INVESTIGATIONS OF THE ECOLOGY AND CONTROL OF PEST MOSQUITOES IN FRESHWATER WETLANDS AT HOMEBUSH BAY, SYDNEY, NSW

Thesis submitted by

Karen J. Willems B.Sc.

For the degree of Master of Science in Medicine
Faculty of Medicine
University of Sydney
March 2001

PREFACE

All of the investigations described, and the results embodied in this thesis are my own work, except where accreditation and due acknowledgment have been accorded.

None of this work has been previously submitted to any University for the award of any degree.

Karen J. Willems

April 2001

SUMMARY

An understanding of the ecology of freshwater mosquitoes in wetland habitats is essential in devising strategies and practices that will maintain populations at an acceptable level for nearby human communities.

The seasonal activity and relative abundance of the freshwater and saltmarsh species at Homebush Bay, in Sydney, NSW was determined by collecting adults with dry-ice baited Encephalitis Virus Surveillance (EVS) light traps from 1995/1996 to 1998/1999. A total of 28 species, in six genera, were collected over the study period. The two most abundant species in the area were the saltmarsh mosquitoes *Ochlerotatus vigilax* and *Culex sitiens*, which made up 51.7% and 12.4%, respectively of the total collection (347,989 mosquitoes). *Culex annulirostris*, a major pest and vector of numerous arboviruses, was the most abundant freshwater species, representing approximately 10% of the total number of mosquitoes collected, followed by *Cx. quinquefasciatus*, *Cx. australicus*, *Aedes notoscriptus*, *Anopheles annulipes* s.l., *Coquillettidia linealis* and *Oc. alboannulatus*.

Freshwater larval populations were sampled using a replicated dipping technique and the type of habitat (i.e. marsh, small pond, etc) and habitat variables such as size, water depth at the sample point, vegetation density and vegetation type were recorded at each site. A total of 16 species was collected in freshwater habitats, with 12 of the 16 considered freshwater mosquitoes. The most abundant species detected in larval surveys throughout the study period were *Culex annulirostris*, *Cx. australicus* and *Cx. quinquefasciatus,* and to a lesser extent *Oc. alboannulatus* and *An. annulipes* s.l. Marsh and wetland habitats with dense (>70%) vegetative growth produced

significantly more larvae compared to other habitats, indicating the need for management of vegetation in areas of dense growth. There was tendency for larval density to decrease as depth increased but the difference just failed to reach statistical significance.

In the last year, studies were undertaken to confirm head capsule width as a measure of larval instar and the stadial duration of *Cx. annulirostris* larvae from the first to the fourth instar in the summer and early spring. Developmental time for December, January, February and March was 12.0, 9.0, 9.3 and 17.0 days, respectively, and was shown to be dependent upon average minimum temperature. The developmental time to the fourth instar was the most important to consider with regard to timing of treatment with the larvicide *Bacillus thuringiensis israelensis* (*B.t.i.*) since larvae must ingest the product and larvae stop feeding before pupation.

Two laboratory experiments were conducted to compare the efficiency of *Pseudomugil signifer*, a native larvivorous fish species abundant in the waterways of Homebush Bay, with that of *Gambusia holbrooki*, an introduced noxious species previously used for mosquito control. The two species performed similarly in feeding trials with increasing sizes (first to fourth instar) and increasing densities (50, 100 and 200) of *Cx. annulirostris* larvae. In the simulated vegetation trials, *P. signifer* performed marginally better than *G. holbrooki* in containers with no 'vegetation' and in medium to high density 'vegetation'.

Tadpoles of four endemic anurans, *Limnodynastes peronii*, *Lim. tasmaniensis*, *Litoria aurea* and *Lit. peronii* were investigated in the laboratory to determine if they

consumed mosquito larvae. Results showed that their consumption levels were very low, with the best predator, *Lit. aurea*, consuming five out of 10 larvae in a 24 hour period.

Continued monitoring of freshwater adult and larval populations during the mosquito breeding season and a knowledge of influential habitat, meteorological and other environmental factors that contribute to mosquito abundance are essential to the success of mosquito management programs at Homebush Bay. The results presented in this thesis demonstrate the diversity of species in the local freshwater mosquito fauna, their associations with particular habitats, the importance of design, and water and vegetation management for the reduction of larval densities of the major pest species in constructed wetlands, the potential for the use of the fish *P. signifer* as a biological control agent in medium to large ponds, and the contribution of endemic larval anurans as opportunistic predators of mosquito larvae in small ponds.

ACKNOWLEDGEMENTS

I wish to thank my supervisor, Assoc. Prof. Richard C. Russell (Dept. of Medicine, University of Sydney) and co-supervisor, Dr. Allan Meats (School of Biological Sciences, University of Sydney). I especially thank Richard Russell for the opportunity, guidance and encouragement to undertake and complete these studies. I also thank Merilyn Geary (Dept. of Medical Entomology, ICPMR, Westmead Hospital) for her technical assistance and the supply of *Culex annulirostris* laboratory stock. I thank my friends and colleagues at the Dept. of Medical Entomology, especially Cameron Webb, for their support. Advice and assistance with statistical analysis for the various studies was provided by Dr. Karen Byth (Westmead Millennium Institute) and her contribution is gratefully acknowledged.

Chapter 3. For the duration of my candidature, Cameron Webb shared with me in the task of monitoring the adult populations at Homebush Bay and I wish to thank him for his assistance. Cameron's responsibility was for the mosquitoes associated with the saline wetlands. I undertook the adult and larval surveillance and all the investigations associated with the freshwater wetlands.

The Olympic Co-ordination Authority provided the funding for this thesis, and I wish to thank Kim McCallum, Barbara Sanders, Kathryn O'Flynn and Kerry Darcovich for their logistical support and assistance.

Finally, I wish to thank my husband, Bryen Willems, my children, Jacqueline and Jake, and my parents, Kenneth and Jenny Irvin, whose love, encouragement, support and patience made this thesis possible.

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CHAPTER 1

INTRODUCTION

Mosquitoes associated with freshwater wetlands in Australia can pose significant pest and public health risks (Russell 1996). Certain species associated with these habitats are of particular concern as they are the major inland vectors of arboviruses causing polyarthritis and encephalitis, and potential vectors of malaria. The most important inland arbovirus vector is *Culex annulirostris* Skuse since it is a vector of Ross River (RR), Barmah Forest (BF), Murray Valley encephalitis (MVE) and Kunjin viruses (KUN) (Russell 1995). In recent times, the conservation of natural wetlands and construction of artificial wetlands for the purpose of wastewater storage, treatment and aesthetic landscaping has raised concern for the possible public health risks associated with the mosquito populations that colonise these habitats. In Sydney, the capital city of New South Wales, a major land use development at Homebush Bay has been occurring, involving local mosquito concerns.

The Homebush Bay area is located close to the geographic heart of Sydney and with the redevelopment of the site for the 2000 Summer Olympic Games, extensive freshwater wetlands were installed adjacent to high density residential housing, sporting facilities and recreational parklands. The wetlands were designed to improve the water quality of urban stormwater and provide important habitat for a number of rare and endangered bird and amphibian species (OCA 1997). Concern was raised as to the potential increase in the importance of mosquitoes associated with the newly created habitats and the resulting pest nuisance and public health risks. In conjunction with the changing land uses of Homebush Bay, there would be a dramatic increase in the residential population as well as visitors to the site, increasing the need to provide an environment free of nuisance mosquitoes.

The Olympic Co-ordination Authority (OCA) developed an Environmental Strategy for the Homebush Bay area that incorporated the principles of Ecologically Sustainable Development (ESD) for the conservation of species, the conservation of resources and for pollution control (OCA 1995). Because of the ecological importance of the freshwater wetlands, management strategies designed to suppress pest mosquitoes were required for the freshwater ecosystem.

1.1 Assessment of mosquito risk in freshwater habitats

Assessing the risks presented by mosquitoes in wetland habitats involves knowledge of the species present, their vector status, local environmental conditions and exposure of human communities (Russell 1999). Surveillance of the habitat will initially give an indication of the nature and extent of the mosquito problem and regular sampling will provide baseline data for comparisons over time (Russell 1993). Prior to sampling, preparation of a map incorporating potential breeding sites, residential, industrial and recreational areas, and topography and vegetation types is useful in planning for surveys (Russell 1993). Climatic factors such as rainfall, wind speed and direction, and temperature all affect mosquito populations and should be collected and correlated with larval and adult abundances (AMCA 1998).

Once potential breeding sites have been selected, routine weekly larval surveillance using a replicated dip sampling technique should be conducted at established sampling points around the perimeter of the wetland habitat (Russell 1996). The larval abundance measured as number of larvae per dip will serve to indicate the need for larval control measures and/or the effectiveness of larval control measures (Russell 1993).

Adult populations should be routinely monitored (every 1-2 weeks) using dry-ice baited light traps for the purpose of determining the pest or vector species present, with further processing of the mosquitoes to detect infection with pathogens (Russell 1996). Monitoring of adult populations can verify the productivity of the larval habitats and indicate the need for larval control or perhaps adult control if there is also verified activity of mosquito-borne pathogens (Russell 1993, AMCA 1998).

A review of the biology (ecology and behaviour) of the principal mosquito species associated with freshwater wetlands will be presented in Chapter 2.

1.2 Mosquito management in artificial wetlands

Constructed wetlands make use of dense macrophyte stands that are useful in optimising the removal of nitrogen and phosphorus (Verhoeven 1996) and are typically shallow to provide for greater plant growth responses (Meney 1996). Shallow depths and dense macrophyte stands however, also provide greater opportunities for mosquito larvae by offering refuge from predators and nutrients for development (Berkelhamer and Bradley 1989; Orr and Resh 1990; Walton *et al.* 1990, Russell 1996). The management of mosquitoes in artificial wetlands must take an integrated approach that incorporates aspects of wetland design, habitat manipulation, chemical agents in the event of large populations of mosquitoes, and biological agents.

1.2.1 *Wetland design*

If possible, artificial wetlands should be established in an open area where wind action will create physical disturbance of the water surface and located away from the human community so as to be outside the flight range of the important local mosquito species (Russell 1999). The construction of steep banks (with a slope of 3:1) also discourages mosquito breeding.

Walton and Workman (1998) compared the population abundance and species richness of larval mosquitoes in one-phase marsh habitats (with continuous vegetation) and in three-phase marsh habitats (with two vegetation regions separated by a region of deep, open water). Results of the study demonstrated that the one-phase marsh contained proportionally more third and fourth instar larvae than the three-phase marsh, implying that larval mosquito mortality rates were significantly lower in thickly vegetated marshes.

Batzer and Resh (1992), using a series of experimental ponds with three depths (20 cm, 40cm and 60 cm), demonstrated that depths of 60 cm resulted in significantly lower mosquito larval densities than 20 and 40 cm water depths.

1.2.2 *Habitat manipulation*

Managing vegetation and manipulating water levels are useful tools for mosquito management in wetland habitats (Russell 1999). For example, Batzer and Resh (1992) have shown that reduction of plant cover by 50%, using the technique of vegetative mowing, concentrated mosquito larvae along the upland edge of the wetlands so that management of mosquitoes was limited to the edge habitats. Manipulating water levels by flooding habitats to a depth greater than 60 cm will result in lower larval densities (Batzer and Resh 1992).

1.2.3 Chemical control

This is useful for short term control to reduce human contact with pest or vector species during periods of heavy breeding and while cost effective, prolonged use may lead to the development of resistance in mosquito populations (AMCA 1998). Generally, temephos, methoprene, *Bacillus thuringiensis israelensis* (*B.t.i.*) and *B. sphaericus* (*B.s.*) have been recommended for use in freshwater wetlands (AMCA 1998).

Temephos, an organophosphate larvicide, has been used in mosquito control programs in eastern Australia but due to acute toxic effects on the planktonic larval life stages of some crustacean species, and to adults of other sensitive species (Mortimer and Chapman 1995, Brown *et al.* 1996) it is no longer used in estuarine systems. It is still recommended for use in freshwater wetlands, but it is moderately to highly toxic for fish and birds, and recommended application rates must be adhered to (AMCA 1998).

Methoprene, an insect growth regulator, interferes with normal development and prevents larvae emerging as adults. Mortality is delayed until the fourth larval instar or pupae, thereby providing food for various aquatic fauna (Service 1995). Higher dosage rates are required in polluted water, deep water, habitats with emergent and submergent vegetation and flowing water (Mulla 1995).

Bacillus thuringiensis israelensis (*B.t.i.*) is a spore forming bacteria of the family Bacillaceae and was isolated in Israel by Goldberg and Margalit (1977). The high degree of toxicity for *Aedes, Culex,* and *Anopheles* mosquitoes, easy production and formulation, and adequate storage properties of the product have made this bacterial larvicide a commercial success (Federici 1995). A drawback of *B.t.i.* is that it has reduced larvicidal activity in polluted habitats (Karsh *et al.* 1991).

Bacillus sphaericus (*B.s.*) is also a spore forming bacteria that has demonstrated excellent control against *Culex* larvae (Lacey *et al.* 1984, Lord 1991, Mulla *et al.* 1984, Mulla *et al.* 1988). Compared with *B.t.i.*, it has demonstrated a greater larvicidal persistence in organically rich environments and an apparent ability to recycle in larval cadavers (Des Rochers and Garcia 1984, Matanmi *et al.* 1990). Lord (1991) demonstrated that *B.s.* applied to dry floodwater habitats in pellet form resulted in 100% control after ponds were flooded. Later, after habitats dried out and were reflooded, *B. sphaericus* was found to be persistent in the pond water.

1.2.4 Biological control

In keeping with the principles of ESD, the OCA devised an Integrated Pest Management Strategy (IPM) in 1998 incorporating the use of biological control agents for the control of mosquitoes (OCA 1999). Biological control involves the use of natural enemies to control a pest organism. The use of larvivorous fish was considered the most appropriate option for use against mosquitoes in the medium (100 m^2 - 2000 m^2) to large (>2000 m^2) wetlands at Homebush Bay.

Gambusia holbrooki (Girard) has been widely used as a control agent in Australia but its effectiveness has been considered questionable (Lloyd 1984, Lloyd 1990a). The species, endemic to the southern USA and Central America, was first introduced to Australia in 1926 as a mosquito control agent (Lloyd and Tomasov 1985) and is now widespread throughout Australia. It is known to consume beneficial invertebrates as well as mosquito larvae (Hoy *et al.* 1972, Hurlbert *et al.* 1972, Bence and Murdoch 1983, Kramer *et al.* 1988, Walton and Mulla 1991), and in Australia it has been implicated in the decline of several native fish species (Lloyd 1990a). Laboratory and field studies in Australia have shown that *G. holbrooki* consumes frog eggs and tadpoles, and also damages the dorsal fin and tail of several tadpole species (Blyth 1994, Webb 1994, Morgan and Buttemer 1996, Webb and Joss 1997).

Native fish have been promoted as preferable to *G. holbrooki* since they are adapted to the local conditions, easy to locate, and are not associated with ecological disturbances (Lloyd 1990b). *Philypnodon grandiceps* (Krefft), *Philypnodon* sp., *Pseudogobius olorum* (Sauvage), *Pseudomugil signifer* (Kner) and *Hypseliotris compressa* (Krefft) have been collected in the waterways of Homebush Bay (The Ecology Lab 1994a, 1994b) and are known to consume mosquito larvae (Durre 1991, Larson and Hoese 1996).

The larvivorous freshwater fish *Pseudomugil signifer*, or Pacific Blue-eye, is abundant in the estuarine waterways of Homebush Bay and is easily acclimatised to freshwater conditions. For these reasons, *P. signifer* was a prime candidate for investigations to determine its efficiency in consuming mosquito larvae compared to the exotic pest species, *G. holbrooki*.

Anecdotally, tadpoles are considered to consume mosquito larvae and be possible biological control agents, however there have been no scientific investigations on local species to substantiate this belief. Besides the endangered Green and Golden

Bell Frog, *Litoria aurea* (Lesson), there are several species of anurans known to be breeding in the wetlands at Homebush Bay (M. Christy, Australian Museum, personal communication). They are *Limnodynastes peronii* (Duméril and Bibron) *Limnodynastes tasmaniensis* Günther and *Litoria peronii* (Tschudi) and one or more of these may be useful as a control agent for the temporary ponds in the area.

The main objectives of this research project were a) to investigate seasonal activity and relative abundance, and potential adult pest status of the major freshwater mosquitoes in the Homebush Bay area; b) to determine the mosquito productivity of various habitats, and determine habitat characteristics associated with increased larval density; c) to examine the potential of the native fish, *P. signifer* as a biological control agent; and d) to investigate the endemic frog species, *Lim. peronii*, *Lim. tasmaniensis*, *Lit. aurea* and *Lit. peronii* as potential biological control agents. The thesis begins with an overview summary of the knowledge of seven major freshwater mosquito species in Chapter 2. The results of the seasonal abundance study of the freshwater mosquito fauna are presented in Chapter 3, and the field studies of *Cx. annulirostris* larval development in Chapter 4. The laboratory experiments investigating the fish *P. signifer*, and the four endemic frog species are presented in Chapters 5 and 6 respectively, and a Concluding Discussion in Chapter 7. The information provided by these investigations will be useful for determining long-term management strategies and operations for the freshwater mosquitoes inhabiting the area.

CHAPTER 2

OVERVIEW OF THE MAJOR LOCAL FRESHWATER MOSQUITO SPECIES

The implementation of control strategies for pest mosquitoes of public health importance requires a thorough understanding of the mosquito fauna, their biology and the ecological interactions. A total of 59 mosquito species has been collected from coastal New South Wales since 1987 (Clancy and Russell 1997), with only a few considered as pests or disease vectors. Seven species endemic to Homebush Bay and significant for their pest potential or involvement in pathogen transmission are reviewed. Lee *et al.* (1982, 1987, 1988, 1989) have discussed these species earlier, but this summary review includes recent literature.

2.1 *Ochlerotatus alboannulatus* (Macquart)

Macquart (1850) first described *Oc. alboannulatus* as *Culex albo-annulatus*. Skuse (1889) incorrectly synonymised the species with *Culex* [i.e. *Ochlerotatus*] *camptorhynchus*, however not long after, Theobald (1901) removed Skuse's synonymy of *Cx. camptorhynchus*. Over the next few years, it was placed in the genera *Culicelsa* (Theobald 1907), *Ochlerotatus* (Edwards 1912) and *Aedes* (Cooling 1924). Edwards (1924) placed *Oc. alboalboannulatus* in the subgenus *Finlaya*. Reinert (2000) recently revised the taxonomy of mosquitoes in the genus *Aedes*, raising the subgenus *Ochlerotatus* to generic rank. The subgenus *Finlaya* was assigned to the genus *Ochlerotatus*, resulting in the name *Ochlerotatus alboannulatus* for the species.

2.1.1 Distribution

Ochlerotatus alboannulatus occurs in QLD (south of the Tropic of Capricorn), most of NSW, VIC (excluding the northwestern corner of the state), SA, TAS and southern WA (Britten 1958, Waterhouse 1959, Dobrotworsky 1965, 1966, Lee *et al.* 1982, Marks 1982b).

2.1.2 Biology and ecology

Larvae colonize ground pools and rock pools in rural environments (Dobrotworsky 1959). From 1951 to 1958, in Colo Vale, New South Wales (NSW), Lee *et al.* (1982) observed that they prefer to breed in small temporary pools that are earthbound or partially earthbound rock pools. Marks (1982b) noted that larvae were also found in semipermanent swamps, tyres and water troughs in partial sunlight. Dobrotworsky (1959) observed that *Oc. alboannulatus* preferred diffuse sunlight, however he found larvae during the summer in a pool fully exposed to the sun at an altitude of 5400 feet on the Bogong High Plains. They are able to colonize permanent water all year round (Dobrotworsky 1959). Eggs are laid singly and can withstand desiccation (Marks 1982b). Eggs that are deposited along the edge of receding pools in the spring are able to remain in a dormant state until autumn rains flood the eggs, hatching within the next twelve hours (Dobrotworsky 1959). Larvae may be associated with *Oc. notoscriptus* Skuse and *Oc. rubrithorax* (Russell 1993).

