The Biology of Mosquitoes

1 Life cycle
The mosquito life cycle begins with an adult female laying eggs. Aquatic immature stages called larvae emerge and develop through four moults (instars), increasing in size until the final moults when it reaches the non-feeding pupal stage (See Figure b & c). Inside the pupa the adult mosquito develops (either a male or female) and the terrestrial/aerial adult stage emerges from a split in the back of the pupal skin (See Figure a & d). The adult mosquitoes feed, mate, and the female develops eggs to complete the cycle and begin the next generation.

Some species of mosquito have only one generation per year. Others have several generations during a single season of favourable climatic conditions. Some continue to breed throughout the year, but may be more abundant in warmer seasons - this depends on the local environment, particularly temperature and rainfall.


1.1 The Egg
Mosquito females oviposit their eggs on or near water bodies. They are almost transparent when first laid, but gradually darken to brown or black as they mature. Eggs of Culicine mosquitoes (e.g. *Culex* and *Aedes*) are usually elongate-oval in shape with the anterior end rounded and the posterior bluntly pointed. Anopheline eggs (e.g. *Anopheles*) are more cigar-shaped with flotation structures on each side.

The eggs are laid singly or in clusters and this can vary depending on the genus. *Aedes* species lay their eggs as single units and deposit them on moist substrate
such as rock surfaces, moist earth and the inside wall of tree holes or containers above the receding water level. They also lay eggs under debris and in crevices in soil and dry mud, where they will be subsequently flooded. These eggs are able to withstand desiccation, and can survive long periods until they are submerged by water, at which time they begin to hatch.

Egg rafts of Culex and Coquillettidia spp. float on top of the water surface, while Mansonia spp. egg rafts can be found attached to the underside of leaves or twigs, just below the water surface. Mansonia species eggs differ from other species in that they have one end extended into a spike. Egg rafts cannot withstand desiccation and are usually associated with permanent or semi-permanent water bodies. They will hatch after about two days on the water and without constant water, they desiccate and die.
1.2 The Larva
The larval stage must have an aquatic habitat in which to complete its development to the pupal stage. The adult female selects an appropriate larval habitat when she deposits her eggs. They are able to discern physical and chemical properties of different collections of water and choose between sites available. Factors including shade, temperature, salinity, water quality and the texture of the substrate (Aedes species), may influence the female in her search for an appropriate oviposition site. Various combinations of these factors can be identified as being characteristic of a typical breeding site of a particular species. Therefore there are a range of diverse habitats where mosquito larvae can be found, which is dependent on species type as well as environmental factors.

*Culex quinquefasciatus* larvae hatching from eggs

The larvae hatch from the eggs and grow through four instars before developing into a pupa. Between each stage they moult their rigid outer skin so they can increase in size. The discarded skin is termed an exuvium/exuvia (singular) or exuviae (plural). The larval instar level is determined by the size of the head capsule, not the body length.

Most larvae feed on microscopic organisms in the water and bottom detritus, either by filtering water through their mouth brushes or by grazing with specially adapted mouth appendages. Some larvae are predatory (*Aedes (Ochlerotatus) alternans* and *Toxorhynchites* sp.) and their mouth brushes are modified so they are strong enough to grasp prey.
Some species feed habitually at the surface (Anopheles), some in the middle range below the surface (Culex) and others typically feed on the bottom of the habitat (Aedes).

Larvae breathe air from openings (spiracles) at the tail end of the body, generally through a structure termed a siphon (See Figure). They hang below the water surface with only the tip of the siphon exposed to the air. They can remain motionless on the bottom for some time, but need to return to the surface for air to prevent suffocation.

One of the two main exceptions to breathing behaviour occurs within larvae of the genera Mansonia and Coquillettidia. They attach to plants below the surface of the water after hatching, using a specially adapted piercing siphon and obtain their oxygen directly from the plant tissues. Larvae of these two genera do not visit the water surface during their development and usually feed by filtering food particles from the surrounding water with their mouth brushes.
The second main exception in breathing behaviour occurs within the genus *Anopheles*, whose larvae do not have a siphon or breathing tube. Species of this genus lie alongside the surface of the water to breathe.

