2.6 Managing mosquitoes in constructed freshwater wetlands

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Abstract

Mosquitoes associated with freshwater habitats can play a significant role in the transmission of disease-causing pathogens, in particular Ross River virus and Barmah Forest virus. In some regions of Australia, they may also transmit the potentially fatal Murray Valley encephalitis virus. While naturally occurring freshwater habitats can be diverse, locally important freshwater habitats are represented by the constructed wetlands increasingly associated with urban developments. Key mosquito species involved in the transmission of pathogens, as well as nuisance-biting impacts, include *Coquillettidia linealis*, *Culex annulirostris*, *Culex quinquefasciatus* and *Mansonia uniformis*. In addition, there is a suite of mosquito species associated with ephemeral habitats, particularly those in coastal swamp forests and inland flood plains that can pose substantial pest and public health risks when environmental conditions are suitable. A range of strategies are available to manage the risks associated with these mosquitoes but, most importantly, consideration should be given to the risks associated with constructed and rehabilitated wetlands early in the planning and design phase. Notwithstanding the use of insecticides to reduce mosquito populations, there is a range of factors that can be incorporated into the design, construction and maintenance of freshwater wetlands in urban environments that can reduce pest mosquito risks. It will be critical that there is an appropriate monitoring program that supports the wetland management plan. Constructed wetlands represent an important resource for sustainable urban development but authorities must ensure that they do not increase local mosquito-borne disease risks.

Introduction

Mosquitoes associated with natural and constructed freshwater wetlands have the potential the pose serious pest (i.e. nuisance biting) and public health (i.e. transmission of human disease causing pathogens such as Ross River virus and Barmah Forest virus) risks. However, the suitability of wetlands for mosquitoes is dependent on a wide range of factors (Russell 1999). The factors that may increase risk and require assessment may include, but are not limited to, size, design function (e.g. waste-water treatment, water storage, wildlife refuge), aquatic macrophyte communities and predatory fish and macroinvertebrate communities (Russell and Kuginis 1998). Most importantly, the relative actual and potential impact of a wetland in the local area will be highly dependent on extant mosquito populations.

Constructed wetlands are becoming increasingly common in coastal regions of SE QLD, NSW and Victoria. They can take many forms and are considered a key component of Water Sensitive Urban Design (WSUD) strategies to conserve water resources in urban environments (Victorian Stormwater Committee 2006). These wetlands may be designed for stormwater or wastewater treatment, water storage, wildlife conservation, passive recreation, community education or simply aesthetic appeal. Although these wetlands are generally small, given their proximity to the community (Figure 2.6.1), they may increase the relative risk of mosquito impacts. In some instances they may substantially influence the diversity of local mosquito populations, the environmental drivers of mosquito abundance and public health risks associated with mosquito-borne pathogens (Russell 1999).

Wetland managers and local authorities have a duty of care to ensure that these wetlands do not increase local mosquito populations. Notwithstanding the increased pest and public health risks, the association of wetlands with unusually large mosquito population may create barriers in the community to embracing wetland conservation initiatives. Mosquitoes are a natural part of Australia's freshwater wetlands and, while some activity of mosquitoes should be expected during the warmer months of the year, steps should be taken to minimise their impacts.

The management strategies required to address the mosquito risks associated with constructed wetlands are often site-specific (Russell and Kuginis 1998). A range of strategies are available to control mosquito populations (Mosquito Control Association of Australia 2008; Becker *et al.* 2010) but there will be limitations for their use in and around constructed wetlands. The critical issue will be the general design, construction and maintenance principles that can be incorporated into wetland management that can minimise mosquito production (Russell 2001; Walton 2011). Most importantly, constructed wetlands should have a well-funded management plan, supported by site-specific monitoring, that incorporates consideration of future mosquito risk and how those risks will be managed. The unique opportunities for engagement and education of the local community on the values of wetlands is provided by the proximity of constructed wetlands within urban developments (Figure 2.6.2). It is important that wetlands are not a cause of pest mosquito populations to erode that value.

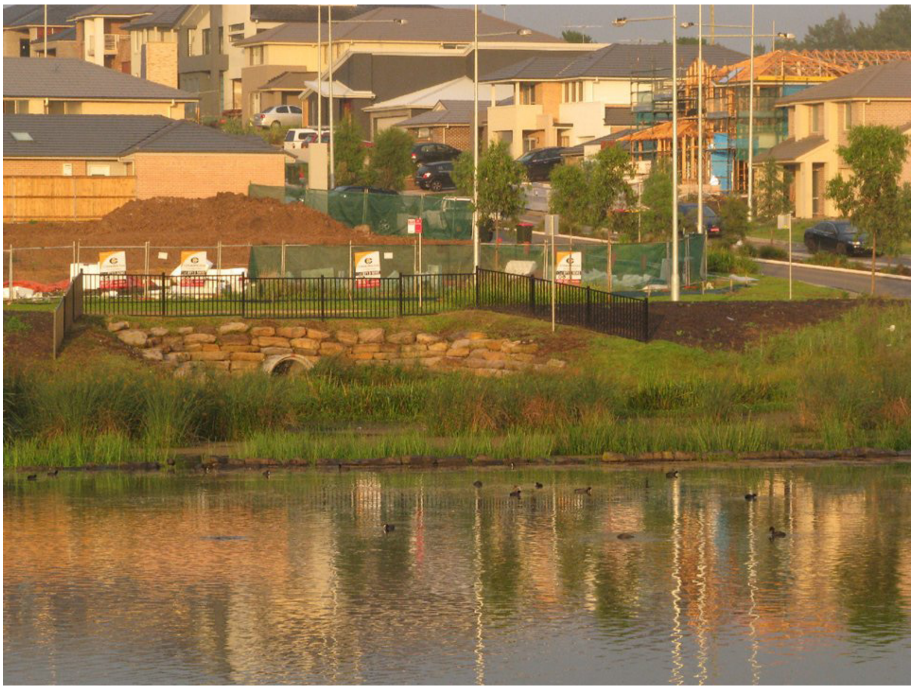

Figure 2.6.1. Constructed wetlands are becoming an increasingly common component of new residential developments to assist with stormwater storage and treatment as well as providing habitat for wildlife. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

What health risks are posed by mosquitoes associated with constructed wetlands?

Ross River virus (RRV) and Barmah Forest virus (BFV) are the two mosquito-borne pathogens that cause the most human illness in Australia (Russell and Kay 2004). Symptoms can vary greatly between individuals but most commonly include fever and rash, infection with either of these viruses may result in a condition known as polyarthritis, with arthritic pain in the ankles, fingers, knees and wrists. Generally, the rash tends to be more pronounced with BFV infection but the arthritic pain is greater and longer lasting with RRV infection (Russell and Kay 2004).

Figure 2.6.2. Constructed wetlands can serve many purposes, as well as water treatment and conservation, they provide opportunities for the community to engage with the local environment as well as providing improved passive recreation areas. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

In addition, Murray Valley encephalitis virus (MVEV) and Kunjin virus (KUNV) may be a concern in some regions, particularly west of the Great Dividing Range. There is no evidence that there is extensive activity of these viruses along the east coast of Australia. Symptoms associated with MVEV infection vary from mild to severe to fatal, with symptoms almost invariably including a sudden onset of fever, anorexia and headache, while vomiting, nausea, diarrhoea and dizziness may also be experienced along with lethargy and irritability. Many who survive the encephalitic syndrome will have some residual mental or functional disability. Infection with KUNV is much rarer. The disease is milder and there are no known human fatalities resulting from the infection (Russell 1998). However, during a major outbreak amongst horses in southeast Australia during early 2011, almost 1000 horse fatalities were recorded (Roche *et al.* 2013).

