes and oviposition cups, and counted the number of egg rafts in each cup. Because a new generation of mosquitoes was used for each experiment and another water collection made, we did not sum the number of egg rafts per treatment across the two experiments, which were analysed separately (n=10 for each experiment, with 10 mosquitoes in each replicate). The number of egg rafts laid in each type of wetland water was compared using a two-tailed paired *t*-test (SPSS Statistics 2013).

Results

Gravid *Cx. annulirostris* females oviposited significantly more egg rafts in the fish-free wetland water in both February (t = 3.80; df = 9; $p < 0.01$) and March (t = 13.82; df = 9; $p <$ 0.01) compared to water from wetlands with abundant *G. holbrooki* populations (Figure 7.2). The mean (± SE) total number of egg rafts laid in each cage in February was 8.3 ± 0.54, and in March was 9.3 ± 0.26. In February 6.9 (± 0.97) egg rafts from each cage were laid in water from the fish-free wetland, and in March 8.7 (± 0.40) egg rafts from each cage were laid in water from the fish-free wetland.

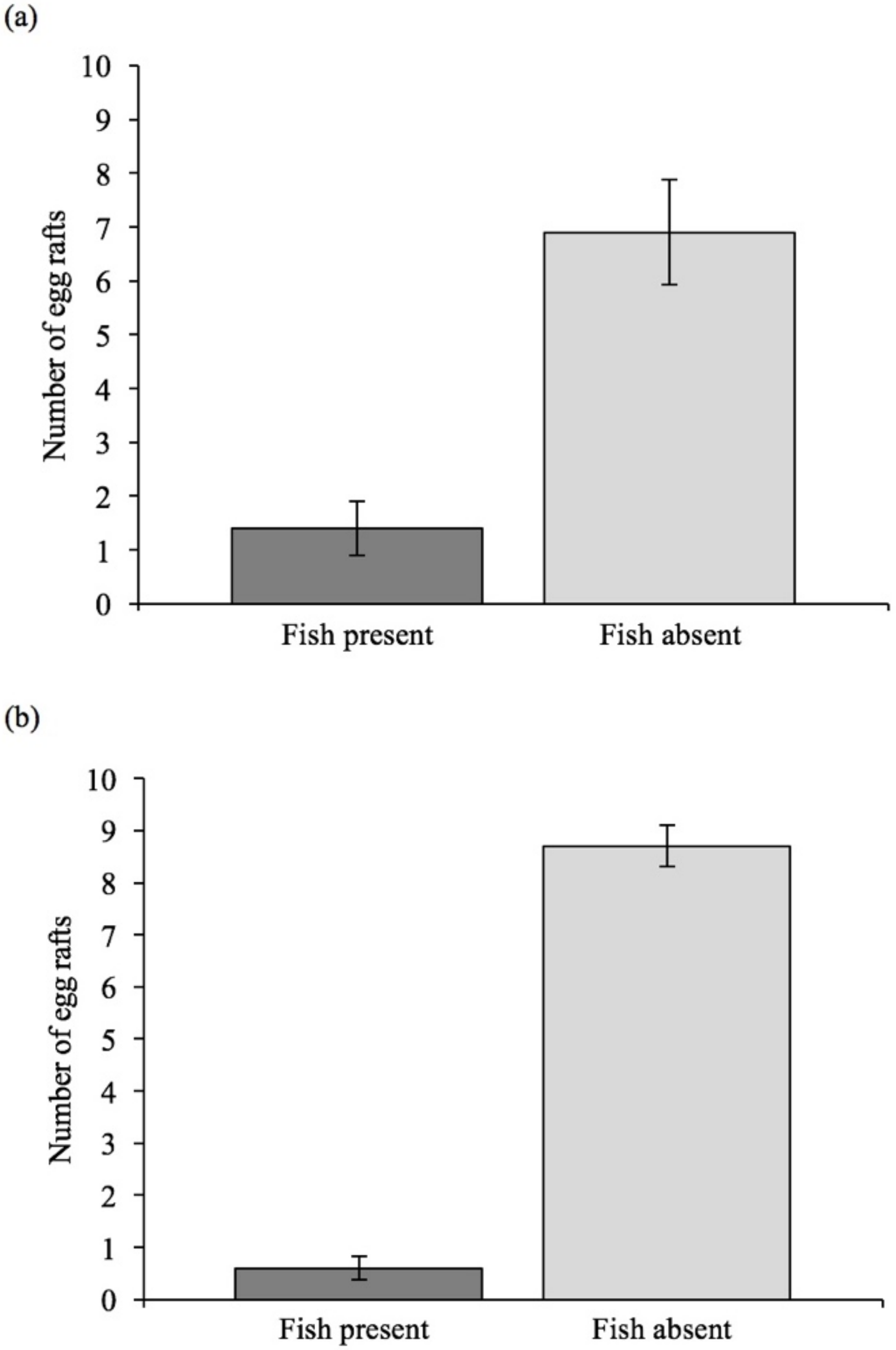


Figure 7.2. Number of egg rafts (± SE) laid by gravid *Culex annulirostris* in water collected from wetlands with and without invasive *Gambusia holbrooki* in (a) February (n=10) and (b) March (n=10). Each experiment used a new generation of mosquitoes, new collection of wetland water and different fish-free sample wetland.

Discussion

Although laboratory responses may vary under different environmental conditions (Van Dam and Walton 2008), the strong preferences observed over our two experiments, along with the shared vegetation composition across the wetlands and close proximity of the wetlands, suggest that chemical cues relating to the recent removal of *G. holbrooki* in wetlands influenced where female *Cx. annulirostris* chose to oviposit. This effect on oviposition behavior remained in the absence of visual and mechanical cues, though the precise chemical cues responsible for mediating this interaction between *Cx. annulirostris* and *G. holbrooki* remain unknown. To minimise public health risks associated with this key mosquito species and other pest and vector species it is vitally important that we understand their responses to the construction of new habitats near human populations (Webb 2013).

There are numerous factors potentially influencing mosquito oviposition (for a review of these factors see Kramer and Mulla 1979, Bentley and Day 1989). Using field-collected water in our laboratory experiments enabled us to present mosquitoes with water containing a variety of chemical compounds that would not be present if water had been artificially conditioned in a laboratory environment as is often done (Allan et al. 2005, Van Dam and Walton 2008, Hurst et al. 2010). Future experiments may reveal further insights by combining these methods – collecting water from fish-free wetlands and conditioning some of this water with *Gambusia*, which would be removed prior to the experiment, generating fish-conditioned water that may still possess the variety of chemical compounds derived from the natural habitat.

Sensitivity of different species of gravid mosquitoes to fish presence may depend on the likelihood of predation (Van Dam and Walton 2008), however oviposition responses to different species of fish and invertebrate predators cannot be generalized across families of potential predators (Why et al. 2016). Management of urban wetlands to reduce potential mosquito risks to humans may benefit from further investigation of species-specific relationships between mosquito species and native and non-native larvivorous fish species, and the differences in the semiochemicals mediating these responses. Interestingly, our study demonstrated that a larvivorous fish species can influence oviposition behavior of a mosquito species with which it shares no evolutionary history, in contrast to many suggestions that fish would not be a strong selective agent if not frequently encountered in natural habitats and therefore presenting a significant risk of predation (Bentley and Day 1989, Van Dam and Walton 2008). These results suggest there may be similarities between the chemical cues produced by native and non-native fish species, which provides an interesting avenue for further research.

In addition to chemical cues indicating presence of potential fish and invertebrate predators (Blaustein et al. 2004, Walton et al. 2009), some species of gravid mosquitoes may also detect and respond to the presence of conspecific and heterospecific eggs (Allan and Kline 1998) or the type of aquatic plants present (Webb et al. 2012). The ability for mosquitoes to detect and respond to the presence of predators, conspecifics and environmental features may also vary due to differences in gene expression, sexual development rate and other physiological measures that occur under laboratory conditions when compared to wild populations (Lainhart et al. 2015). For example, odour-mediated host preferences have been demonstrated to vary between laboratory colonies of *Cx. annulirostris*, though this is likely due to host availability rather than heritability (Williams 2002). Therefore, the effects of colonisation on host preferences are likely to be minimal especially for generalist species such as *Cx. annulirostris* (Williams 2002), and host preferences can persist following hundreds of laboratory generations (McCray and Schoof 1970). Even so, investigating the effect of colonisation on innate responses such as avoidance of co-evolved and novel predators could be an interesting area for further research.