Ochlerotatus alboannulatus has the potential to be a minor pest in areas surrounded by or adjacent to sylvan habitats (Russell 1993). Adults are vicious day-biters (Dobrotworsky 1959), with peak biting near sunset (Lee *et al.* 1954). They are known to feed on marsupials and rabbits (Lee *et al.* 1957), birds, other than domestic poultry

(Dyce and Lee 1962), humans, cattle, sheep, dog and fowl (Lee *et al.* 1982). Human disease associations

2.1.3 Arboviruses

Ochlerotatus alboannulatus has been shown to be susceptible to Ross River (RR) virus in laboratory studies (Kay and Aaskov 1989). The virus has been isolated from *Oc. alboannulatus*, but only in the Leschenault and Capel-Busselton regions of Western Australia (WA) (Lindsay *et al.* 1997). It is not known to have any other human disease associations. It is unlikely to be an important vector, however it may be involved in persistence of the virus through sylvan cycles (Russell 1988).

Vector competence studies by Kay *et al.* (1989) demonstrated the species was a poor vector of Murray Valley encephalitis (MVE) virus.

2.2 *Ochlerotatus notoscriptus* (Skuse)

Skuse (1889) originally described *Ochlerotatus notoscriptus* as *Culex notoscriptus*. Over the next two decades, it was placed in the genera *Stegomyia*, *Scutomyia* and *Ochlerotatus* respectively (Theobald 1901, 1905, Edwards 1912). Many generic changes based on new evidence by others prompted Edwards (1922) to place the species in *Aedes* and the subgenus *Finlaya*. Recent taxonomic revisions of the genus *Aedes* (Reinert 2000) resulted in the reassignment of the species to the genus *Ochlerotatus*.

2.2.1 Distribution

Ochlerotatus notoscriptus is distributed in all states of Australia as well as the southern half of Papua New Guinea, New Caledonia, New Zealand, Indonesia and

Torres Strait Islands (Belkin 1968, Lee *et al.* 1982, Russell 1993). In New Zealand, the distribution of *Oc. notoscriptus* appeared to be restricted to areas surrounding present or former seaports on the North Island (Belkin 1968), and on occasion was collected alive from vessels arriving at Auckland from Sydney, suggesting that it was an introduced species (Graham 1939). Surveys in 1993/1994 however, confirmed that the species had spread south of the Northern Island (Laird 1995).

Surveys of areas in NSW thought to be receptive to re-introduction or maintaining remnant populations of *Ae. aegypti* were undertaken between 1976-1984 (Russell *et al.* 1984, Russell and Bryan 1985). The resulting data demonstrated an absence of *Ae. aegypti* but an increase in the abundance of *Oc. notoscriptus* in NSW since the 1930's. Laboratory experiments designed to test the hypothesis that *Oc. notoscriptus* may have competitively displaced *Ae. aegypti* in larval habitats were conducted by Russell (1986c). Although the hypothesis was not supported, findings showed that *Oc. notoscriptus* may have an advantage in cooler areas where the species is more abundant.

2.2.2 Biology and ecology

Ochlerotatus notoscriptus is a domestic and peri-domestic pest species that can be found breeding in artificial containers such as gutters, tyres, pot-plant saucers, tin cans and bottles near human habitation and in hollows of trees and creekline rockpools in sylvan environments (Bancroft 1908, Marks 1982b, Russell 1993). Marks (1982b) also notes that larvae are commonly found in artificial containers that contain rotting vegetation. Eggs are laid singly along the water line of receptacles (Bancroft 1908) and they are able to resist desiccation for several months (Cooling 1924). Russell (1993) notes that in southeastern Australia and depending on the environment, larvae can be found in association with *Oc. rubrithorax, Cx. quinquefasciatus* Say, or *Tripteroides atripes* (Skuse).

Russell (1993) believes the species to be the major domestic pest in southeastern Australia. Biting activity occurs throughout the 24-hour period in shaded areas and peak biting generally occurs near sunrise and sunset (Lee *et al.* 1982, Russell 1993). Precipitin tests performed by Lee *et al.* (1954) tested positive for humans, rabbit, fowl, marsupial, dog and cattle. In host preference trials by Kay *et al.* (1979), females collected in animal-baited stable traps were attracted to calves, kangaroos, humans, dogs and pigs.

2.2.3 Human disease associations

2.2.3.1 Arboviruses

In laboratory studies, Doggett and Russell (1997) demonstrated that *Oc. notoscriptus* was capable of transmitting two Barmah Forest (BF) virus isolates, BF18295 and BF23554. In the field, BF virus has been isolated from *Oc. notoscriptus* in urban Brisbane, Queensland (QLD) (Russell 1995).

Ochlerotatus notoscriptus has been shown to be susceptible to Ross River (RR) virus in lab studies (Kay and Aaskov 1989). Later, transmission of RR virus isolates from Bourke and Batemans Bay was demonstrated with *Oc. notoscriptus* collected from Sydney (Doggett and Russell 1997). However, transmission studies from other areas have not been as successful. *Ochlerotatus notoscriptus* from the Sunshine Coast, QLD was unable to transmit the RR virus strain B94/20 (Ryan and Kay 1997) and *Oc. notoscriptus* from Brisbane, although moderately susceptible to infection, was considered an inefficient vector of the B92/20 isolate, (Watson and Kay 1997). In New Zealand, *Oc. notoscriptus* was assessed for its ability to transmit the RR virus considering increased virus activity in the Pacific and the possible introduction of the virus to the country by viraemic travellers (Maguire 1994). Using the F9073 strain, transmission was unsuccessful, although mosquitoes carried high titres of virus at the time of the transmission attempt. In the field, *Ochlerotatus notoscriptus* has yielded RR virus isolates from Darwin, NT (Whelan and Weir 1992), the NSW central and south coasts (Doggett *et al.* 1999) and northern inland NSW (Russell *et al.* 1997), and from suburban Brisbane in QLD (Russell 1995).

Edge Hill virus has been isolated from *Oc. notoscriptus* in Sydney, NSW (Russell *et al.* 1998).

Stratford virus was isolated from the species in suburban and outer western semi-rural areas of Sydney, NSW (Doggett *et al.* 1999).

In laboratory experiments, McLean (1953) demonstrated that *Oc. notoscriptus* was capable of carrying Murray Valley encephalitis virus for ten days. To date however, no isolates of the virus have been recovered from *Oc. notoscriptus* in the field (Russell 1995).

2.2.3.2 Filariasis

Ochlerotatus notoscriptus was found naturally infected with dog heartworm (*Dirofilaria immitis*) and was designated a primary vector based on the vector potential index (Russell 1985b). Laboratory studies have demonstrated that it probably is a more important vector than *Cx. quinquefasciatus* in the urban environment (Russell 1990), and has a very high vector efficiency index (number of third stage microfilariae/ number of microfilariae), although high microfilarial densities cause increased mortality (Russell and Geary 1992, Russell and Geary 1996).

2.3 *Anopheles annulipes* Walker sensu lato (s.l.)

This species was first described by Walker (1856) as *Anopheles annulipes*. The taxon is a species complex that includes at least four species in NSW and 10 species Australia wide (Booth and Bryan 1986). The following discussion will refer to *An. annulipes* in the broad sense or sensu lato (s.l.) unless otherwise stated.

2.3.1 Distribution

This taxon is distributed throughout mainland Australia, TAS, King and Flinders Islands in the Bass Strait, Torres Strait Islands, Lord Howe Island, Papua New Guinea, D'Entrecasteaux Islands and Irian Jaya (doubtful) (Lee *et al.* 1987).

2.3.2 Biology and ecology

An. annulipes s.l. is an ever-present species, breeding in a variety of permanent and temporary ground and rock pools, usually fresh but sometimes brackish water (Lee *et al.* 1987). Larvae have also been found in tubs and fishponds located near houses (Lee and Woodhill 1944). The species is typically associated with pools containing green filamentous algae (Lee and Woodhill 1944). In many parts of Australia, breeding is continuous throughout the year, and in cooler climates it over-winters as larvae (Lee *et al.* 1987). In southeastern Australia, Russell (1987) found that the taxon is present throughout the year and most abundant in mid to late summer at Echuca, VIC and Appin, NSW. Depending on habitat, associated species in southeast Australia are *Oc.*

alternans (Westwood), *Oc. camptorhynchus* (Thomson), *Oc. notoscriptus, Oc. rubrithorax*, *Oc. vigilax* (Skuse), *Cx. annulirostris* Skuse, *Cx. cylindricus* Theobald, *Cx. fergusoni* (Taylor) and *Cx. quinquefasciatus* (Russell 1993).

Anopheles annulipes s.l. is rarely a pest of humans (Russell 1993). It is attracted to humans (but they are not the preferred hosts), rabbits, marsupials, cattle and other domestic animals, fowl and wild birds (Lee *et al.* 1954, Dyce and Lee 1962). Foley and Bryan (1991) proposed that the attraction of the taxon to a wide range of hosts might be a result of mixed populations of more than one sibling species that have an attraction to a specific host. Females attack during the day when close to the larval habitat but more often at night (Lee *et al.* 1987). According to Fenner and Ratcliffe (1965), the species was recorded travelling five to six kilometres in WA, and 16 km in the Murrumbidgee Irrigation Area of NSW. Later, Bryan *et al.* (1991) recaptured sibling species 'A' five km from the release site in dispersal studies at Griffith, NSW.

2.3.3 Human disease associations

2.3.3.1 Arboviruses

Barmah Forest virus has been isolated from *An. annulipes* s.l. from the north inland region of WA (Broom *et al.* 1992).

Isolates of Ross River virus has been recovered from *An. annulipes* s.l. in the Murray Valley region of NSW and VIC (Russell 1986e, Russell 1995, Russell *et al.* 1997, Doggett *et al.* 1999, Doggett *et al.* 2000), and the north inland and southwest regions of WA (Lindsay *et al.* 1989, Russell 1995).

Records of Sindbis virus isolations from *An. annulipes* s.l. have been reported from the north inland regions of NSW, and from the Murray Valley of NSW and VIC (Russell 1986e, Russell 1995, Russell *et al.* 1997, Doggett *et al.* 1999), and from the north inland region of WA (Broom *et al.* 1992).

Trubanaman (TRU) virus was first isolated from the species in 1965 at Kowanyama, north QLD (Doherty *et al.* 1968). The virus has been recovered from north inland and south coast of NSW (Vale *et al.* 1985, Russell *et al.* 1992, Russell 1995) as well as the Murray Valley of NSW and VIC (Russell 1986e, Russell 1995). Additional isolates of TRU have been recovered from the species in the southwest regions of WA (Mackenzie *et al.* 1994).

Murray Valley encephalitis virus was isolated from *An. annulipes* s.l. in north inland region of WA (Broom *et al.* 1992).

2.3.3.2 Malaria

Roberts (1943) demonstrated that experimental transmission of *Plasmodium vivax* and *P. falciparum* with *An. annulipes* s.l. was possible and later, Mackerras and Roberts (1947) reconfirmed the earlier findings but concluded that the species wasn't necessarily an important vector in nature. Lee *et al.* (1987) states that *An. annulipes* s.l. was the presumed vector in the past of sporadic malaria cases in the southern states of Australia.

2.3.3.3 Filariasis

In 1934, G. Heydon demonstrated in laboratory experiments that *An. annulipes* s.l. could be an intermediate host of *Wuchereria bancrofti*, the nematode parasite causing lymphatic filariasis (Backhouse and Heydon 1950). Filariasis had disappeared from Australia by the 1940s (Mackerras 1958, Boreham and Marks 1986), and there is no current concern for this disease.

Anopheles annulipes s.l. has been found naturally infected with dog heartworm (*Dirofilaria immitis*) (Russell 1985b), however laboratory studies have determined that it was not a very competent vector (Russell 1990).

2.4 *Coquillettidia linealis* (Skuse) sensu stricto

Skuse (1889) first described *Coquillettidia linealis as Culex linealis*. Generic changes occurred over the next century with the literature placing the species in the genera *Culicelsa* (Cooling 1914), *Culicada* (Ferguson 1916), *Taeniorhynchus* (Edwards 1924), *Mansonia* (Edwards 1932) and eventually *Coquillettidia* (Knight and Stone 1977).

2.4.1 Distribution

Coquillettidia linealis occurs in NSW, QLD, SA, VIC and possibly TAS (Russell 1993). The undescribed species, *Cq.* species near *linealis* occurs in NSW, VIC, WA, TAS, and possibly SA (Russell 1993).

2.4.2 Biology and ecology

Although very little is known about larval biology, the species has been assumed to be associated with marginal reeds in permanent water bodies (Russell 1993). Attempts to observe larval development in the laboratory failed after larvae died before reaching the second instar (Dobrotworsky 1965).

It is a nuisance pest in some coastal and inland areas though not of great concern (Russell 1993). Adults bite during the day, evening and night and are common from late spring throughout the summer with peak abundance varying with the region (Russell 1993). It is known to disperse at least 5 km from the larval habitat (Russell 1988). Based on precipitin tests, it is known to feed on humans, sheep, dog, rabbit, marsupial and fowl (Lee *et al.* 1988).

2.4.3 Human disease associations

2.4.3.1 Arboviruses

Barmah Forest virus has been isolated from the species on the east coast of VIC (Russell 1995) and central coast of NSW (Russell *et al.* 1995).

Isolates of Ross River (RR) virus have been recovered from *Cq. linealis* on the central coast, south coast and south inland regions of NSW (Gard *et al.* 1973, Vale *et al.* 1985, Cloonan and Russell 1986, Russell *et al.* 1991, Russell *et al.* 1992, Doggett *et al.* 1995). Russell *et al.* (1991) consider this species to be a vector of at least secondary importance for RR virus in coastal NSW.

Viruses of less importance, such as Edge Hill virus, Gan Gan virus and Trubanaman virus have all been recovered from *Cq. linealis* on the south coast of NSW (Vale *et al.* 1985, Russell *et al.* 1991, Vale *et al.* 1992).

2.5 *Culex annulirostris* Skuse sensu stricto

Skuse (1889) was the first to describe *Culex annulirostris,* the Common Banded Mosquito. It is a member of a species complex with at least two forms in Australia that have been confused in the past (Lee *et al.* 1989). Marks (1982a) was able to distinguish between the members of the complex, determining the two forms as *Cx. annulirostris* sensu sensu (s.s.) and the 'Normanton sp.' that is now known as *Cx. palpalis* (Taylor). The following discussion will refer to *Cx. annulirsotris* s.s.

2.5.1 Distribution

Culex annulirostris occurs in NSW, NT, QLD, SA, VIC, WA and possibly TAS (Russell 1993). It also occurs outside of Australia in southeast Asia and the Pacific regions (Lee *et al.* 1989).

2.5.2 Biology and ecology

Kay *et al.* (1981) described the larval habitats of *Cx. annulirostris* as temporary freshwater pools lined with grass, freshwater swamps and lagoons; it can be found in association with *Cx. sitiens* in saline pools and may displace *Ochlerotatus vigilax* when the saline pools are diluted with freshwater runoff; larvae can colonize effluent pools along the grassy margins. In North QLD, Marks (1963) found larvae colonizing a pondage dam, seepage pools and a flooded pasture at Parada. In addition, Russell *et al.* (1983) collected *Cx. annulirostris* larvae from a drainage line of raw sewage effluent in Darwin. Eggs are laid on the surface of the water and are not resistant to drying out (Kay *et al.* 1981). In southeastern Australia, larvae have been found in association with *Aedeomyia venustipes* (Skuse), *An. annulipes* s.l., *Cx. australicus* (Dobrotworsky and Drummond), *Cx. cylindricus* and a few floodwater *Aedes* species (Russell 1993).

Populations in the Murray Valley were found to increase from late October until February, and then decline in March and April (McDonald *et al.* 1980; Russell

1986a). McDonald *et al.* (1980) conducted laboratory experiments on *Cx. annulirostris* from the Murray Valley in VIC to determine the effect of temperature on population growth potential. Larval survival was greatest at 25°C. The period for complete juvenile development ranged from 8.57 days at 35°C to 37 days at 15°C. Development from egg to adult required 196 day-degrees above 9.7°C with incubation temperatures between 15 and 30°C. The population growth potential was positive at 20°C, 25°C and 30°C, the greatest being 25°C. Population growth was negative at 15°C. The minimum threshold temperature for population growth was estimated to be 17.5°C.

Mottram *et al.* (1986) investigated the effects of temperature on eggs and immature stages on *Cx. annulirostris* from Brisbane. At 15°C and 40°C eggs failed to hatch and larvae died in the first instars. It was determined that the best temperatures for egg hatching and survival was 25°C and 30°C. The period for development from first instar to adult ranged from 7.1 days at 35°C to 25.2 days at 20°C. The minimum threshold temperature for development was 15.6 ± 2.5°C.

In laboratory investigations, Russell (1986b) determined the time from blood feeding to oviposition of *Cx. annulirostris* by using females collected from the field in Appin, NSW. The collected females were fed on human blood and held in an environmental cabinet set to the average monthly temperature and photoperiod until oviposition occurred. The duration of the cycle was 4-12 days at 14°-24.6°C.

Rae (1990) investigated survival and development of immature stages of *Cx. annulirostris* from Townsville, QLD at constant temperatures in the laboratory and at fluctuating temperatures in the field. Survival from the first instar to adults ranged from 5.5% at 18°C to 96.5% at 35°C. In the field, mortality increased by 60% due to predators. The calculated developmental threshold temperature was determined to be 13.7°C and the thermal constant was 129 day-degrees.

In field investigations, Mottram and Kettle (1997) studied the rates of development and survival of *Cx. annulirostris* larvae in a temporary rain-filled pool, a semi-permanent pool and a flooded grassland site. The temporary pool gave rise to the greatest survival and the fastest development.

Kay *et al.* (1986) reared and maintained *Cx. annulirostris* from Brisbane and Mildura on nutrient rich diets and observed autogeny in 8.5% and 5.1% respectively of the females. Russell (1986b) however, found no evidence of autogeny in adult females reared from field collected larvae in Appin, NSW.

An earlier dispersal study near Echuca, VIC, demonstrated that *Cx. annulirostris* was capable of dispersing up to 7 km (Russell 1986d). Parity decreased with distance from the breeding site, showing that nullipars travelled further than parous females. Two later field studies in Griffith, NSW, by O'Donnell *et al.* (1992) and Bryan *et al.* (1992) demonstrated that *Cx. annulirostris* was able to disperse 8.7 and 12 km respectively. However, Bryan *et al.* (1992) did not detect any significant differences between parity rates of the recaptured females and a sample of the females at the release point.

Russell (1985a) observed swarming of *Cx. annulirostris* males at Appin, NSW between October 1979 and April 1980. Swarming commenced approximately 25 minutes before sunset and lasted 60 minutes after sunset. It also associated with markers such as low shrubs or bare patches of earth on grass-covered ground.

Culex annulirostris is a major pest of inland riverine areas in southeastern Australia during the summer months (Russell 1993). It is active at night in the early evening and in the pre-dawn period (Kay *et al.* 1981). Adults feed on cattle, rabbits, horses, cats, dogs, domestic birds, buffalo, sheep, mice, brush-tailed possum and humans (Kay *et al.* 1981). At Conjola State Forest, NSW, Russell (1987) demonstrated a seasonal shift in feeding behaviour from bird to mammal between January and February 1982, and February and March 1983.

2.5.3 *Human disease associations*

2.5.3.1 Arboviruses

Barmah Forest (BF) virus was first recovered from *Cx. annulirostris* from the Murray Valley region of VIC during the MVE epidemic of 1974 (Marshall *et al.* 1982). The virus has been isolated from *Cx. annulirostris* on the coast and inland regions of NT (Standfast *et al.* 1984, Whelan and Weir 1992) and in QLD from Brisbane, southeast and south inland regions (Doherty *et al.* 1979, Russell 1995). In NSW, BF virus has been recovered from the species in the west of the state (Russell 1995) and in WA from the north inland, northwest, and southwest regions (Lindsay *et al.* 1995).