Both MVEV and KUNV viruses have a natural endemic cycle in northern Australia which involves water birds as the vertebrate host and the freshwater mosquitoes as the major vectors (Russell and Kay 2004). Epidemic activity of the viruses in the southeast of Australia is rare and has been associated with excessive rainfall and flooding. Above average rainfall and subsequent flooding, particularly under the influence of prevailing La Nina weather patterns, increases both bird and mosquito populations. However, it is still uncertain whether the viruses are introduced occasionally to the southeast from the north or whether either or both are endemic in inland areas at undetectable levels and only become evident with periods of intense bird and mosquito breeding (Russell 1998; Spencer *et al.* 2011).

The diseases resulting from infection with RRV, BFV, MVEV and KUNV are "notifiable diseases" in Australia and human infection is only recorded in the official statistics following confirmation of infection with a blood test. Cases of human infection with MVEV or KUNV are extremely rare, even more so in southeast Australia (Knox *et al.* 2012). However, there are, on average, approximately 5,000 notifications of human disease caused by RRV and BFV combined per year across Australia (Russell and Kay 2004).

The drivers of mosquito-borne disease in Australia can be complex and it is difficult to predict local outbreaks of disease (Jacups *et al.* 2008). Transmission cycles generally require the presence of suitable reservoir hosts (mostly birds and/or

Figure 2.6.3. The immature stages of the freshwater mosquito Culex quinquefasciatus. (Photo: Stephen Doggett, Medical Entomology, Pathology West – ICPMR Westmead.)

mammals) as well as abundant mosquito populations. In most coastal areas, estuarine mosquito species play the most important role in pathogen transmission. Away from the coast, as well as along the coast at times of favourable environmental conditions, the freshwater mosquito populations drive mosquito-borne disease risk (Kelly-Hope *et al.* 2004). However, some studies have suggested that proximity to ephemeral and coastal swamp forests (that may fluctuate between brackish water and freshwater dominated), more so than freshwater wetlands, are stronger predictors of RRV (Muhar *et al.* 2000)

Mosquito-borne disease risk is generally lower in metropolitan areas compared to rural regions. This is primarily due to a lack of suitable habitats capable of producing substantially large mosquito populations but also a lack of suitable reservoir hosts. There have been clusters of locally acquired human illness resulting from RRV in western Sydney (Amin *et al.* 1998; Brokenshire *et al.* 2000) and concern has been raised as to the increased relative importance of freshwater mosquitoes if constructed wetlands become more widespread in metropolitan areas, creating additional habitats for mosquitoes and wildlife (Russell 1998). However, assessing the public health risks associated with constructed wetlands located in urban environments can be difficult as site-specific factors relating to mosquito and reservoir host abundance and diversity must be investigated (Johnson *et al.* 2012).

Mosquito biology

Mosquitoes are small blood sucking insects that belong to the family of flies called Culicidae (Order Diptera). They have a relatively short but complex life cycle consisting of eggs, four aquatic larval stages (instars), a pupal stage and an adult stage (Becker *et al.* 2010).

Depending on the species, eggs are laid either on the water surface (usually with eggs in the form of a floating raft) or on a frequently inundated substrate (usually singly or in small groups). Most mosquito species associated with constructed freshwater wetlands will lay eggs as floating rafts on the water surface. These rafts may contain over 300 eggs. For species that lay floating egg rafts, the eggs hatch in approximately 48h but for some mosquitoes that lay desiccation resistant eggs individually, particularly for species associated with inland flood plains, the eggs can remain viable until favourable environmental conditions occur (e.g. above average rainfall and/or major flooding of inland rivers).

Larvae (commonly called wrigglers) that hatch from eggs will feed continuously on aquatic particulate matter (Figure 2.6.3). The immature stages of the majority of mosquito species breathe at the water surface through a "snorkel-like" structure called a siphon. They develop through four different instars or moults until the final larval stage develops into a pupa (commonly called tumbler) from which the adult mosquito emerges approximately 2 days later. The length of larval development is primarily dependent on water temperature and the availability of food. During the warmer months, it generally takes 7–10 days from the hatching of larvae to the emergence of adults.

There are groups of mosquitoes that modified their immature development to exploit specific niches in freshwater wetlands. *Coquillettidia* spp. and *Mansonia* spp. have modified siphons that allow them to attach to submerged parts of aquatic plants. This is typically to stem or roots of either emergent or floating macrophytes. Development of these larval stages can take many months and studies have shown that the immature stages will "over winter" when temperatures fall below a threshold temperature.

Adult mosquitoes may live for up to 3 weeks but the lifespan of the male mosquito is much shorter. Both adult male and female mosquitoes will feed on nectar and plant fluids, but only the female feeds on blood. The blood meal provides nutrients required for egg development. Mosquitoes identify potential blood meals by detecting carbon dioxide, body heat and the "smell" produced from the chemical cocktail of compounds found on the host's skin. While some mosquito species have specific host preferences (e.g. birds, mammals, amphibians), many are generalist feeders and will readily bite humans. It is important to note that very rarely do mosquitoes emerge from the wetlands as adults infected with pathogens, mosquitoes will almost always need to bite an infected animal before becoming infected, and subsequently, infective.

After feeding, the female will find a resting place to digest the blood meal and develop eggs before flying off to deposit them in a suitable habitat. This process may take many days. It is typically not until the eggs have been laid and the mosquito seeks out another blood meal that transmission of pathogens can occur. For the mosquito to transmit a pathogen, the salivary glands of the individual must be infected. When the mosquito finds a host, they will inject a small amount of saliva to assist blood feeding and it is this route of pathogen transmission that results in the infection of a new host. If the salivary glands are not infected, the mosquito cannot transmit the pathogen. There are complex relationships between pathogens and mosquitoes, not all species can transmit pathogens.

What mosquitoes may be associated with constructed wetlands?

There is a wide range of mosquito species that may be associated with constructed wetlands. While relatively few mosquito species are associated with estuarine and brackish water habitats, there are dozens of mosquitoes that may be found in freshwater habitats directly and indirectly associated with constructed wetlands. Some of these species can pose substantial pest risk (Table 2.6.1). While not all mosquitoes will pose a substantial pest or public health risk, under favourable environmental and/or climatic conditions, there is always a risk that there will be site-specific mosquito issues resulting from unusual population abundances of a mosquito species generally considered to be of lower risk.

The diversity and abundance of mosquitoes will vary geographically as well as seasonally. However, there are a number of commonly encountered mosquitoes that may be associated with constructed freshwater wetlands and associated habitats.

Anopheles annulipes can be a nuisance-biting pest and, historically, has been associated with the local transmission of malaria (Ewald *et al.* 2008; Russell 2009). Larvae are closely associated with permanent freshwater habitats, in particular those habitats that contain floating mats of algae. The larvae exploit the shallow water above the algal mats that provide refuge from fish and other mosquito predators. The abundance of this species is determined by seasonal rainfall, as well as the availability of suitable habitat. Although this mosquito can occasionally be abundant, it is considered only a secondary pest species.

Aedes notoscriptus is an important nuisance-biting pest species and has been associated with the transmission of RRV and BFV but will not be associated with wetlands directly. Larvae are found in water holding containers such as rainwater tanks, block gutters, drains, discarded tyres, pot plant saucers etc. Given the proximity of residential areas to constructed wetlands, this species may be present around the wetlands and care should be taken to disassociate any local nuisance-biting impacts caused by this species to the wetland itself.

Coquillettidia linealis has the potential to be a locally important nuisance-biting pest but little is known of the importance of its role in arbovirus transmission. Both RRV and BFV have been isolated from field-collected specimens (Doggett *et al.* 2009)

Table 2.6.1. The habitat associations and public health risks associated with key mosquito species directly associated with constructed freshwater wetlands in Australian.