Given that *Cx. annulirostris* can detect and respond to the presence of fish, it is possible that gravid females move to nearby habitats that lack fish for oviposition (Angelon and Petranka 2002). *Culex annulirostris* is known to disperse more than 7 km from larval habitats (Russell 1986). Productivity of a single wetland habitat may also be driven by a range of other factors, such as vegetation type and abundance, presence of invertebrate and other vertebrate mosquito predators, surrounding land use, water quality and water permanence. Wetland managers must therefore also consider surrounding habitats when assessing potential mosquito risks, and further research on the effects of surrounding land use and urban habitat connectivity on mosquito risks would better inform future management decisions.

Our findings highlight that management of urban wetlands to benefit threatened species may also affect the ecological interactions of non-target species, with potential implications for public health risks. We have shown that *G. holbrooki* presence likely exerts a strong influence on oviposition behavior of *Cx. annulirostris*, which has implications for our understanding of ecological interactions in constructed urban wetlands, and management of these environments for their multiple goals.

Acknowledgements

This work was conducted under Western Sydney Local Health District Animal Ethics Committee Protocol No.2002. We thank Karen Willems and David Lilly for their assistance in conducting these experiments; Jenny O’Meara for her assistance gaining access to field sites, and Ryan Leonard for thoughtful feedback on earlier versions of this manuscript.

We would also like to acknowledge the Wann-gal people, the traditional custodians of the land on which this work was conducted.

This work was conducted in part with support from a Holsworth Wildlife Research Endowment awarded to J. Hanford.

References

Allan, S. A., U. R. Bernier, and D. L. Kline. 2005. Evaluation of oviposition substrates and organic infusions on collection of *Culex* in Florida. Journal of the American Mosquito Control Association **21**:268-273.

Allan, S. A., and D. L. Kline. 1998. Larval rearing water and preexisting eggs influence oviposition by *Aedes aegypti* and *Ae. albopictus* (Diptera: Culicidae). Journal of Medical Entomology **35**:943-947.

Angelon, K. A., and J. W. Petranka. 2002. Chemicals of predatory mosquitofish (*Gambusia affinis*) influence selection of oviposition site by *Culex* mosquitoes. Journal of Chemical Ecology **28**:797-806.

Bentley, M. D., and J. F. Day. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. Annual Review of Entomology **34**:401-422.

Blaustein, L., M. Kiflawi, A. Eitam, M. Mangel, and J. E. Cohen. 2004. Oviposition habitat selection in response to risk of predation in temporary pools: Mode of detection and consistency across experimental venue. Oecologia **138**:300-305.

Bureau of Meteorology. 2017. Climate Data Online. http://www.bom.gov.au/climate/data/. Accessed 10 March 2017.

Doggett, S. L., J. Clancy, J. Haniotis, C. E. Webb, L. Hueston, M. Marchetti, E. Blyth, S. Howard, D. E. Dwyer, and R. C. Russell. 2009. Arbovirus and vector surveillance in NSW, 2004/05-2007-08. Arbovirus Research in Australia **10**:28-37.

Haas, R. C., M. V. Thomas, and G. L. Towns. 2003. An assessment of the potential use of *Gambusia* for mosquito control in Michigan. Fisheries Technical Report 2003-2. Michigan Department of Natural Resources.

Hamer, A. J., S. J. Lane, and M. J. Mahony. 2002. The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia. Oecologia **132**:445-452.

Hurst, T. P., B. H. Kay, M. D. Brown, and P. A. Ryan. 2010. *Melanotaenia duboulayi* influence oviposition site selection by *Culex annulirostris* (Diptera: Culicidae) and *Aedes notoscriptus* (Diptera: Culicidae) but not *Culex quinquefasciatus* (Diptera: Culicidae). Environmental Entomology **39**:545-551.

Kumar, R., P. Muhid, H. U. Dahms, L. C. Tseng, and J. S. Hwang. 2008. Potential of three aquatic predators to control mosquitoes in the presence of alternative prey: A comparative experimental assessment. Marine and Freshwater Research **59**:817-835.

Lainhart, W., S. A. Bickersmith, M. Moreno, C. T. Rios, J. M. Vinetz, and J. E. Conn. 2015. Changes in genetic diversity from field to laboratory during colonization of *Anopheles darlingi* Root (Diptera: Culicidae). The American Journal of Tropical Medicine and Hygiene **93**:998-1001.

Lee, D. J., M. M. Hicks, M. L. Debenham, M. Griffiths, E. N. Marks, J. H. Bryan, and R. C. Russell. 1984. The Culicidae of the Australasian Region. Australian Government Publishing Service Press, Canberra.

McCray, E. M., and H. F. Schoof. 1970. Laboratory behaviour of *Culex pipiens quinquefasciatus* and the effects of TEPA, METEPA, and apholate upon its reproduction. Mosquito News **30**:149-155.

Mitsch, W. J., J. G. Gosselink, C. J. Anderson, and L. Zhang. 2009. Wetland Ecosystems. Wiley, New York.

O'Meara, J., and K. Darcovich. 2008. *Gambusia* control through manipulation of water levels in Narawang Wetland, Sydney Olympic Park 2003-2005. Australian Zoologist **34**:285-290.

Russell, R. C. 1986. Dispersal of the arbovirus vector *Culex annulirostris* Skuse (Diptera: Culicidae) in the Murray Valley of Victoria, Australia. General and Applied Entomology **18**:5-9.

Russell, R. C. 1999. Constructed wetlands and mosquitoes: Health hazards and management options—an Australian perspective. Ecological Engineering **12**:107-124.

SPSS Statistics. 2013. Version 22.0.0.0. IBM Corporation.

Van Dam, A. R., and W. E. Walton. 2008. The effect of predatory fish exudates on the ovipostional behaviour of three mosquito species: *Culex quinquefasciatus*, *Aedes aegypti* and *Culex tarsalis*. Medical and Veterinary Entomology **22**:399-404.

Walton, W. E., A. R. Van Dam, and D. A. Popko. 2009. Ovipositional responses of two *Culex* (Diptera: Culicidae) species to larvivorous fish. Journal of Medical Entomology **46**:1338-1343.

Webb, C., S. Doggett, and R. Russell. 2016. A Guide to Mosquitoes of Australia. CSIRO Publishing, Clayton South, Victoria.

Webb, C., and J. Joss. 1997. Does predation by the fish *Gambusia holbrooki* (Atheriniformes: Poeciliidae) contribute to declining frog populations? Australian Zoologist **30**:316-324.

Webb, C. E. 2013. Managing mosquitoes in constructed freshwater wetlands.*in* S. Paul, editor. Workbook for Managing Urban Wetlands in Australia. Sydney Olympic Park Authority, Sydney Olympic Park.

Webb, C. E., A. Ironside, and S. Mansfield. 2012. A comparison of oviposition preference in the presence of three aquatic plants by the mosquitoes *Culex annulirostris* Skuse and *Culex quinquefasciatus* Say (Culicidae: Diptera) in laboratory tests. General and Applied Entomology **41**:21-26.

Why, A. M., J. R. Lara, and W. E. Walton. 2016. Oviposition of *Culex tarsalis* (Diptera: Culicidae) differs on water conditioned by potential fish and insect predators. Journal of Medical Entomology **53**:1093-1099.

Willems, K. J., C. E. Webb, and R. C. Russell. 2005. A comparison of mosquito predation by the fish *Pseudomugil signifier* Kner and *Gambusia holbrooki* (Girard) in laboratory trials. Journal of Vector Ecology **30**:87-90.

Williams, C. R. 2002. Behavioural heterogeneity in the mosquito *Culex annulirostris* Skuse in South Australia. University of South Australia.