The time taken for development through the larval stages is dependent on a number of environmental factors, the most important of which is temperature. Availability of food and the extent of larval crowding within the habitat are also important.

During favourable summer conditions, *Anopheles* species may complete larval development in 7-10 days, *Aedes* species may complete larval development in as little as 4-5 days and *Culex* species may require at least 7-10 days. Low temperatures usually delay development and may cause cessation of growth and induce a over-wintering of larvae in some species.

Identification of larvae is most easily accomplished with mature larvae, i.e. the fourth instar and microscopic examination is usually required. However, there are some genus characteristics that enable partial identification in the field. For example, *Anopheles* species’ lack of a siphon and larvae lying flat at the surface of the habitat when breathing or resting, distinguishes them from *Culex* and *Aedes* species which have siphons and hang suspended from the surface. *Culex* species typically have longer siphons than *Aedes* species, which also can help assist in recognising the different genera in the field, however this can really only be achieved with experience and should always be checked under the microscope.

The larvae of *Mansonina* and *Coquillettidia*, although not commonly collected because of their attachment to submerged aquatic vegetation, can be identified as being from one of these two genera either by their attachment to a plant or if separated from the vegetation, by their modified siphon.
1.3 The Pupa

After the 4th larval instar completes its development, it moults into a non-feeding but highly mobile stage called the pupa. Within the body casing of the pupa, the immature tissues are breaking down and adult tissues are forming.

The pupa breathes through a pair of tube-like organs (trumpet) situated at the ‘head’ end of the comma-shaped body.

Identification of pupae is only possible using microscopy. However, as with the larvae, some groups can be distinguished by their behaviour. *Mansonia* and *Coquillettidia* species for example, are different from other mosquitoes in that their pupae (like their larvae), obtain oxygen from plant tissues below the water surface, using modified trumpets (See Figure 9b).
Coquillettidia linealis pupa breathing through plant stem.

The duration of the pupal stage again is dependent on temperature but is generally of the order of 2-3 days for Anopheles, Aedes and Culex species. Once the adult tissues have developed and it is time for emergence, the pupa swims to the water surface and stretches itself out to full length and the pupal skins splits along the back and the teneral adult mosquito emerges above the water surface (see photo below).

After emerging from the pupal casing, the adult mosquito rests on the water surface for a short time, to allow its wings and body to dry, before flying off in search of nourishment and a mate. Male mosquitoes develop faster than females, and are usually the first to emerge.
1.4 The Adult
After emerging from the pupal casing, the adult mosquito rests on the water surface for a short time allowing its wings and body to dry, before flying off in search of a mate.

In a single generation, the males of a species usually develop marginally more quickly than the females, and males are usually first to emerge from the larval habitat. This is not always noticeable in the field where generations may overlap. Male mosquitoes do not normally travel far from the breeding site and feed on plant juices, sugars from flowers and fruit nectars.

The adult female also seeks out a sugar meal of nectar or similar plant juices to replenish expended energy reserves and then mates with a male, usually near a breeding site at dusk. Female mosquitoes mate only once, as the sperm packet introduced by a male during the mating act is sufficient for the female to fertilise all batches of eggs she subsequently produces.

For egg production, female mosquitoes require protein via a blood meal. A few species can develop the first batch of eggs using nutritional reserves carried over from the larval stage, this is called autogeny. They usually require a blood source to produce the second and subsequent batches.

The preferred source of a blood meal can vary widely between mosquito species. In
general terms, mosquitoes are attracted to a warm-blooded host by a combination of factors; carbon dioxide, a product of respiration is an important attractant as are various body odours and chemicals such as lactic acid. These seem to be the longer range attractants. At closer distances, temperature can be a factor, as can visual perception at very close proximities.

Some species may take several blood meals to acquire sufficient protein for egg production. The female searches for secluded refuge where she can rest undisturbed, digest the blood meal and develop a batch of eggs. She will then fly off in search of additional blood meals to repeat this process. Subsequent blood meals may be taken the night of oviposition if a host is nearby, otherwise a day or more may elapse before the next feed.