Mosquito species	Habitat associations	Public health risks
Anopheles annulipes	Freshwater wetlands with preference for thick algal mats. Occasionally also found in brackish water habitats.	Occasional nuisance biting pest and vector of RRV, BFV, MVE.
Coquillettidia linealis	Permanent well vegetated wetlands and sedgelands.	Nuisance biting pest when abundant and may play a role in transmission of RRV and BFV.
Coquilettidia xanthogaster	Permanent well vegetated wetlands and sedgelands.	Nuisance biting pest when abundant role in transmission of pathogens unclear.
Culex annulirostris	Permanent well vegetated wetlands and ephemeral ground pools in grasslands.	Severe nuisance biting pest and vector of RRV, BFV, MVEV & KUNV. The most important freshwater pest species Australia.
Culex australicus	Permanent well vegetated wetlands and ephemeral ground pools in grasslands.	Bird biting mosquito that does not pose a risk to humans but immature stages may be abundant in wetlands.
Culex quinquefasciatus	Freshwater wetlands containing high organic content as well as stormwater and waste-water structures.	Nuisance biting pest common in urban areas and may play a role in transmission of RRV, BFV, MVEV, KUNV.
Mansonia uniformis	Freshwater wetlands with preference for habitats associated with floating vegetation.	Severe nuisance-biting pest and a vector of RRV and BFV.

and laboratory tests have demonstrated that the mosquito can transmit both viruses (Jeffery *et al.* 2002). The larvae have a modified siphon that, instead of connecting to the water/air interface to breathe, attaches to the roots and/or stems of aquatic vegetation to obtain air. As a consequence, dense aquatic macrophyte growth in natural and/or constructed wetlands can increase the suitability of a wetland for this species. The closely related *Coquillettidia xanthogaster* is also often abundant close to freshwater wetlands.

Culex annulirostris is the most important pest mosquito associated with freshwater habitats in Australia (Figure 2.6.4). This is particularly the case throughout inland areas of Queensland, New South Wales and Victoria, particularly in the major river basins and irrigation areas (Russell 1996). As well as being a nuisance-biting pest, this species has been associated with outbreaks of RRV and BFV as well as being regarded as the primary vector of MVEV and KUNV (Kay *et al.* 1984; Russell and Kay 2004). Testing of field-collected specimens regularly yields RRV and BFV in NSW (Doggett *et al.* 2006). Larvae are commonly collected from a range of freshwater habitats from flooded grasslands to permanent, well-vegetated wetlands (Lee *et al.* 1984). This mosquito is becoming of greater concern as constructed freshwater wetlands are increasingly incorporated into urban developments (Russell 1999).

Culex australicus may be abundant in wetlands but it does not represent an important pest. This mosquito preferentially bites birds and, as a consequence, is unlikely to play a direct role in the transmission of mosquito-borne pathogens. Most importantly, this species may cause concern if abundant mosquito larvae are detected in the wetlands but if appropriate monitoring and identification of specimens is undertaken, no unnecessary mosquito control activity will be initiated.

Culex molestus is thought to have been introduced into Australia in the 1940s and is now common in urban areas across all southern regions of Australia (Kassim *et al.* 2013). While little is known

Figure 2.6.4. *Culex annulirostris* is one of the most important nuisance-biting pests and vectors of mosquito-borne pathogens associated with freshwater habitats in Australia. (Photo: Stephen Doggett, Medical Entomology, Pathology West – ICPMR Westmead.)

Figure 2.6.5. *Culex quinquefasciatus* is an internationally important pest mosquito associated with freshwater habitats ranging from highly polluted wetlands to constructed water-holding containers and stormwater infrastructure. (Photo: Stephen Doggett, Medical Entomology, Pathology West – ICPMR Westmead.)

of its current importance in the transmission of mosquito-borne pathogens in Australia, this and closely related species are associated with outbreaks of West Nile virus overseas (Farajollahi *et al.* 2011) and may pose a potential public health risk here (Jansen *et al.* 2013). The larvae of the mosquito are closely associated with subterranean habitats, particularly in urban areas (Kassim *et al.* 2012). This has implications for stormwater infrastructure such as septic tanks and other underground water storages. The larvae may not be found within the wetlands itself but surrounding built structures, these can often be cryptic and difficult to sample.

Culex quinquefasciatus is one of the most widespread mosquitoes internationally (Farajollahi *et al.* 2011). It is a common pest species in urban areas, usually biting indoors at night. This is the mosquito species most likely responsible for disturbing sleep by "buzzing" around the bedroom (Figure 2.6.5). Although in Australia it is not considered to play an important role in the transmission of pathogens, but RRV, BFV, MVEV and KUNV have been isolated from field collected specimens of this mosquito in the field. The larvae of this mosquito are usually associated with, but not limited to, habitats with a high organic content such as drains, sullage pits, septic tanks and other water holding and water storage areas. In rural areas, large populatons of this mosquito can be associated with sewerage treatment plants, or other facilities, where highly polluted water is present. This mosquito may also be associated with constructed freshwater wetlands, including the water management structures (such as gross pollutant traps, pipelines and drains).

Mansonia uniformis is typically associated with permanent freshwater habitats with abundant floating vegetation. Little is known of its importance in the transmission of mosquito-borne pathogens, although it has been shown to transmit RRV in laboratory tests (Russell 2002), but it can be a severe nuisance-biting pest when populations are abundant. (Russell 1996).

How do I know if mosquitoes are a problem in my wetland?

Mosquito monitoring is critical to assessing the mosquito risks associated with constructed wetlands. When planning the construction of a wetland, it is even more critical to assess mosquito populations both before construction and after construction so that there is an ability to measure the relative change in mosquito populations. It is not only abundance that may change but diversity as the vegetation structure and composition of

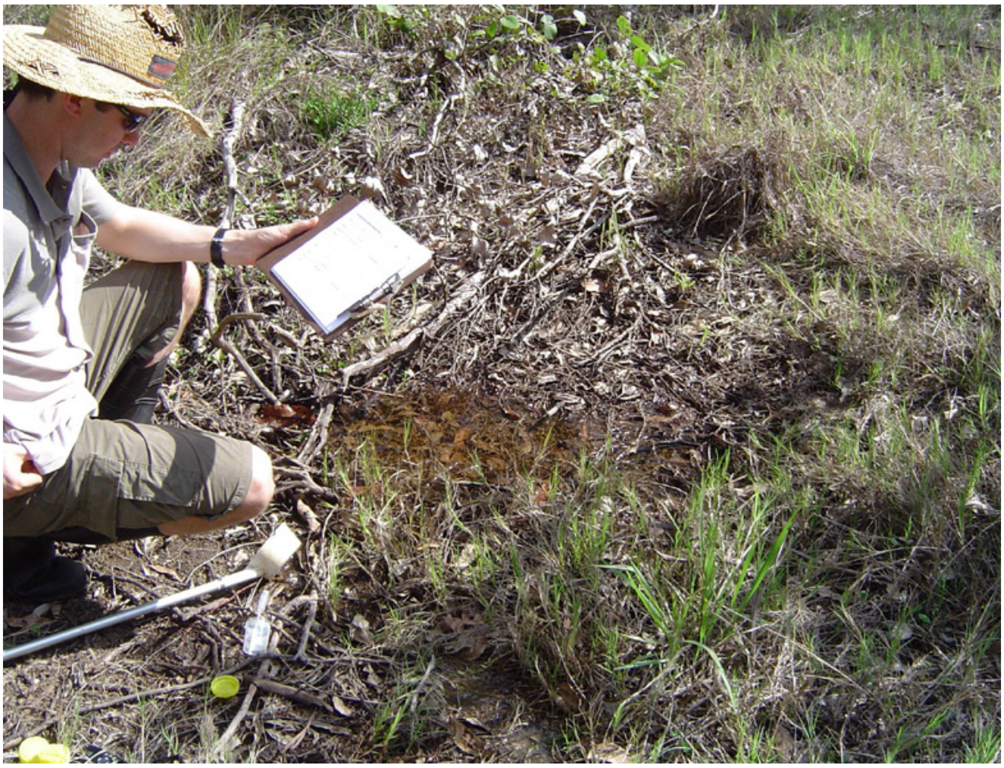

Figure 2.6.6. Sampling of immature mosquito populations is an important component of mosquito monitoring and risk assessment. Not only do mosquitoes associated with the constructed wetlands need to be sampled but also surrounding permanent and ephemeral habitats. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

the wetlands may influence the mosquito species present. It is also important to document the extant mosquito populations so that an assessment of the site-specific change in mosquito habitats may be represented by the constructed wetlands with respect to surrounding habitats (Barker-Hudson *et al*. 1993; Hearnden and Kay 1995).