Ross River (RR) virus has been isolated from *Cx. annulirostris* in northeast and northwest coastal and inland regions of the NT (Standfast *et al.* 1984, Whelan and

Weir 1992). From QLD, isolates were recovered from Brisbane and from the north and south inland regions (Doherty *et al.* 1971, Doherty *et al.* 1979, Ritchie *et al.* 1997). Isolates have been recovered in NSW on the north and central coasts, north and south inland regions and in several locations throughout the Murray Valley of NSW and VIC (Gard *et al.* 1973, Woodroofe 1976, Marshall *et al.* 1982, Russell 1986e, Russell *et al.* 1992, Russell *et al.* 1997, Doggett *et al.* 1999, Doggett *et al.* 2000). In WA, RR virus isolates were recovered from the species in coastal and north inland regions (Wright *et al.* 1981, Lindsay *et al.* 1992).

The first recorded isolation of Sindbis (SIN) virus from mosquitoes in Australia was in 1960 from *Cx. annulirostris* in Kowanyama (formerly Mitchell River Mission), north QLD (Doherty *et al.* 1963, Doherty 1972). Isolates have been reported from the northern inland regions of WA (Liehne *et al.* 1976, Liehne *et al.* 1981, Broom *et al.*1989, Broom *et al.* 1992), and from the southwest regions of WA (Lindsay *et al.* 1992). Isolates have also been recorded from the Darwin area and inland regions of the NT (Doherty *et al.* 1977, Whelan and Weir 1992). SIN virus has been recovered from the species in NSW from the north and south inland regions and in the Murray Valley region of NSW and VIC (Woodroofe 1976, Marshall *et al.* 1982, Russell 1986e, Russell *et al.* 1992, Russell *et al.* 1997, Doggett *et al.* 1999, Doggett et al. 2000).

Edge Hill virus was isolated from *Cx. annulirostris* for the first time at Edge Hill (Cairns) in northern QLD (Doherty *et al.* 1963) and later in north and south regions of QLD (Doherty *et al.* 1979). Isolates from NSW have been recorded from the northwest and the Murray Valley (Marshall *et al.* 1982, Russell *et al.* 1997, Doggett *et al.* 1999).

Gan Gan virus has been recovered from the species in south inland regions of QLD (Doherty 1977, Doherty *et al.* 1979), and north and south inland regions of NSW (Russell 1995, Russell *et al.* 1997).

Trubanaman virus was recovered from *Cx. annulirostris* for the first and only time in northern WA (Liehne *et al.* 1976).

Alfuy virus has been isolated from the species in the north inland region of WA (Russell 1995).

Kokobera virus was first recovered from *Cx. annulirostris* in 1960 and 1961 in Kowanyama, north QLD (Doherty *et al.* 1963). Later isolates were recovered in north and south regions of QLD (Doherty *et al.* 1968, Doherty *et al.* 1979) and the northwest and south inland (Murray Valley) regions of NSW (Woodroofe 1976, Russell *et al.* 1997, Doggett *et al.* 1999). Isolates from *Cx. annulirostris* have also been reported in north inland regions of WA (Liehne *et al.* 1981, Russell 1995) and in the NT (Standfast *et al.* 1984).

Kunjin virus was first recovered from *Cx. annulirostris* in 1960 in Kowanyama, north QLD (Doherty *et al.* 1963) and later in south QLD (Doherty *et al.* 1968, Doherty *et al.* 1979). It has been isolated from the species in NSW from north inland regions and in the Murray Valley of NSW and VIC (Marshall *et al.* 1982, Russell *et al.* 1992). It has also been isolated from the species in the north inland region of WA (Liehne *et al.* 1976, Liehne *et al.* 1981, Broome *et al.* 1989). In the NT, it has been isolated from the Darwin area and inland (Whelan and Weir 1992).

Murray Valley encephalitis (MVE) virus was first recovered from *Culex annulirostris* in Kowanyama, northern QLD, in 1960 (Doherty *et al.* 1963). Additional isolates from QLD were recovered from the northern and southern regions of the state (Doherty *et al.* 1979). Isolates have been recovered from *Cx. annulirostris* in the Murray Valley of NSW and VIC (Marshall *et al.* 1982). WA has reported many isolations of MVE virus from the north inland regions of the state (Liehne *et al* 1976, Liehne *et al.* 1981, Wright *et al.* 1981, Broom *et al.* 1989, Broom *et al.* 1992). NT also has a record of virus from the species (Whelan and Weir 1992).

2.5.3.2 Filariasis

The species has been found naturally infected with dog heartworm (*Dirofilaria immitis*), and laboratory studies have demonstrated that it is probably an important vector in the rural and peri-urban environment (Russell 1985b, Russell 1990).

2.6 *Culex australicus* Dobrotworsky and Drummond

Culex australicus was first described by Dobrotworsky and Drummond (1953). It is a member of the pipiens subgroup, which also includes *Cx. quinquefasciatus*, *Cx. globocoxitus* Dobrotworsky, and *Cx. molestus* Forskal in Australia (Sirivanakarn 1976). However, Belkin (1968) and Lee *et al.* (1989) point out that *Cx. molestus* in Australia may not be the *Cx. moletus* of Forskal.

2.6.1 Distribution

It is a native species distributed throughout mainland Australia and TAS except for hot, wet regions of the continent, north of 17°S (Marks 1965, 1982b). In VIC, it occurs in mountainous country with altitudes up to 5,000 feet as well as flat country (Dobrotworsky 1965).

2.6.2 Biology and ecology

Marks (1965) noted the following habitats for *Culex australicus* in QLD. The species occupies remote habitats as well as semi-domestic areas and occurs in sunlit or shaded water, although rarely in deep shade. It has been collected from springs, tea tree swamps, hoof prints at the edge of waterholes, lagoons and swiftly moving water draining from irrigated sugarcane crops. It can also rapidly colonise leafy or grassy rain-filled pools and wheel ruts.

In VIC, larvae has been found in great abundance in fresh-water swamps, brackish water, small to large pools, artificial containers and channels, but rarely in pools totally shaded. In the Mildura area, larvae may be found during the winter, however south of the Dividing Range, females hibernate in the winter (Dobrotworsky 1965). In WA, Britten (1958) regularly collected the species in vegetated freshwater pools and only occasionally in stagnant pools or saline water. Dobrotworsky (1965) considers the species eurygamous and heterodynamic.

It is found in association with *Cx. annulirostris* and *An. annulipes* s.l. in vegetated open pools, *Oc. sagax* and *Oc. theobaldi* in pools with no vegetation, *Oc. alboannulatus*, *Oc. notoscriptus* and *Oc. rubrithorax* in rock pools and *Oc. notoscriptus* and *Cx. quinquefasciatus* in containers (Russell 1993).

The species does not normally bite humans, preferring birds and rabbits, and therefore is unlikely to be of concern as a pest (Dobrotworsky 1965, Russell 1993). Precipitin tests have tested positive for rabbit, fowl, humans, cattle, sheep and dog (Lee *et al.* 1989). At Conjola State Forest, NSW, truck traps demonstrated that the species was most active during the two hours after sunset (Russell 1987).

2.6.3 Human disease associations

Although the species does not bite humans, it may be of significance in human disease as a vector in wildlife and maintenance or amplification cycles (Russell 1988).

2.6.3.1 Arboviruses

Ross River (RR) virus has been recovered from *Cx. australicus* in the Murray Valley (Russell 1986e) and from WA in the southwest and from the coast (Lindsay *et al.* 1992). Laboratory studies by Ryan and Kay (1997) demonstrated that *Cx. australicus* from the Sunshine Coast, QLD were able to carry the RR virus strain B94/20 but no transmission experiments were attempted.

Sindbis virus has been isolated from the species in the Murray Valley region of VIC and also from the north inland and south inland (Murray Valley) regions of NSW (Russell 1986e, Russell *et al.* 1992, Russell 1995).

Kunjin virus has been isolated from the species in the Murray Valley region (Marshall 1988).

Murray Valley encephalitis virus has been recovered from *Cx. australicus* in the Murray Valley of NSW (Marshall *et al.* 1982).

2.6.3.2 Filariasis

Culex australicus was found with natural infections of dog heartworm (*Dirofilaria immitis*) in NSW but was not considered an important vector (Russell 1985b).

2.7 *Culex quinquefasciatus* Say

Thomas Say (1823) was the first to describe the species, calling it *Culex 5-fasciatus*. Another name, *Cx. fatigans*, determined a few years later for the same species, became the widely accepted name, although earlier in the century Dyar and Knab (1909) proposed synonymy with *quinquefasciatus*. In an attempt to resolve the conflict surrounding the use of *fatigans* or *quinquefasciatus*, several authors reviewed the issue (Stone 1957, Belkin 1977) and determined that *quinquefasciatus* should be the name used for the species. An exhaustive list of regional synonyms is given by Lee *et al.* (1989).

2.7.1 Distribution

It is distributed throughout temperate, subtropical and tropical regions of the world (Lee *et al.* 1989). It is distributed in NT, WA, SA, QLD, NSW and VIC (however it is not permanently established south of the Central Highlands) although it is absent from TAS (Dobrotworsky 1966, Russell 1993).

2.7.2 Biology and ecology

It is a domestic breeding species, usually colonising polluted water in diverse artificial containers such as drains, sumps, gully-traps, vases, tins, tyres, septic tanks as well as natural containers such as tree holes and ground pools (Marks 1982b). According to

Dobrotworsky (1966), it is a stenogamous, anautogenus species and is homodynamous over much of its range except in VIC. Woodhill (1936) determined in laboratory experiments that larvae could complete their development in tap and distilled water, provided that fish food was available, but could not survive in seawater. In southeastern Australia, the species can be found in association with *Oc. notoscriptus* in clean water in containers (Russell 1993).

Culex quinquefasciatus is considered a major domestic pest in many urban regions (Russell 1993). Biting activity occurs during the middle of the night principally indoors, and the species is prevalent during the warmer months (Russell 1993). From precipitin tests, Lee *et al.* (1954) found that the species feeds on humans, horse, dog, cattle and fowl. Kay *et al.* (1979) used stable traps to collect mosquitoes for host-preference studies and by a hand-held aspirator from daytime resting sites to determine host-feeding patterns at Kowanyama in northern QLD and Charleville (Murray-Darling basin), QLD. Results showed that *Cx. quinquefasciatus* preferred humans and that no seasonal shifts were evident from the host-preference or host-feeding studies. The study also showed that *Cx. quinquefasciatus* fed on amphibians and reptiles.

2.7.3 Human disease associations

2.7.3.1 Arboviruses

Ross River virus was recovered from the species in the northwestern and north inland regions of WA (Lindsay *et al.* 1992, Broom *et al.* 1995), but Kay *et al.* (1982) have shown in laboratory studies that it is a poor vector of the virus.

Barmah Forest virus has been isolated from the species from the north inland regions of WA (Lindsay *et al.* 1994).

Sindbis virus has been isolated from the species in northern QLD (Doherty *et al.* 1973, Doherty *et al.* 1979), and from the north region of WA (Broome *et al.* 1989).

Kunjin virus has been isolated from the species in north QLD (Doherty *et al.* 1979), but Kay *et al.* (1982) have shown in laboratory studies that it is a poor vector of the virus.

Murray Valley encephalitis (MVE) virus was isolated from the species in the north region of WA (Liehne *et al.* 1981). Carley *et al.* (1973) demonstrated replication of MVE in *Cx. quinquefasciatus* from Brisbane although transmission was not attempted since it was relatively insensitive to experimental infection. Kay *et al.* (1982), using *Cx. quinquefasciatus* from various regions of QLD from northern VIC and from north coast of NT, found that the colonies were poorly susceptible and transmission to chickens was insignificant.

2.7.3.2 Filariasis

Early investigations of lymphatic filariasis by Walker (1924) determined that *Cx. quinquefasciatus* was an efficient host of *W. bancrofti* in QLD and later Heydon (1931) showed that filariae reached maturity in the species. However, Boreham and Marks (1986e) concluded that *Cx. quinquefasciatus* was not an efficient field vector since transmission ceased when contact was eliminated between infected persons and mosquitoes.

Culex quinquefasciatus has been found naturally infected with dog heartworm (*Dirofilaria immitis*), but further studies demonstrated that it was a vector of secondary importance (Russell 1985b, Russell 1990).

CHAPTER 3

SEASONAL ACTIVITY AND RELATIVE ABUNDANCE OF THE LOCAL MOSQUITOES

3.1 Introduction

The Homebush Bay area, located 16 km west of the central business district of Sydney, comprises approximately 760 hectares of land that includes saline and freshwater wetlands, mangrove forests, eucalypt woodlands, two major creeks, sporting facilities and high-density residential housing. At one time the largest estuarine complex in the Sydney Region (Clarke and Benson 1988), only small remnants of the wetlands remain today. Major areas of freshwater habitat were removed during remediation and development of the area and replaced with large artificial wetlands designed to manage water volume and quality and provide habitat for local and visiting fauna. There was concern that the design of the wetlands would favour production of important freshwater pest and vector mosquito species. Therefore, this investigation was designed to identify the major freshwater pest and vector species in the area, and monitor the temporal and spatial abundance of mosquitoes associated with the freshwater habitats so that appropriate management strategies could be determined.

3.2 Materials and methods

3.2.1 Historical land usage

Clarke and Benson (1988), Godden Mackay (1990), Anonymous (1996), the Australian Heritage Commission (1997) and Waste Service NSW (1999) have documented the changes that have occurred at the Homebush Bay area over the last 200 years, as briefly described below.

John Blaxland originally acquired a 522 hectare land grant along the Parramatta River in 1807 and named it 'Newington' after his family estate in Kent; he lived there until his death in 1845. Large tracts of forest vegetation were cleared for orchards and grazing during this time. In 1882, 352 hectares of land were resumed by the government for a powder magazine. After Federation in 1901, the magazine was taken over by the New South Wales Navy and subsequently by the Royal Australian Navy; this area encompassed 259 hectares of land.

In 1810, D'arcy Wentworth acquired a 370 hectare grant between Powells Creek and Haslams Creek, naming the property 'Home Bush'. Wentworth cleared extensive areas of forest vegetation and drained swamps during the mid 1820's. A new inlet called Wentworth Bay was created in 1891 by the construction of a sea wall along the northern perimeter of Newington and into Homebush Bay.

The NSW Government acquired 368 hectares of the Home Bush Estate for the establishment of the State Abattoir in 1907. By 1910, a rail line was constructed to link the Abattoir to the main trunk line and during this time the site was levelled and excavated. Located at the head of the bay, the State Brickworks was established in 1911, creating two large pits during its period of operation. Along Haslams Creek and Powells Creek, levee banks were constructed and in 1915, land was reclaimed for resting paddocks. The Homebush Abattoir and the State Brickworks were closed in 1988.

Tipping rights granted to private and local government waste operators, beginning in the early 1900's, allowed extensive areas of Homebush Bay to be used as a waste repository. In the 1950's, Wentworth Bay was reclaimed and by 1962, Haslams Creek and Powells Creek were straightened with bund walls, using industrial and domestic waste as fill. In preparation for site remediation in the mid 1990's, a major 2 year investigation of the landfill profile determined that at least 9 million cubic metres of landfill had been spread across 220 hectares of land.

In the mid to late 1980's, the Australia Centre, a privately owned business park, the State Sports Centre and Bicentennial Park were established as part of a long-term plan by the government to redevelop the Homebush Bay area. After Australia successfully won the bid to host the year 2000 Olympic Games, the decision was made to use the area for the development of major sporting facilities. The Olympic Co-ordination Authority (OCA), formed in 1995 to oversee development of the site in preparation for the Olympics, incorporated the NSW Government's concept of ecologically sustainable development (ESD) for the Homebush Bay area. Numerous ecological studies were commissioned by the Commonwealth Department of the Defence and the OCA over the next few years to evaluate the remaining ecosystems and to ensure their protection and augmentation (OCA 1997).

3.2.2 Study area

For the purpose of this investigation, the study area was divided into three sites during the first three seasons of surveillance (FIGURE 3.1). The sites consisted of Newington RANAD, the Olympic Development Site and Bicentennial Park. Site boundaries changed in Newington RANAD during the last season in preparation for final stages of development for the Olympics, with further subdivision into North Homebush Bay, the Residential Development Site and the Hill Road Freshwater Wetlands (FIGURE 3.2).

3.2.2.1 Bicentennial Park (BP)

This area contained extensive areas of mangrove habitat, saltmarsh and mudflats, a saline waterbird refuge, large permanent ponds, small freshwater wetlands, and parklands. It was bound by Bennelong Road to the west, and Australia Avenue to the south, and Homebush Bay Drive to the east.

3.2.2.2 Olympic Development Site (ODS)

During the four-year study period, this area underwent major changes with preparation and construction of the main venues for the Olympics as well as the Royal Agricultural Society Showgrounds. A network of small to medium sized ponds were constructed to provide refuge for the Green and Golden Bell Frog, *Litoria aurea*. A major creekline, Boundary Creek, was located at the southern end of the site, next to the States Sports Centre. The area was bounded by Hill Road to the west, the M4 to the south and Bennelong Road to the east.

3.2.2.3 Newington RANAD (N)

This area consisted of extensive saltmarsh, mudflats, mangrove habitats, eucalypt forest, freshwater marsh habitat and two small creeklines. After the second year, the freshwater marshes and creeklines were removed for redevelopment. From 1995 to 1998, the boundaries were Parramatta River to the north, Jamieson Street to the west, and the M4 motorway to the south and Hill Road to the east. During the last season, the area was subdivided as described below.

3.2.2.4 North Homebush Bay (NHB)

This area consisted of subdivided Newington RANAD land that was handed over by the Department of the Defence to NSW National Parks and Wildlife during 1998/1999. Included in this location were the extensive areas of saltmarsh and mangrove habitats and eucalypt woodland that subsequently became named Silverwater Nature Reserve. The area was bounded by Parramatta River to the north, Jamieson Street to the west, Holker Road to the south and Hill Road to the east.

3.2.2.5 Residential Development Site (RDS)

This location included Newington RANAD land that was subdivided in 1999 and handed over by the Department of the Defence to the OCA for the development of residential housing called Newington Village, and for the athlete's village. It was bounded by Holker Road to the north, Silverwater Industrial Road to the west, the M4 motorway to the south and the Hill Road Freshwater Wetlands to the east.

3.2.2.6 Hill Road Freshwater Wetlands (HR)

This location contained subdivided RANAD land handed over to the OCA for the development of artificial freshwater wetlands designed for stormwater control and water retention for irrigation of parklands. It was bounded by North Homebush Bay to the north, the Residential Development Site to the west, the M4 to the south and Hill Road to the east.

3.2.3 Weather

The Bureau of Meteorology provided daily rainfall and temperatures (maximum and minimum) during the four study periods. During the first year, 1995-1996, weather data from Observatory Hill, Sydney were used. Data from an all weather station located at the Olympic Stadium (site number 066195) were used for the remaining three years.

FIGURE 3.1 Map of Homebush Bay area for the 1995/1996 period with locations of adult trap sites shown in red, larval sites shown in blue and marsh habitats shown in green.

