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Managing mosquitoes in coastal wetlands

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Abstract

The mosquitoes associated with estuarine wetlands represent some of the most important pest species in Australia. The saltmarsh mosquito, *Aedes vigilax*, and the southern saltmarsh mosquito, *Aedes camptorhynchus*, are both adapted to estuarine conditions and are often exceptionally abundant during periods of favourable environmental conditions. In those regions where abundant populations occur, these species play a locally significant role in the transmission of disease-causing pathogens, in particular Ross River virus and Barmah Forest virus. A range of strategies are available to manage the risks associated with these estuarine mosquitoes. These may include the use of control agents targeting the aquatic immature mosquitoes and/or habitat modifications that reduce the suitability of estuarine habitats. Local authorities must remain mindful of these strategies as an increased abundance of mosquitoes in association with wetland construction and rehabilitation projects may be a concern. While the ecological importance of these estuarine mosquitoes has not been fully demonstrated, any mosquito management program must consider the indirect impacts of mosquito population suppression, particularly on insectivorous vertebrates that may utilise mosquitoes as a food supply. Mosquitoes are a natural part of estuarine wetlands and living with the risks associated with mosquito-borne disease requires balance between reducing the nuisance-biting and public health risks while minimising any adverse environmental or ecological impact of mosquito management activities.

Introduction

Mosquitoes are a natural component of coastal estuarine wetlands. Given the threats to coastal wetlands posed by urbanisation and climate change, considerable effort is being placed on the conservation and rehabilitation of these environments. Unfortunately, mosquito-borne disease is a concern for authorities in coastal Australia. The most important mosquito species involved in transmission of mosquito-borne pathogens in coastal regions are mosquitoes associated with estuarine wetlands (Russell 1998) and there is a potential threat that these risks may increase in the future under the influence of a changing climate (Russell 2009). Notwithstanding the public health risks associated with these mosquitoes, nuisance-biting impacts from biting insects associated with estuarine wetlands can be substantial and may have wide ranging impacts on local communities (Ratnayake *et al.* 2006).

Local authorities charged with the task of managing coastal estuarine wetlands must be mindful of the risks associated with mosquitoes. These mosquitoes can often disperse widely from wetlands, resulting in impacts far beyond the boundaries of the wetlands themselves (Webb and Russell 1999; Vally *et al.* 2012). However, despite the potential pest and public health risks, it must be remembered that these estuarine mosquitoes are Australian native animals and are a natural part of estuarine ecosystems. These mosquitoes may even serve an important ecological role in some regions as a potential food sources for insectivorous birds, mammals and arthropods. In particular, recent studies have indicated that mosquitoes, along with moths, provide food for some species of insectivorous bats in coastal environments (Gonsalves *et al.* 2013).

While there is a range of strategies are available to reduce mosquito risk (Mosquito Control Association of Australia 2008; Becker *et al.* 2010), not all strategies will be considered appropriate under all circumstances. The use of insecticides, biological control and habitat modification may all reduce the production of adult mosquitoes from the wetlands, insecticides may also be used to reduce the impacts of these adult mosquito populations beyond the wetlands themselves. While the most commonly used mosquito control agents currently have been shown not to have direct adverse impacts on non-target organisms (Russell and Kay 2008), and broadscale mosquito control has been shown to reduce the risks of mosquito-borne disease (Tomerini *et al.* 2011), a balance between the benefits to human health, amenity of the local community and environmental health is required when mosquito management strategies are implemented.

What health risks may be posed by local mosquitoes?

Ross River virus (RRV) and Barmah Forest virus (BFV) are the two mosquito-borne pathogens that cause the most human illness in Australia. While the symptoms can vary greatly between individuals and 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 pronouced with BFV infection but the arthritic pain is greater and longer lasting with RRV infection (Russell and Kay 2004). The disease resulting from infection with these pathogens are "notifiable diseases" in Australia and, given the potential variability in symptoms, human infection is only recorded in the official statistics following confirmation of infection with a blood test. There are, on average, approximately 5,000 notifications of human disease caused by these two viruses combined per year across Australia (Russell and Kay 2004). The diseases are not fatal but can be seriously debilitating.

There is a common misbelief that mosquito-borne disease risk is only a problem for northern Australia. However, major outbreaks of illness resulting from RRV and BFV infection have occurred in southern states including the NSW south coast, Victoria, Tasmania and southern Western Australia (Russell 1998; Russell and Kay 2004).

The drivers of mosquito-borne disease in coastal Australia can be complex with transmission cycles generally requiring the presence of suitable reservoir hosts (mostly birds and/or mammals) as well as abundant mosquito populations. While some debate surrounds the reservoir hosts of BFV, the locally significant hosts of RRV are generally considered to be macropods (i.e. kangaroos and wallabies) (Russell 2002). As a consequence, regardless of the abundance of mosquitoes, in the absence of suitable reservoir host populations, public health risks associated with mosquitoes will remain low.

The risks of arbovirus transmission in metropolitan areas is generally lower than in rural areas as habitats capable of producing substantially large mosquito populations are low, as is the abundance of suitable reservoir hosts. Given that it is the coastal areas where urban developments are expanding closer to estuarine wetlands, contact between mosquitoes, wildlife, pathogens and the community is increasing the risks of mosquito-borne disease. However, human to mosquito to human (thus occurring without the involvement of an animal) transmission of arboviruses is suspected to have occurred in some circumstances within urban environments (Ritchie *et al*. 1994).