It is important to note that complaints from the local community to authorities on the level of nuisance-biting activity will not provide a suitable measure of mosquito populations. There are many factors that may influence the number of complaints, not only the abundance of mosquitoes. In some regions, the proposal of a constructed wetland may be perceived by the community as creating a mosquito problem and these misconceptions may motivate a disproportionate response from residents. There is no substitute for good data collection on local mosquito populations as a basis for mosquito risk assessment and mosquito management strategies in constructed or rehabilitated wetlands.

An assessment of nuisance-biting and public health risks associated with a constructed wetlands should commence before the construction, modification or rehabilitation of a wetland is undertaken (Midge Research Group of Western Australia 2007; Byun and Webb 2012). Ideally, the services of a professional entomologist should be engaged to provide advice on the likely mosquito risk that may be posed by the wetland. An assessment of risk should include the proposed design of the wetlands as well as consideration of nearby mosquito habitats (e.g. estuarine wetlands, bushland areas, urban habitats) and current and future land use adjacent to the wetland (e.g. residential or recreational development). Input into design elements of the wetlands during the design phase can be greatly beneficial, as can the collection of data on extant mosquito populations. This baseline information will be critical in assessing future changes in pest and public health risks.

Management of mosquitoes is most effectively undertaken in response to the results of monitoring of immature and adult populations. It is important that specimens are correctly identified by an experienced entomologist as often the most abundant mosquitoes may not be directly associated with the wetland habitats and alternative management strategies may be required.

Immature populations

Sampling immature mosquito populations associated with constructed wetlands is critical to inform management decisions. Some mosquito species likely to be associated with constructed wetlands may also be associated with nearby or non-wetland habitats (e.g. septic tanks, drains, backyard habitats etc) so detection of adult mosquitoes does not confirm their presence in the wetlands. Additionally, constructed wetlands can contain a complex matrix of habitats, even within the most likely areas of mosquito activity (e.g. the shallow macrophyte zone) the spatial

Figure 2.6.7. Adult mosquitoes can be easily sampled using carbon dioxide-baited light traps. These traps specifically target host-seeking female mosquitoes, providing an effective measure of local nuisance-biting impacts and an opportunity to test these specimens for the presence of pathogens. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

The distribution of mosquito larvae is not evenly distributed (Yadav *et al*. 2012; Walton *et al.* 2012; Walton *et al*. 2013).

Immature mosquito populations can be sampled in a variety of ways (Mosquito Control Association of Australia 2008; Silver 2008) but most commonly are sampled using a net or "dipper" (i.e. typically a 200-300ml container) (Figure 2.6.6). Alternatively, as the use of a "dipper" is not always appropriate in wetlands dominated by emergent and/or floating vegetation, emergent traps (i.e. a floating trap to collects adult mosquitoes as they emerge from the water) may be required. For mosquitoes whose immature stages attach to submerged vegetation, alternative sampling measures are required (Silver 2008) For the purpose of on-going monitoring of local mosquito populations, there should be consistency in the strategic use of sampling devices used.

collection, and correct identification, of mosquito larvae is the only reliable method of identifying the breeding habitats and determine the spatial and temporal distribution of productive mosquito breeding sites. While there are taxonomic keys (e.g. Russell (1993)) available for the identification of immature mosquito stages, these keys are generally based on 4th instar larvae and to adequately record the diagnostic features, specimens need to be mounted on slides. It can often be easier for immature stages to be returned to the laboratory and reared through until development is complete and specimens can be identified as adults.

Adult mosquito populations

Adult mosquito populations are generally sampled using dry-ice baited light traps. The most commonly used traps in Australia are known as Encephalitis Virus Surveillance (EVS) traps (Rohe and Fall 1974) (Figure 2.6.7). These traps consist of an insulated "billy" can, a small motorised fan and collection receptacle. Dry-ice blocks or pellets are used as an attractant to draw in host seeking female mosquitoes that are subsequently drawn through the fan into the catch bucket or bag. Additional chemicals, such as octenol, can be added to traps to increase collections of Aedes spp. (Webb *et al.* 2004) but are generally not required when general information on abundance and diversity is required.

A network of traps would normally be operated around the existing, or proposed, wetland. Traps are typically hung in vegetation and operated overnight with the frequency of trapping dependent on the current state of the wetland, actual or perceived mosquito populations and environmental conditions. When adult mosquito trapping is undertaken around newly constructed wetlands that may have minimal terrestrial vegetation. Traps can be set on fences or specially installed posts but when operated in exposed and wind swept areas, they typically collect smaller numbers of mosquitoes. Care should be taken to ensure that relatively small numbers of mosquito collections are a true representation of mosquito abundance and not adversely impacted by the exposed nature of the study site. Mosquito collections can be returned to the laboratory and killed by placing into a freezer for approximately 20 minutes. The dead specimens can then be identified using taxonomic keys such as Russell (1993). In addition, collections can be tested to determine if mosquitoes are infected with any pathogens (Doggett *et al.* 2009; van den Hurk *et al.* 2012)

To measure the relative spatial and temporal abundance of local mosquito populations, a network of traps is usually operated around the wetland to sample mosquitoes dispersing from and into a wetland and local area (Webb and Russell 1999). The exact number of traps will be dependent on a range of factors including the suitability of vegetation surrounding the wetland and the diversity of wetland habitats themselves that must be sampled. Additional traps may also be operated at increasing distances from breeding habitats to identify dispersal patterns of pest species and identify areas of greatest pest impacts. This additional trapping may provide important information on the relative impact of mosquitoes associated with the constructed wetlands compared to in nearby estuarine, brackish water or "backyard" mosquitoes.

The timing and frequency of mosquito population sampling is an important consideration since mosquitoes have short life cycles and their abundance closely linked to the environmental factors. Generally, the abundance of freshwater mosquitoes is driven by local rainfall and temperature. However, in the case of constructed wetlands that may be artificially filled or where water is recirculated between ponds, changes in mosquito abundance may occur independent of rainfall.

There are few quantitative measures of mosquito abundance that determine that a wetland has a "mosquito problem". As mosquitoes are a natural part of Australia's wetlands, it should be expected that there will be mosquitoes present and active during the warmer months. The critical issue is the relative impact of these populations and if these populations are considered to be unusually large from a local perspective. Building a data set on local mosquito populations is critical and will allow a comparison of changing mosquito abundance with seasonal variability in environmental factors (Webb and Russell 1999).

How can wetlands be designed and managed to reduce mosquito risk?

While the design of constructed wetlands can be tailored to site-specific requirements, they generally fall into one of two categories, subsurface flow or surface flow systems (there may also be both elements present at some sites). Subsurface flow systems rarely pose a mosquito problem unless water inflows exceed hydraulic capacity or the subsurface media becomes blocked with sediments and surface water persists for five days or more. Surface Flow systems are the more common type in Australia, and are a higher risk of providing suitable conditions for pest mosquitoes.

The design, operation and maintenance of constructed wetlands will be primarily determined by the objective of local authorities. It is often difficult to balance the design considerations required to allow the wetland to meet its specific function (e.g. remove pollutants in stormwater, provide wildlife refuge, and improve aesthetic appeal for new urban developments), while keeping mosquito productivity to a minimum. As the management of mosquitoes associated with these wetlands must be integrated into the overall management of the wetland, there are a number of constraints imposed on mosquito management (Table 2.6.2).