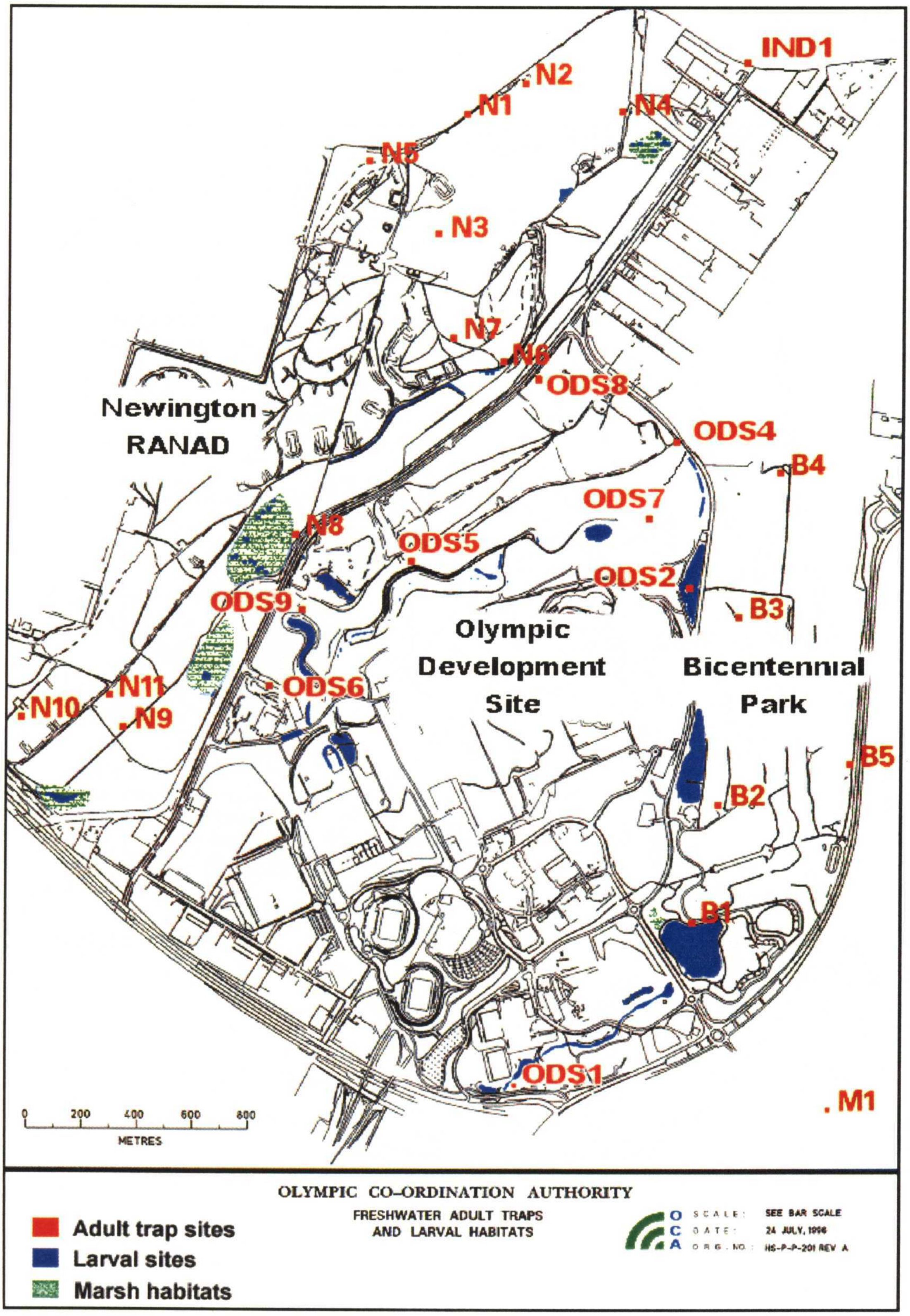
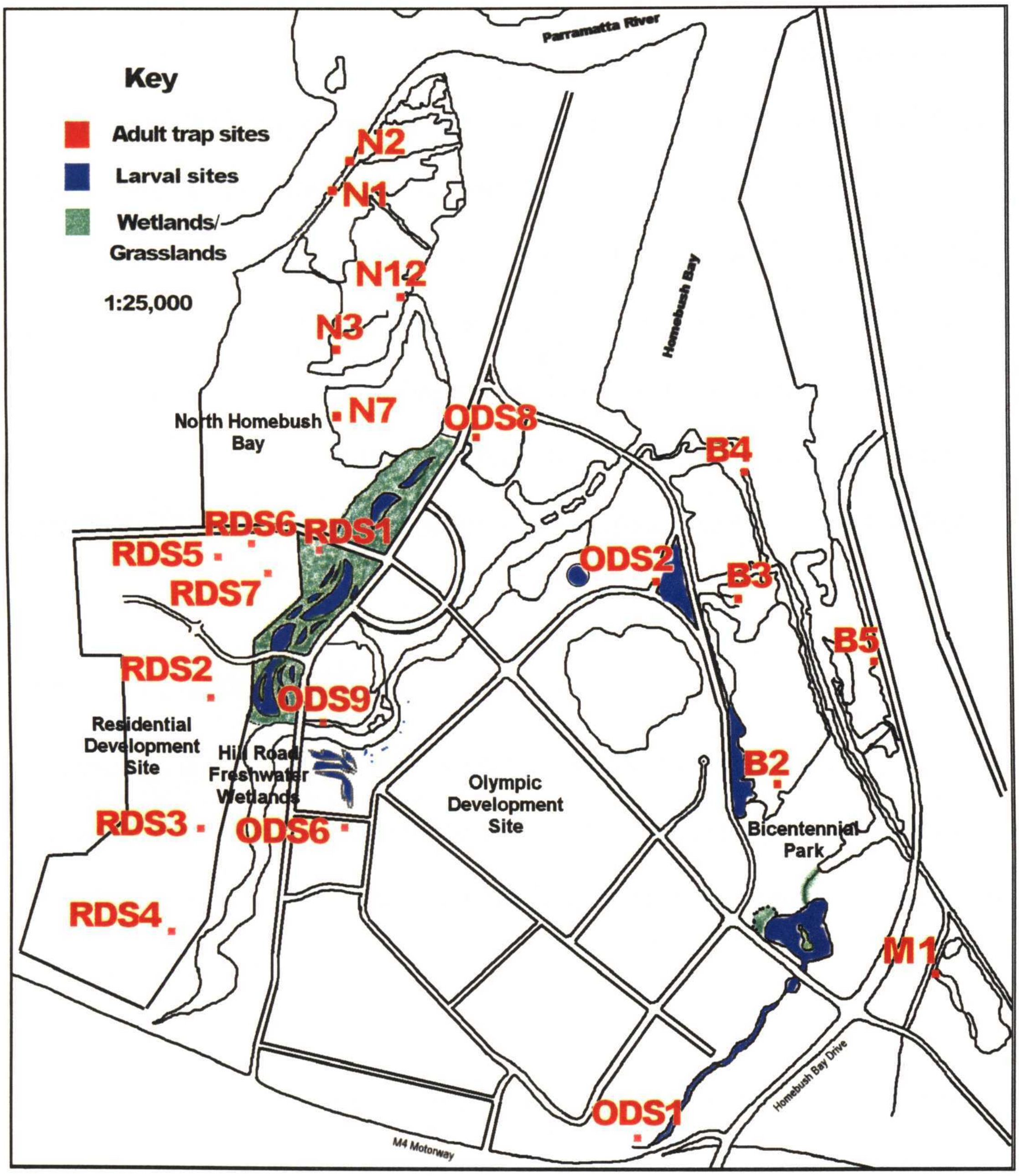


FIGURE 3.2 Map of Homebush Bay area for the 1998/1999 period with locations of adult trap sites shown in red, larval sites shown in blue and wetlands/grasslands shown in green.



3.2.4 Habitat types

Habitats in the area were classified as marsh habitats, large permanent ponds, creeks, constructed ponds and wetlands, man-made drainage systems and ephemeral pools. Some of the freshwater habitats were enduring features of the Homebush Bay area while others were created and/or destroyed due to development and construction during the time of the study. The descriptive words 'constructed' and 'man-made' were used to describe habitats that were created during the redevelopment of the area to provide refuge for native fauna or treating and/or receiving stormwater. Water bodies varied in size from small (< 100 m^2), medium (100 - 2000 m^2) to large (> 2000 m^2). Water depths were classified as shallow (< 15 cm), intermediate (15 cm - 35 cm), and deep (> 35 cm). Vegetation density was described as an estimation of the percentage vegetation cover in a one metre square quadrat. The categories were described as bare (no vegetation), sparse (< 30%), patchy (30% - 70%), and dense (>70%).

3.2.5 Larval surveillance

Quantitative sampling of larval populations was undertaken at weekly intervals using a standard dipping technique (Service 1993) of five random dips per one metre square sample point with a 300ml dipper. Larvae were identified using the taxonomic key of Russell (1993) and quantified.

Surveillance continued from October to June during the 1995/1996 and 1996/1997 seasons, October to May during the 1997/1998 season, and September to May during the 1998/1999 season. The locations of the sample sites during the first season and the last season are shown on FIGURES 3.1 and 3.2, respectively. The sample date, type of habitat (ie. marsh, small pond, ditch, etc.) and habitat variables such as size, water depth at the sample point, vegetation type and vegetation density were recorded at each site.

The influence of these variables on the presence or absence of *Cx. annulirostris* larvae was analysed by logistic regression analysis. Linear regression analyses (see APPENDIX for an explanation of logistic regression analysis) were then used to determine the independent predictors in habitats with larvae present. The program used to perform the analyses was the Statistical Package for Interactive Data Analysis (SPIDA), Version 6. Data from the 1995/1996 season (pre-development) and the 1998/1999 season (post-development) were used in the analysis.

3.2.6 Adult surveillance

In the Homebush Bay area, dry ice baited Encephalitis Virus Surveillance (EVS) light traps (Rohe and Fall 1979) were used to survey adult mosquito populations. They were set out weekly from July 1995 to June 1996, October 1996 to June 1997, November 1997 to June 1998, and September 1998 to June 1999. TABLE 3.1 shows the year of operation of each of the adult mosquito trap sites at Homebush Bay. Adults were identified using the taxonomic key of Russell (1993) and data were recorded by date, location and species. FIGURE 3.1 shows the trap locations during the first year of the study and FIGURE 3.2 shows the trap locations during the last year. The traps were retained at fixed locations each year, however some trap locations were discontinued due to construction interference or because they were no longer considered valuable for surveillance purposes. Two traps were set out at the extreme boundaries of the study site to determine how far adults were dispersing, but they were not operated every year of the study. One trap (IND1) was set out on the north side of Homebush Bay, next to Parramatta River and a second trap (M1) was set out in Mason Park. Core trap sites (four in Bicentennial Park and four in Newington) were retained throughout the four year study period to allow for comparison of the mean mosquito abundances between the years by single factor analysis of variance (ANOVA). Over a four year period, using core trap data, weekly trap collections of *Cx. annulirostris* were pooled to generate mean weekly abundance values. These mean values were regressed on average minimum and maximum temperature and on total rainfall using autoregressive time series analysis. Cross correlation plots of first differences between the mean abundance data and minimum and maximum temperature and rainfall were used to identify any lagged associations (data not shown). The software program used was SPSS for Windows, Version 10.0.

TABLE 3.1 Fixed trap sites and year of operation at Homebush Bay.

Trap Site	1995/1996	1996/1997	1997/1998	1998/1999
N1	•	•	•	•
N2	•	•	•	•
N3	•	•	•	•
N4	•	•		
N5	•			
N6	•			
N7	•	•	•	•
N8	•	•		
N9	•	•		
N10	•	•		
N11	•	•		
N12		•	•	•
ODS1	•	•	•	•
ODS2	•	•	•	•
ODS4	•	•	•	
ODS5	•			
ODS6	•	•	•	•
ODS7	•	•	•	
ODS8	•	•	•	•
ODS9	•	•	•	•
IND1	•			
B1	•	•	•	
B2	•	•	•	•
B3	•	•	•	•
B4	•	•	•	•
B5	•	•	•	•
M1	•		•	•
RDS1				•
RDS2				•
RDS3				•
RDS4				•
RDS5				•
RDS6				•
RDS7				•
Total	26	22	18	22

3.2.7 *Arbovirus surveillance*

Arbovirus surveillance was undertaken from 1996 until 1999. From December 1996 to May 1997 and December 1997 until April 1998, mosquitoes from three trap sites in Newington RANAD and one site in Bicentennial Park were tested for arbovirus infection. During the last year, three trap sites in North Homebush Bay were used for arbovirus surveillance from January 1999 until April 1999 and two additional trap sites in Bicentennial Park were used from March 1999 until April 1999 to provide larger numbers for testing.

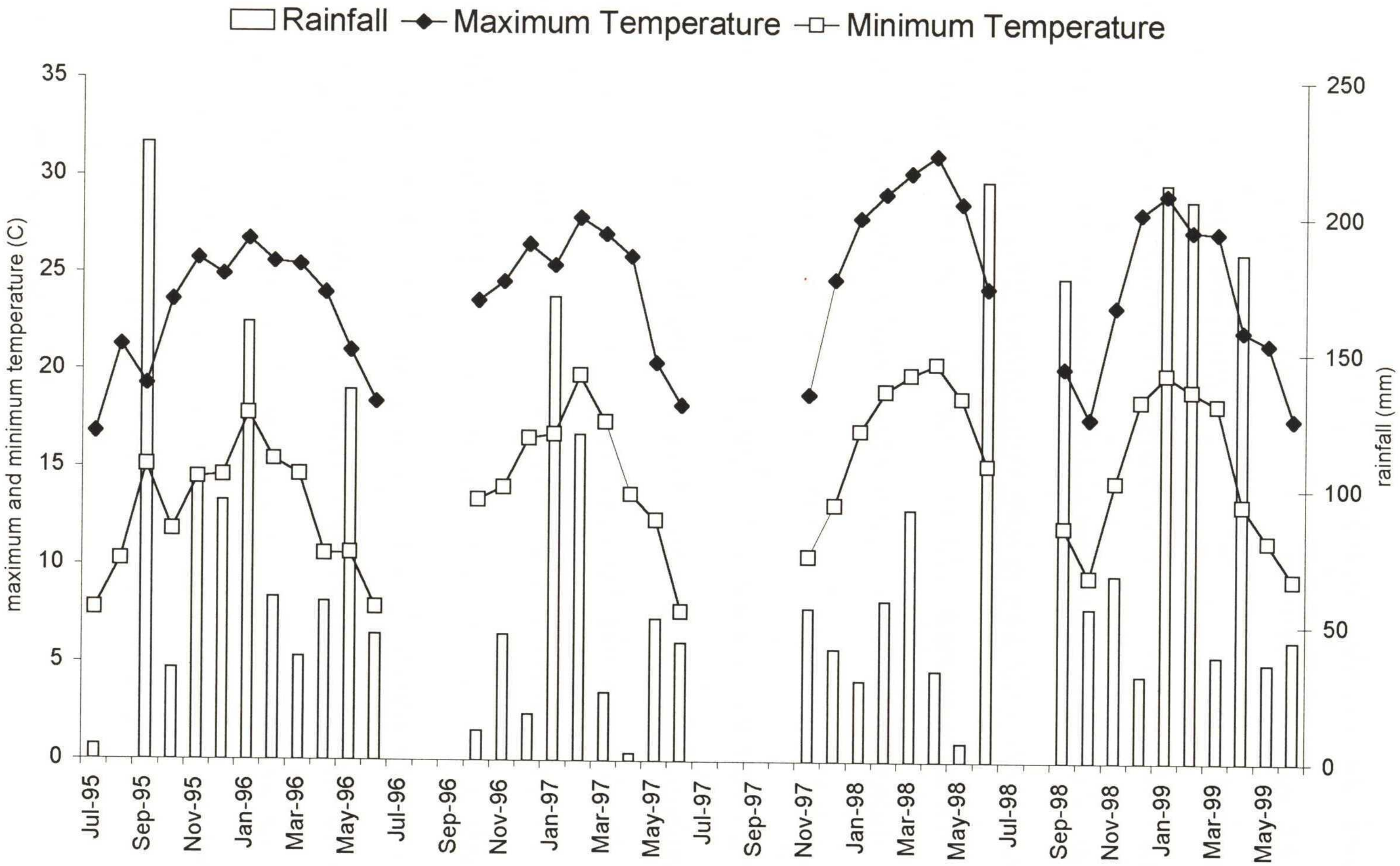
Adults were collected weekly as described for adult surveillance with the exception of using collection buckets instead of nets on the EVS traps to provide for greater survival of the mosquitoes and viability of the virus. The mosquitoes were sorted in pools of 25 by trap site, species, sex and whether or not blood-fed. Mosquitoes were then tested by the Arbovirus Research Section of the Department of Medical Entomology at Westmead Hospital using procedures outlined in Russell *et al.* (1997).

3.3 Results

3.3.1 *Weather*

The mean maximum and minimum temperature and the total rainfall for each month of the four year period are shown on FIGURE 3.3. The amount of rainfall from December to March, the period of peak abundance of *Cx. annulirostris*, was greatest during 1998/1999 season and least during the 1997/1998. The highest maximum and minimum temperatures also occurred during the 1997/98 season. The first season, 1995/1996, had the coolest maximum and minimum temperatures.

FIGURE 3.3 The mean maximum and minimum temperature and total rainfall for each month of the 1995 -1999 surveillance period.



3.3.2 Habitat types

3.3.2.1 Marsh habitats

Marsh habitats were located in low-lying flood plains of Haslams Creek, along the edges of the saltmarsh and in the northeast corner of the former Newington RANAD in a landfill area. All marsh habitats had dense vegetation, shallow to intermediate depth, and were small to medium in size. These areas developed over time with an accumulation of water from freshwater runoff or seepage from land higher up and were the most productive larval habitats while they existed. Generally, vegetation such as *Bolboschoenus caldwellii*, *Phragmites australis, Schoenoplectus validus* or *Typha* sp. formed dense stands within marshy areas. Submerged or partially erect vegetation such as *Alternanthera philoxeroides*, *Paspalum paspalodes* and *Persicaria decipiens* formed dense mats of floating vegetation. Marsh habitats attracted a diverse mosquito fauna (TABLE 3.2). Species such as *Oc. alboannulatus*, *Cx. annulirostris, Cx. australicus* and *Cx. quinquefasciatus* readily colonised these areas. *Coquillettidia linealis* is commonly associated with marginal reeds in permanent water bodies (Russell 1993) and most likely occurred in some areas but larvae were not detected with the larval survey technique. Minor species such as *Cx. bitaeniorhynchus*, *Cx. halifaxii* Theobald and *Cx. orbostiensis* Dobrotworsky colonised marsh habitats from time to time. The saltmarsh species *Oc. vigilax* and *Cx. sitiens* were occasionally detected when adult numbers were elevated. All of these sites were removed after the first two years of surveillance to make way for redevelopment of the area.

3.3.2.2 Large permanent ponds

Several large, deep ponds in Newington, the ODS and Bicentennial Park, were established many years before the remediation works began. All but one contained the larvivorous fish, *G. holbrooki* and no larvae were detected in sites with this fish. The solitary pond without fish was destroyed after the first year, however, minor densities of *An. annulipes* s.l., *Cx. annulirostris, Cx. australicus* and *Cx. quinquefasciatus* were detected breeding amongst fringe vegetation (TABLE 3.2).

3.3.2.3 Creeks

Prior to 1993, Boundary Creek, located south of the States Sports Centre, was a concrete lined channel that flowed through a landfill area (Waste Service 1999). By 1993, the creek was diverted and concrete channels replaced with a series of pools and riffles with gabion retaining walls. Patchy stands of *Typha* sp. developed at each bend of the creek. The depth of the creek varied from intermediate to deep depending on rainfall. Small numbers of *An. annulipes* s.l., *Cx. australicus* and *Cx. annulirostris* were detected on occasion during the first year of the survey within the *Typha* sp. stands (TABLE 3.2). *Gambusia holbrooki,* became abundant in Boundary Creek during the second, third and fourth year of the survey and consequently, larvae were no longer detected. In Newington, narrow creeklines constructed prior to redevelopment drained freshwater runoff from the Haslams Creek floodplain. They were fringed with dense *Typha* sp. growth and contained *G. holbrooki*. The depth ranged from shallow to deep with water remaining in the deep end year round. After periods of low rainfall, shallow sections of these creeklines were reduced to isolated pools. *An. annulipes* s.l., *Cx. australicus, Cx. annulirostris* and *Cx. quinquefasciatus* were detected on occasion in isolated pools with no fish (TABLE 3.2).