Mosquito biology

Despite the diversity of mosquitoes in Australia, and the range of habitats in which they're found, the basic life cycle is similar for most species. This is the case for mosquitoes associated with estuarine wetlands.

They 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). In the case of estuarine mosquitoes, eggs can remain viable until favourable environmental conditions occur. On hatching, the young larvae (commonly called wrigglers) feed continuously on aquatic particulate matter although the immature stages of some mosquitoes are predatory and will consume other mosquito larvae. The immature mosquitoes grow 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.

On average, a female mosquito 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 extra nutrients provided by the blood meal is required specifically 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 pathogens, the salivary glands of the individual must be infected. When the 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. For example, for the 300 mosquito species in Australia, only one species can transmit dengue viruses (the distribution of this mosquito is limited to Far North Queensland) but over 20 species can transmit RRV (Russell and Kay 2004).

What mosquitoes are associated with estuarine wetlands?

Estuarine wetlands are harsh environments but some mosquito species have adapted to these often highly saline and ephemeral habitats. The saltmarsh mosquito (*Aedes vigilax*), southern saltmarsh mosquito (*Aedes camptorhynchus*), Hexham grey mosquito (*Aedes alternans*) and banded saltmarsh mosquito (*Culex sitiens*) are the most common species found in tidally influenced estuarine wetlands. A much wider range of species can be found in the brackish water habitats that adjoin many estuarine wetlands but, even though some of these species may represent locally important pest species, their importance of often over shadowed by the substantially more abundant estuarine mosquitoes (Table 3.6.1).

Aedes vigilax

Aedes vigilax (Figure 3.6.1) is one of the most important pest mosquitoes in Australia. As well as being a severe nuisance-biting pest, it has also been shown to play an important role in the transmission of mosquito-borne pathogens (Russell 1998). It is

Table 3.6.1. The habitat associations and public health risks associated with key mosquito species associated with estuarine wetlands in Australian.

Mosquito species	Habitat associations	Public health risks
Aedes alternans	Tidally influenced saltmarsh but can also be found in freshwater habitats. Larvae are predatory and feed on other mosquito larvae.	Potential nuisance-biting pest but is not considered an important vector of RRV or BFV.
Aedes camptorhynchus	Can be found in saline habitats but more common in brackish-freshwater habitats such as sedgelands to flooded grasslands adjacent to estuarine wetlands.	Severe nuisance biting pest and vector of RRV and BFV. One of the most important pest species in coastal Victoria, Tasmania, SA and southern WA.
Aedes vigilax	Tidally influenced saltmarsh but also other saline and brackish water habitats such as flooded sedgelands and coastal swamp forests. Travels many kilometres from larval habitats.	Severe nuisance biting pest and vector of RRV and BFV. One of the most important pest species in coastal regions of NSW, QLD, NT and WA.
Culex sitiens	Permanently inundated saline to brackish habitats, including saltmarsh and mangroves.	Bird feeding mosquito and not considered a nuisance-biting pest. Not considered an important vector of disease.
Verrallina funerea	Saline and brackish habitats including coastal swamp forests and the margins of saltmarsh. Does not travel far from larval habitats.	Severe nuisance-biting pest and a vector of RRV and BFV.

found in the majority of coastal regions of Australia, with the exception of Tasmania, and molecular studies have shown that there may be genetic differences between populations between different regions (Puslednik *et al.* 2012).

The mosquito is closely associated with tidally influenced habitats within estuarine wetlands (Webb and Russell 1997) (Figure 3.6.2). The mosquito is generally less common in upper saltmarsh communities dominated by sedges and rushes and, although the mosquito will lay eggs within mangroves (specifically at the base of pneumatophores), it is more commonly found in association with Sarcocornia quinqueflora and Sporobolus virginicus (Kay and Jorgensen 1986; Gislason and Russell 1997) (Figure 3.6.3). It is, however, important to note that that modifications to either saltmarsh or mangrove communities through restricted tidal flows and drainage can greatly increase the productivity of these habitats (Figure 3.6.4). In some circumstances, the production of abundant *Aedes vigilax* populations can be a symptom of degraded wetlands. This is particularly the case for mangrove communities where restricted tidal flows resulting from habitat modifications (e.g. sea walls, levee banks, pathways, rail lines etc) reduce the frequency of flushing events and distribution of predators (e.g. fish) and consequently increase the suitability of habitats for mosquitoes (Webb and Russell 1999). Well flushed mangrove habitats typically do not provide suitable conditions for *Aedes vigilax* (Figure 3.6.5).

The key areas within the wetlands that produce the most *Aedes vigilax* are determined by a range of complex issues. It is not only the presence of preferred plant species but also the local tidal inundation regimes and availability of suitable depressions and/or pools within the habitats. The mosquito has the ability to develop its first batch of eggs without the need for blood (a phenomenon known as autogeny) (Hugo *et al.* 2003), ensuring that the next generation of mosquitoes is ensured within suitable habitats. The eggs of *Aedes vigilax* can remain viable in the environment for long periods of time, possibly years. Hatching is triggered by inundation of the wetlands by tides or major rainfall events. During the warmer months, the immature mosquitoes can complete their

Figure 3.6.1. The saltmarsh mosquito, *Aedes vigilax*. (Photo: Stephen Doggett, Medical Entomology, Pathology West – ICPMR Westmead.)