As mentioned above, the oviposition site (and thus, the larval habitat) may be characteristic for a particular species. However, although we know that mosquitoes have sense organs which allow them to choose between physical and chemical features of an aquatic site, the determining features important to the mosquito may not always be apparent.

Identification of adult mosquitoes is very complex, even to genus level and microscopes are required. However, the sex of mosquitoes caught in the field can often be determined by eye – if they stop flying around for a second! Adult males differ from females in that they have long palps protruding from their head next to their proboscis, and very bushy antennae compared to those of the females. An exception to this occurs in the *Anopheles* genus, which has both sexes with long palps, but the males still have the more bushy antennae. Resting positions also vary between these genera (see diagram).
The life span of adult mosquitoes is not well known. Some species apparently live one or two months during the summer, although under unfavourable conditions this period may be greatly reduced. Adults that hibernate during winter may live for six months or more. In laboratory conditions, *Aedes aegypti* adults have lasted as much as 240 days (about eight months).

All stages in the life cycle of a mosquito are dependent upon a number of environmental factors for their survival and development. Some common and measurable environmental factors, such as wind, light, temperature, rainfall, and humidity, have a known relationship to the survival of mosquitoes and can be used as the basis of an index for use in surveillance and control.

2. Habitats

The range of habitats utilised by mosquitoes is extremely diverse. With over 3000 species worldwide, mosquitoes have evolved to utilise almost any aquatic system in most parts of the world.

2.1 Larvae

An internationally accepted mosquito breeding habitat classification lists the following 11 larval habitats:

1. Flowing stream
2. Ponded stream
The majority of mosquito species can be classified as either Container Breeders or Groundwater Breeders. Container breeders generally utilise smaller habitats such as tree holes, leaf axils and coconut shells. However they have adapted well to artificial habitats, often found in discarded rubbish, tyres, tin cans, plastic sheeting as well as items that are in use, oil drums, buckets and guttering. Some containers may provide more permanent habitat, such as drain sumps and rock pools, but classification of those habitats may be debatable. Container breeders are often more commonly associated with populated areas as these generally provide a much greater opportunity for breeding.

Groundwater breeders utilise more expansive habitats; swamps, marshes, lake edges field drains and mangroves etc. Groundwater breeders may be found in and around urban areas, although often their habitat will not occur within cities. An example of this involves saltmarsh habitats which often occur adjacent to urban areas. One of the key characteristics of saltmarsh species is often a long flight range, so the habitat existing outside of urban environments does not necessarily provide protection for the hosts within the city.

There are also some species whose behaviour allows for breeding in both container and groundwater e.g. *Culex gelidus* an important vector of Japanese Encephalitis.

Temperature plays a vital role in larval mosquito population dynamics. In tropical regions where there is no significant cold season, the seasonal pattern of mosquito population changes is related to the supply of water and rainfall. A slight rise in the level of water may cause an increase in mosquito production by re-establishing the less frequently inundated oviposition sites and increasing the number of temporary bodies of water. Excessively heavy rainfall and runoff during flood conditions may have a flushing effect and reduce the numbers of mosquitoes in the area. Such a reduction in the larval mosquito population is normally of a relatively short duration.

2.2 Adults

Adult mosquitoes will utilise different habitat for different purposes. In general however, males will remain near the breeding habitat and only travel short distances to some source of a sugar feed (nectar, fruit etc). Females will generally seek shelter from the environment, somewhere with little air movement, often dark, with sugar feeds nearby but also within distance of blood sources and breeding habitat. This will be significantly affected by the flight range of the mosquito, species with a short flight range will be found near to the breeding habitat, while mosquitoes with a longer flight range may be found sheltering several kilometres from the nearest breeding habitat.
Many mosquitoes prefer vegetation to rest in but domestic mosquitoes such as *Aedes aegypti* will be found in dwellings, resting in closets and under tables etc.