3.3.2.4 Constructed ponds

Numerous ponds and wetlands were created during the development period to receive and treat stormwater runoff as well as to provide refuge for native fauna. The most diverse mosquito fauna was detected in the constructed ponds (TABLE 3.2). Small ponds (<100 m^2), within the ODS, were created specifically for the Green and Golden Bell Frog, and were designed with a shallow, gently sloping end and a steep, deeper end. Due to their small size, these ponds would regularly dry out during the hotter months and on occasion, landscaping contractors would top up the ponds with recycled water. In this same area, medium ponds (100 m^2 - 2000 m^2) and large ponds (> 2000 m^2), retained water all or most of the year, depending on their size. Native emergent vegetation, such as *S. validus*, *Carex petrie* and *Gahnia sieberana,* was transplanted around the perimeter of ponds shortly after creation, and by the next growing season, patchy to dense vegetation was well established, sometimes with vegetation growing throughout the centre of small and medium ponds. Weed species (ie. species not planted) such as *Cyperus difformis*, *Ludwigia peploides montevidensis*, *Myriophyllum verrucosum*, *Paspalum paspalodes*, *Persicaria decipiens*, *Potamogeton crispus*, *Triglochin striata* and *Typha* spp. occasionally colonised edge habitats or grew throughout the centre of shallow ponds. These plant species were associated with increased larval densities.

Ochlerotatus alboannulatus was the first species to colonise small frog ponds while they were bare. After the establishment of vegetation, *An. annulipes* s.l., *Cx. annulirostris, Cx. australicus*, and on occasion, *Cx. quinquefasciatus* were detected. Minor species such as *Ad. venustipes* (Skuse), *Cx. bitaeniorhynchus*, *Cx. halifaxii*, *Cx. orbostiensis* and *Ur. pygmaea* Theobald were also detected on occasion. The saltmarsh species, *Oc. vigilax,* occasionally colonised frog ponds when adult numbers were high.

A one hectare water treatment wetland was constructed within the ODS and was designed with a shallow perimeter and a deep, open centre. Monostands of *Bolboschoenus fluviatilis*, *Cyperus exaltatus*, *Eleocharis sphacelata*, *S. validus* and *P. australis* were transplanted around the perimeter, providing refuge for frog and bird fauna. After establishment of dense vegetation, the site supported *An. annulipes* s.l., *Cx. annulirostris*, and the minor species *Cx. bitaeniorhynchus* and *Cx. squamosus* (Taylor) (TABLE 3.2).

The extensive 20 hectare Hill Road freshwater wetland was developed during the final year of the study period. After transplantation of thousands of native wetland plants, the area became quickly established with dense vegetation. The dominant wetland plants were *Baumea articulata*, *B. caldwellii*, *B. fluviatilis*, *C. exaltatus*, *S. mucronatus* and *S. validus*. The area finally comprised 27 medium sized ponds, no deeper than 50cm in the centre with gently sloping banks, three large deep ponds used for irrigation and a large feature pond with shallow edges. No larvae were detected in the large ponds that supported *G. holbrooki*. *Anopheles annulipes* s.l. and *Cx. annulirostris* were consistently detected along the perimeter of the smaller ponds. However, after heavy rains in February 1999, larger ponds that supported *G. holbrooki* overflowed into some of the smaller adjacent ponds. After this event, *G. holbrooki* became established in these ponds and consequently eliminated larval populations.

3.3.2.5 Man-made drainage systems

Prior to redevelopment, drainage systems on site consisted of isolated shallow earthen lined ditches with culverts. Later, interconnecting drainage systems were constructed with pebble or boulder-lined water retention areas as well as earthen lined ditches with culverts. There was one gross pollutant trap (GPT), south of the States Sports Centre. Interconnecting drainage systems downstream of stormwater retention basins usually contained *G. holbrooki*. Overall, *Oc. alboannulatus*, *An. annulipes* s.l., *Cx. annulirostris*, *Cx. australicus* and *Culex quinquefasciatus* were detected in sites without fish (TABLE 3.2). Minor densities of *Ad. venustipes*, *Cx. halifaxii* and *Cx. sitiens* were also detected.

3.3.2.6 Ephemeral pools

Small, shallow ephemeral pools would form on occasion after abundant rainfall, lasting a few days to several weeks at a time. Site restrictions prevented surveillance of ephemeral pools in the former Newington RANAD after February 1997. Ephemeral pools in the Olympic Development Site or Hill Road Wetlands were sampled from 1995 to 1999. Many of these pools formed over terrestrial vegetation or, if they were the result of construction, were earthen-lined. *Ochlerotatus alboannulatus*, *Oc. procax* (Skuse), *An. annulipes* s.l., *Cx. annulirostris*, and *Cx. australicus* were usually detected (TABLE 3.2). *Aedeomyia venustipes* and *Cx. halifaxii* were detected in low densities.

TABLE 3.2 Larval species of mosquitoes recorded from the various freshwater habitats at Homebush Bay.

Species	Marsh habitats	Large, deep ponds	Creeks	Constructed ponds	Drainage systems	Ephemeral pools
Ad. venustipes				●	●	
Oc. alboannulatus	●			●	●	●
Oc. alternans	●					
Oc. procax						●
Oc. vigilax	●			●		
An. annulipes s.l.	●	●	●	●	●	●
Cx. annulirostris	●	●	●	●	●	●
Cx. australicus	●	●	●	●	●	●
Cx. bitaeniorhynchus	●			●		
Cx. halifaxii	●			●	●	●
Cx. orbostiensis	●			●		
Cx. quinquefasciatus	●	●	●	●	●	
Cx. sitiens	●				●	
Cx. squamosus				●		
Ur. pygmaea				●		

3.3.3 Larval mosquito abundance

A total of 16 species was collected as larvae during the four year surveillance period (TABLE 3.3). Twelve of the 16 were considered freshwater species. An additional 14 species, *Ochlerotatus australis* (Erichson), *Oc. notoscriptus, Oc. eidsvoldensis* Mackerras, *Oc. flavifrons* (Skuse), *Oc. mallochi* Taylor, *Oc.* nr. *monocellatus* Marks, *Oc. multiplex* (Theobald), *Oc. rubrithorax* (Macquart), *Oc. vittiger* (Skuse), *An. atratipes* Skuse, *Cq. linealis, Cx. molestus* Forskal, *Cx. postspiraculosus* Lee and, *Ma. uniformis* (Theobald) were collected in adult surveys but not in larval collections (TABLE 3.3).

In general, dip densities of the major species from each area were low throughout the four year surveillance period (TABLE 3.4). Dip densities could not be compared between the years since sites were destroyed and/or created each year of the study.

3.3.3.1 1995/1996

Culex annulirostris and *Cx. australicus* were the most abundant species sampled in BP and N the first season. The most productive sites were marsh sites and vegetated predevelopment ponds. Low densities of *Oc. alboannulatus, Cx. annulirostris, Cx. australicus* and *Cx. quinquefasciatus* were detected in the frog ponds located in the ODS.

3.3.3.2 1996/1997

In the second season, *Cx australicus* was the most abundant species detected in BP and N. By February and March, site restrictions in N prevented larval surveillance within the most productive sites of the area, artificially lowering the dip densities of *Cx. annulirostris*. In BP, shallow wetlands produced low densities of Cx. *australicus* in autumn and early spring but by summer, all sites had dried out and remained so for the rest of the season.

3.3.3.3 1997/1998

The third season was very dry, resulting in reduced larval densities throughout the area. However, in the ODS, a large permanent water quality pond produced minor breeding of *Cx. annulirostris* along the shallow, vegetated edges. Also, additional drainage systems were established throughout the ODS, resulting in an increase in *Cx. quinquefasciatus* larval densities in that area. Access to freshwater sites in N was restricted for the entire season as the area continued to be under redevelopment. Shallow habitats in BP remained dry for most of the season, however when they were inundated, numerous larvivorous fish were detected and there were few larvae.

3.3.3.4 1998/1999

In the fourth year, *Cx. australicus*, most abundant in the spring, was the most common species detected as larvae in BP. In the summer months, habitats were dry or harboured *G. holbrooki*. *Culex annulirostris* was detected in the shallow ponds of the HR wetlands between November and January. Heavy rains in February flooded the larger ponds, resulting in the dispersal of *G. holbrooki* into the larval habitats and the eventual reduction in mosquito larvae. In the ODS, shallow habitats remained dry during the summer. Minor breeding of *Cx. annulirostris* occurred in the ODS treatment wetland. Densities of the remaining species were negligible throughout the area.

A typical seasonal distribution of the major freshwater species from all habitats at Homebush Bay is shown in FIGURE 3.4 using data from the 1995/1996 season.

Ochlerotatus alboannulatus was present all year with small peaks in spring and autumn, however densities fluctuated as habitats dried. *Anopheles annulipes* s.l. was detected all year round in larval surveys at low densities. *Culex annulirostris* was usually first detected in late spring with densities rising to a peak in the summer months and declining in autumn. *Culex australicus* densities peaked in the spring and gradually declined for the remainder of the season. *Culex quinquefasciatus* occurred through all season except in February when habitats dried out after periods of low rainfall.

Results of logistic regression analysis to determine which habitat variables were significant predictors are shown in TABLE 3.5. In the first year, the odds of finding a larva increased by an average multiplicative factor of 2.96 for each increase in vegetation density ($P<0.001$). Compared to dense vegetation, the odds of finding a larva in patchy vegetation were only 0.64, and the odds of finding a larva in sparse or bare vegetation just 0.22 and 0 respectively.

Using generalised estimating equations, it was found that in habitats with larvae, dense vegetation was the only independent predictor ($P=0.04$) with an increase of 5.3 larvae per dip compared to sparse vegetation. There was no significant difference between patchy and sparse vegetation. At the univariate level, depth was an independent predictor with less larvae in the deep habitats compared to the shallow habitats ($P=0.05$). However, when used in the multivariate model, depth was no longer statistically significant.

The last year, the only independent predictor was sample week. The odds of finding a larva decreased by a factor of .85 (P=0.003) as each week progressed (less likely to find larvae in autumn). Considering habitats that had larvae, size was the only independent predictor (P=0.003) with density increasing by an average of 0.73 larvae per dip for each categorical increase in size. At the univariate level, there was a tendency for larval density to decrease as depth increased but the difference just failed to reach statistical significance (P=0.06).

TABLE 3.3 The presence (1) and absence (0) of mosquito species collected as larvae (L) and adults (A) during the four year surveillance period at Homebush Bay.

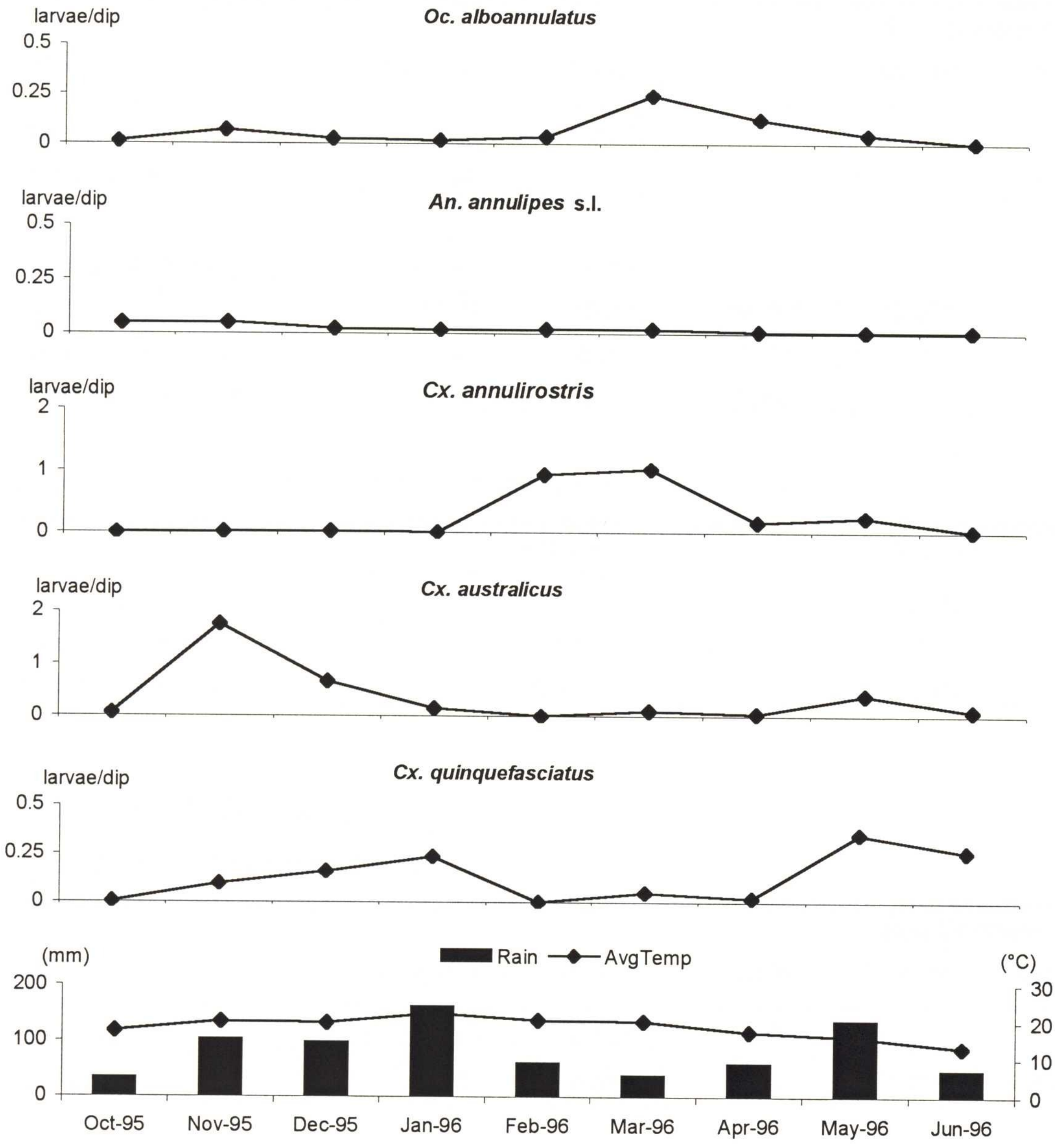
	1995/1996		1996/1997		1997/1998		1998/1999	
Species	L	A	L	A	L	A	L	A
Aedeomyia venustipes	0	0	1	1	1	1	0	0
Ochlerotatus alboannulatus	1	1	1	1	1	1	1	1
Oc. alternans	1	1	1	1	1	1	1	1
Oc. australis	0	1	0	1	0	0	0	0
Oc. camptorhynchus	0	1	0	1	0	1	1	1
Oc. eidsvoldensis	0	0	0	0	0	0	0	1
Oc. flavifrons	0	1	0	1	0	0	0	1
Oc. mallochi	0	1	0	1	0	1	0	0
Oc. nr. *monocellatus*[1]	0	0	0	0	0	0	0	1
Oc. multiplex	0	0	0	0	0	1	0	0
Oc. notoscriptus	0	1	0	1	0	1	0	1
Oc. procax	1	1	0	1	0	1	0	1
Oc. rubrithorax	0	1	0	1	0	0	0	0
Oc. vigilax	1	1	1	1	1	1	1	1
Oc. vittiger	0	1	0	1	0	1	0	0
Anopheles annulipes s.l.	1	1	1	1	1	1	1	1
An. atratipes	0	1	0	0	0	0	0	0
Coquillettidia linealis	0	1	0	1	0	1	0	1
Culex annulirostris	1	1	1	1	1	1	1	1
Cx. australicus	1	1	1	1	1	1	1	1
Cx. bitaeniorhynchus	1	1	0	1	1	0	1	1
Cx. halifaxii	1	1	1	1	1	1	0	0
Cx. molestus	0	1	0	1	0	1	0	1
Cx. orbostiensis	1	1	0	1	0	1	1	1
Cx. postspiraculosus	0	0	0	0	0	0	0	1
Cx. quinquefasciatus	1	1	1	1	1	1	1	1
Cx. sitiens	1	1	1	1	1	1	1	1
Cx. squamosus	0	0	0	0	0	0	1	0
Mansonia uniformis	0	1	0	1	0	1	0	1
Uranotaenia pygmaea	0	0	0	0	0	0	1	0

[1] Collected during the surveillance period but not as a part of routine surveillance.

TABLE 3.4 Larval dip densities of major species from Bicentennial Park (BP), Newington (N), the Olympic Development Site (ODS) and Hill Road Wetlands (HR) for each year of the investigation at Homebush Bay.

	1995/1996			1996/1997			1997/1998		1998/1999		
	BP	N	ODS	BP	N	ODS	BP	ODS	BP	ODS	HR
Oc. alboannulatus	<0.1	<0.1	0.1	0.1	0.1	<0.1	0	<0.1	<0.1	<0.1	0
An. annulipes s.l.	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Cx. annulirostris	0.2	1.0	0.1	0.1	<0.1	0.1	0.1	0.3	<0.1	<0.1	0.3
Cx. australicus	0.5	0.9	0.1	0.9	1.8	0.1	<0.1	<0.1	0.3	<0.1	0
Cx. quinquefasciatus	<0.1	0.2	0.1	0.1	0.2	0.1	0	0.9	<0.1	<0.1	0

FIGURE 3.4 Seasonal distribution of the major freshwater species at Homebush Bay using dip densities (larvae/dip) from all habitats during the first year, 1995/1996, including monthly rainfall and average temperature. Note variable Y-axis.



(mm) = millimetres rainfall; (°C) = Celcius

TABLE 3.5 Results of logistic regression analysis and generalised estimating equations to determine which habitat variables were significant predictors of larval density.

1995/1996 - Pre-development

Habitats likely to contain larvae

Name	Coefficient	P-value	Odds	95% Confidence Lower	95% Confidence Upper
Constant (No vegetation)	-4.39	<0.001			
Vegetation	1.08	<0.001	2.96	2.03	4.31

Constant (Dense veg.)	-0.32	0.30			
Bare	-18.61	<0.001	<0.001	<0.001	
Sparse vegetation	-1.53	0.001	0.22	0.09	0.52
Patchy vegetation	-0.45	0.31	0.64	0.27	1.52

Significant predictors in ponds with larvae

	Coefficient	P-value
Constant (Sparse veg.)	0.54	<0.001
Patchy vegetation	0.23	0.59
Dense vegetation	5.32	0.04

1998/1999 - Post-development

Habitats likely to contain larvae

Name	Coefficient	P-value	Odds	95% Confidence Lower	95% Confidence Upper
Constant	2.17	0.104			
Week	-0.16	0.003	0.85	0.77	0.95

Significant predictors in ponds with larvae

Name	Coefficient	P-value
Constant	-0.60	0.15
Size	0.73	0.003

3.3.4 Adult mosquito abundance

A total of 347,989 freshwater and saltmarsh adult mosquitoes were collected in all traps during the four year study period, comprising six genera and 28 species (TABLE 3.6). New species records for Homebush Bay were established for *An. atratipes* in 1995/1996, *Ad. venustipes* in 1996/1997, *Oc. mallochi* in 1997/1998 and *Oc. eidsvoldensis*, *Oc.* nr. *monocellatus*, and *Cx. postspiraculosus* in 1998/1999. The saltmarsh species, *Oc. vigilax* (51.7 %) was the most abundant species collected over the four-year period, with *Cx. sitiens* (12.4 %) the next most common species. *Culex annulirostris* was the most abundant freshwater species amongst the total mosquito fauna (10.5 %), followed by *Cx. quinquefasciatus* (9.7 %), *Cx. australicus* (5.7 %), *Oc. notoscriptus* (2.2 %), *An. annulipes* s.l. (2.0 %), *Cq. linealis* (1.0 %) and *Oc. alboannulatus* (0.2 %). Proportionally, *Cx. annulirostris* was the most abundant freshwater species collected in Newington for each year of the study. In Bicentennial Park, *Cx. annulirostris* was the most abundant except for the second year when *Cx. quinquefasciatus* dominated the trap catch. In the Olympic Development Site, *Cx. annulirostris* dominated the trap catch in the first and fourth year while *Cx. quinquefasciatus* was most abundant in the second and third year. Although the Industrial area (1995/1996) and the Residential Development Site (1998/1999) traps were operated for only one season, *Cx. quinquefasciatus* was the most abundant freshwater species collected in these traps. In Mason Park, the dominant species changed from *Cx. annulirostris* in the first year, to *Cx. quinquefasciatus* in the third year and *Cx. australicus* in the fourth year.