The suitability of wetlands for mosquitoes is dependent on a wide range of factors that include the size, design function (e.g. waste-water treatment, water storage, wildlife refuge) and location of the wetlands; aquatic macrophyte communities; predatory fish and macroinvertebrate communities; extant mosquito populations and wetland and/or mosquito management plans (Russell 1998, Walton *et al.* 1998; Knight *et al.* 2003; Walton 2011). The management strategies required to address the mosquito risks associated with constructed wetlands are often site-specific. However, there are general design, construction and maintenance principles that can be incorporated into wetland management that can minimise mosquito production.

Wetland location and buffer zones

Where possible, the wetland should be located away from the community beyond the flight range of local pest mosquitoes, thereby creating a buffer between the mosquitoes and people. While there are likely to be site-specific factors that determine the actual dispersal range of pest mosquitoes, *Culex annulirostris* has been shown to disperse over 12km (Russell 1986), *Coquillettidia linealis* over 5km (Russell 1988) and Anopheles annulipes approximately 1km (Bryan *et al.* 1991) from wetlands. Given propensity of these key freshwater mosquito species can disperse long distances there is unlikely to be sufficient available land to accommodate buffer zones around newly constructed wetlands.

Where buffer zones can be incorporated into the location of constructed wetlands, consideration must be given to the surrounding terrestrial vegetation. Buffer zones should be kept clear of significant vegetation that may be likely to afford harbourage to mosquitoes. Vegetation within the buffer zone can negate the potential benefits of the buffer zone by providing “stepping stones” across those open areas that increase the likely movement of mosquitoes as well as increase the survivorship of mosquitoes by providing a humid refuge from heat and wind.

The most desirable design of buffer zones to minimise the movement of mosquitoes is to minimise refuge for mosquitoes and maximise exposure to wind disturbance. With this in mind, buffer zones can be incorporated into the design of residential and/or recreational developments by including walkways, cycle paths and the construction of roadways around the boundary of “buffer zone” (i.e. roadways form buffers between

Table 2.6.2. Constraints and compromises required to balance mosquito risks and associated risk management strategies in constructed wetlands.

Constraints or compromise	Why?	Mosquito risk	Risk reduction strategies
Wetland located in urban area close to community	Local water storage and/or recycling purposes or to create an aesthetic amenity or wildlife habitat that will increase land and lifestyle values	Residential allotments close to wetlands and/or local community will be within dispersal range of mosquitoes from wetland.	Where possible, locate wetlands away from high density residential areas to enable a buffer between mosquitoes and community
Wetlands may receive relatively polluted water and maintain a high organic content	Wetlands may be placed to reduce the flow of nutrients/pollution in local waterways	High organic content of water increases production of some mosquitoes directly with increased nutrients and indirectly with increased vegetation growth and decreased predator populations	Pre-treatment of waste-water will reduce organic content. Reduces suitability for some mosquitoes and unsuitable conditions for mosquito predators.
Wetland must have stormwater structures such as gross pollutant traps	These structures are required for retention macropollutants, including floatables, and exclusion from wetland proper	Accumulation of organic material (e.g. garden waste/lawn clippings) and rubbish (e.g. plastic bottles) can increase the suitability of habitats for mosquitoes	An appropriate maintenance regime of stormwater structures is required to minimise accumulation of sediments, rubbish

Table 2.6.2. (cont.) Constraints and compromises required to balance mosquito risks and associated risk management strategies in constructed wetlands.

Constraints or compromise	Why?	Mosquito risk	Risk reduction strategies
Wetland must have abundant aquatic vegetation	Aquatic vegetation assist the trapping of sediments and removal of nutrients	Greater densities of aquatic vegetation result in higher risk of creating productive mosquito habitat	A preference for non-invasive macrophytes will reduce risk of creating favourable mosquito habitats
Wetlands should be shallow	Shallow water provided suitable conditions of vegetation growth	Shallow water (depths less than 30cm) creates more favourable conditions for mosquitoes	The provision of deeper water areas that provide refuge for predator populations may reduce production of mosquitoes
Water movement throw wetland must be slow	Rate of sediment deposition and nutrient removal will be greater with slow water movement	Wetlands are more suitable for mosquitoes without water	It may be impractical to move water through the wetland system sufficiently to reduce mosquito production alone but may assist in reducing the suitability of habitats for mosquitoes
Wetland should have gentle sloping edges	Authorities may require easy and safe access to the edge of the wetlands for vegetation maintenance	A gentle slope at wetland margin facilitated a wider macrophyte zones and consequently, may provide extra habitats for mosquitoes.	Steeper sloped wetland margins will reduce the suitability of habitats for mosquitoes. In some instances, a vertical edge to the wetlands may further reduce suitability of habitats for mosquitoes.

vegetation and residential allotments). In addition, sporting fields and other 'open' active and/or passive recreation space can be incorporated into the "buffer zone".

Where "buffer zones" are used for wildlife conservation and/or as wildlife corridors for movement of kangaroos and wallabies, there may be an increase in RRV risk as these are key reservoir hosts for the virus (Russell 2002). In addition, the forest or grassland within the "buffer zones" may provide habitat for pest mosquitoes associated with ephemeral ground pools that form after rain. A suite of species may be found in such habitats, including those also found in association with the constructed wetlands.

In reality, many constructed wetlands will be incorporated into new residential, industrial or recreational developments. These wetlands are making a valuable contribution to both water and wildlife conservation. Studies from North America have indicated that thoughtful integration of constructed wetlands with recreational facilities can increase residential property values (Lee and Li 2009). While the opportunity to incorporate "buffer zones" is inappropriate, the advantages of integrating the constructed wetlands with other developments is an opportunity to develop funding models for wetland management plans where stakeholders contribute to the cost of monitoring and maintenance programs. With an ongoing funding base, local authorities may be better placed to ensure that the wetland not only continues to meet its objectives but minimise the production of mosquitoes.

Wetland design

Constructed freshwater wetlands often contain various component zones, and these can be variously classified and many characteristics of

Figure 2.6.8. Constructed wetlands may contain many different components, each bringing with them a different suite of mosquito risk factors. All components of a wetland, as well as surrounding habitats, should be taken into account when reviewing design plans and mosquito monitoring programs. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

each component can vary from site to site (Figure 2.6.8). However, there is a range of mosquito species, and resulting management options, that may be associated with each of these components directly and indirectly associated with constructed wetlands (Table 2.6.3).

In general, constructed wetlands should be sited in open areas where wind action produces surface waves that disrupt larval respiration, discourage mosquito oviposition (egg-laying) and inhibit the algae and floating plants that provide refuge for immature mosquitoes from predators (Russell 1999). The benefits of wave action can be further increased by situating the long axis of the wetland in line with prevailing summer wind directions or ensuring the wetland is large and circular in nature (Midge Control Group 2007). Where the open areas of wetlands are designed as sedimentation traps, they should be deep to restrict the invasion of aquatic macrophytes. While deep water (more than 300mm in depth) will generally be unfavourable for mosquitoes (Russell 1999), if large areas of floating vegetation are able to become established, suitable conditions for mosquitoes may be created.

Ponds with simple shapes and a low edge to area ratio are less likely to be productive of mosquitoes. A complex wetland with shorelines that promote heterogeneity of the vegetation zones and the presence of small coves and inlets provides for growth of dense vegetation, and accumulation of floating debris. Under these circumstances, mosquito larvae are protected from wave action and predators. Wetlands with generally linear shorelines provide less area of refuge for larvae from predators than do convoluted shorelines but such linear wetlands may not be conducive to slowing water flows to assist pollutant removal.