### 3 Hosts

Mosquitoes will utilise almost any land-based animal large enough to provide it with a blood feed. Some species are adaptable while others are quite host specific. Hosts include:
- Birds
- Mammals
- Reptiles
- Amphibians

The choice of host species for blood feeding is an important factor in disease transmission. *Aedes aegypti* is an urban mosquito with a preference for biting man. It’s ability to transmit dengue combined with a close association with human populations make it the most significant vector in dengue outbreaks. However with diseases like Japanese encephalitis where two host species are required for the disease cycle, a less specific mosquito species, such as *Culex annulirostris* is a better vector.

### 4 Behaviour

Male and female adult mosquitoes are usually present in about equal numbers following emergence. Typically the male mosquitoes reside near the breeding sites and have a shorter lifespan than females. Females may travel some distance to find a blood source. Only the female mosquitoes blood feed in order to obtain protein to produce fertile eggs.

Flight habits vary considerably; *Aedes aegypti*, arguably the most highly domesticated mosquito, typically flies very short distances (usually less than 500 metres). In studies some individuals have flown less than 35m from the water body they emerged from in their entire lifetime, while *Aedes vigilax* will comfortably travel 5-10km for sugar and blood feeds and may travel upwards of 300km in jetstream wind-assisted migrations. Although a coastal species, *Ae. vigilax* has been found as far inland in Australia as Alice Springs following a migration dispersal.

The possible flight range of Anopheline mosquitoes varies considerably, depending on the species and circumstances in search of food and shelter. Generally they will fly less than 3km, but they have been known to fly 30 kilometres in temperate climates with wind assistance.

Times of activity vary from species to species. Some species are active during the day (diurnal or day-biting) and others only at night (nocturnal or night-biting) with many more active at dawn and dusk (crepuscular).
5 Diseases

Mosquitoes are the most important group of blood sucking insects that cause nuisance and transmit diseases to humans and other warm blooded animals. The nuisance and annoyance caused by mosquitoes is not easy to translate into economic value, however it is as vectors of disease that mosquitoes are most often of concern.

Vector mosquitoes and the parasites and pathogens that they transmit, are recognised to have played an important role in the development and dispersal of the human race, being responsible for some events that have shaped the course of history. Although vaccines, chemoprophylaxis, chemotherapy, genetics and vector control measures are becoming more sophisticated, even now, none of the major mosquito-borne diseases of the world can be said to be under complete control.

Mosquitoes are responsible for transmitting three types of human pathogenic organisms:
- Arboviruses – viruses causing diseases such as dengue, Yellow fever and various encephalitides. (The term arbovirus is derived from arthropod-borne-virus)
- Plasmodia – protozoans which are the cause of malaria
- Filarial worms – nematodes that cause lymphatic filariasis

Mosquitoes can act as transmitters or vectors of pathogens or parasites by both mechanical and biological means. Mechanical transmission occurs where the pathogen has no biological association with the vector, i.e. the pathogen is picked up from one source and deposited in another location. This occurs when the pathogen is carried passively on the biting mouthparts of a mosquito which has fed on an infected host and the pathogen passes passively into a second host at a subsequent feeding. This is most likely to occur where mosquitoes are interrupted during feeding and where any pathogens on the mouthparts remain viable for a short time and are introduced to another host during a subsequent attempt to feed to repletion. Although mechanical transmission of pathogenic organisms occurs via mosquitoes with some animal diseases, biological transmission is predominant for human parasites.

Biological transmission refers to the situation where the pathogen or parasite undergoes a period of development and/or multiplication within the vector (which acts as a true intermediate host and is essential for the completion of the cycle) before being passed on to another host following this incubation period (sometimes called the ‘intrinsic’ incubation period to differentiate it from the incubation period in the vertebrate host which is the ‘extrinsic’ incubation period). There are three systems that apply:

1. The pathogen develops and multiplies, e.g. malarial parasites – there is sexual union of the blood stages in the mosquito gut, encystation in the gut wall, multiple sporozoite formation in the cyst and movement of the sporozoites to the mosquito’s salivary glands as infective stages for introduction into a new host during subsequent feeding.

2. The pathogen develops only, e.g. filarial parasites – the microfilarial blood stages taken into the mosquito gut escape from the gut and develop through three stages in the mosquito’s tissues before entering the head as infective stages for introduction into a new host during subsequent feeding.
3. The pathogen multiplies only, e.g. arboviruses – virus particles taken in with blood into the mosquito gut invade the gut cells, disseminate and multiply in body tissues and penetrate the salivary glands to be introduced into a new host during subsequent feeding.