The proportion of mosquitoes represented by a particular species could not be directly compared between years using the total trap catch since the number of traps varied.

For this reason, core trap sites (four in the former Newington RANAD and four in Bicentennial Park) were used to compare between the years. Plots of the monthly abundance of the seven major freshwater species collected from July 1995 to June 1999 are shown in FIGURE 3.5 along with rainfall data. The mean monthly abundances with significant differences between the years are shown in TABLE 3.7.

Low numbers of *Oc. alboannulatus, Oc. notoscriptus* and *An. annulipes* s.l. were collected every month (July to June the first year) during the four year period. Significantly fewer of these species were collected in the third year compared to other years.

Adults of *Cq. linealis* were collected from October to June during the first year with a peak in January. The species showed a similar tendency the second year but with significantly decreasing numbers over the third and fourth seasons.

Culex annulirostris was recorded generally from October to June each year with abundance peaks in January or February, except for the 1996/1997 season, when numbers peaked in March. Fewer numbers were collected in the third year but the difference was not significant.

Adults of *Cx. australicus* were collected every month of the first year, with a peak in October and December the first year, a peak in February the second year, and a peak in October the last year. Significantly less were collected in the third year. *Culex quinquefasciatus* was collected every month of the four-year period with population peaks in the summer months. Relative abundance of this species significantly decreased in the last year of the study.

Results of time series analysis of the observed abundance of *Cx. annulirostris* adults showed that weekly rainfall from the third week prior (RainLag3) and the mosquito abundance from the previous week (MPTNLag1) significantly predicted mosquito numbers. The summary of the analysis and resulting equation is shown in TABLE 3.8. The plot of the observed and predicted abundance from the 1995-1999 core trap data is shown on FIGURE 3.6.

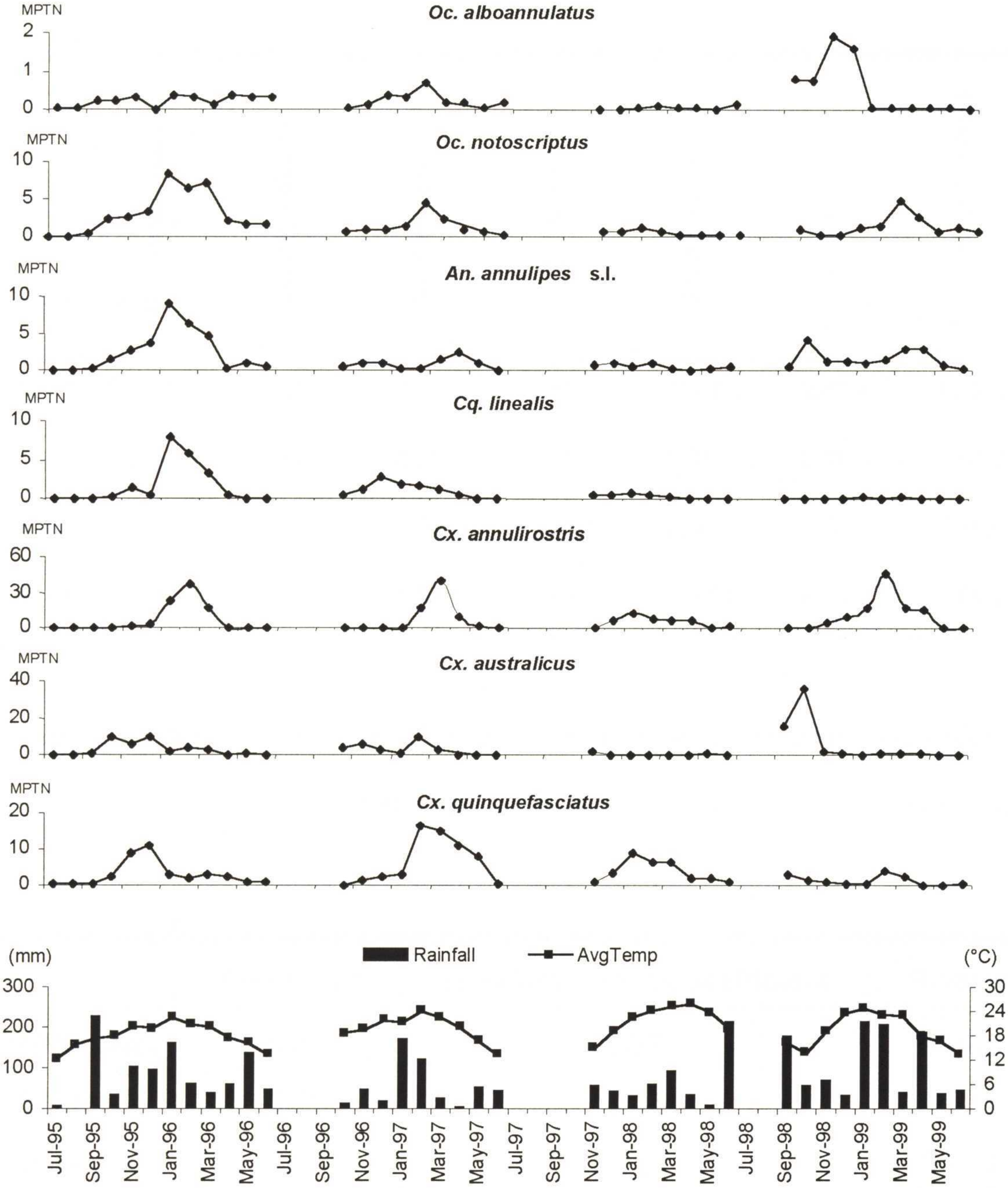
TABLE 3.6 Annual activity of the mosquito species (percentage of the total adults) collected from 1995 to 1999 by area at Homebush Bay, NSW.

	BP				ODS				N (*NHB)				MP			Industrial	RDS
	95-96	96-97	97-98	98-99	95-96	96-97	97-98	98-99	95-96	96-97	97-98	98-99*	95-96	97-98	98-99	95-96	98-99
Trap sites per area	5	5	5	4	8	7	7	5	11	10	5	5	1	1	1	1	7
Species																	
Ad. venustipes			<0.1			<0.1	<0.1				<0.1						
Oc. alboannulatus	0.3	0.2	<0.1	0.1	0.1	<0.1		0.2	0.5	0.3	<0.1	0.7	0.2		0.2	0.1	0.1
Oc. alternans	4.1	0.6	0.4	3.3	3.1	0.3	0.5	0.9	1.5	0.4	0.1	0.6	4.9	2.1	3.5	1.6	0.2
Oc. australis						<0.1			<0.1								
Oc. camptorhynchus	0.3	0.6	<0.1	0.6	<0.1	0.3	0.1	0.5	0.3	0.9	0.1	1.0	0.2	<0.1	0.3		0.2
Oc. eidsvoldensis				<0.1				<0.1				<0.1			0.1		
Oc. flavifrons		<0.1			<0.1	<0.1		<0.1	<0.1			0.1					
Oc. mallochi											<0.1						
Oc. nr. monocellatus[1]												<0.1					
Oc. multiplex	<0.1				<0.1		<0.1			<0.1							
Oc. notoscriptus	3.4	1.4	0.5	2.1	2.8	2.0	0.5	2.9	3.2	0.9	0.1	1.0	0.5	0.8	3.3	11.5	0.6
Oc. procax	<0.1	<0.1		<0.1	0.1	<0.1			0.3	0.1	<0.1	0.3				0.1	
Oc. rubrithorax	0.1									<0.1							
Oc. vigilax	65.2	72.2	81.7	41.4	29.5	50.3	77.5	31.6	36.5	55.1	86.3	35.4	48.8	90.8	56.1	2.7	18.6
Oc. vittiger	0.0				<0.1		<0.1		0.1	<0.1	<0.1						
An. annulipes	2.7	0.5	0.1	1.6	2.8	0.3	<0.1	2.8	9.3	2.4	0.2	2.6	3.1	<0.1	0.7	0.2	0.7
An. atratipes					<0.1												
Cq. linealis	1.2	0.7	0.3	0.1	1.8	0.6	0.1	0.2	6.8	2.9	0.1	0.1		0.1		0.3	0.0
Cx. annulirostris	11.0	4.4	2.1	8.2	30.7	7.8	1.8	15.7	19.6	12.2	2.1	13.5	18.0	0.9	9.1	3.9	17.4
Cx. australicus	3.2	1.8	0.3	6.9	6.3	6.2	1.4	12.2	7.0	5.3	0.2	6.6	6.9	0.1	15.0	11.1	6.7
Cx. bitaeniorhynchus		<0.1		0.1	<0.1	<0.1		0.1	<0.1			<0.1					
Cx. halifaxii	<0.1				<0.1	<0.1					<0.1						
Cx. molestus	1.0	0.3	0.3	0.5	3.5	2.6	1.1	1.5	4.2	0.9	0.4	1.4	0.5		0.2	26.3	0.9
Cx. orbostiensis	0.1			<0.1	0.1	0.1	<0.1	0.1	0.1		<0.1	0.1	0.5			0.1	
Cx. postspiraculosus				<0.1													
Cx. quinquefasciatus	3.1	6.0	1.9	1.3	8.8	15.2	6.7	11.9	6.5	8.3	1.6	1.2	13.7	2.7	2.2	39.7	34.2
Cx. sitiens	4.1	11.3	11.9	33.8	10.4	14.2	10.2	19.5	4.0	10.3	8.7	35.6	2.8	2.3	9.3	2.5	20.4
Ma. uniformis	0.1	0.1	0.4	<0.1	<0.1	<0.1	0.1	0.0	<0.1	<0.1	<0.1			0.3			
Total/year	10844	14477	12182	12319	29634	31039	42342	9083	39883	39199	60553	32669	611	2743	1960	4458	3993

[1] Collected during the surveillance period but not as a part of routine surveillance.

BP = Bicentennial Park; ODS = Olympic Development Site; N = Newington RANAD; NHB = North Homebush Bay;
MP = Mason Park; Industrial = Industrial area; RDS = Residential Development Site

FIGURE 3.5 Monthly abundance (mosquitoes per trap night) of the seven major freshwater species at Homebush Bay from October 1995 to June 1999 in the eight core traps with rainfall and average temperature data. Note variable Y-axis.



MPTN = mosquitoes per trap night
(mm) = millimetres
(°C) = Celcius

TABLE 3.7 Comparison of mean number of mosquitoes per trap night for the seven major species collected from the eight core trap sites during 1995/1996 to 1998/1999 at Homebush Bay by analysis of variance. Numbers of each species followed by different letters are significantly different ($P<0.05$) for values between years.

Year	*Oc. alboannulatus*	*Oc. notoscriptus*	*An. annulipes s.l.*	*Cq. linealis*	*Cx. annulirostris*	*Cx. australicus*	*Cx. quinquefasciatus*
95-96	0.29 a	4.15 a	3.55 a	2.63 a	10.73 a	2.87 a	4.08 a
96-97	0.28 a	1.52 b	0.99 b	1.18 a	8.87 a	2.89 a	7.57 b
97-98	0.04 b	0.52 c	0.54 c	0.31 b	5.30 a	0.57 b	4.20 a
98-99	0.49 a	1.75 b	1.52 b	0.06 c	14.50 b	4.99 a	1.04 c

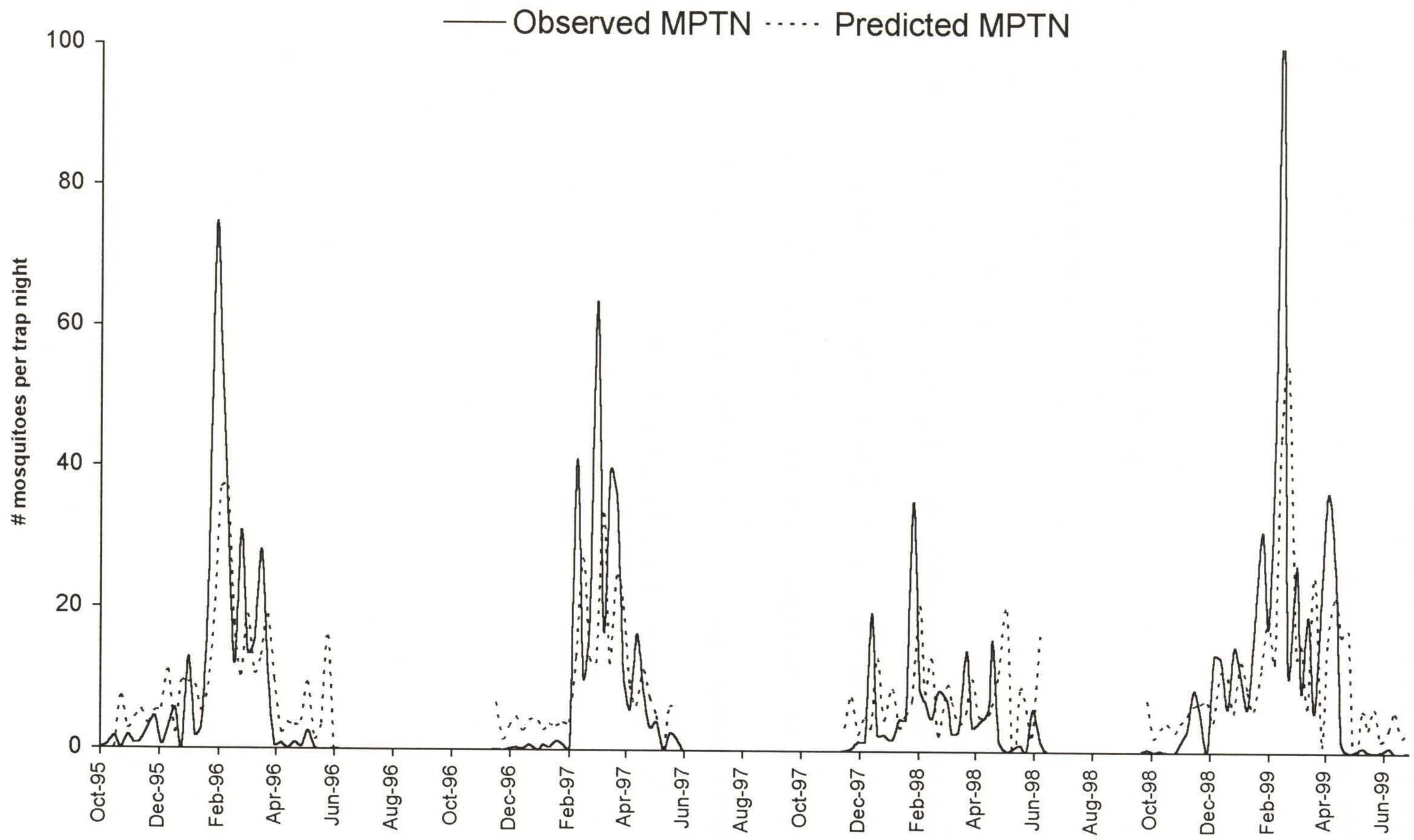
TABLE 3.8 Results of autoregressive time series analysis using the four year *Culex annulirostris* core trap data and showing the relationship between rainfall and relative abundance.

	Coefficient	Standard error of coefficient	P-value
MPTNLag1	0.51	0.07	<0.001
RainLag3	0.11	0.03	0.001
Constant	6.50	2.17	0.003

MPTNPredicted = 6.502 + 0.107[RainLag3] + 0.505[MPTNLag1]

MPTNLag1=mosquito abundance from the previous week
RainLag3=weekly rainfall from the third week prior

FIGURE 3.6 Observed and predicted abundance of *Culex annulirostris* based on core trap data from 1995 to 1999 using an autoregressive time series model.



Observed MPTN = mosquitoes per trap night observed values; Predicted MPTN = mosquitoes per trap night predicted values.

3.3.5 Arbovirus activity

A total of 79,042 mosquitoes were processed for virus isolation over the four year period (TABLE 3.9). *Ochlerotatus vigilax* was the dominant species collected and processed (72%), followed by *Cx. annulirostris* (10%) and *Cx. sitiens* (10%). The remaining species comprised 8% in total.

One Ross River virus isolate was obtained from a pool of 25 *Oc. vigilax* collected on 16 February 1999 from North Homebush Bay.

From the collections of 10 December 1996, a virus isolate was obtained from a pool of eight *Oc. camptorhynchus* from North Homebush Bay but could not be identified. The unknown isolate exhibited cytopathic effects (CPE) consistent with virus activity, however there was no cross-reactivity with broad alpha- and flavivirus monoclonal antibodies. The CPE was not due to mycoplasma infection or pool toxicity.

TABLE 3.9 The number of mosquitoes processed (in pools of ≤ 25) for arbovirus isolation using four trap sites in Homebush Bay, December 1996 - April 1999.

	1996/1997		**1997/1998**		**1998/1999**	
Species	**# tested**	**positive Pools**	**# tested**	**positive pools**	**# tested**	**positive pools**
Ad. venustipes	-	-	3	0	-	-
Oc. alboannulatus	118	0	6	0	17	0
Oc. alternans	122	0	69	0	324	0
Oc. camptorhynchus	185	1 (Unk)	25	0	14	0
Oc. flavifrons	-	-	-	-	13	0
Oc. mallochi	-	-	1	0	-	-
Oc. notoscriptus	290	0	87	0	209	0
Oc. procax	45	0	3	0	39	0
Oc. vigilax	18503	0	31964	0	6191	1 (RR)
Oc. vittiger	-	-	1	0	-	-
An. annulipes s.l.	744	0	78	0	439	0
Cq. linealis	848	0	54	0	9	0
Cx. annulirostris	3592	0	1095	0	3496	0
Cx. australicus	693	0	50	0	71	0
Cx. molestus	210	0	135	0	91	0
Cx. orbostiensis	35	-	4	0	13	0
Cx. quinquefasciatus	646	0	497	0	66	0
Cx. sitiens	1727	0	2498	0	3713	0
Ma. uniformis	2	0	7	0		-
Total	27760	0	36577	0	14705	1
Total 1996/1999	79042					

Unk = unknown

RR = Ross River virus

3.4 Discussion

During four year investigation at Homebush Bay a total of 30 species (16 as larvae, 28 as adults) was recorded. The number of species collected as adults at Homebush Bay represents approximately 46% of the 59 species collected by the NSW Mosquito Monitoring Program from 22 coastal locations in NSW since 1987 (Clancy and Russell 1997).

Several freshwater species were collected in adult surveys but not in larval surveys. *Ochlerotatus notoscriptus*, a domestic species, typically breeds in tree holes and artificial containers (Russell 1993). This species was not recorded in larval surveys since their habitats were not part of the routine surveillance. *Culex molestus* larvae are indistinguishable from *Cx. quinquefasciatus* and may have been collected but recorded as *Cx. quinquefasciatus*. *Coquillettidia linealis* and *Ma. uniformis* larvae, commonly associated with submerged water plants (Russell 1993), were not detected but the routine dip-sampling larval surveillance method was not suitable for sampling their population. Other species not collected as larvae, such as *Oc. eidsvoldensis*, *Oc.* nr. *monocellatus*, *An. atratipes* and *Cx. postspiraculosus,* were rare in adult collections and it is most likely their larval abundance was very low in the field.

Although extensive areas of breeding habitat were removed over time, *Cx. annulirostris* continued to be the most abundant freshwater species collected from traps in N (NHB in 1998/1999) in each year of the study. Freshwater pools fed by concrete-lined drains were located on the southeastern edge of the mangrove saltmarsh. These sites were the most productive breeding sites in N and were not removed during remediation. Access became restricted to these sites and they could not be properly sampled, but most likely they were the source for the continued production of *Cx. annulirostris* in the area.