Chapter 8:

General discussion

Understanding the relationships between urban wetland traits, aquatic biodiversity and mosquitoes is essential for both conservation of urban wildlife and management of potential public health risks. My research contributes to a growing body of knowledge highlighting that many assumptions about the relationships between mosquitoes and freshwater urban wetlands may not be supported. Perceptions that urban wetlands are inevitable sources of mosquito problems can lead to inclusion of traits during wetland planning and construction that not only minimise mosquito habitat, such as hard vertical edges and minimal vegetation, but also minimise habitat for other wildlife such as birds and frogs, and potentially reduce water quality improvement value (Hansson et al. 2005, Hamer and Parris 2011, Murray et al. 2013). I have shown that increased aquatic biodiversity is not necessarily linked to increased mosquito abundance (Chapter 3, Hanford et al. 2019b), which complements other research linking increased urban wetland cover to reduced prevalence of mosquito-borne West Nile virus (Johnson et al. 2012) and dengue occurrence, even though the mosquitoes that transmit dengue viruses are not directly associated with wetlands (de Jesús Crespo et al. 2018), and a general trend towards healthy wetlands causing fewer mosquito problems (Carver et al. 2015).

While there is no doubt that mosquitoes have the potential to be major nuisance biting pests and transmit pathogens that cause significant disease globally, this concern should not provide the only impetus for design and management considerations of wetlands in urban contexts. My research confirms findings in other urban contexts that factors such as traits of surrounding land uses (Chapters 4 and 5, Claflin and Webb 2017, Hanford et al. 2020a, Hanford et al. 2020b) and wetland management regimes (Chapter 6, Jiannino and Walton 2004, Walton 2012) are essential considerations during wetland design for reducing the potential for mosquito problems and in plans of management for mitigating existing mosquito problems. There is a clear need for multidisciplinary research and cross-agency collaboration to provide the best outcomes from urban wetland management.

Cross-disciplinary research combining medical entomology and wetland ecology, though a relatively small field of research (Chapter 2), is providing invaluable insights into low-cost, proactive design solutions to minimise mosquito risks. Considering wetland traits such as vegetation type and configuration (Walton and Workman 1998, Thullen et al. 2002, Walton et al. 2012, Popko and Walton 2013), water inlet and outlet type (Chapter 3, Hanford et al. 2019b), and surrounding land use (Chapter 4, Claflin and Webb 2017, Hanford et al. 2020b) can shape mosquito abundance and identity at urban wetlands. Understanding the (often non-target) effects of wetland management regimes (Chapter 6, Jiannino and Walton 2004, Hanford et al. 2020c) will also provide valuable guidance to practitioners, though improved methods for ensuring this information reaches across traditional research communities are required (Chapter 2).

Integrating fine scale research with landscape scale management

Wetlands are often studied at coarse landscape scales, while mosquito research is typically focused on finer scales, targeting key species. Both fields tend to be very place-based. Wetland design and management strategies for a specific context or climate may not be suitable for another area, and problematic mosquito species are often geographically-specific due to climatic and habitat availability factors. The place-based nature of both research fields, rather than being a hinderance to broader application of understanding of wetland and mosquito relationships, emphasises the value to be gained by improved integration. While it is possible to study relationships between each wetland and mosquito species individually, identifying general trends which can then be tested in other contexts and places will continue to advance our understanding of wetland and mosquito ecology and management.

My research has demonstrated the potential benefits to combining the fine scale and landscape scale to inform management of urban areas. Taking a fine scale species-specific approach to assessing potential mosquito risks associated with a particular wetland is essential to inform landscape scale management. Mosquitoes as a group are highly diverse and generally have very specific larval habitat requirements (Webb et al. 2016). Considering the mosquito assemblage as a whole can mask mosquito responses to wetland and surrounding land use traits (Chapter 3, Hanford et al. 2019b). For example, the dominance of a container-inhabiting mosquito species in collections from an urban wetland would confound management of wetland traits to reduce potential mosquito risks, as the dominant mosquito species would be utilising nearby habitats rather than the wetland itself. Considering wetland-specific mosquito species that are using the wetland habitat allows identification of mosquito relationships with wetland traits that can be managed to reduce mosquito abundance.

Mosquitoes can also have unexpected distributions in urban areas, such as the presence of the saltmarsh mosquito species *Ae. vigilax* in freshwater wetlands in Sydney (Chapter 5, Hanford et al. 2020a). Understanding these fine scale patterns is essential for both management of individual wetlands to reduce site-specific mosquito risks, and also management and planning at the landscape scale which can in turn shape fine scale mosquito responses. This integration across research scales and research communities offers the greatest co-benefits for multiple species, including humans, and meeting multiple wetland management objectives.

Public perceptions and mosquito management

Managing stormwater using green infrastructure such as wetlands is highly valued by the community, who recognise the multiple benefits such systems provide. Communities are frequently not only willing to pay for wetlands as green infrastructure, but are also willing to volunteer their time to maintain it (Ando et al. 2020), even though public perceptions of a ‘good’ wetland vary and are often mismatched to ecological value (Dobbie and Green 2013, Scholte et al. 2016). The community also strongly values mosquito abatement in urban areas, even when there is no immediate disease risk, in order to maintain outdoor amenity (Dickinson and Paskewitz 2012, Darbro et al. 2017). Values held by residents surrounding an urban wetland are therefore an essential component of adaptive wetland and mosquito management, to maintain local support for these habitats and ensure residents' quality of life is not negatively affected by urban conservation efforts. Understanding links between mosquitoes and urban wetlands is a fundamental component of this adaptive management process.

Given that many problematic mosquito species in urban areas are more closely associated with water-holding containers than wetland habitats (e.g. *Ae. notoscriptus* and *Ae. aegypti* are known to breed in containers within or close to human environments) (Webb et al. 2016), public awareness and education are also critical for maintaining support for urban wetland habitats, and provision of ecological information can shape community values and landscape preferences (Straka et al. 2016). The increasing popularity of citizen science (e.g. Mozzie Monitors in Australia, Invasive Mosquito Project in the U.S., Mosquito Alert in Spain) can provide a valuable pathway for achieving this (Palmer et al. 2017, Braz Sousa et al. 2020).

Urban wetlands have unique challenges

The negative impacts of urbanisation on biodiversity are well-known and documented for multiple species and habitats (Walsh et al. 2005, McKinney 2008). While urban areas can also be valuable conservation resources (Chester and Robson 2013, Hassall and Anderson 2015, Locke and McPhearson 2018), challenges such as impaired water quality, flashy hydrology, lack of connectivity and invasive species all have detrimental impacts on most native aquatic wildlife trying to persist in urban areas (Walsh et al. 2005, Hamer and Parris 2013, Mackintosh et al. 2015).

The impacts of these urban-specific challenges on mosquitoes are largely unknown, though there is a sizeable research base documenting the sensitivity of mosquitoes to their surrounding habitat, for example their ability to detect and respond to the presence of potential predators and competitors when laying their eggs (Chapter 7, Bentley and Day 1989, Why et al. 2016, Hanford et al. 2019a). Furthermore, attempts to mitigate the impact of damaging processes on other (more charismatic?) wildlife, such as native species of fish and amphibians, can have unintended and unforeseen consequences for mosquitoes, and potentially public health and wellbeing. To most effectively manage mosquito threats in urban areas, non-target impacts of common management regimes, especially those that have potential to undermine public support for urban conservation, should be more thoroughly evaluated (Chapter 6, Hanford et al. 2020c).

My research has also demonstrated that despite the many pressures urban wetlands face, diverse assemblages of aquatic animals, including sensitive and rare macroinvertebrates, are able to persist (Chapters 3 and 6, Hanford et al. 2019b, Hanford et al. 2020c). Their persistence not only confirms assertions by others that urban wetlands are valuable conservation resources (Le Viol et al. 2009, McKinney et al. 2011, Chester and Robson 2013), but also that actions to mitigate common urban stressors, such as invasive species, should not be assumed to have positive outcomes for all native taxa.

Future research opportunities

Given the capacity for degraded urban wetlands to support rare and threatened taxa including insects, amphibians, birds and mammals, greater understanding of the implications of wetland management regimes and wetland and landscape traits on the conservation values of these habitats is urgently needed. Future research into possible alternative stable states of urban wetlands, for example where an endangered native species is able to persist in the presence of an invasive species, and how management of both these species may affect this balance, would be highly beneficial when developing plans of management for urban wetlands.