Concern for public safety around wetlands is an important consideration and often results in the incorporation of gently sloped wetland banks. To discourage access to wetlands, spiky, thorny or otherwise impenetrable terrestrial vegetation can be planted, but this strategy is not always desirable. The recommended bank slope of wetlands to minimise mosquito breeding is from 2.5H:1V to 4H:1V, and slopes should not be planted with grasses that may trap water and provide habitat for mosquitoes (alternatively, grasses should be regularly cut to minimise habitat available).

Steep slopes can restrict the density of vegetation, and reduce the area of shallow water, minimising suitable mosquito habitat by maximising the access of predatory fish and exposing larvae to surface water disturbance that may increase larval mortality. If the recommended bank steepness cannot be maintained for safety or other considerations, a vertical 'lip' between 100 - 300mm may be used at the water margin, allowing more gradual slopes above and below the vertical edge (Figure 2.6.9). While effective, this strategy will not be effective if water levels cannot be maintained at appropriate levels.

It can be beneficial if water level management capabilities are incorporated into the design of the wetland. However, while lowering and raising the water level can be can be detrimental to *Anopheles* spp. *Coquillettidia* spp. *Culex* spp. and *Mansonia* spp. it may promote some Aedes species adapted to wetlands with fluctuating water levels (Russell 1999). Similarly, periodic draining can effectively interrupt mosquito production, and a wetland

Table 2.6.3. Components of constructed wetlands and associated mosquito risk and management option.

Component	Mosquito Risk	Management options
Inlet Zone (Stormwater pipes, GPT, detention basin)	*Culex quinquefasciatus*	Maintenance schedule to reduce accumulation of debris, rubbish and sediments.
Riffle Zone	*Anopheles* spp. *Aedes* spp. *Culex quiquefasciatus*	These structures to slow water flow and dissipate energy should be designed not to allow small reservoirs of water to persist. These areas inaccessible by predators. Algal mats will encourage *Anopheles* spp.
Macrophyte Zone	*Anopheles* spp. *Aedes* spp. *Culex* spp. *Coquillettidia* spp. *Mansonia* spp.	Mosquito risk reduced by ensuring non-invasive plant species, maintenance of vegetation at sparse densities and encourage mosquito predators. Ensuring minimal water level fluctuations will reduce suitability for *Aedes* spp.
Floating Macrophyte Islands	*Coquillettidia* spp. *Mansonia* spp.	Ensure that island is located in deep water to allow predator access.
Deepwater Zone	*Anopheles* spp. *Culex* spp.	Minimise algal cover that provides habitat for *Anopheles* species. Encourage predator populations.
Subsurface Flows	*Culex quinquefasciatus* *Culex annulirostris*	Design and maintenance to ensure carrying capacity of wetland is not exceeded and reduce risk of surface water pool creation.
Bioretention Swales	*Aedes* spp. *Culex annulirostris Culex quinquefasciatus*	Inflow/evaporation rates should ensure surface water is not maintained for more than 5 days. Sedimentation of swales may reduce infiltration rates over time and create mosquito habitat
Outlet/Spillway	*Anopheles* spp. *Aedes* spp. *Culex quiquefasciatus* *Culex annulirostris*	Water flows should not be allowed to accumulate as surface pools or retained in spillway or behind weir structures. Algal mats will encourage *Anopheles* spp.
Terrestrial Habitats (i.e. bushland, grassland)	*Anopheles* spp. *Aedes* spp. *Culex* spp.	Ephemeral ground pools may provide habitat for pest mosquitoes not directly associated with wetland. Site-specific management strategies required.
Built Environment	*Aedes notoscriptus* *Culex molestus* *Culex quinquefasciatus*	Above and below ground water holding structures should be screened, sealed or removed where possible to limit opportunities for pest mosquitoes

system with a number of bays in parallel allows for some cells to be drained for mosquito management while others remain flooded and continue to provide treatment of polluted waters. Prescribed times for drying of wetlands must be decided with a view to site-specific issues such as mosquito species, vegetation types, seasonal factors but the incorporation of water level management capabilities will greatly assist future mosquito management.

Water management

There are various ways in which water management can assist in reducing the suitability of constructed wetlands for mosquitoes. As well as water quality, water movement, water levels, circulation and aeration may all provide site-specific strategies for reducing mosquito production.

The type and quality of inflows will directly influence mosquito populations as well as indirectly through impacts to the plants and animals associated with the wetland. With regard to wetlands receiving waste-water flows, secondary

Figure 2.6.9. Wetlands that contain large areas of open water will not be suitable for mosquitoes due to disturbance from wind and wave action. A hard, vertical edge to the wetland further reduces the suitability of the habitats for mosquitoes. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

Figure 2.6.10. The mosquito important habitats for mosquitoes within a constructed wetland are the shallow, will vegetated macrophyte zones. These habitats can vary substantially depending on the design of the wetland and abundance and diversity of aquatic vegetation. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

treatment may be required to substantially reduce nutrient load and other pollutants. Highly organic water flows may trigger the growth of algal mats that, in turn provide habitats for mosquitoes. Studies from California indicate that wetlands that receive inflows that are continuously or intermittently low in organic content actually support mosquito populations that are more diverse but less abundant (Walton 2011).

Water circulation within the wetland can indirectly assist in reducing mosquito productivity. As well as increasing the suitability of the wetlands for vertebrate and invertebrate predators, it reduces the risk of algal mat formation and helps maintain healthy macrophytes. The form of aeration and/or circulation itself is unlikely to have a major impact on mosquitoes directly. Fountains can provide some disturbance to the water surface and may either drown immature mosquitoes or inhibit oviposition by mosquitoes. However, this strategy is only going to be of limited use, particularly if wave action doesn't impact the margins of the wetlands due to vegetation.

Another design element that can be useful for both vegetation management and mosquito control is the ability to manipulate water levels. The ability to completely drain a wetland, or component of wetland, can assist in the control of both pest vertebrates as well as mosquitoes. If abundant populations of Culex spp. mosquitoes are present, the build up of populations can be interrupted by draining the wetland.

Aquatic vegetation

Shallow water macrophyte zones are the most common site of mosquito production in constructed wetlands (Figure 2.6.10). Studies in North America have identified positive correlations between vegetation density and the abundance of mosquito larvae (Walton 2012). However, the design of macrophyte beds and the species present may also influence the abundance of mosquitoes (Walton *et al.* 2012; Walton *et al.* 2013). Comparable studies are not available from Australia but given the similarities between the wetlands,

Table 2.6.4. Qualitative assessment of potential mosquito risk associated with aquatic macrophytes.

Plant group	Plant name	Mosquito Risk		
		High	Med	Low
Emergent	*Alisma* spp. (e.g. Water Plantain)			+
	Sagittaria spp. (e.g. Arrowhead)			+
	Cyperus spp. (e.g. Giant Sedge)		+	
	Typha spp. (e.g. Cumbungi)	+		
	Phragmites spp. (e.g. Common Reed)	+		
	Bolboschoenus spp. (e.g. Clubrush)		+	
	Eleocharis spp (e.g. Common Spikerush)		+	
	Persicaria spp. (e.g. Slender Knotweed)	+		
Floating	*Azolla* spp. (e.g. Water Fern)		+	
	Eichhornia spp. (e.g. Water Hyacinth)	+		
	Lemna spp. (e.g. Duckweed)		+	
	Potamogeton spp. (e.g. Pondweed)			+
	Salvinia spp. (e.g. Salvinia)	+		
	Ranunculus spp. (e.g. Buttercups)	+		

and their resident mosquito species, in California compared to Australia, it would not be unexpected for similar trends to occur here also.