In BP, smaller, shallow wetlands were inundated by spring rains each season but in summer most habitats dried out. Consequently, *Cx. australicus* was the most abundant species in larval surveys since few habitats were available for *Cx. annulirostris* in the summer months. *Culex annulirostris* usually dominated the trap catch of freshwater species except in the second year of the study, when numbers of *Cx. quinquefasciatus* were greater. The *Cx. quinquefasciatus* may have been emanating from the suburban areas or development areas adjacent to BP but access restrictions prevented confirmation. Larval surveys indicated that *Cx. australicus* was the most abundant species colonising the habitats within the park at that time, but, *Cx. australicus* larval populations appear to be not well indicated by EVS trap sampling of adults (R. Russell, Dept. of Medical Entomology, personal communication).

In the ODS, *Cx. annulirostris* dominated the trap catch of freshwater species during the first and last year and *Cx. quinquefasciatus* was most abundant in the second and third year. Larval densities of the major species in the area were generally low each year of the study. In the first year, *Cx. annulirostris* adults most likely were dispersing from the north N marsh habitats. In the last year, habitats in the HR wetlands and the treatment wetland in the ODS provided increased sources for the species and consequently greater numbers were collected from the ODS.

The Industrial area trap, operated only for the first year, and the Mason Park trap, operated for the first, third and fourth year, were used to investigate dispersal of adult mosquitoes from the major habitats. *Culex annulirostris* adults comprised 9-18% of the total catch during the three years of operation at Mason Park indicating that the species was dispersing well to the southeast since there were no developmental sites for the species near the trap location. However, the species comprised only 4% of the collections in the Industrial area trap during the 1995/1996 season.

The seven traps in the Residential Development Site, operated during the last year, collected more *Cx. quinquefasciatus* than any other freshwater species. This is most likely a reflection of the increase of drainage systems constructed throughout the area although access to the site for surveillance of these habitats was prohibited and this could not be confirmed.

The adult core trap data shows that in 1997/1998, significantly fewer *Oc. alboannulatus*, *Oc. notoscriptus*, *An. annulipes* s.l., *Cq. linealis* and *Cx. australicus* adults were collected. *Culex annulirostris* showed a similar tendency for numbers to decline but the difference was not significant. The 1997/1998 season was exceptionally dry and most temporary habitats favourable for production of *Oc. alboannulatus* and *Oc. notoscriptus* dried out.

The combination of marsh habitat removal and low rainfall brought about a decline in the numbers of *An. annulipes* s.l. from 1995/1996 to 1997/1998. There was a slight increase in numbers during 1998/1999 as new wetland habitats were established in the ODS and adjacent to Hill Road.

Coquillettidia linealis showed a steady decline from the first to fourth season, coinciding with the removal of substantial areas of their reed habitat.

The abundance of *Cx. annulirostris* was adversely affected by low rainfall and removal of habitat in the third year, as was that of other species. The establishment of treatment wetlands in the ODS and the HR areas, along with increased rainfall in January and February of the last year, brought about an increase in habitat and consequently an increase in numbers from the previous season. Adult populations of *Cx. annulirostris* from VIC and NSW have been shown to appear and disappear in association with mean weekly threshold temperature of 17.5 °C (McDonald *et al.* 1980, Russell 1986b, Clancy and Russell 1992). The activity of the species in this study revealed the same seasonal pattern.

The abundance of *Cx. quinquefasciatus* fluctuated with the abundance of rainfall. However in the last season, the most productive site, the GPT, was removed for roadworks and populations declined accordingly.

At the beginning of the investigation, in 1995/1996, habitats with patchy and dense vegetation were most likely to have larvae, and dense vegetation was the only significant predictor. In the final year, habitats influencing the results were primarily marsh habitats in Newington. In 1998/1999, the likelihood of finding a larva decreased as autumn approached and size of the habitats was the only significant predictor. Vegetation was not taken into account since only habitats with patchy and dense vegetation were available for sampling. The HR wetlands primarily influenced the results in the last year. The artificial wetlands established in the last year of the study are similar to the highly productive marsh habitats removed after the first year.

In 1995/1996 and 1998/1999, there was a tendency for larval density to decrease as depth of the habitat increased but it was not significant at the multivariate level. Overall, the analysis showed that patchy to dense vegetation produced greater larval densities of mosquitoes compared to less vegetated habitats. For purposes of mosquito management therefore, new habitats should be maintained so that vegetation is restricted to the margins. Weekly larval surveillance of the HR wetlands could provide information for pond levels to be drawn down at an appropriate time so that larval population increases can be managed.

Overall, the effectiveness of *G. holbrooki* as a predator on mosquito larvae was often demonstrated when habitats with the fish were found not to support larval populations of mosquitoes.

Weekly rainfall from the third week prior to the sample period, and mosquito abundance from the previous week, were the only significant predictors of adult *Cx. annulirostris* populations. The result from this analysis is in contrast to findings by Dhileepan (1996), who investigated the relationship between *Cx. annulirostris* abundance and various meteorological factors in the Murray Valley of Victoria. He found positive correlations between *Cx. annulirostris* abundance and temperature but not rainfall since rainfall was low during the summer months. In general, the plot of predicted values (FIGURE 3.6) based on regression analysis of the 1995-1999 data, followed the peaks and troughs of the observed values but did not reach the magnitude of the observed values. In practice the model would perform better as the parameter estimates used in prediction could be updated on a weekly basis. Despite the results, the model could be used as a rough estimate to predict *Cx. annulirostris* abundance and therefore as a mosquito management tool for determining timing of control agent applications.

Ross River (RR) virus is the most important annually active arbovirus in Australia (Russell 1998). This was the first isolate recorded from *Oc. vigilax* in inner urban Sydney. Large numbers of adults of the major coastal vector *Oc. vigilax* were present at the time of the isolate collection, following abundant rainfall and high tides in January and February. During the same season, 1998/1999, Stratford virus was isolated from other species in suburbs of Sydney, from *Oc. procax* and *Oc. notoscriptus* in Pennant Hills, from Oc. *notoscriptus* in Parramatta, and from *Oc. notoscriptus* in Werrington (Doggett *et al.* 1999).

The RR isolate acquired at Homebush Bay was not completely unexpected in light of an outbreak of RR virus infection in the north-western fringe of Sydney in early 1997 (Amin *et al.* 1998) and in early 1999, further activity occurred in the far western suburbs (Brokenshire and Reynolds 2000). The ecology of the virus in Australia is thought to require native mammalian hosts such as wallabies and kangaroos to maintain the cycle and perhaps horses as amplifying hosts (Vale *et al.* 1991, Russell 1998), however none of these animals remain in the Homebush Bay area. Therefore if transmission should occur, it may be a result of mosquitoes feeding on infected humans travelling through the area. This is believed to have been the case during the RR virus outbreaks in Perth in 1988/1989 and 1991/1992 (Lindsay *et al.* 1992) and in

Brisbane in 1992 and 1994 (Ritchie *et al.* 1997). With urbanisation of the Homebush Bay area, the number of humans coming into contact with mosquito vectors will increase rapidly, highlighting the need for continued mosquito and arbovirus surveillance and measures to minimising mosquito breeding in the area.

CHAPTER 4

DETERMINATION OF STADIAL DURATION OF IMMATURE *CULEX ANNULIROSTRIS* IN THE FIELD

4.1 Introduction

Culex annulirostris, a major pest species and vector of numerous arboviruses (Russell 1995), breeds abundantly in the vegetated small to large ponds at Homebush Bay. Peak populations of the species occurred from December to March, depending on temperature and availability of habitat (Chapter 3).

The ability to reliably grade larval instars and knowledge of the stadial duration of immatures in the field is crucial for the accurate application of *Bacillus thuringiensis israelensis* (*B.t.i.*) larvicide since it must be ingested by the larvae to kill them, and therefore must be applied prior to larvae reaching the late fourth instar.

McDonald *et al.* (1977) reported on head capsule width of *Cx. annulirostris* larvae derived from different habitats and varied environmental conditions. This present study set out to determine if head capsule width changed over time in the varying field conditions at Homebush Bay and, additionally, if siphon length could be used as a determinant of instar.

McDonald (1978), Rae (1990) and Mottram and Kettle (1997) have investigated developmental rates of *Cx. annulirostris* larvae under field conditions in Mildura, VIC, Townsville, North QLD and Brisbane, QLD, respectively, and have shown that rates vary according to location. Therefore, it was appropriate to investigate the influence of field temperature on developing *Cx. annulirostris* larvae in the Hill Road freshwater wetlands under the local conditions at Homebush Bay for the purpose of mosquito management.

4.2 Materials and methods

The Hill Road freshwater wetlands were chosen for the study site since the area was known to support larval populations of *Cx. annulirostris* (Chapter 3) and were located in an area that was secure from tampering. Three cages, constructed of white buckets (26.5 cm diameter) with fine mesh windows (250 μm) to allow for water exchange, were placed along the margin of a large reservoir (approximately 22000 m^2). Margins were heavily vegetated and shallow and water levels along the bank varied from 10 to 15 cm of water, depending on rainfall or water usage. Pond water was used for irrigation on occasion so that water level fluctuated during the course of the investigation.

Culex annulirostris colony larvae, originally from stock of the Murray Valley strain (McDonald *et al.* 1977), were used in the studies. Eggs hatched in 1.6 ± 0.05 days at a water temperature of 25° C and 85% humidity (data not shown). Three hundred larvae were placed into predator exclusion cages (described below) at a density of 0.54 larvae/cm^2 on day zero within four hours of hatching. Approximately 0.1 g of fish pellets were crushed and placed in the cages on day zero (the same day larvae were placed in the field) and then every other day until larvae reached fourth instar to provide optimum conditions for growth. In February, an additional three cages were placed in the field and left unfed to determine the influence of added food on head capsule size and developmental time. Three control containers, each with 12.5 cm of pond water (average of variable field depths) and 300 larvae were placed in a temperature-controlled room (23° C average water temperature) to ensure that the pond water had no detrimental effect on larvae.

Water and air temperature were taken at the time of field collections (approximately midday) with a hand-held mercury thermometer. Daily water samples were taken back to the laboratory and measured for pH. Maximum and minimum air temperatures were obtained from the Bureau of Meteorology's Homebush weather station (site number 066195) for the length of the experimental period.

A sample of 30 larvae (10 larvae per cage) was collected on day one (24 hours after larvae were placed into the field) and every day after until no larvae were remaining. Larvae were preserved in 70% ethyl alcohol and taken to the laboratory for measurement. To test for possible shrinkage in the preservative, a sample of 30 live *Cx. annulirostris* larvae from the Westmead Hospital colony stock were immobilised in cold water and measured, then later remeasured after being preserved in 70% ethyl alcohol. The data were analysed using a paired t-Test (Microsoft® Excel 97 software program).

In the laboratory, head capsule width was measured at the widest section of the head and siphon length was measured from the base to the apex using a 50x eyepiece micrometer. The mean and range of head capsule width and siphon length for each instar were compared between the months. Two factor analysis of variance (ANOVA) was used to test for significance between head capsule widths, between siphon lengths and between February fed and unfed cohorts of the same instar between months. If there was a significant interaction between instar and month, the effects of month was examined separately for each instar. Microsoft® Excel 97 was the software program used to perform the ANOVA. Larvae were classified according to instar and data recorded per cage so that at the end of the month, there were three data points for each instar duration.

The cumulative number of days of each instar was determined by the time half the larvae took to reach the instar (t_{50}). Environmental factors were averaged over time according to the duration of each instar. Multiple regression analysis was used to determine if a significant relationship existed between the fourth instar developmental period and the environmental factors. The cumulative t_{50} from each of the experimental containers was regressed on mean minimum and maximum air temperature, water temperature, and ambient temperature to investigate the influence of each of these factors on fourth instar developmental time throughout the entire study period (12 data points). The software program used was SPSS for Windows, Version 10.0.

4.3 Results

4.3.1 Test for shrinkage

Results of the paired t-Test showed that there was no significant difference (P= 0.40) between head capsule widths of larvae immobilised by cooling and larvae in 70% ethyl alcohol. Therefore the methodology used had no adverse effect on the results.

4.3.2 Head capsule width

Ranges of head capsule widths for first instar, second instar, third instar and fourth instar are shown in TABLE 4.1, and all of the ranges remained discrete between consecutive instars. There was a significant interaction between instar and month ($P<0.001$), therefore the effect of month was examined separately for each instar using single factor ANOVA (TABLE 4.2). The second and third instar differed significantly ($P<0.05$) between the months.

4.3.3 Siphon length

Siphon length ranges overlapped between consecutive instars in January, February and March (TABLE 4.3). There was a significant interaction between instar and month ($P<0.001$). The effect of month was examined separately for each instar using single factor ANOVA (TABLE 4.4). The fourth instar differed significantly ($P<0.05$) between months.

TABLE 4.1 Range of head capsule widths (mm) of first, second, third and fourth instar *Culex annulirostris* larvae for each month.

Instar	December	January	February	March
First	0.28 – 0.36	0.26 – 0.34	0.26 – 0.34	0.26 – 0.34
Second	0.42 – 0.52	0.42 – 0.54	0.42 – 0.56	0.40 – 0.52
Third	0.66 – 0.82	0.64 – 0.84	0.70 – 0.88	0.66 – 0.82
Fourth	1.08 – 1.30	1.04 – 1.32	1.10 – 1.30	1.00 – 1.30

TABLE 4.2 Mean head capsule widths (mm) and standard deviation of first, second, third and fourth instar *Culex annulirostris* larvae. The effect of month was examined separately for each instar.

Instar	December	January	February	March	P-value
First	0.32 ± 0.01	0.30 ± 0.02	0.30 ± 0.002	0.31 ± 0.001	=0.24
Second	0.47 ± 0.01	0.47 ± 0.01	0.50 ± 0.01	0.48 ± 0.002	<0.05
Third	0.75 ± 0.01	0.78 ± 0.001	0.79 ± 0.02	0.78 ± 0.002	<0.05
Fourth	1.20 ± 0.01	1.20 ± 0.02	1.22 ± 0.004	1.20 ± 0.02	=0.13

TABLE 4.3 Range of siphon lengths (mm) of first, second, third and fourth instar *Culex annulirostris* larvae for each month.

Instar	December	January	February	March
First	0.12 – 0.34	0.14 – 0.46	0.14 – 0.38	0.08 – 0.36
Second	0.42 – 0.58	0.44 – 0.60	0.36 – 0.62	0.42 – 0.56
Third	0.66 – 0.90	0.70 – 0.96	0.64 – 1.00	0.60 – 0.98
Fourth	1.10 – 1.70	1.14 – 1.70	1.24 – 1.80	0.90 – 1.86

TABLE 4.4 Mean siphon lengths (mm) and standard deviation of first, second, third and fourth instar *Culex annulirostris* larvae. The effect of month was examined separately for each instar.

Instar	December	January	February	March	P-value
First	0.23 ± 0.01	0.23 ± 0.02	0.23 ± 0.01	0.23 ± 0.01	=0.99
Second	0.51 ± 0.01	0.50 ± 0.01	0.52 ± 0.02	0.52 ± 0.01	=0.37
Third	0.80 ± 0.01	0.80 ± 0.01	0.84 ± 0.05	0.84 ± 0.01	=0.11
Fourth	1.37 ± 0.02	1.40 ± 0.03	1.53 ± 0.04	1.50 ± 0.04	<0.05

4.3.4 Head capsule widths of February fed and unfed cohorts

There was only a slight variation in range of the same instar between fed and unfed cohorts (TABLE 4.5) but the addition of food significantly increased ($P<0.05$) the mean head capsule widths of fed cohorts (TABLE 4.6 and TABLE 4.7).

4.3.5 Developmental rates

Developmental time from first to fourth instar was shortest in January (9.0 days) and longest in March (17.0 days). The mean water temperatures of the month for the duration of the experiment, the mean minimum temperatures for each stage of development and the mean developmental times of *Cx. annulirostris* larvae over a four month period are shown on TABLE 4.8.

The only independent predictor of the median number of days to reach and complete the fourth instar was average minimum temperature, explaining 93% of the variability (TABLE 4.9). The linear relationship between the number of days to reach and complete the fourth instar and average minimum temperature is shown in FIGURE 4.1 but only seven of the 12 points are visible. The calculated regression equation of fourth instar developmental time on average minimum temperature is: $Y = -4.842X + 107.8$ ($n=12$, $P<0.001$).

The addition of food slightly shortened the duration of the first, second and fourth instar and pupal stage (TABLE 4.10). However, the difference was not significant ($P=0.10$) (TABLE 4.11).

TABLE 4.5 Ranges for head capsule widths (mm) of February fed and unfed cohorts of *Culex annulirostris* larvae.

Instar	Fed	Unfed
First	0.30 – 0.32	0.30 – 0.34
Second	0.42 – 0.56	0.42 – 0.56
Third	0.70 – 0.88	0.70 – 0.84
Fourth	1.14 – 1.30	1.12 – 1.32

TABLE 4.6 Mean head capsule widths (mm) and standard deviation of February fed and unfed cohorts of *Culex annulirostris* larvae.

Instar	Fed	Unfed
First	0.30 ± 0.002	0.31 ± 0.001
Second	0.50 ± 0.01	0.48± 0.004
Third	0.80 ± 0.02	0.78 ± 0.01
Fourth	1.23 ± 0.004	1.21 ± 0.01

TABLE 4.7..Results of two factor analysis of variance between February fed and unfed cohorts of *Culex annulirostris* larvae.

Source	DF	MS	P-value
Sample	3	0.96	<0.05
Columns	1	<0.001	<0.05
Interaction	3	<0.001	=0.15
Within	16	<0.001	
Total	23		

TABLE 4.8 Median developmental time of *Culex annulirostris* larvae, water temperature and minimum temperature (for the duration of each instar) over a four month period.

Month	Water Temp (°C)	Min Air Temp (°C)	Instar	Cumulative Age (Days)
December		20.3	1^{st}	2.0
		21.1	2^{nd}	5.0
		20.5	3^{rd}	6.7
		20.0	4^{th}	12.0
	27.7	19.8	P	13.0
January		22.2	1^{st}	2.0
		21.3	2^{nd}	3.3
		20.6	3^{rd}	5.0
		20.1	4^{th}	9.0
	25.8	19.9	P	10.0
February		19.5	1^{st}	2.3
		19.6	2^{nd}	4.0
		20.1	3^{rd}	7.0
		20.4	4^{th}	9.3
	26.4	20.0	P	10.7
March		17.8	1^{st}	4.0
		18.3	2^{nd}	6.3
		18.4	3^{rd}	14.7
		18.5	4^{th}	17.0
	23.0	18.4	P	19.3

1^{st}=first instar
2^{nd}=second instar
3^{rd}=third instar
4^{th}=fourth instar
P=pupae

TABLE 4.9 Summary of multiple regression model to determine independent predictor of length of *Culex annulirostris* fourth instar developmental time.

Variable	B	T	Prob>|t|	R^2
Constant	107.80	13.09	<.001	.93
Avg Min Temp	-4.84	-11.63	<.001	

FIGURE 4.1 Relationship between the length of *Culex annulirostris* fourth instar developmental time (days) and average minimum temperature (°C).