Figure 3.6.2. An example of saltmarsh habitat on the north coast of NSW that provides suitable conditions for *Aedes vigilax*. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

Figure 3.6.3. An example of saltmarsh habitat in the Hunter region of NSW that provides suitable conditions for Aedes vigilax. The most suitable "hot spots" for this mosquito in estuarine wetlands are these pools surrounded by *Sarcocornia quinqueflora* and *Sporobolus virginicus* that are only filled by the highest spring tides. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

development in less than a week. Consequently, population increased in *Aedes vigilax* can be predicted based on the environmental drivers of temperature, rainfall and tides from regions across Australia (Webb and Russell 1999; Jacups *et al.* 2008; Yang *et al.* 2008; Jacups *et al.* 2009; Hu *et al.* 2010).

Many studies have investigated the spatial and temporal egg-laying behaviour by *Aedes vigilax* in estuarine wetlands. The highest densities of egg shells are often concentrated in the zone beneath vegetation rather than more open areas and, on a larger scale, it is likely there would be a concentration of eggs in an area of "preferred inundation" (Dale *et al.* 1986; Ritchie 1994). This zone of "preferred inundation" can be variable but generally, it is found in the zone only inundated by the highest tides of the month (i.e. spring tides). More regular inundation of the wetland creates relatively unsuitable conditions for *Aedes vigilax* as eggs may not have sufficient period of drying and suitable "maturation" to occur and nor suffer damage by moving water, debris or predators.

While it may seem counter intuitive, the most suitable environmental conditions for *Aedes vigilax* occur during summers with below average rainfall. These conditions, typically occurring during El Nino weather patterns (i.e. hot and dry summer along the east coast of Australia) when the wetlands dry completely between tidal inundation events. This drying process ensures that habitats remain predator free, allow access of mosquitoes to idea egg-laying sites and provide an opportunity for deposited eggs to "mature". Conversely, during prevailing La Nina weather patterns (i.e. relatively cool summers with above average rainfall) the wetlands will generally remain inundated for longer periods of time, allowing the persistence of predator populations (i.e. fish) while prohibiting access to preferred egg-laying sites by the mosquitoes.

Nothwithstanding the potential for exceptionally large populations of *Aedes vigilax* to occur during periods of favourable environmental conditions, the mosquito can disperse many kilometres from the wetlands. There have been numerous qualitative reports of *Aedes vigilax* adults being collected long distances (e.g. over 20km) from the nearest saline breeding grounds (Lee *et al.* 1984) and the results of genetic analysis of *Aedes vigilax* populations

Figure 3.6.4. An example of mangrove habitat at Sydney Olympic Park that provides suitable conditions for estuarine mosquitoes. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

Figure 3.6.5. An example of mangrove habitats at Sydney Olympic Park. Mangrove habitats that are frequently flushed by tides rarely provide suitable conditions for estuarine mosquitoes. It is only when tidal flows and the intrusion of predatory fish into the wetlands are restricted that conditions become more suitable for pest mosquitoes. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

Figure 3.6.6. The southern saltmarsh mosquito, *Aedes camptorhynchus*. (Photo: Stephen Doggett, Medical Entomology, Pathology West – ICPMR Westmead.)

suggest that the mosquito is dispersing widely from local wetlands and resulting in little diversity amongst local populations in SE QLD (Chapman *et al*. 1999). Mark-release-recapture experiments have found that marked mosquitoes were recaptured between 1km and 5km from release points. The active dispersal of mosquitoes from estuarine wetlands can have a dramatic impact on nuisance-biting and public health risks in the nearby residential areas (Webb and Russell 1999; Vally *et al*. 2012).

Aedes camptorhynchus

In southern states, *Aedes camptorhynchus* (Figure 3.6.6) generally replaces *Aedes vigilax* as the major pest species, it is a serious biting pest and vector of arboviruses including RRV and BFV (Russell and Kay 2004; Barton and Kay 2009; Carver *et al*. 2011). Although often found in the same habitats as *Aedes vigilax*, *Aedes camptorhynchus* is typically more commonly associated with less saline conditions such as flooded brackish water and freshwater marsh and pastures located immediately behind saltmarsh and mangrove wetlands (Webb and Russell 2001; Kokkinn *et al*. 2009). The habitats that appeared to be preferred by this species are those more strongly influenced by rainfall beyond the regular direct influence of tidal inundation, although some tidal flooding may occasionally occur (Barton *et al*. 2004; Kokkinn *et al*. 2009). The species is not restricted to coastal areas and has been recorded from freshwater habitats extended distances from brackish habitats (Dobrotworsky 1965).

Like many *Aedes* spp., the eggs of *Aedes camptorhynchus* are desiccation resistant (Lee *et al*. 1984; Bader and Williams 2011) and, as is the case for *Aedes vigilax*, eggs are typically laid at the base of vegetation. Laboratory studies have demonstrated that these desiccation resistant eggs will remain viable for up to 15 months under ideal conditions (Bader and Williams 2011). However, it is interesting that the eggs will not always hatch when inundated, hatching appears to be trigger by a range of factors that may reflect the evolution of adaptive strategies to harsh estuarine environments where the occurrence of suitable environment conditions (e.g. rainfall or tidal inundation of wetlands) may be irregular. As a result, highly variable seasonal abundance of *Aedes camptorhynchus* is often observed (Barton *et al.* 2004).