Aquatic macrophytes primarily provide refuge for immature stages of mosquitoes from predators (e.g. fish, macroinvertebrates) and wind generated wave action while also providing an oviposition site. There is limited information available on the associations between specific vegetation types and mosquito productivity but a qualitative assessment can be made based on the likelihood of providing suitable conditions for mosquitoes based on growth forms and other biological and ecological characteristics (Table 2.6.4). However, the species of greatest concern are *Typha* spp. and *Phragmites* spp. that are prone to wetland invasion and dense growth. These species may “clog” wetland systems, creating refuge for mosquito larvae and restricting access of predators. Also, dead plant material increases the organic content of the water, increasing the suitability of the habitat for mosquito species such as *Culex annulirostris* and *Culex quinquefasciatus*. Floating plants such

Figure 2.6.11. Dense stands of aquatic vegetation, particularly of invasive *Typha* spp. or *Phragmites* spp., can increase the suitability of habitats for mosquitoes.

Figure 2.6.12. The suitability of macrophyte zones for mosquitoes can be reduced by locating the vegetated shallow water areas away from the wetlands edge. In addition, the inclusion of a hard vertical edge around the wetland margins further reduces the suitability of mosquitoes in the macrophyte zone by increasing access of predatory fish and other macroinvertebrates. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

as water hyacinth (*Eichhornia crassipes*), salvinia (*Salvinia molesta*) and duckweed (*Lemna* spp.) all have the potential to increase the suitability of wetlands, particularly open water areas that would otherwise not provide suitable conditions. Recent simulated field tests in the laboratory have shown that *Culex annulirostris* preferentially lays eggs in association with *Salvinia molesta* compared *Eichhornia crassipes* and *Cyperus haspens* (Webb *et al.* 2012). It is, however, worth noting that in situations where floating vegetation is particularly thick, mosquitoes may not have access to water to lay eggs.

The most productive areas of macrophyte zones are typically the margins of the wetlands where vegetation is dense and water relatively shallow. This is particularly the cases when water levels are high (during periods of above average rainfall) and inundating 'terrestrial' vegetation. Similarly, during dry periods, isolated pools may become disconnected to the main water body, limiting the movement of predatory fish and macroinvertebrates, as well as minimising any impact of wave action. Studies have shown that where there is greater isolation of smaller pools in the wetland system, greater mosquito productivity is likely to result (Chase *et al.* 2009).

The design of macrophytes zones can vary greater and there are options that can increase or decrease opportunities for mosquitoes. Densely vegetated macrophyte zones close to the edge of a wetland are most likely to support mosquitoes (Figure 2.6.11). However, movement of the macrophyte zone away from the edge, including a deeper water area between the macrophytes and edge can assist access of predators, as well as the impacts of wind and wave disturbance, in reducing the suitability of habitats for mosquitoes (Figure 2.6.12).

Barriers between the macrophyte zone and the deeper water sections of the wetlands can often be incorporated into the wetland design. These barriers are useful in that they can limit the movement of invasive macrophytes. However, they can also

Figure 2.6.13. Structural barriers between the shallow macrophyte zones and deeper/open water components of wetlands will restrict the movement of aquatic plants but may reduce the impact of wind and wave disturbance, as well as movement of predators, into the macrophyte zone to reduce mosquito populations.

limit the impact of wind and wave action, as well as providing some obstruction to the movement of predatory fish and macroinvertebrates, and may inadvertently increase the suitability of the macrophyte zone for mosquitoes (Figure 2.6.13).

Inflow and outflow structures

Stormwater structures featuring sub-surface water flow and storage capabilities are generally not suitable sites for mosquito production unless mosquitoes can gain access to the standing water. Mosquitoes that exploit these habitats, such as *Culex molestus* and *Culex quinquefasciatus* are adept at using these habitats. The production of mosquitoes from stormwater inlet pits, gross pollutant traps or surface storage areas can be avoided by ensuring that the structures are self draining, are shallow enough to encourage evaporative drying, and that the accumulation of sediments and organic material is maintained at low levels. As these sites are often isolated from the main water body of the wetland, predator access is limited, further enhancing condition for mosquitoes.

It is important to note that mosquito production, particularly *Culex quinquefasciatus*, can occur within storwater pipes. Adult mosquitoes will seek out structures like stormwater pipes as a resting site during the day due to the protected cool and humid conditions provided. During periods of low flow through pipes, small pools of very shallow water can persist and mosquito larvae can complete their development. Adult mosquitoes may disperse within stormwater pipelines away from the wetland. Adult mosquitoes then emerge in areas some distance from the wetland and with no alternative immature habitats available. The source of the perplexing abundance of mosquitoes can often be very difficult to identify and, at times, difficult to control.

The key to the suitability of GPTs for mosquitoes is the permanent retention of standing water in these structures and mosquitoes can use even small quantities of water. However, in a regularly maintained GPT that minimises the quantity of sedimentation and accumulation of floating rubbish, the suitability of these structures for mosquitoes is reduced. The sediments and rubbish often provide a refuge for larval mosquitoes and increases the likelihood mosquitoes can complete their development.

Bioretention swales

Bio-retention swales, in association with wetlands, can provide habitat for mosquitoes but typically only if the carrying capacity of the structure has been overestimated (i.e. stormwater flows are greater than predicted and results in standing water remaining on the surface for longer periods than desirable) or a build-up of sediments overtime reduces the infiltration rate and storage capacity of the wetlands and/or basin. The retention of stormwater flows, particularly following major rainfall events or during periods of above average rainfall, as standing water for periods of more than 5 days during summer or 7 days during spring or autumn will provide suitable conditions for mosquitoes. Water flows through structures following rainfall and/or ponding of water for short periods does not represent a risk of mosquito production from swales or basins.

Rainwater tanks

While not a component of constructed wetlands, rainwater tanks are increasingly being used in to assist water storage in residential and recreational areas. Any building structure (e.g. amenities block) associated a newly constructed wetland may have above ground water storages associated with them. There has been much debate recently surrounding the role of rainwater tanks in the abundance of local mosquito populations, activity of mosquito-borne disease and by providing habitats for exotic mosquito species that may be associated with the transmission of tropical diseases including dengue (Russell 2009; Beebe *et al.* 2009).

The main concern regarding rainwater tanks is that they may increase the available habitat for *Aedes notoscriptus*, increasing nuisance-biting rates (Kay *et al.* 2008) and potential transmission of RRV and BFV (Doggett and Russell 1997). Rainwater tanks have historically been identified as locally significant mosquito habitats. However, these tanks vary dramatically from modern tanks that come in a wide range of shapes and sizes and made from a number of different materials, most commonly molded polyethylene or steel. These modern tanks are designed to reduce their suitability as mosquito habitats through the use of sturdy screens on all openings. In Queensland, where dengue mosquitoes have been shown to be associated with unscreened rainwater tanks, regulations specify that brass, copper, aluminum or stainless steel gauze not coarser than 1 mm should be fitted to all openings.

The most important consideration is that tanks are properly installed and that any openings (such as inflow, outflow and access points) are completely screened to prevent entry by mosquitoes. Similarly, during maintenance checks of tanks, screens should be checked to ensure that they're intact.

What mosquito control agents would be suitable for constructed wetlands?

If constructed wetlands are producing abundant mosquito populations, investigations should be undertaken to determine the factors driving this abundance with a view to developing long-term solutions through wetland management strategies (e.g. water levels and circulations, vegetation). In some circumstance, short-term reliance on mosquito control agents will be required. All mosquito control agents should be registered with the Australian Pesticides and Veterinary Medicines Authority (APVMA) and be provided with recommended application rates against target species in specific habitats. The application of mosquito control agents may also require approvals from local authorities and the appropriate legislation should be considered when developing, even on a short-term basis, a mosquito control program.

Pre-emptive control of mosquitoes has been shown to reduce the risks of mosquito-borne disease (Tomerini *et al.* 2011) but the control agents and strategies for their use to control freshwater mosquitoes will differ substantially from site to site (Breitfuss *et al.* 2005; Russell and Kay 2008). The active ingredients of the most commonly used mosquito control agents in Australia are discussed below.