Irrespective of the particular cycle, once a mosquito vector has picked up a pathogen, the vector needs to survive for at least the period of time required by the pathogen to complete its development cycle or multiply to the point that the mosquito becomes infective, before that mosquito can be involved in the transmission of the pathogen to the new host. This intrinsic incubation period varies with pathogen and temperature, but in general is in the order of 1-2 weeks. Thus the mosquito must survive for at least 1-2 weeks for it to become infective and therefore mosquito longevity is a critical factor in the dynamics of transmission of disease pathogens.

Some of the diseases spread by mosquitoes are associated with animal reservoirs and are called zoonoses (e.g. Yellow fever, viral encephalitides, Brugian filariasis), while others involve only human reservoirs (e.g. dengue, malaria, Bancroftian filariasis). In all cases, the crucial factor in transmission to man (the epidemiology of the disease) is the amount and type of contact between the mosquito vector and the human host. The incidence and prevalence of disease in an area will depend upon the presence of the disease, susceptible vectors and the amount of human-vector contact. The latter is a product of interaction between habitat and behaviour of the mosquito vector and the habitat, and behaviour of the human host. The more often that a potentially infective mosquito intrudes into the human environment or that the humans intrude into the natural environment where mosquitoes harbour pathogenic organisms, the greater the risk of initiating an urban outbreak or epidemic.

Mosquito borne diseases are complicated communicable diseases as they involve the vector as an additional component of the disease system. Social, behavioural, environmental and immunological factors may affect the human component, yet with vector involvement further influences impinge on the system and still more may arise if the disease is a zoonosis, involving other vertebrates as well as humans. Such a complex system may seem formidable, however the more complex the system, the greater the number of opportunities exist for disrupting the disease cycle.

Not all mosquitoes can or do act as vectors for all or any pathogens.

6. Exotic Mosquitoes

6.1 Why are we concerned about exotic mosquitoes?

New Zealand has a small number of species, meaning there are a number of underutilised mosquito habitats. There is the potential for exotic species to exploit these habitats, with very little local competition from our own mosquitoes. New Zealand has no endemic diseases of public health significance and the population is largely unaware of mosquitoes and their roles as vectors of disease. Therefore the population is highly susceptible to exotic mosquito-borne diseases, both in terms of the lack of inherent resistance and ignorance, regarding controlling mosquito numbers to prevent epidemics.
First there would be a potential risk of transmission of disease; not only to humans but to animals and birds. There would of course be nuisance biting from any human biting species that arrived, to a degree most New Zealanders would not be familiar with. There is also the cost of control and mitigation of all types from monitoring, to sprays to repellents.

There are about 2700 species of mosquito described in the world at present, as well as over 1000 arboviruses which are known to cause disease in humans, animals or both. Even Australia, our neighbour has over 300 species of mosquito as well as several mosquito-borne diseases including Dengue, Ross River virus, Barmah Forest virus, Murray Valley Encephalitis and Kunjin virus.

Unwanted Organisms.

- Aedes (Stegomyia) aegypti – Yellow fever mosquito
- Aedes (Stegomyia) albopictus- Asian tiger mosquito
- Aedes (Stegomyia) polynesiensis
- Aedes (Stegomyia) scutellaris
- Culex gelidus – frosty mosquito
- Culex annulirostris – common banded mosquito
- Culex pipiens pallens - common house mosquito
- Culex sitiens
- Aedes (Finlaya) japonicus – Asian rockpool mosquito
- Aedes (Ochlerotatus) vigilax - Saltmarsh mosquito
- Aedes (Ochlerotatus) camptorhynchus - Southern Saltmarsh mosquito
- Aedes (Finlaya) sierrensis - western tree hole mosquito
- Aedes (Finlaya) atropalpus
- All species from the genus Anopheles

Other than the above the following exotics have also been intercepted in the last 9 years

- Aedes (Mucidus) alternans - scotch grey mosquito
- Culex fuscocephala Aedes vexans
- Culex australicus Aedes cooki
- Uranotaenia novobscura Aedes vittiger
- Tripterooides bambusa Toxorhynchites speciosus
- Verrallina funerea Anopheles albimanus
- Culex ocossa Mansonia titillans
- Culex australicus

* These species have been intercepted already in the last 12 months (July 2011 to June 2012)
* These species have not been intercepted in NZ since July 2001
6.2 Pathways

Increased imports and international travel has meant there are several pathways of entry for mosquitoes into NZ.