There is still much to learn about the interaction between mosquitoes associated with urban wetlands, the wildlife associated with these environments, and the adjacent human populations. While my research has demonstrated that there may be mosquitoes of public health significance associated with urban wetlands, the actual risk of mosquito-borne disease will be influenced by both mosquito abundance and diversity as well as the activity of vertebrate reservoirs of pathogens such as Ross River virus, Barmah Forest virus, and West Nile (Kunjin) virus. Research into the host-feeding preferences of urban wetland-associated mosquitoes, or the response of mosquitoes to urban wetlands assessed as favourable to urban wildlife, may provide further insights to the calculation of public health risks. I have demonstrated that characteristics of landscapes surrounding urban wetlands may influence the abundance and identity of mosquitoes, but further research to elucidate the dispersal patterns of wetland-associated mosquitoes across urban landscapes is required, especially given the emphasis on "greening" metropolitan areas.

Many of the favourable wetland characteristics required for the persistence of wildlife such as birds, amphibians and reptiles in urban areas are well studied. To balance these traits with traits that may minimise the potential for emerging mosquito issues requires further research into relationships between mosquito species of pest and public health significance and wetland and urban landscape traits. This would not only facilitate early mitigation of potential mosquito issues, but also inform wetland design and management to maximise benefits to urban wildlife while supporting human health and wellbeing. Critical to facilitating this research is the development of new approaches to studying urban wetlands and their associated mosquito populations. There are clear challenges in the sampling of immature mosquitoes associated with these habitats and new technologies, such as the incorporation of eDNA methodologies, may prove useful once they become a more cost-effective option. Similarly, the rise of unpiloted aircraft (a.k.a. drones) to undertake visual mapping of wetlands may also provide much greater understanding of these environments.

Conclusions

My research has demonstrated that freshwater urban wetlands can provide multiple benefits to humans and the environment without creating urban mosquito problems. While delivering co-benefits can be challenging and requires multidisciplinary approaches across fine to coarse spatial scales, the potential returns are substantial. We need wetlands in our cities, not just to manage our stormwater, but also for the multitude of health and wellbeing benefits they provide and biodiversity and ecosystem functions they support. These functions are essential for long-term human survival and maintaining the quality and liveability of urban environments. While people may not like mosquitoes, they are an intrinsic component of wetland systems. Management of mosquitoes is critical to successful integration of wetlands into urban landscapes, and with appropriate design and planning, healthy wetlands and reduced mosquito risks need not be mutually exclusive goals.

References

Ando, A. W., C. L. Cadavid, N. R. Netusil, and B. Parthum. 2020. Willingness-to-volunteer and stability of preferences between cities: Estimating the benefits of stormwater management. Journal of Environmental Economics and Management **99**:102274.

Bentley, M. D., and J. F. Day. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. Annual Review of Entomology **34**:401-422.

Braz Sousa, L., S. R. Fricker, S. S. Doherty, C. E. Webb, K. L. Baldock, and C. R. Williams. 2020. Citizen science and smartphone e-entomology enables low-cost upscaling of mosquito surveillance. Science of the Total Environment **704**:135349.

Carver, S., D. P. Slaney, P. T. Leisnham, and P. Weinstein. 2015. Healthy wetlands, healthy people: Mosquito borne disease.*in* C. M. Finlayson, editor. Wetlands and Human Health. Springer, Dordrecht.

Chester, E. T., and B. J. Robson. 2013. Anthropogenic refuges for freshwater biodiversity: Their ecological characteristics and management. Biological Conservation **166**:64-75.

Claflin, S. B., and C. E. Webb. 2017. Surrounding land use significantly influences adult mosquito abundance and species richness in urban mangroves. Wetlands Ecology and Management **25**:331-344.

Darbro, J., Y. Halasa, B. Montgomery, M. Muller, D. Shepard, G. Devine, and P. Mwebaze. 2017. An economic analysis of the threats posed by the establishment of *Aedes albopictus* in Brisbane, Queensland. Ecological Economics **142**:203-213.

de Jesús Crespo, R., P. Méndez Lázaro, and S. H. Yee. 2018. Linking wetland ecosystem services to vector-borne disease: Dengue fever in the San Juan Bay Estuary, Puerto Rico. Wetlands **39**:1281-1293.

Dickinson, K., and S. Paskewitz. 2012. Willingness to pay for mosquito control: How important is West Nile virus risk compared to the nuisance of mosquitoes? Vector-Borne and Zoonotic Diseases **12**:886-892.

Dobbie, M., and R. Green. 2013. Public perceptions of freshwater wetlands in Victoria, Australia. Landscape and Urban Planning **110**:143-154.

Hamer, A. J., and K. M. Parris. 2011. Local and landscape determinants of amphibian communities in urban ponds. Ecological Applications **21**:378-390.

Hamer, A. J., and K. M. Parris. 2013. Predation modifies larval amphibian communities in urban wetlands. Wetlands **33**:641-652.

Hanford, J. K., D. F. Hochuli, and C. E. Webb. 2019a. Oviposition behavior of *Culex annulirostris* (Diptera: Culicidae) is affected by the recent presence of invasive *Gambusia holbrooki* (Cyprinodontiformes: Poeciliidae). Journal of Medical Entomology **56**:1165-1169.

Hanford, J. K., D. F. Hochuli, and C. E. Webb. 2020a. Observations of an unexpected abundance of estuarine mosquitoes associated with an urban freshwater wetland. Australian Zoologist doi: 10.7882/AZ.2020.014.

Hanford, J. K., C. E. Webb, and D. F. Hochuli. 2019b. Habitat traits associated with mosquito risk and aquatic diversity in urban wetlands. Wetlands **39**:743-758.

Hanford, J. K., C. E. Webb, and D. F. Hochuli. 2020b. Human population density is linked to abundance of container-inhabiting mosquitoes at urban wetlands. In Review.

Hanford, J. K., C. E. Webb, and D. F. Hochuli. 2020c. Management of urban wetlands for conservation can reduce aquatic biodiversity and increase mosquito risk. Journal of Applied Ecology **57**:794-805.

Hansson, L. A., C. Bronmark, P. A. Nilsson, and K. Abjornsson. 2005. Conflicting demands on wetland ecosystem services: Nutrient retention, biodiversity or both? Freshwater Biology **50**:705-714.

Hassall, C., and S. Anderson. 2015. Stormwater ponds can contain comparable biodiversity to unmanaged wetlands in urban areas. Hydrobiologia **745**:137-149.

Jiannino, J. A., and W. E. Walton. 2004. Evaluation of vegetation management strategies for controlling mosquitoes in a southern California constructed wetland. Journal of the American Mosquito Control Association **20**:18-26.

Johnson, B. J., K. Munafo, L. Shappell, N. Tsipoura, M. Robson, J. Ehrenfeld, and M. V. K. Sukhdeo. 2012. The roles of mosquito and bird communities on the prevalence of West Nile virus in urban wetland and residential habitats. Urban Ecosystems **15**:513-531.

Le Viol, I., J. Mocq, R. Julliard, and C. Kerbiriou. 2009. The contribution of motorway stormwater retention ponds to the biodiversity of aquatic macroinvertebrates. Biological Conservation **142**:3163-3171.

Locke, D., H., and T. McPhearson. 2018. Urban areas *do* provide ecosystem services. Frontiers in Ecology and the Environment **16**:203-205.

Mackintosh, T. J., J. A. Davis, and R. M. Thompson. 2015. The influence of urbanisation on macroinvertebrate biodiversity in constructed stormwater wetlands. Science of the Total Environment **536**:527-537.

McKinney, M. L. 2008. Effects of urbanization on species richness: A review of plants and animals. Urban Ecosystems **11**:161-176.

McKinney, R. A., K. B. Raposa, and R. M. Cournoyer. 2011. Wetlands as habitat in urbanizing landscapes: Patterns of bird abundance and occupancy. Landscape and Urban Planning **100**:144-152.

Murray, C. G., S. Kasel, R. H. Loyn, G. Hepworth, and A. J. Hamilton. 2013. Waterbird use of artificial wetlands in an Australian urban landscape. Hydrobiologia **716**:131-146.

Palmer, J. R. B., A. Oltra, F. Collantes, J. A. Delgado, J. Lucientes, S. Delacour, M. Bengoa, R. Eritja, and F. Bartumeus. 2017. Citizen science provides a reliable and scalable tool to track disease-carrying mosquitoes. Nature Communications **8**:916.