Bacillus thuringiensis israelensis

The bacterium *Bacillus thuringiensis israelensis* (*Bti*) produces a protein crystal which contains a number of microscopic pro-toxins that, when ingested, are capable of destroying the gut wall and killing mosquito larvae. Control agents containing Bti have been used widely in Australia since the late 1980s and is considered the mainstay of mosquito control programs throughout the country (Russell and Kay 2008).

There are limitations to the use of *Bti*, primarily due to the relatively short period, less than 3 days, of effective control. It is highly specific to mosquito larvae and very few non-target effects, and no known resistance, have been recorded when the product is applied and recommended rates. In Australia, it has been used to successfully reduce pest mosquito populations in both saline and freshwater habitats but this product does have some disadvantages in that the efficacy is reduced in habitats with a high organic content. For waste-water treatment wetlands, *Bti* is not likely to be a suitable control agent.

Bacillus sphericus

Bacillus sphericus (*Bs*) was first registered for use against mosquitoes in Australia in 2005 (Russell and Kay 2008) and it is considered most appropriate for use in freshwater habitats to control populations of Culex annulirostris and Culex quinquefasciatus with residual activity of up to 3 weeks (Brown *et al.* 2004). Interestingly, the residual activity provided by *Bs* has been attributed to bacterial spores replicating in the cadavers of mosquito larvae. Consequently, greater residual control can be achieved with *Bs* if it is first applied to the wetlands when immature mosquitoes are active. If abundant immature stages of *Culex* spp. were detected in a wetland, *Bs* may be a suitable product to employ.

s-methoprene

The most widely used mosquito control agent in Australia against freshwater pest species is the insect growth regulator, s-methoprene. This product is a synthetic mimic of juvenile hormone produced by insect endocrine systems and has been shown to be an effective control agent of freshwater pest mosquitoes, including Anopheles spp., Coquillettidia spp. and Culex spp., without adversely effecting the non target organisms (Russell and Kay 2008). When absorbed by the larvae, development is interrupted and larvae fail to successfully develop to adults, usually dying in the pupal stage or the adults die shortly after emerging. This is a great benefit of s-methoprene as it maximises the presence of mosquito larvae in the aquatic ecosystem long enough to provide food for predators. Furthermore, s-methoprene has the operational advantage that there are sustained release formultions that can provide control for several months. One of the disadvantages of s-methoprene is that it is relatively expensive compared to the alternative products such as *Bti* or *Bs*.

Biological control

Mosquitoes are no doubt consumed by a range of vertebrate and invertebrate predators in and around the wetlands (Caprinera 2010) and predators have been explored as potential biological control agents of mosquitoes (Becker *et al.* 2012). A number of specific organisms have been investigated to determine their suitability as effective predators of mosquito larvae including invertebrates (e.g. Diptera and Coleopteran larvae, Copepods, Crustaceans, Notonectids, Odonates) and vertebrate predators (e.g. fish).

Commonly referred to as the 'mosquitofish', *Gambusia holbrooki* (eastern Gambusia) was introduced to Australia from South America in the 1920s to address mosquito-borne disease risks. Since that time, the fish has spread, and been spread by humans, to most of the waterways in Australia. There is some debate as to the effectiveness of *Gambusia holbrooki* as a mosquito control agent, and in Australia the species has been implicated in significant adverse impacts on aquatic native fauna, particularly fish and amphibians (Webb and Joss 1997; Howe *et al.* 1997). The species is now classified as a noxious pest and should never be introduced to control mosquitoes.

Studies in Queensland investigated the potential mosquito control capacity of a range of native fish species including *Melanotaenia duboulayi* (crimson-spotted rainbowfish), *Retropinna semoni* (Australian smelt), *Pseudomugil signifer* (Pacific blue-eye), *Craterocephalus stercusmuscarum* (fly-specked hardyhead), *Hypseleotris galii* (firetail gudgeon), *Hypseleotris compressa* (empire gudgeon) and *Ambassis marianus* (estuary perchlet) (Hurst *et al.* 2004). Many of these fish demonstrated the potential to be as effective as *Gambusia holbrooki* in their consumption of mosquito larvae. This is also supported by laboratory studies demonstrating that native fish can consume comparable numbers of immature mosquitoes compared to *Gambusia holbrooki* (Willems *et al.* 2005a). Even in the presence of other aquatic invertebrates and tadpoles, the fish exhibited a strong preference for larvae of *Culex annulirostris* over both alternative prey species with *Melanotaenia duboulayi* consumed the most mosquito larvae of all fish tested in the simulated field tests (Hurst *et al.* 2006a).

With regard to mosquito control, studies using *Melanotaenia duboulayi* and *Hypseleotris galii* found that complete control of *Culex annulirostris* was not achievable when larval densities were high (Hurst *et al.* 2006b). However, the authors proposed that when used in conjunction with the mosquito control agent *Bacillus sphericus* to assist in reducing the density of immature mosquitoes, long term control could be achieved. Furthermore, studies demonstrated that the presence of this *Melanotaenia duboulayi* deterred oviposition by *Culex annulirostris* (Hurst *et al.* 2010).

With regard to control of mosquitoes in association with constructed wetlands, native fish will always be the preferred option over introduced species. However, in urban environments, it may be difficult to exclude exotic species such as *Gambusia holbrooki*. In situations where habitats may be suitable for native fish, the use of endemic species may play a role in integrated mosquito control strategies. In Queensland, some local governments have established fish breeding programs and have been distributing fish throughout the community. They have found that the distribution of fish has provided additional opportunities to raise awareness of mosquito and mosquito-borne disease issue (Moffat 2005).

There is often a misconception that tadpoles of Australian frogs will consume mosquito larvae. Studies testing the rates of predation of tadpoles of four Australian frog species, *Limnodynastes peronii*, *Limnodynastes tasmaniensis*, *Litoria aurea* and *Litoria peronei*, indicated that none of the four species were actively preying on immature stages of *Culex annulirostris* in laboratory tests (Willems *et al.* 2005). While tadpoles may not hold potential for mosquito control in Australia, there are indications that they may have indirect impacts on mosquito larvae. Laboratory tests have shown that immature stages of *Culex quinquefasciatus* had reduced rates of survival, growth and development, and smaller size at metamorphosis, when they were raised with tadpoles of *Limnodynastes peronii* (Mokany and Shine 2002). This adds further evidence that maintaining a diverse aquatic ecosystem in constructed wetlands may assist in reducing the production of mosquitoes.

While many animals associated with freshwater wetlands eat adult mosquitoes (e.g. frogs, birds), insectivorous bats are most commonly described as potential mosquito control agents. It has been demonstrated that smaller bats (e.g. *Vespadelus* spp.) are more likely to eat mosquitoes (Gonsalves *et al.* 2013a) and that these bats will change their foraging activity in response to both habitat and prey abundance (Gonsalves *et al.* 2013b; Gonsalves *et al.* 2013c). However, there is currently no evidence that any bat species relies exclusively on mosquitoes as a food source and many larger bats may only consume mosquitoes infrequently (Gonsalves *et al.* 2013a). There is no evidence to support the claim that predation by bats will assist in the reduction of local mosquito populations.

Summary

Mosquito management should be an important objective in the planning, design, operation and maintenance of constructed wetlands. Pest and disease hazards caused by mosquitoes are important issues that must be addressed by those responsible for constructed wetlands. To create wetlands in areas endemic for mosquito-borne pathogens, and which might otherwise not have habitats producing large numbers of mosquitoes, could have severe public health consequences unless mosquito control strategies are incorporated in or anticipated for the wetlands.

Although mosquito management principles may appear to be incompatible with engineering and water quality objectives and operations of wetlands, practical compromises are feasible. More integrated research on the production of mosquitoes by constructed wetlands in various regions of Australia is needed, so that interaction between the various stakeholders can lead to informed and practical solutions, and appropriate mosquito management can become an integral part of constructed wetland technology.

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