The seasonality of this mosquito differs from *Aedes vigilax* in that it tends to be more abundant in spring and autumn as opposed to summer (Williams *et al.* 2009). In regions where these two estuarine species are found, the peaks in population abundance rarely overlap (Russell 2002). These differences in seasonal abundance can be pronounced in areas such as southern NSW where the species is far more abundant in the cooler spring months as opposed to the warmer summer months (Russell *et al.* 1992; Webb and Russell 2001).

Aedes alternans

Aedes alternans is a large sandy coloured mosquito species closely associated with estuarine wetlands. Immature stages are often collected from estuarine wetlands but they can also be found in fresh-water habitats (Lee *et al.* 1984; Webb and Russell 2001). Although common in coastal regions, and a known nuisance-biting pest, *Aedes alternans* is generally considered a less important pest species compared with other estuarine mosquitoes such as *Aedes vigilax* and *Aedes camptorhynchus* as the population abundance is relative low. The population abundance of this species remains low as the immature stages are predatory and mostly rely on an abundance of immature stages of *Aedes vigilax* to generate large populations (Lee *et al.* 1984). Like all predator/prey relationships, the abundance of prey larvae is a substantial driver of predator populations but those predator populations will always remain substantially lower than the prey.

Although mosquito-borne viruses such as RRV have been isolated from this mosquito (Ritchie *et al.* 1997), laboratory studies suggest that *Aedes alternans* is not an effective vector of RRV (Wells *et al.* 1994) and is, consequently, not considered a major public health concern.

Culex sitiens

The highly ephemeral nature of estuarine wetlands generally favours *Aedes* species. However, *Culex sitiens* is one mosquito that is commonly found in permanently inundated saline to brackish habitats (Lee *et al.* 1984; Webb and Russell 1997) with studies indicating that survival of immature stages is generally lower in highly saline habitats (Mottram *et al.* 1994). This mosquito lays its eggs as floating rafts on the water surface and, unlike species such as *Aedes camptorhynchus* and *Aedes vigilax* that can be abundant early in the season due to a reserve of desiccation resistant eggs in the wetlands, *Culex sitiens* must steadily build up populations over the summer. As a result, this mosquito is generally more common during the late summer and autumn, and under favourable environmental conditions, can generate abundant populations (Webb and Russell 1999). However, as this mosquito preferentially feeds on birds, it is rarely considered a serious pest. While it isn't thought to play a role in the transmission of RRV to humans (Fanning *et al.* 1992) but it is important to note that arboviruses have been isolated from this mosquito (Ritchie *et al.* 1997) and it may play a role in spreading viruses between local bird populations.

Verrallina funerea

A mosquito species found at the margins of estuarine wetlands is *Verrallina funerea*. This species is considered a relatively important nuisance-biting mosquito and is common in northern NSW and Queensland where, in some locations, it can be one of the most commonly collected species in mosquito surveillance programs (Ryan *et al.* 1999; Ryan and Kay 2000; Jeffery *et al.* 2005). Studies have also shown that this mosquito may play a role in local transmission of RRV and BFV (Jeffrey *et al.* 2006). The species lays desiccation resistant eggs in brackish to freshwater ground pools within flooded coastal swamp forests and she-oak woodlands (Figure 3.6.7) with a stronger preference for those areas occasionally flooded by tides (Ryan and Kay 2000). Although *Verrallina funerea* is generally not considered to disperse widely from larval habitats, pest impacts are limited when compared with more widely dispersing saltmarsh mosquitoes such as *Aedes vigilax* and *Aedes camptorhynchus*. However, coastal developments have brought people closer to their habitats, increasing the relative impact of these mosquitoes in recent years and there is qualitative evidence that the mosquito can use corridors of vegetation to move into residential areas adjacent to their preferred habitats.

Other estuarine and brackish water mosquitoes

There are a number of mosquito species that may be occasionally found in estuarine wetlands. Under some circumstances, these species may cause localised pest impacts but they are generally not considered serious concerns for public health. A range of *Aedes*, *Anopheles*, *Coquillettidia*, *Mansonia* and *Verrallina* species associated with temporary and semi-permanent freshwater and, occasionally, brackish-water ground pools in coastal swamp forests and coastal floodplain wetlands adjoining

Figure 3.6.7. An example of coastal swamp forest habitat on the north coast of NSW that provides suitable conditions for estuarine and brackish water mosquitoes. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

estuarine wetlands. These habitats are usually inundated by rainfall and would rarely, if ever, be flooded by tides. During periods of above average rainfall, when the saltmarsh may be inundated by considerable rainfall runoff, some of the mosquitoes associated with freshwater habitats may be collected. The pest impacts posed by these species will vary regionally as well as with seasonal rainfall but will, generally, always be overshadowed by the pest impacts of saltmarsh mosquitoes.