Shipping
New Zealand imports a large number of goods, from cars to food stuffs. The large number of vehicles and containers that arrive at our borders contain endless sites for mosquitoes to stowaway in. There have been several discoveries of exotic mosquito adults in containers in the last few years but the bulk of interceptions have been related in some way to used vehicles/machinery.

Aircraft
This mode of transportation is the second major pathway which, as well as transporting a wide variety of import goods, is also responsible for bringing countless travellers and their luggage into NZ. Interception specimens have been found in airfreighted goods and in cabins of aircraft entering NZ for engineering work.

Postage
The posting of goods is another pathway, associated with both shipping and aircraft. There is a continuous supply of materials entering the country.

Generally mosquito interceptions take the form of larval specimens, probably due to the greater visibility of the habitat compared to individual mosquito adults.
6.3 Mosquito Control

The most widely used approach for mosquito abatement and prevention of mosquito-borne diseases worldwide continues to be the application of chemical pesticides to aquatic larval sources (larvicides) and into the air for adult mosquito control (adulticides). Such applications are done with aircraft-mounted, truck-mounted or manual equipment. Two popular methods for pesticide application are spreading of granular formulations and fogging with small amounts of concentrated insecticides broken into very small particles, Thermal Fogging and Ultra-Low Volume, or ULV.

In the years following World War II, highly effective and persistent insecticides such as DDT, an organochlorine, were used to control both mosquito larvae and adults. The worldwide malaria eradication effort of the 1950s and 1960s was based on treatment of interior walls of houses to kill indoor resting female anopheline mosquitoes. This program was responsible for the complete disappearance of malaria in some countries and the reduction of human cases in others. Unfortunately, a combination of factors, including resistance to DDT, increasing costs, and political instability, eventually doomed the program, and malaria has returned to nearly all the formerly malaria-free areas at incidences as high as or higher than before.

Organochlorines were phased out in most areas of the world and replaced by newer classes of conventional insecticides, such as organophosphates (e.g., malathion), carbamates (e.g., carbaryl), and synthetic pyrethroids (e.g., resmethrin). Some of the same problems that arose with DDT (resistance, environmental safety) have occurred with nearly all the classes of synthetic organic insecticides, and few chemical companies are developing new products for mosquito control. This has led to the use of pesticides known collectively as third-generation insecticides. These include synthetic materials that affect mosquito development (insect growth regulators), microbial insecticides such as Bacillus thuringiensis israelensis (Bti) and chitin inhibitors, such as diflubenzuron. The use of oils to kill mosquito larvae predates the use of synthetic organic chemicals, and such use continues. Third-generation pesticides are more expensive than conventional pesticides but generally are less toxic to humans and other vertebrates. Because many are highly specific for mosquitoes, they are less disruptive to the environment.

The future of insecticides for mosquito abatement is uncertain. Physiological resistance to pesticides in general has been a problem since the introduction of DDT, and resistance is now beginning to show up even among third-generation products, including Bacillus sphaericus (Bs), a microbial insecticide, and altosid, an insect growth regulator. The greatest threat to the continued use of pesticides for mosquito control is economics. The costs involved in conducting vertebrate and environmental safety tests on new pesticides are rarely justified on the basis of a relatively small market for public health pesticides. Consequently, few products based on new active ingredients have become available over the past 10—15 years.

Source reduction, e.g., the management of standing water to avoid mosquito development, is an important tool in mosquito control. In the early days of mosquito control, source reduction usually meant draining of swamps and marshes, and vast areas of wetlands were permanently lost. As appreciation of the value of wetlands incre