Popko, D. A., and W. E. Walton. 2013. Small-stature emergent macrophytes and crepuscular sprinkler disturbance reduce mosquito abundance in wetland mesocosms. Journal of Vector Ecology **38**:379-389.

Scholte, S. S. K., M. Todorova, A. J. A. van Teeffelen, and P. H. Verburg. 2016. Public support for wetland restoration: What is the link with ecosystem service values? Wetlands:1-15.

Straka, T. M., P. E. Lentini, L. F. Lumsden, B. A. Wintle, and R. van der Ree. 2016. Urban bat communities are affected by wetland size, quality, and pollution levels. Ecology and Evolution **6**:4761-4774.

Thullen, J. S., J. J. Sartoris, and W. E. Walton. 2002. Effects of vegetation management in constructed wetland treatment cells on water quality and mosquito production. Ecological Engineering **18**:441-457.

Walsh, C. J., A. H. Roy, J. W. Feminella, P. D. Cottingham, P. M. Groffman, and R. P. Morgan. 2005. The urban stream syndrome: Current knowledge and the search for a cure. Journal of the North American Benthological Society **24**:706-723.

Walton, W. E. 2012. Design and management of free water surface constructed wetlands to minimize mosquito production. Wetlands Ecology and Management **20**:22.

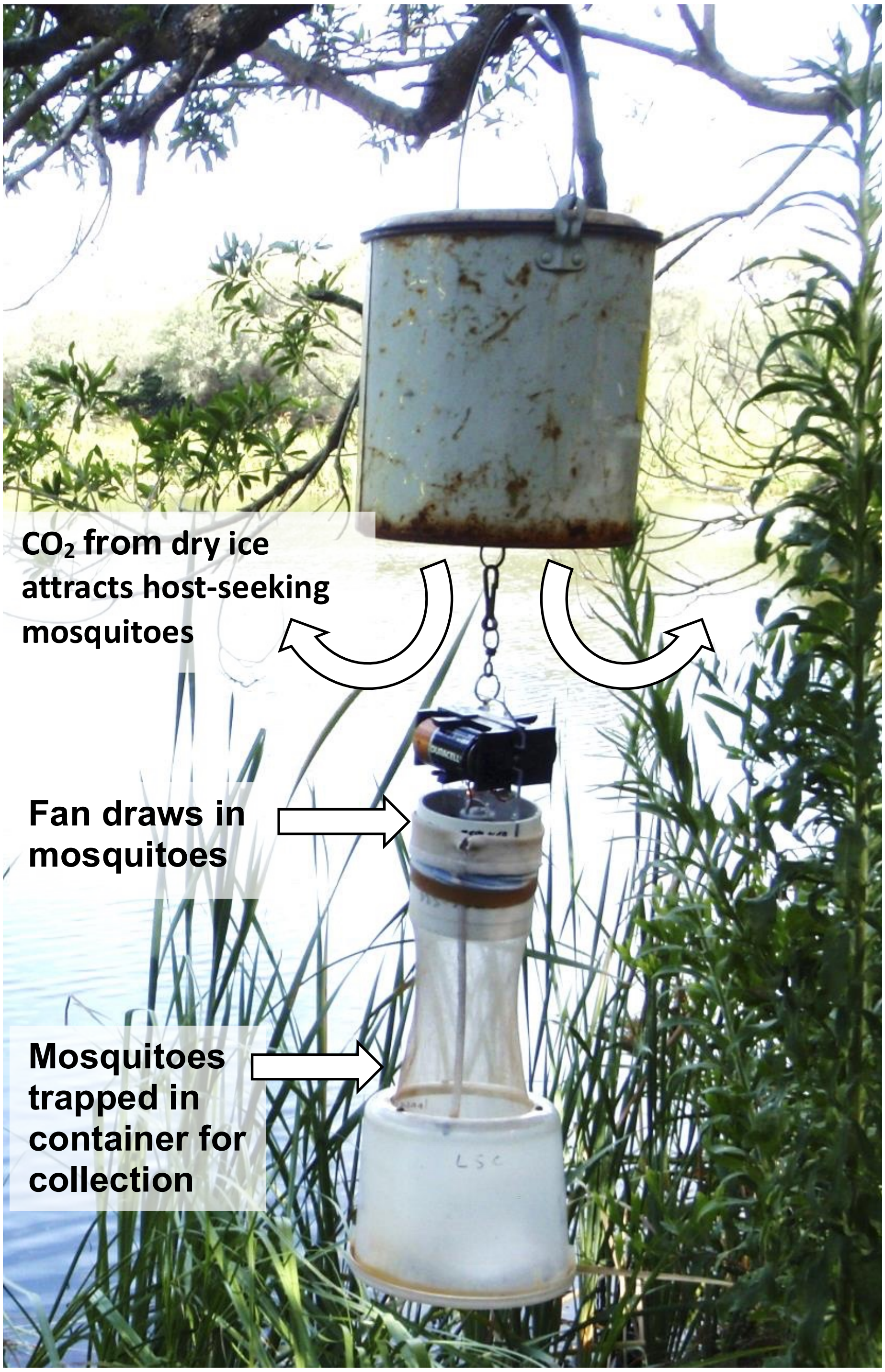
Walton, W. E., D. A. Popko, A. R. Van Dam, A. Merrill, J. Lythgoe, and B. Hess. 2012. Width of planting beds for emergent vegetation influences mosquito production from a constructed wetland in California (USA). Ecological Engineering **42**:150-159.

Walton, W. E., and P. D. Workman. 1998. Effect of marsh design on the abundance of mosquitoes in experimental constructed wetlands in southern California. Journal of the American Mosquito Control Association **14**:95-107.

Webb, C., S. Doggett, and R. Russell. 2016. A Guide to Mosquitoes of Australia. CSIRO Publishing, Clayton South, Victoria.

Why, A. M., J. R. Lara, and W. E. Walton. 2016. Oviposition of *Culex tarsalis* (Diptera: Culicidae) differs on water conditioned by potential fish and insect predators. Journal of Medical Entomology **53**:1093-1099.

Appendix 1



Supplementary Figure 1.1. Encephalitis Virus Surveillance (EVS) trap used to sample host-seeking mosquitoes at wetland sites. Approximately 500g of dry ice pellets are placed in the insulated tin above, which has holes in the bottom. As the dry ice sublimates it releases carbon dioxide that attracts host-seeking mosquitoes, which are sucked into the container below by a small fan.

Supplementary Table 2.1 . Physical characteristics of selected study wetlands. GPT=gross pollutant trap.

Site	LATITUDE	LONGITUDE	Perimeter Complexity Index	Wetland Perimeter Length (m)	Wetland Surface Area (ha)	Inlet Type	Outlet Type	Immediate Surrounding Land use
BAN 06	-33.938015	150.979076	1.67	538	0.83	GPT	pipe/drain	park/grass
BAN 07	-33.910507	150.995196	1.80	307	0.23	channel/creek	overflow/spillway	bushland
BAN 08	-33.905888	150.999080	1.06	200	0.29	pipe/drain	overflow/spillway	park/grass
BLA 02	-33.753113	150.880408	1.63	158	0.08	pipe/drain	pipe/drain	residential
BLA 03	-33.751722	150.838165	1.69	412	0.47	GPT	channel/creek	park/grass
CAN 01	-33.943575	151.052497	2.55	696	0.59	GPT	pipe/drain	park/grass
CAN 02	-33.915350	151.119923	1.26	159	0.13	GPT	pipe/drain	residential
FAI 05	-33.880384	150.873617	1.16	130	0.10	channel/creek	channel/creek	park/grass
FAI 06	-33.887680	150.883596	1.16	171	0.17	pipe/drain	channel/creek	park/grass
FAI 08	-33.873444	150.960505	2.30	517	0.40	pipe/drain	channel/creek	park/grass
HOL 01	-33.811193	150.931220	1.36	248	0.27	pipe/drain	channel/creek	park/grass
HUR 02	-33.980777	151.042838	1.23	235	0.29	pipe/drain	overflow/spillway	park/grass
KOG 02	-33.977918	151.088255	1.11	269	0.47	pipe/drain	pipe/drain	park/grass
MAR 02	-33.926717	151.163248	1.33	332	0.50	pipe/drain	pipe/drain	residential
ROC 01	-33.963600	151.145771	2.65	653	0.48	pipe/drain	pipe/drain	park/grass
SUT 01	-34.066739	151.016455	1.43	246	0.23	GPT	channel/creek	bushland
SUT 03	-34.010547	151.012580	1.17	145	0.12	GPT	pipe/drain	residential
WOL 01	-34.196826	150.978052	1.40	262	0.28	pipe/drain	pipe/drain	park/grass

WOL 02	-34.328910	150.896185	2.12	450	0.36	channel/creek	channel/creek	bushland
WOL 03	-34.345983	150.899328	1.59	270	0.23	channel/creek	pipe/drain	residential
WOL 07	-34.505178	150.882421	1.75	444	0.51	channel/creek	channel/creek	residential
WSP 01	-33.882759	150.857588	1.34	347	0.54	channel/creek	channel/creek	bushland
WSP 03	-33.876070	150.854457	1.07	111	0.09	channel/creek	channel/creek	bushland

Supplementary Table 1.2. Aquatic macroinvertebrates collected at each study wetland in summer.