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

A well designed monitoring program is essential to assessing the mosquito risks associated with estuarine wetlands. While mapping vegetation and the extent of tidal inundation may provide a guide to the potential suitability of the local wetland to mosquitoes such as *Aedes vigilax*, there is no substitute to sampling the local immature and adult mosquito populations (Webb and Russell 2012). It is important to note that maintaining a record of complaints and/or feedback to local authorities on the level of nuisance-biting activity will not provide a suitable measure of mosquito populations. Information of this nature is considered an unreliable basis for the design of mosquito risk assessment and mosquito management strategies.

Immature populations

While sampling adult mosquito populations will provide a measure of loal mosquito abundance and diversity, it is not possible to identify key mosquito habitats without sampling immature mosquitoes. There are strong habitat associations between estuarine mosquitoes and habitats. However, those habitats cannot be assessed based on the presence of vegetation communities alone and, as well as consideration being given to local tidal conditions, the abundance of mosquitoes should be quantified to guide mosquito risk management (Webb and Russell 2012).

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). The actual size and design of this

Figure 3.6.8. The foundation for effective mosquito management is a good surveillance program. The most commonly used mosquito traps in Australia use carbon dioxide to attract mosquitoes. The abundance and diversity of mosquitoes collected provides an assessment of mosquito risks and the effectiveness of mosquito control programs. Specimens can also be tested to determine if they are infected with any mosquito-borne pathogens. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

sampling device is not critical. However, for the purpose of on-going monitoring of local mosquito populations, there should be consistency in both the size and shape of the sampling device as well as the way it is used to sample mosquitoes. Studies have shown that individual field operators may bring subtle differences and biases to their sampling methodologies. As a consequence, it is important that appropriate training is provided to field staff to ensure consistence in operational procedures.

A network of sampling sites that includes a number of replicate samples across a range of habitats types will form an important baseline measure with which to base mosquito management decisions (Webb and Russell 1999; Webb and Russell 2001).

The 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 3.6.8). 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. Traps are typically hung in vegetation and operated overnight. Traps set in exposed and wind swept areas typically collect smaller numbers of mosquitoes. 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 estuarine mosquitoes in nearby residential or recreational areas compared to freshwater, brackish water or "backyard" mosquitoes (Webb and Russell 1999; Vally *et al.* 2012).

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. There are also differences in the seasonal abundance of the three most common estuarine mosquitoes, *Aedes camptorhynchus*, *Aedes vigilax* and *Culex sitiens*. This is particularly the case *Aedes vigilax* with population abundance closely associated with tidal inundation of the wetlands by tides or rainfall (Webb and Russell 1999). Where weekly sampling of mosquito populations is not possible, monitoring programs that require a comparison of mosquito abundance between wetlands must ensure that populations of *Aedes vigilax* are sampled at similar periods in conjunction with tidal and/or rainfall events. As mosquito larvae can hatch from eggs and complete development to adults within approximately 7 days during the warmer months, sampling should be undertaken approximately 10 days follow wetland inundation to gain a measure of peak mosquito abundance.

There can be substantial differences in the abundance of mosquitoes relatively to short-term changes in tidal cycles (Gonsalves *et al.* 2013).

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 estuarine 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).

Mosquito population management

Reducing the nuisance-biting and public health risks associated with mosquito populations produced from estuarine wetlands should follow the principles of Integrated Pest Management (IPM). The basis for any integrated pest management program is a multidisciplinary strategy built on a site-specific mosquito-monitoring program (Webb and Russell 2001; de Little *et al.* 2012). There doesn't need to be a reliance on insecticide treatments of wetlands but a consideration of all available strategies (i.e. habitat modification, insecticides, biological control, community education) within a regional context that may offer the best approach (Webb and Russell 2005; Webb and Russell 2007; Webb and Russell 2012).

It would not be possible, or desirable, with methods currently available to eradicate mosquitoes entirely from coastal Australia. No control strategy is 100% effective and there would be financial and operational limitations in many regions to target all habitats. However, there are options available for the use of control agents and habitat modification that have been shown to be effective and environmentally sustainable. All strategies will have advantages and disadvantages (Table 3.6.2) but through the prioritising of key mosquito "hot spots", it is possible to design a mosquito management program and reduces nuisance biting impacts (Webb and Russell 1999; Webb and Russell 2001) and minimised public health risks (Tomerini *et al.* 2011).

It is important to note that any mosquito control activities should be undertaken with products approved for use against mosquitoes and registered with the Australian Pesticides and Veterinary

Table 3.6.2. Advantage and disadvantages of mosquito management strategies for estuarine wetlands.

Strategy	Advantages	Disadvantages
Environmental modifcation	• Potential long term solution without reliance on routine application of control agents • May have secondary benefits for rehabilitation of degraded wetlands (e.g. restore tidal flushing) • Potentially cost effective	• May be legislative restrictions for application to some estuarine wetlands • Potentially expensive due to site-specific matters • May not significantly reduce overall mosquito populations • May impact some elements of wetland ecosystem (e.g. increased soil moisture, reduced crab populations, increase encroachment of mangroves) • May require regular maintenance to maintain effectiveness
Bti	• Proven effective control agent • Minimal non-target impacts • Easy to assess treatment success and reapplication possible if treatment fails	• Reapplication required to control each generation of mosquitoes • Small window of application for effective treatment • Larvae quickly removed from ecosystem • No residual control • For environmentally sustainable programs, aerial applications are the ideal strategy and may be expensive
Methoprene	• Proven effective control agent • Minimal non-target impacts • Sustained release formulations provide residual efficacy (i.e. reapplication frequency reduced) • Larvae are retained in ecosystem for longer periods	• Potentially expensive • Time consuming to apply sustained release formulations • Treatment assessment requires collection of pupae • No opportunity for reapplication if treatment fails
Biological control	• Introduction of native fish complementary to other wetlands management objectives • Very acceptable to community	• Not appropriate for highly saline and/or ephemeral habitats • Only fish species endemic to the local area can be released • Community may feel mislead if introductions do not reduce mosquito impacts
Adulticides	• Ground based applications may be effective in small areas (e.g. isolated communities close to wetlands) • Harbourage/barrier treatments potentially effective for home owners close to wetlands • Useful strategy in emergence response to disease epidemics	• Difficult to achieve effective long term control • Treatments may need to be repeated at daily intervals • Potentially significant non-target impacts • Expensive and requires operational capacity of local authorities • May not be acceptable to community

Medicines Authority (APVMA). The application of mosquito control agents and/or the modification of estuarine wetlands will require approvals from local authorities (Webb *et al.* 2009) and the appropriate legislation should be considered when developing a local mosquito management program.

Controlling adult mosquito populations

Adult mosquito control is rarely undertaken in Australia. For the most part, it is limited to periods of epidemic virus activity or when exceptional levels of nuisance-biting impacts are experienced (e.g. post-flooding). The most commonly used products are permethrin or synthetic pyrethroid based, products. Known as adulticides, these products are typically applied as either a "fog" or "mist" delivering very small droplet sizes. Adulticides can be expensive, their effectiveness is dependent on favourable weather, multiple treatments are often required and potential non-targets are a concern as these products are not specific to mosquitoes. Where estuarine mosquitoes are a problem, adulticides may be a very ineffective way to control mosquito populations as they disperse widely from the wetlands. As a routine management option, adulticides are not recommended.

In recent years, another insecticide application strategy is increasingly used to reduce the nuisance-biting impacts of biting midges and mosquitoes associated with estuarine wetlands is with "barrier treatments" or "harbourage treatments". This strategy most commonly involves the application of the synthetic pyrethroid, bifenthrin. The product provides a residual layer of pesticide that kills resting mosquitoes and is currently registered for treating mosquito resting places (i.e. internal and external areas of domestic, commercial, public and industrial buildings). While some limited studies have shown that biting insect populations immediately around treated areas can be reduced (Hurst *et al.* 2012), there are, however, some environmental concerns surrounding the widespread use of this product, in particular for non-target insects and aquatic organisms. There are warnings on the label that the product is toxic to bees, fish and aquatic organisms and that mud, sand, mangroves and other aquatic habitats should not be directly treated or exposed to spray drift.

Controlling immature mosquito populations

Given that the major mosquito pests associated with estuarine wetlands can widely disperse from local habitats, the most effective way to reduce mosquito risk is to target immature stages of mosquitoes. Historically, control agents used in estuarine wetlands have had the potential to cause non-target impacts and/or contribute to the development of insecticide resistance in local populations.

Prior to World War II, petroleum oils were commonly used (Bertram 1927) although the extent to which it was used in estuarine wetlands is not clear. These oils were then replaced by organochlorides (e.g. DDT) until the late 1960s when organophosphates (e.g. temephos) become the most commonly used larvicide. Concerns regarding the development of resistance and the potential for non-target impacts saw the reduced the use of temephos during the early 1990s in favour of more environmentally sensitive control agents (Russell and Kay 2008).

The most commonly used control agent in estuarine wetlands is *Bacillus thuringiensis israelensis* (*Bti*). This product is available in a small number of commercial formulations and is registered for use against mosquitoes by the APVMA. The bacteria produce a protein crystal that contains a number of microscopic pro-toxins which, when ingested, are capable of destroying the gut wall and killing mosquito larvae.

The greatest benefit of *Bti* is that it is highly specific to mosquito larvae, and very few non-target effects have been recorded when the product is applied at the recommended rates. The product does, however, have some disadvantages in that there is little residual activity, and efficacy is reduced in habitats with a high organic content or when applied when larval populations are young or nearing pupation. This has operational implications for the use of this product in estuarine wetlands. For the most effective results, wetlands must be monitored for inundation resulting from tides or rainfall and treatment must be undertaken when immature populations are most susceptible (Webb and Russell 2001; de Little *et al.* 2011). As the temporal abundance of estuarine mosquitoes can be highly variable in response to environmental factors, it is difficult to predict the need for treatment based on tidal and rainfall data alone. A site-specific monitoring program is essential.

The insect growth regulator, s-methoprene, is a synthetic mimic of the juvenile hormone produced by insect endocrine systems and is becoming increasingly common for the control of estuarine mosquito populations (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. A range of commercial formulations (including liquid, slow-release solid pellets and briquets) have been approved for use against estuarine mosquitoes by APVMA. Although generally more expensive than *Bti*, there are some advantages to the use of this product due to the availability of the sustained release formulations that may provide longer periods of residual control in some habitats. For areas that may be difficult to access or where the production of mosquitoes may be difficult to predict, treatment with s-methoprene will be useful. One of the drawbacks to the use of s-methoprene is that, as it does not kill the immature stages, assessing the effectiveness of treatments can be difficult and generally requires the collection of pupae to be returned to the laboratory to record emergence rates.