CLASS	ORDER	FAMILY	SUBFAMILY	GENUS	BAN 06	BAN 07	BAN 08	BLA 02	BLA 03	CAN 01	CAN 02	FAI0 5	FAI0 6	FAI0 8	HOL 01	HUR 02	KOG 02	MAR 02	ROC 01	SUT 01	SUT 03	WOL 01	WOL 02	WOL 03	WOL 07	WSP 01	WSP 03
Arachnida	Acarina	Arrenuridae		Arrenurus			3																		1		
		Hydrachnidae		Hydrachna	14	18	14					9			2		43	10	1				5	3	11	7	23
		Oribatida																									
Bivalvia	Veneroida	Corbiculidae					1																				
		Sphaeriidae													3												
Crustacea	Decapoda	Atyidae		Paratya	11																				10	4	
	Isopoda	Scyphacidae		Haloniscus										1													
Gastropoda	Cerithimorpha	Thiaridae																									
	Hygrophila	Lymnaeidae											3				1			1			2				4
		Physidae		Physa	21	1	6	1	1			25	9	2	4		13			1			4	10	9	3	
		Planorbidae										1	1							1							2
		Planorbidae (Ancyclidae)							1															1	5		
	Hypsogastropoda	Tateidae																									
Hirudinea	Arhynchobdellida										1						1										
	Rhynchobdellida	Glossiphoniidae			1		6	19			4	40	2		15	7		16	8		20	20	2	5		1	
Insecta	Coleoptera	Chrysomelidae				2									1								4				2
		Dytiscidae				3							1	1		1	1						1			1	2
		Elmidae											2														
		Hydrophilidae				3				1				1									1		3		1
		Nanophyidae		Austronanodes		5	1																				2
		Scirtidae												3				1					1		5		

CLASS	ORDER	FAMILY	SUBFAMILY	GENUS	BAN 06	BAN 07	BAN 08	BLA 02	BLA 03	CAN 01	CAN 02	FAI0 5	FAI0 6	FAI0 8	HOL 01	HUR 02	KOG 02	MAR 02	ROC 01	SUT 01	SUT 03	WOL 01	WOL 02	WOL 03	WOL 07	WSP 01	WSP 03
	Diptera	Ceratopogonidae	Ceratopogoninae		1									2	2	3		7					10			2	5
		Chironomidae	Chironominae		9	68	21	89	57	92	81	43	16	59	32	2	1	9	42	36		17	8	14	86	3	1
			Orthocladinae		4		5			1	2	3		6	10			6	1			18	3	1	2	1	
			Podonominae																								
			Tanypodinae																						1		
		Culicidae	Anophelinae																				2		1		
			Culicinae								2																
		Empididae																									
		Ephydridae									1																
		Muscidae				1																					
		Stratiomyidae		Odontomyia	1	6	1							6		2											3
		Syrphidae		Eristalis																							
		Tabanidae				1																					
		Tipulidae					1																5	1			
	Ephemeroptera	Baetidae		Centroptilum			9								2												
				Cloeon																							
		Caenidae		Tasmanocoenis	5	1	16																			2	
	Hemiptera	Belostomatidae		Diplonychus																							
		Corixidae		Agraptocorixa			4		2						11			1	4			1					
				Diaprepocoris																	3						
				Sigara		5		1					5					14		3		1		1			1
				Corixidae (juv)	16		21			1	7	12			10	1			4	4		44		9		1	7
		Gerridae		Limnogonus																							1

CLASS	ORDER	FAMILY	SUBFAMILY	GENUS	BAN 06	BAN 07	BAN 08	BLA 02	BLA 03	CAN 01	CAN 02	FAI0 5	FAI0 6	FAI0 8	HOL 01	HUR 02	KOG 02	MAR 02	ROC 01	SUT 01	SUT 03	WOL 01	WOL 02	WOL 03	WOL 07	WSP 01	WSP 03
				Tenagogerris																							
				Gerridae (other)																							
		Hebridae		Hebrus																							
		Mesoveliidae		Mesovelia									1					2							1		
		Micronectidae		Micronecta	52		8		2	49	9	1	1		20							19		8	1		
		Naucoridae		Naucoris		4		6						12				4									
		Nepidae		Ranatra				1																	2		
		Notonectidae		Anisops	20	1	3	2					1		1			23				18					15
				Enithares			13																				3
				Paranisops																							
		Pleidae		Paraplea			3					3	2				1	9									1
		Veliidae		Drepanovelia																							3
				Lacertovelia														1									
				Microvelia											1												1
	Lepidoptera	Crambidae																					2				
	Odonata	Aeshnidae		Adveraeschna			1											1						2			
		Coenagrionidae		Argiocnemis									1	1	2												
				Austroagrion											1												
				Caliagrion																					1		
				Ischnura		2	21	10	1	14				5	24	6	3	8			2	9	2	6	1	15	1
				Pseudagrion						1																	
				Coenagrionidae (juv)	2							2													1		

CLASS	ORDER	FAMILY	SUBFAMILY	GENUS	BAN 06	BAN 07	BAN 08	BLA 02	BLA 03	CAN 01	CAN 02	FAI0 5	FAI0 6	FAI0 8	HOL 01	HUR 02	KOG 02	MAR 02	ROC 01	SUT 01	SUT 03	WOL 01	WOL 02	WOL 03	WOL 07	WSP 01	WSP 03
		Corduliidae		Hemicordulia		2								1		3				3			4				1
				Procordulia											6												
		Lestidae		Austrolestes																							
		Libellulidae		Agrionoptera																							
				Diplacodes										1									1				1
				Nannophlebia																							
				Orthetrum										1													
				Potamarcha			1																				
				Rhyothemis																							
				Tramea																							
				Zyxomma																					1		
				Libellulidae (juv)								1										1		1			
		Macromiidae		Macromia																							
		Megapodagrionidae		Austroargiolestes																							
	Trichoptera	Calamoceratidae		Anisocentropus																					1	2	
		Calocidae		Cal. B.																						13	
		Ecnomidae		Ecnomus			3			5															1	1	
		Hydroptilidae		Hellyethira						3						1					8	1				2	
		Leptoceridae		Condocerus																							
				Lectrides																							
				Notalina			4																			1	
				Notoperata																							
				Oecetis			1																	1	1	8	

CLASS	ORDER	FAMILY	SUBFAMILY	GENUS	BAN 06	BAN 07	BAN 08	BLA 02	BLA 03	CAN 01	CAN 02	FAI0 5	FAI0 6	FAI0 8	HOL 01	HUR 02	KOG 02	MAR 02	ROC 01	SUT 01	SUT 03	WOL 01	WOL 02	WOL 03	WOL 07	WSP 01	WSP 03
				Triplectides		3	31					3		28						4		1		1	37	26	
Oligochaeta						27	1	42	1		4	5	10	17	4	6	5	15	25		20	4	6	10	3		2
Tubellaria	Tricladida	Dugesiidae			13	4	8			13	8	10	10	6	12	12	8	6	13		21		2	12	1	10	7

Supplementary Table 1.3. Aquatic macroinvertebrates collected at each study wetland in autumn.