Habitat modification

Historically, dramatic modifications were made to coastal wetlands to reduce local mosquito populations. Draining and filling saltmarshes, as well as the construction of levee banks and sea walls to reclaim land for agricultural and industrial purposes, may have reduced mosquito populations but these approaches had significant adverse impacts on the local environment. Such dramatic approaches are not undertaken today but it is interesting to note that as local authorities attempt to rehabilitate some of these degraded wetlands, the potential to increase local mosquito-borne disease risk is a concern.

Advances in habitat modification techniques have shown that the suppression of mosquito populations is possible without reliance on the use of insecticides or jeopardising the flora, fauna or ecological function of the wetland itself. In some cases, unusually large mosquito populations may be identified as a symptom of degraded wetlands and improving the health of the wetland may reduce mosquito productivity (Webb and Russell 1997; Webb and Russell 1999; Turner and Streever 1999).

A range of modification strategies are available but the key is to increase the frequency of tidal flushing, improve the drainage of water, and maximise access of fish to productive mosquito habitats. These factors combine to create less favourable conditions for estuarine mosquitoes. The most common form of habitat modification currently practiced on saltmarshes is the construction of shallow, spoon-shaped channels that connect pools and depressions on the saltmarsh, and allow improved exchange of tidal water over the marsh without severely impacting the hydrology of the marsh. This strategy, known as runnelling, has been shown to reduce mosquito productivity in saltmarsh habitats dominated by *Sarcocornia quinqueflora* and *Sporobolus virginicus* (Dale 1993; Dale and Knight 2012).

While studies have shown that runnels reduce mosquito breeding in estuarine wetlands (Dale and Knight 2012) without major adverse impacts to the wetlands (Dale 2008), the promotion of increased tidal inundation of the saltmarsh and the potential for increased mangrove propagule dispersal and, consequently, mangrove colonisation at higher elevations on the marsh (Breitfuss *et al.* 2003). The intrusion of mangroves into the saltmarsh may represent a potentially significant adverse impact to the wetlands (Saintilan and Rogers 2013). Given the scrutiny currently given to management of coastal wetlands, it is unlikely that large scale runnelling projects will be undertaken in the future. However, in those areas where runnels current exist, it is important to ensure that they remain functional as, if they become block with sediment and mangrove seedlings, the runnel may be converted into a series of isolated pools and enhance habitat conditions for mosquitoes.

Biological control

An effective biological control strategy through the specific release of mosquito predators into estuarine wetlands is unlikely to be effective. A number of native fish (e.g. *Pseudomugil signifer* (Pacific Blue-eye), *Hypseleotris compressa* (Empire Gudgeon) and some *Melanotaenia* species (Rainbowfish)) have been identified that may be appropriate for mosquito control in Australia. However, given the highly ephemeral nature of habitats in which key pest species are found, the specific introduction of fish is not a suitable management strategy, particularly in the upper saltmarsh (Morton *et al.* 1988). However, there are still substantial gaps in our knowledge regarding the role of fish in the control of immature mosquito populations in mangrove habitats (Griffin and Knight 2012). While native fish introductions alone will not significantly reduce mosquito populations, they do provide an important component of integrated mosquito management and may provide a valuable link to the wider community promoting environmentally sensitive mosquito management.

Case study: Mosquito management at Sydney Olympic Park

Estuarine wetlands close to urban centres pose the greatest mosquito risk. As well as the mosquitoes typically associated with these habitats, given the likely modifications that have occurred to the wetlands, they may be producing unusually large populations. One of the largest areas of estuarine wetland in Sydney is located at Sydney Olympic Park, NSW. The wetlands are located within the Parramatta River estuary approximately 20km from the CBD of Sydney and are comprised of extensive areas of saltmarsh and mangrove habitats. Many of these areas had become badly degraded as a result of restricted tidal flushing and ineffective drainage of rainfall resulting from many decades of modifications (e.g. sea wall constructions and other raised infrastructure).

Symptomatic of the degraded wetlands are unusually large mosquito populations. The issue of nuisance-biting mosquitoes was first documented in the 1920s with *Aedes vigilax* identified as the most significant pest species. While the public health risks associated with these mosquito populations are low, given the absence of suitable reservoir hosts (i.e. macropods), RRV has been isolated from mosquitoes on occasion. More significant, however, are the nuisance-biting impacts extended into nearby residential areas. Mosquito populations were studied in the late 1980s and identified the estuarine wetlands as the source of unusually large *Aedes vigilax* populations. During the mid-1990s, the impact of nuisance-biting mosquitoes in the suburbs of Parramatta, Ryde and Concord was so great that it became a political issue and public meetings to discuss the issue were commonly held during the summer.

Developing a mosquito management program

Prior to 1998, there was a relatively ad hoc approach to mosquito control with ground based application of the organophosphate insecticide temephos to some wetlands. This was often on a weekly or bi-weekly basis during summer but also in response to community complaints. There was no integrated mosquito monitoring program in place to inform or assess treatment effectiveness.

In conjunction with the redevelopment of the Sydney Olympic Park site and surrounding suburbs, there was a need identified to develop an integrated, ecologically sustainable strategy to reduce mosquito risk. Two key approaches were taken that included the rehabilitation of the most degraded areas of estuarine wetlands and a seasonal program of mosquito control agent application. These two aspects of the mosquito management strategy were supported by a seasonal mosquito monitoring program of adult and immature mosquito populations.

Mosquito monitoring

Investigations into the local mosquito fauna identified over 30 species of mosquito associated with a range of estuarine, brackish water and freshwater habitats. As well as being associated with the local wetlands, there were mosquitoes associated with man-made structures also. Research findings indicated that population increases of *Aedes vigilax* were triggered by tides tides over 1.8m but that the magnitude of those increases is dependent on the number and actual height of tides over 1.8m. While predictions of tidal heights provide a good guide to the timing of potential increases, variation between actual and predicted tide heights can result in unexpected, or more extensive than expected, inundation of the wetlands. In addition, rainfall events in which 50 mm or more rainfall recorded within three days is typically sufficient to trigger a hatch of mosquitoes. Populations are, consequently, likely to be significantly greater if there is a major rainfall event or if rainfall and tidal flooding occur at the same time.

Between the months of November and May each year, adult mosquito populations are sampled weekly at approximately 15 fixed trap sites across Sydney Olympic Park. The abundance and diversity of mosquitoes is recorded and compared to long-term averages so that an assessment of relatively nuisance-biting impacts can be assessed. The information gathered also provides information on pest mosquitoes associated with non-estuarine habitats that may be responsible for any unusual nuisance-biting impacts.

Figure 1. An example of coastal swamp forest habitat on the north coast of NSW that provides suitable conditions for estuarine and brackish water mosquitoes. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

Larvicide treatments

The current treatment program was established in 1998 and combines aerial (helicopter) and ground based application of the biorational control agent, *Bti* (Figure 1). The larvicide is generally applied via helicopter but some areas may require application from the ground. The mosquito control program is based on established operating procedures with recommendations for control agent applications based on the extent of wetland inundation and abundance of newly hatched larvae at fixed monitoring sites across the estuarine wetlands. Comparing the pre- and post-treatment larval densities in each habitat as well as changes in the relative abundance of adult mosquitoes assesses the effectiveness of the treatment program. On average, a reduction of between 80-95% of immature mosquitoes following treatment is recorded. Since the program commenced in 1998, there have been no reports of any non-target impacts.

Habitat rehabilitation

One of the key mosquito habitats in the local area was a stand of degraded mangroves. The lack of effective flushing led to stunted growth of mangroves, which in turn led to incomplete canopy cover and establishment of saltmarsh vegetation (the primary oviposition site for saltmarsh mosquitoes) around those pools. Few predatory fish could gain access to these habitats. Sampling of these habitats routinely found high densities of *Aedes vigilax* and *Culex sitiens* during the summer months.

A tidal restoration project was designed, while being informed by data on mosquito populations, involving the construction of primary and secondary channels throughout the mangroves to improve tidal flushing (i.e. increase the frequency of tidal flushing events and increase the volume of water entering and exiting the mangroves in each tidal flushing event) (Figure 2). It was hoped that the increased

Figure 2. The rehabilitation of degraded mangroves at Sydney Olympic Park through the reintroduction of tidal flushing significantly reduced mosquito production to the extent that the application of insecticides was no longer required. (Photo: Cameron Webb, Medical Entomology, Pathology West – ICPMR Westmead.)

flushing would improve the health of the wetlands as well as reduce mosquito productivity.

Virtually all mosquito production within the modified wetlands was removed. The reduction in mosquitoes was so significant that the application of mosquito control agents was no longer required. Many of the most productive mosquito 'hot spots' were physically removed as part of the channel construction process. The key factors contributing to the decline in mosquito production, as expected, were increased tidal flushing and more widespread fish movement. As mangrove health improved and the canopy closed in, less suitable conditions were present for mosquito oviposition sites and there was a substantial reduction in the number of pools that held water for sufficient time to allow mosquito larvae to complete development. Permeation of tidal and rainfall into the substrate appears to be more effective post-modification.

Education & Awareness

A key component of the mosquito management program at Sydney Olympic Park has been proactive education and awareness of the local community and stakeholders. Mosquitoes will always be active during the warmer months and, as the control program is not designed to eradicate mosquitoes, appropriate measures should be taken to avoid bites. It is particularly important that appropriate information on personal protection measures (e.g. topical insects repellents) be provided to residents and visitors spending time in the wetland areas (e.g. mangrove boardwalks, bird watching hides). In addition, residents, visitors and stakeholders must be adequately informed of the timing of mosquito control activities as to not alarm or inconvenience those participating in activities close to the wetlands. Strategies to ensure the effective delivery of this information have been a crucial part of the overall mosquito management program.

Summary

Although mosquitoes are a natural part of the estuarine wetland ecosystem, they have the potential to be significant nuisance-biting pests and vectors of disease-causing pathogens. Managing these pest and public health risks can be a major concern for local authorities and may add substantial financial costs to wetland management. While mosquito control may not always be required or considered appropriate, an appreciation of mosquito issues should be carefully considered by wetland managers, and minimisation strategies for mosquito populations. As well as having a duty of care that local wetlands are not impacting the local community, it may prove a substantial impediment to gather support for wetland conservation and rehabilitation projects is it is perceived that they will increase mosquito populations.

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