CLASS	ORDER	FAMILY	SUBFAMILY	GENUS	BAN 06	BAN 08	BLA 02	BLA 03	CAN 01	CAN 02	FAI 06	FAI 08	HOL 01	HUR 02	KOG 02	MAR 02	ROC 01	SUT 01	SUT 03	WOL 01	WOL 02	WOL 03	WOL 07	WSP 01	WSP 03
Arachnida	Acarina	Arrenuridae		Arrenurus																					
		Hydrachnidae		Hydrachna	13	22							1		29	94	2				5	6	5	10	10
		Oribatida														4		1							
Bivalvia	Veneroida	Corbiculidae																							
		Sphaeriidae																							
Crustacea	Decapoda	Atyidae		Paratya	3																		6	7	
	Isopoda	Scyphacidae		Haloniscus																					
Gastropoda	Cerithimorpha	Thiaridae			18																				
	Hygrophila	Lymnaeidae							1		2		3		1	2			5	2		2	2		5
		Physidae		Physa	2	7		3	2		10	1	3		2								10		
		Planorbidae									2	1						6					3		4
		Planorbidae (Ancyclidae)																							
	Hypsogastropoda	Tateidae																			9				
Hirudinea	Arhynchobdellida									1															
	Rhynchobdellida	Glossiphoniidae				1	3	1		4						7	5		3		1	4			
Insecta	Coleoptera	Chrysomelidae			1	2							6						3						
		Dytiscidae													1						2			1	4
		Elmidae																							
		Hydrophilidae				1	2	3	1																
		Nanophyidae		Austronanodes																					
		Scirtidae							1							1							1		
	Diptera	Ceratopogonidae	Ceratopogoninae			8						1	8	1	1	19							4		11

CLASS	ORDER	FAMILY	SUBFAMILY	GENUS	BAN 06	BAN 08	BLA 02	BLA 03	CAN 01	CAN 02	FAI 06	FAI 08	HOL 01	HUR 02	KOG 02	MAR 02	ROC 01	SUT 01	SUT 03	WOL 01	WOL 02	WOL 03	WOL 07	WSP 01	WSP 03
		Chironomidae	Chironominae		29	21	29	115	14	48	4	48	5	3	5	2	66	9	15	7	8	31	91	15	13
			Orthocladinae			1		1		5		2									2		2		
			Podonominae			1																			
			Tanypodinae			2							1								3		4	1	2
		Culicidae	Anophelinae																						
			Culicinae																		1				
		Empididae				1							2												
		Ephydridae																							
		Muscidae																							
		Stratiomyidae		Odontomyia		5								1		1							2		
		Syrphidae		Eristalis		2																			
		Tabanidae																			2				
		Tipulidae					1									1			1				2		
	Ephemeroptera	Baetidae		Centroptilum																					
				Cloeon	1	2							1										2		
		Caenidae		Tasmanocoenis		1																			
	Hemiptera	Belostomatidae		Diplonychus								2				2									
		Corixidae		Agraptocorixa	3		1		1	10			2												
				Diaprepocoris																					
				Sigara		1																			
				Corixidae (juv)	11	23		3	1	19			2				1							1	
		Gerridae		Limnogonus																					2
				Tenagogerris		2																			

CLASS	ORDER	FAMILY	SUBFAMILY	GENUS	BAN 06	BAN 08	BLA 02	BLA 03	CAN 01	CAN 02	FAI 06	FAI 08	HOL 01	HUR 02	KOG 02	MAR 02	ROC 01	SUT 01	SUT 03	WOL 01	WOL 02	WOL 03	WOL 07	WSP 01	WSP 03
				Gerridae (other)												1									
		Hebridae		Hebrus									2												
		Mesoveliidae		Mesovelia									9		5	9					1		1	1	2
		Micronectidae		Micronecta	28				1	11			3									1			
		Naucoridae		Naucoris																					
		Nepidae		Ranatra		1																			
		Notonectidae		Anisops		32				12						16									9
				Enithares									1												4
				Paranisops			3																		
		Pleidae		Paraplea		5							2	1	1	12					1		1		4
		Veliidae		Drepanovelia																					
				Lacertovelia																					
				Microvelia	1											1									2
	Lepidoptera	Crambidae														2									1
	Odonata	Aeshnidae		Adveraeschna																		1			1
		Coenagrionidae		Argiocnemis			1	2				1	2								3	1	1		1
				Austroagrion																					
				Caliagrion																					
				Ischnura	13	5	3	1	1	26		1	1		1	4	13	2		2	1	2	9	3	12
				Pseudagrion																			8		
				Coenagrionidae (juv)																					
		Corduliidae		Hemicordulia	1	1						1		1			3	1	1		5		4		7
				Procordulia													2								

CLASS	ORDER	FAMILY	SUBFAMILY	GENUS	BAN 06	BAN 08	BLA 02	BLA 03	CAN 01	CAN 02	FAI 06	FAI 08	HOL 01	HUR 02	KOG 02	MAR 02	ROC 01	SUT 01	SUT 03	WOL 01	WOL 02	WOL 03	WOL 07	WSP 01	WSP 03
		Lestidae		Austrolestes																			1		
		Libellulidae		Agrionoptera					1							1		10							
				Diplacodes					1																6
				Nannophlebia	1															2					
				Orthetrum		6		3																1	
				Potamarcha																					
				Rhyothemis															1						
				Tramea																					1
				Zyxomma																					
				Libellulidae (juv)		1																			
		Macromiidae		Macromia										1											
		Megapodagrionidae		Austroargiolestes																	1				
	Trichoptera	Calamoceratidae		Anisocentropus																			2		
		Calocidae		Cal. B.																					
		Ecnomidae		Ecnomus																			1		
		Hydroptilidae		Hellyethira																					
		Leptoceridae		Condocerus																			1		
				Lectrides		2																			
				Notalina		7																	1		
				Notoperata																			4		
				Oecetis		1																	6	2	
				Triplectides	1	8			1			4		1				1					26	8	
Oligochaeta					6	2	7	3	3	29	8	1	2	17		14	10	1	20	2	1	9	5		3

CLASS	ORDER	FAMILY	SUBFAMILY	GENUS	BAN 06	BAN 08	BLA 02	BLA 03	CAN 01	CAN 02	FAI 06	FAI 08	HOL 01	HUR 02	KOG 02	MAR 02	ROC 01	SUT 01	SUT 03	WOL 01	WOL 02	WOL 03	WOL 07	WSP 01	WSP 03
Tubellaria	Tricladida	Dugesiidae			5	7							2	1		15	17				8	6		1	9

Supplementary Table 1.4. Mosquitoes collected at each wetland in summer using CO2-baited EVS traps.

SPECIES	BAN 06	BAN 07	BAN 08	BLA 03	CAN 01	CAN 02	FAI 05	FAI 06	FAI 08	HOL 01	HUR 02	KOG 02	MAR 02	ROC 01	SUT 01	SUT 03	WOL 01	WOL 02	WOL 03	WOL 07	WSP 01	WSP 03
Aedes alboannulatus																						1
Aedes camptorhynchus						1																
Aedes mallochi				1																		
Aedes multiplex	12																					
Aedes notoscriptus	201	59	92	51	29	41	79	45	40	25	76	16	87	9	11	83	23	8	16	39	18	10
Aedes procax			1															2				
Aedes rubrithorax															1			1				
Aedes vigilax				2				1			6			4		21				2		1
Anopheles annulipes				1											2			2		1		3
Coquillettidia linealis	2	3	1	2	2				2	1			4	13	1			11	2	88	3	25
Coquillettidia xanthogaster																						1
Culex #32							1															
Culex #52															1				1			
Culex annulirostris	2	2	3	7	1	5	4	7		1	3										3	20
Culex australicus	1			2				7					2		2				1		1	4
Culex bitaeniorhynchus																						8
Culex molestus															1				10			1
Culex orbostiensis	2					1	1						1		1			2		17		2
Culiseta antipodea																						3

Culex quinquefasciatus	77	1	6	3	88	92	22	63	6	73	4	14	25	36	1	17	10		1	8	2	2
Culex sitiens														1								
Mansonia uniformis							1	4	2	1			1					21		1		30

Supplementary Table 1.5. Mosquitoes collected at each wetland in autumn using CO2-baited EVS traps.

SPECIES	BAN 06	BAN 07	BAN 08	BLA 03	CAN 01	CAN 02	FAI 05	FAI 06	FAI 08	HOL 01	HUR 02	KOG 02	MAR 02	ROC 01	SUT 01	SUT 03	WOL 01	WOL 02	WOL 03	WOL 07	WSP 01	WSP 03
Aedes alboannulatus				1																		
Aedes multiplex											2											
Aedes notoscriptus	145	32	44	83	3	7	93	59	28	29	30	6	6	4	5	19	14		6	27	11	8
Aedes rubrithorax																		1				
Aedes vigilax	1										1					1						
Anopheles annulipes		1					1		2	1				2	1		1			2	1	
Coquillettidia linealis	1	4	1	1					3	3	1		3	3				4		49	2	14
Culex annulirostris	33	45	5	82	4	40	109	94	30	23	35	27	23	39		6	1		2	15	58	335
Culex australicus	13	2			3	14	1		3			5		1			1	1				1
Culex bitaeneorhynchus				1				2					4				1				4	35
Culex halifaxii							1															
Culex molestus					1										27							
Culex orbostiensis			1							2			13	1						3	1	2
Culex quinquefasciatus	10	2	5	5	103	23	43	102	5	39	1	21	15	11	4	19	12	1	4		1	6
Culex sitiens					3	4					2	6	19	11								
Mansonia uniformis		9	13	1	1	1	2	24	14	12		1					1	31		1	13	51

Appendix 2

Supplementary Table 2.1. Details of study wetlands locations.

Site ID	Latitude	Longitude	Name	Local Government Area
BAN-06	-33.938015	150.979076	Newland Reserve Wetland	Bankstown
BAN-07	-33.910507	150.995196	Bellevue Reserve Wetland	Bankstown
BAN-08	-33.905888	150.999080	Louisa Reserve Wetland	Bankstown
BLA-03	-33.751722	150.838165	Plumpton Park Wetland	Blacktown
CAN-01	-33.943575	151.052497	Riverwood Wetland	Canterbury
CAN-02	-33.915350	151.119923	Cup and Saucer Wetland	Canterbury
FAI-05	-33.880384	150.873617	Clarevale Wetland	Fairfield
FAI-08	-33.873444	150.960505	De Freitas Wetland	Fairfield
FAI-06	-33.870678	150.867161	Stockdale Wetland	Fairfield
HOL-01	-33.811193	150.931220	Lakewood Estate	Holroyd
HUR-02	-33.980777	151.042838	Webbs Dam	Hurstville
KOG-02	-33.977918	151.088255	Moore Reserve Wetland	Kogarah
MAR-02	-33.926717	151.163248	Tempe Lands	Marrickville
ROC-01	-33.963600	151.145771	Bicentennial Park	Rockdale
SUT-01	-34.066739	151.016455	Engadine Avenue Wetland	Sutherland
SUT-03	-34.010547	151.012580	Mina Road Wetland	Sutherland
WOL-01	-34.196826	150.978052	Gymea Glade Wetland	Wollongong
WOL-02	-34.328910	150.896185	Bulli Wetland	Wollongong
WOL-03	-34.345983	150.899328	Forestview Way Wetland	Wollongong
WOL-07	-34.505178	150.882421	Korrongulla Wetland	Wollongong
WSP-01	-33.882759	150.857588	Abbotsbury Pond	Fairfield
WSP-03	-33.876070	150.854457	WSP Wetland	Fairfield

Appendix 3

Supplementary Table 3.1. Number of adult mosquitoes captured adjacent to drained (n=6) and undrained (n=6) wetlands during each sampling month.

	January		March		April		May	
	drained	undrained	drained	undrained	drained	undrained	drained	undrained
Aedes alternans	279	366	147	142	17	28	0	2
Aedes multiplex	0	0	5	1	1	1	5	1
Aedes notoscriptus	4	7	4	4	2	1	6	0
Aedes procax	0	0	2	0	5	2	3	1
Aedes vigilax	5256	6394	430	585	51	62	17	22
Anopheles annulipes	5	14	35	53	125	101	221	115
Coquillettidia linealis	8	17	4	8	0	3	1	0
Coquillettidia xanthogaster	1	6	5	12	2	2	1	0
Culex annulirostris	758	757	813	994	196	255	13	10
Culex australicus	5	3	9	3	16	8	12	17
Culex bitaeniorhynchus	2	5	0	0	1	1	0	0
Culex molestus	18	7	0	2	1	1	5	9
Culex orbostiensis	6	1	6	1	1	0	0	0
Culex quinquefasciatus	19	86	22	36	53	24	8	7
Culex sitiens	58	22	135	132	231	65	12	2
Mansonia uniformis	1	36	24	30	12	30	2	5
Mimomyia elegans	0	0	0	0	0	0	1	0
Total	**6420**	**7721**	**1641**	**2003**	**714**	**584**	**307**	**191**

Supplementary Table 3.2. Results of Welch two tailed t-tests for water quality parameters between drained and undrained wetlands for each month.

	Sample	t	df	p	r
Salinity	Mar	0.04	9.51	0.97	0.01
Salinity	Apr	0.14	9.16	0.89	0.05
Salinity	May	0.32	9.83	0.75	0.10
Temperature	Mar	-0.22	9.24	0.83	0.07
Temperature	Apr	-0.24	9.68	0.82	0.08
Temperature	May	0.73	9.94	0.48	0.22
Turbidity	Mar	-1.13	9.92	0.29	0.34
Turbidity	Apr	0.84	9.77	0.42	0.26
Turbidity	May	4.08	5.10	0.01	0.87
Oxygen	Mar	-0.21	9.96	0.84	0.07
Oxygen	Apr	0.81	5.57	0.45	0.32
Oxygen	May	0.44	6.07	0.68	0.17
pH	Mar	-0.08	8.72	0.94	0.03
pH	Apr	-1.15	7.95	0.28	0.38
pH	May	-2.20	8.15	0.06	0.61

Supplementary Table 3.3. Abundance of *Anopheles annulipes* and *Culex annulirostris* larvae were strongly linked to macroinvertebrate abundance and several water quality parameters. Several macroinvertebrate variables were also linked to water turbidity and dissolved oxygen.

p-values	*Ae. alternans*	*Ae. vigilax*	*An. annulipes*	*Cx. annulirostris*	*Cx. quinquefasciatus*	*Cx. sitiens*	Macroinvertebrate abundance	EPT abundance	Macroinvertebrate richness
Ostracod abundance	0.516	0.538	0.841	0.273	0.406	0.438			
Macroinvertebrate abundance	0.448	0.867	0.031	0.089	0.745	0.533			
EPT abundance	0.148	0.723	0.233	0.245	0.587	0.973			
Macroinvertebrate richness	0.110	0.837	0.062	0.769	0.292	0.426			
Salinity	0.962	0.597	0.012	0.044	0.228	0.580	0.172	0.547	0.110
Temp	0.885	0.074	0.005	0.084	0.470	0.183	0.775	0.404	0.149
Turbidity	0.229	0.466	0.651	0.036	0.413	0.498	0.018	0.129	0.192
Dissolved oxygen	0.112	0.706	0.068	0.840	0.229	0.167	0.924	0.051	0.038
pH	0.136	0.238	0.002	0.963	0.736	0.598	0.549	0.119	0.309

Kendall's tau	*Ae. alternans*	*Ae. vigilax*	*An. annulipes*	*Cx. annulirostris*	*Cx. quinquefasciatus*	*Cx. sitiens*	Macroinvertebrate abundance	EPT abundance	Macroinvertebrate richness
Ostracod abundance	0.079	-0.074	0.023	-0.123	0.101	-0.093			
Macroinvertebrate abundance	0.092	0.020	0.247	0.190	-0.039	-0.074			
EPT abundance	0.178	-0.043	0.139	-0.133	0.067	-0.004			
Macroinvertebrate richness	0.200	-0.026	0.221	0.034	-0.132	-0.098			
Salinity	-0.007	-0.074	0.330	0.262	-0.170	-0.077	0.161	0.072	0.195
Temp	0.020	0.247	-0.368	0.223	0.101	0.183	-0.034	-0.099	-0.175
Turbidity	0.168	0.101	0.059	0.271	0.115	0.093	0.276	0.180	0.158
Dissolved oxygen	0.222	0.052	0.238	0.026	-0.168	0.190	-0.011	0.232	0.251
pH	0.209	0.164	-0.407	0.006	-0.047	0.073	-0.070	0.185	-0.123