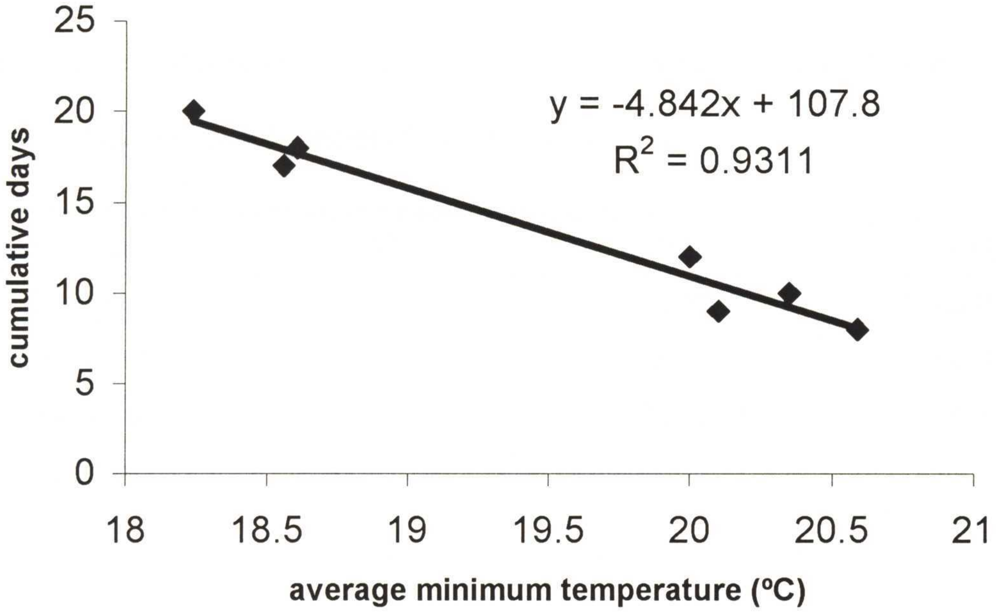


TABLE 4.10 Cumulative duration (days) and standard deviation of *Culex annulirostris* instars for fed and unfed cohorts in February.

	Cumulative Days	
Instar	**Fed**	**Unfed**
First	2.3 ± 0.58	3.0 ± 0.00
Second	4.0 ± 0.00	5.0 ± 1.00
Third	7.0 ± 1.73	7.0 ± 1.00
Fourth	9.3 ± 1.15	10.0 ± 0.00
Pupae	10.7 ± 0.58	11.0 ± 0.00

TABLE 4.11 Results of two factor analysis of variance between the cumulative duration of fed and unfed cohorts of *Culex annulirostris* larvae.

Source	**DF**	**MS**	**P-value**
Sample	4	70.22	<0.001
Columns	1	2.13	=0.10
Interaction	4	0.22	=0.87
Within	20	0.7	
Total	29		

4.4 Discussion

Appropriate timing of larvicide treatment requires the correct identification of the larval instar. *Bacillus thuringiensis israelensis* (*B.t.i.*), the control agent selected for use in the environmentally sensitive wetlands of Homebush Bay, must be ingested by the larvae to kill them and therefore must be applied prior to larvae reaching the late fourth instar when they stop feeding (Christophers 1960). This study showed that head capsule width of the second and third instar differed between the months in varying field conditions but that each stage remained discrete. Siphon length of the fourth instar differed between months and siphon ranges overlapped in January, February and March. Although the addition of food in February increased the mean head capsule width of larvae, the ranges of fed and unfed cohorts did not overlap. Therefore, head capsule width is a reliable method for identifying larval instars regardless of variable field temperatures and nutrition, but siphon length is too variable to be a useful measure.

Earlier larval development studies by McDonald (1978) in Mildura, Rae (1990) in Townsville and Mottram and Kettle (1997) in Brisbane, recorded stadial duration of *Cx. annulirostris* until adult emergence, whereas this study allowed for adults to escape and continued until no more pupae remained in the cages. In this investigation, the most important stage to consider from a control perspective was the time to reach and complete the fourth instar. Duration of first to fourth instar in Mildura, Townsville and Brisbane was 9.37, 12.8 – 15.2, and 10.03 – 10.65 days respectively. Field water temperatures in the Hill Road wetlands during December (27.7°C), January (25.8°C) and February (26.4°C) were similar to the Townsville (27.4°C) and Brisbane (26.0-27.5°C) studies and higher than the Mildura (24.4°C) study. However, in this investigation, water temperatures were taken at midday and most likely were still approaching maximum temperature for the day. Nevertheless, development from first to fourth instar during December (12.0 days), January (9.0 days) and February (9.3 days) were similar to the range of the previous studies. Longer developmental time in March (17.0 days) can be attributed to the lower field temperatures.

In this study, the density of larvae in the predator exclusion cages (0.56 larvae/cm^2 surface area) was greater than the calculated larval densities by surface area in Townsville (0.11 larvae/cm^2) and Brisbane (0.22 larvae/cm^2) but less than Mildura (0.67 larvae/cm^2). Reisen and Siddiqui (1979) showed that higher (4.0 larvae/cm^2) larval densities increased developmental time in studies with *Cx. tritaeniorhynchus* Giles. This most likely did not occur in this investigation, since developmental times were similar to the earlier studies, but the Reisen and Siddiqui (1979) densities were much higher than all Australian studies.

Approximately 48% of the total number of larvae placed in the cages were extracted for head capsule and siphon measurements. The removal of larvae could be considered comparable to loss of larvae to predators in natural situations. Larval survival was reduced 58% by predators in Townsville (Rae 1990), and by 43.2 - 68.7% in Brisbane (Mottram and Kettle 1997). Rae (1990) noted that larvae developed faster in the presence of predators, attributing this result to greater food availability in the containers. It is not known what effect the extraction of larvae had on developmental time in this study. However, larval density in this investigation was 2.5 to 5 times greater than the Brisbane or Townsville study, respectively, and consequently food requirements would be greater. While it may be argued that developmental time was shortened by the extraction of larvae, the higher density of larvae may have counteracted any such effects.

Reisen *et al.* (1989) have shown that adding food to the water shortened the developmental period of *Cx. tarsalis* Coquillet. Although the addition of food increased head capsule width in February fed cohorts, the amount of food (0.1 g every other day) was not enough to shorten developmental time. This result implies that developmental times in December, January and March were not influenced by the addition of this level of food.

Culex annulirostris will colonise a pond within a day of rainfall, which apparently encourages oviposition (McDonald and Buchanan 1981). After rainfall, field surveillance must commence to determine areas of high productivity. Assuming that the mosquito control personnel are able to correctly identify the larval instars, the stage and productivity of the ponds must be noted. Using a regression model based on projected minimum temperatures for the time of year, an estimate of the fourth instar development time may be achieved. The optimal time for treatment (should the need arise) exists when immatures are at the second instar and before the fourth instar to ensure effective application of the control agent.

Mottram and Kettle (1997) clearly demonstrated that developmental time varied according to the type of pool that *Cx. annulirostris* colonised. Water temperature, nutrition and predator density play a key role in larval survivorship (Reisen *et al.* 1989) and each of these factors changes with the size, depth, water quality and age of a pond. The relationship between minimum temperature and developmental time as well as egg production has been observed previously in other *Culex* (Hayes 1975; Hayes and Hsi 1975).

This study used colony stock in a large reservoir pond, and development may have differed with wild caught *Cx. annulirostris* and/or in a smaller pond that had warmer temperatures. Therefore, additional studies using wild caught stock and different breeding sites, such as ephemeral and semi-permanent frog ponds, would be useful in understanding the range of influences of biotic and abiotic factors on length of development.

CHAPTER 5

ASSESSMENT OF THE NATIVE FISH, *PSEUDOMUGIL SIGNIFER* KNER FOR BIOLOGICAL CONTROL OF MOSQUITOES IN ARTIFICIAL WETLANDS

5.1 Comparison of feeding efficiency of *Pseudomugil signifer* Kner and *Gambusia holbrooki* (Girard)

5.1.1 Introduction

For mosquito control in newly created or disturbed habitats without fish, native fish should be preferred over exotic species such as *Gambusia holbrooki* (the mosquito fish) since the former are more suited to local conditions and *G. holbrooki* has adverse impacts on non-target local species (Lloyd 1986). *Pseudomugil signifer* (the Pacific blue-eye) has been nominated as a potential effective predator on mosquitoes (Durre 1991), is found in the waterways of Homebush Bay (The Ecology Lab 1994a), and could be considered as a biological control agent for the wetlands needing mosquito control. However, prior to releasing a native fish for mosquito control, the species should undergo rigorous testing in the laboratory and the field to determine its effectiveness (Lloyd 1986). This investigation was designed to compare the predator efficiency of the native *P. signifer* with that of *G. holbrooki* in consuming mosquito larvae in laboratory trials.

5.1.2 Materials and methods

Pseudomugil signifer were collected from Bicentennial Park and Haslams Creek at Homebush Bay, from brackish water with salinities ranging from 25 ‰ to 30 ‰. Similar sized *G. holbrooki* were collected from Boundary Creek, a freshwater creek draining into Bicentennial Park at Homebush Bay. Fish were collected under NSW Fisheries Collecting Permit No. F9751. Brief descriptions of *P. signifer* and *G. holbrooki* are presented below.

5.1.2.1 Experimental fish

Pseudomugil signifer (FIGURE 5.1) occurs along the northeast and southeast coast drainage divisions of Australia from Cooktown, QLD, to Ulladulla, NSW (Merrick and Schmida 1984) and it has been found on offshore islands such as Fraser Island, Low Isles and Lizard Island (Merrick and Schmida 1984, Allen 1989). It inhabits shallows along the edges of lagoons, streams and coastal lakes (Semple 1986) in fresh to brackish water at temperatures ranging from 15 to 28° C and pH values from 5.5 to 7.8 (Merrick and Schmida 1984). The body is moderately compressed and elongate with a large bright blue eye, and melanophores outline the scales forming a reticulate pattern over the body (Ivantsoff and Crowley 1996). Size varies from 30 mm to 40 mm and the body is semitransparent often with pale olive, yellowish or slightly bluish colouration; the operculum and belly region are silvery and the fins are clear to yellow (Allen 1989). Males have extended dorsal and anal fins that become bright yellow during spawning, but in females and juveniles, dorsal and anal fins are clear, short and rounded (Semple 1986, Ivantsoff and Crowley 1996).

The species has the ability to breed in fresh and saline water (Howe 1987). Females can lay 4 to 9 eggs every day, sometimes lasting over a week, and the eggs take 18 to 21 days to hatch at 22° to 24° C (Merrick and Schmida 1984). Howe (1987) found that spawning activity and egg viability were optimum from November to February, decreasing through March and April, while no spawning occurred from June to August.

Gambusia holbrooki (FIGURE 5.2) occurs throughout estuarine and freshwater systems in NSW, SA, VIC, QLD, and parts of NT and WA (McDowall 1996). Individuals are olive green to brown in colour, with a silvery white belly and a small, upturned and protrusible mouth (McDowall 1996). Size varies from 35 mm for males to 60 mm for females (Allen 1989). The species has one soft-rayed dorsal fin, and in males the anal fin is modified to form the gonopodium, which is used to internally fertilise the eggs in the female (McDowall 1996).

The species prefers warm and gently flowing or still waters along margins and edges of vegetation and can tolerate a wide range of salinities from freshwater to marine (McDowall 1996). They are able to tolerate a wide range of pollutants and can successfully exploit environments that are lethal to other species (Lloyd 1984). They prefer temperatures greater than 25° C; however, warm adapted populations can survive 44° C for short periods and cold adapted populations can withstand temperatures as low as 0.5° C (Lloyd 1984).

It is a live-bearing fish and the breeding period for *G. holbrooki* from different regions of Australia varies somewhat depending on water temperature (Pen and Potter 1991).

In tropical Queensland, the species breeds for 8 months from mid-winter to early autumn (Milton and Arthington 1983) while in the Collie River, Western Australia, it breeds for 6 months from mid-spring to early autumn. The mean fecundity of pregnant females ranged from 22.8 ± 1.02 eggs in tropical Queensland to 47.4 ± 2.76 eggs in the Collie River (Milton and Arthington 1983, Pen and Potter 1991). During the breeding season, there are two groups of pregnant females. The first group of females breed in the spring and live for one year, followed by a summer breeding group that usually die after they are a few months old (Pen and Potter 1991).

FIGURE 5.1 Photograph of *Pseudomugil signifer*

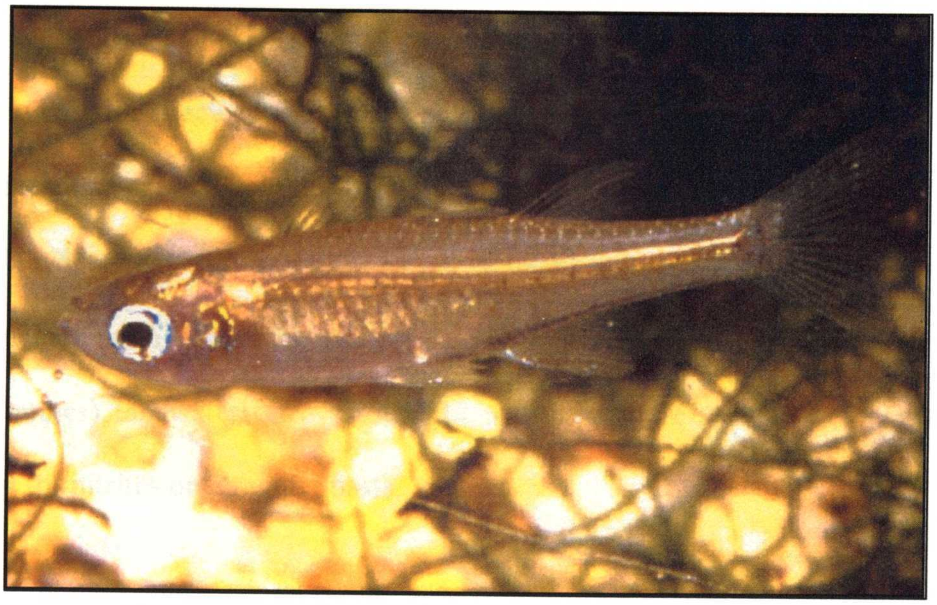


FIGURE 5.2 Photograph of *Gambusia holbrooki*

5.1.2.2 Fish maintenance

The *G. holbrooki* were collected from freshwater, but the brackish-water derived *P. signifer* were acclimatised to freshwater over a two-week period before experimentation. Each species was kept in separate holding tanks with natural lighting at constant temperature of 19° - 21° C. They were fed fish flake (Wardley's Pond Stix®) twice a day and mosquito larvae or blood worms once a week. Water quality was maintained by changing one third of the tank water with conditioned (to remove chloramines) tap water once a fortnight, vacuuming the gravel once a month, and testing for nitrites on a regular basis.

5.1.2.3 Experimental procedures

Since *P. signifer* and *G. holbrooki* are schooling fish (Moyle and Cech 1982, Effie Howe, CSIRO, personal communication), they were tested in groups of five to reduce stress. Experimental trays were 12 cm x 18 cm and contained 1 litre of conditioned water. The experiment was conducted in a temperature-controlled environment (20° - 22° C) with natural lighting.

A measuring tube similar to that of Litvak (1983) was used to measure fish without injury. Measurements were taken from the snout tip to the base of the tail. The mean standard length of *P. signifer* and *G. holbrooki* used in the experiments was 16.11 ± 2.67 mm and 14.85 ± 2.71 mm, respectively. The fish were starved for 24 hours and placed in containers 30 minutes before experimentation. They were exposed to three densities of *Cx. annulirostris* larvae from the laboratory colony at Westmead Hospital, with three replicates at each density. The larval densities were 50, 100 and 200 larvae per tray, corresponding to 1:10, 1:20, and 1:40 predator/prey ratios. The number of mosquito larvae remaining was counted and recorded after 15 minutes, 2 hours, 6 hours, 12 hours and 24 hours. The experiment was repeated for all four larval instars (first, second, third and fourth), representing increasing prey sizes. The same group of five individuals was not used in any two successive experiments within the same larval density. Logistic regression analyses (see APPENDIX 1 for an explanation of odds ratio and logistic regression analysis) were used to determine the independent predictors of the proportion of larvae consumed at 10 minutes and 24 hours. The software program used to perform the analyses was SPSS for Windows, Version 10.0.

5.1.3 Results

5.1.3.1 Fish behaviour

Before experimentation, *P. signifer* usually grouped into a corner of the container, while *G. holbrooki* swam freely throughout the container. However, both species attacked larvae immediately upon introduction into the experimental trays.

5.1.3.2 Larval instars

Over a 24-hour period, 100% of the first, second and third instar were consumed by both fish at the low and medium density (data not shown). The percentage predation over time for *G. holbrooki* and *P. signifer* at the highest density (200 larvae) is shown in FIGURE 5.3. At 15 minutes, *G. holbrooki* and *P. signifer* consumed 100% of the first instar but consumed 25% and 20% respectively of the larger larval sizes. Percentage predation for *G. holbrooki* and *P. signifer* increased over time for the second, third, and fourth instars. At 24 hours, both species consumed almost 100% of the first and second instar, and approximately 90% to 100% of the third instar.

Gambusia holbrooki and *P. signifer* consumed only 30% and 45%, respectively, of the fourth instar within 24 hours.

5.1.3.3 Larval densities

The percentage predation of the fourth instar at the three densities over time for *G. holbrooki* and *P. signifer* is shown on FIGURE 5.4. Each species increased predation across all larval densities. At 15 minutes, *G. holbrooki* consumed 15% of all larval densities. *Pseudomugil signifer* consumed 5% of the lowest density and approximately 12% of the medium and high density. At 24 hours, both species consumed 85% of the lowest larval density. However, *G. holbrooki* and *P. signifer* consumed 35 and 50%, respectively, of larvae at the the highest density.

5.1.3.4 Logistic regression analysis

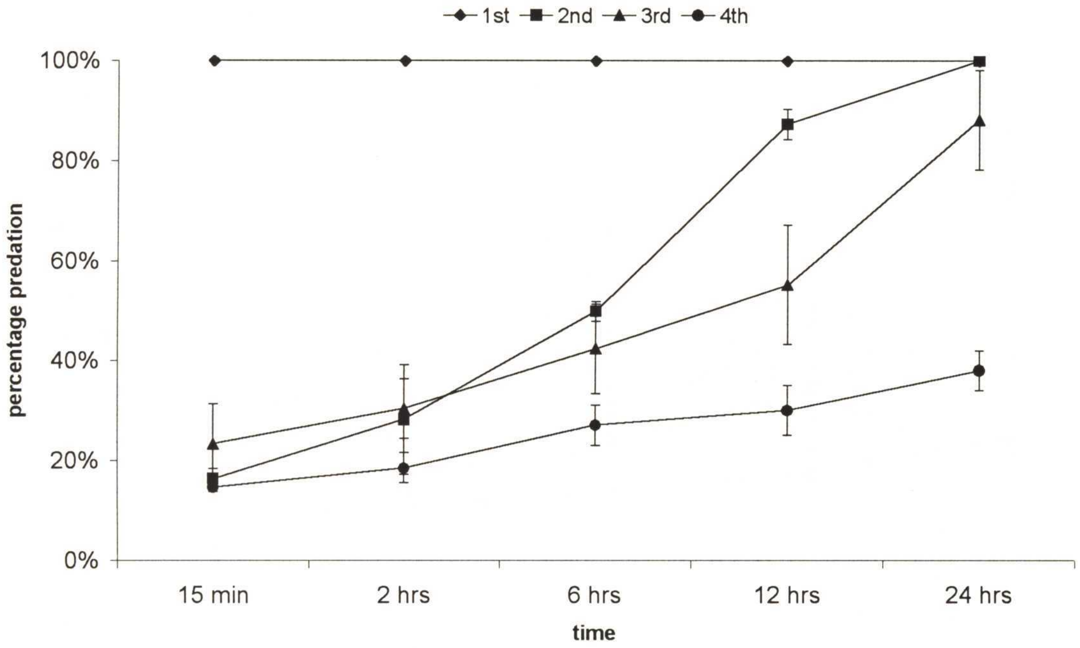
The data were analysed to determine any significant differences between the amount of larvae consumed by each species at 15 minutes and at 24 hours. At 15 minutes (TABLE 5.1), the odds of a larva being eaten by *P. signifer* were only 0.59 times those of being eaten by *G. holbrooki* ($P<0.001$) after adjusting for instar and density. Significantly fewer ($P<0.001$) of second, third and fourth instar larvae than first instar larvae were consumed by both fish after adjusting for species and density. Significantly fewer ($P<0.001$) of the medium and high density larvae were consumed by both species compared with the low density larvae.

At 24 hours (TABLE 5.2), the odds of a larva being eaten by *P. signifer* were 1.75 times greater than those of being eaten by *G. holbrooki* after adjusting for instar and density. As the larval density doubled, the odds of a larva being eaten by either fish decreased by one third ($P<0.001$).

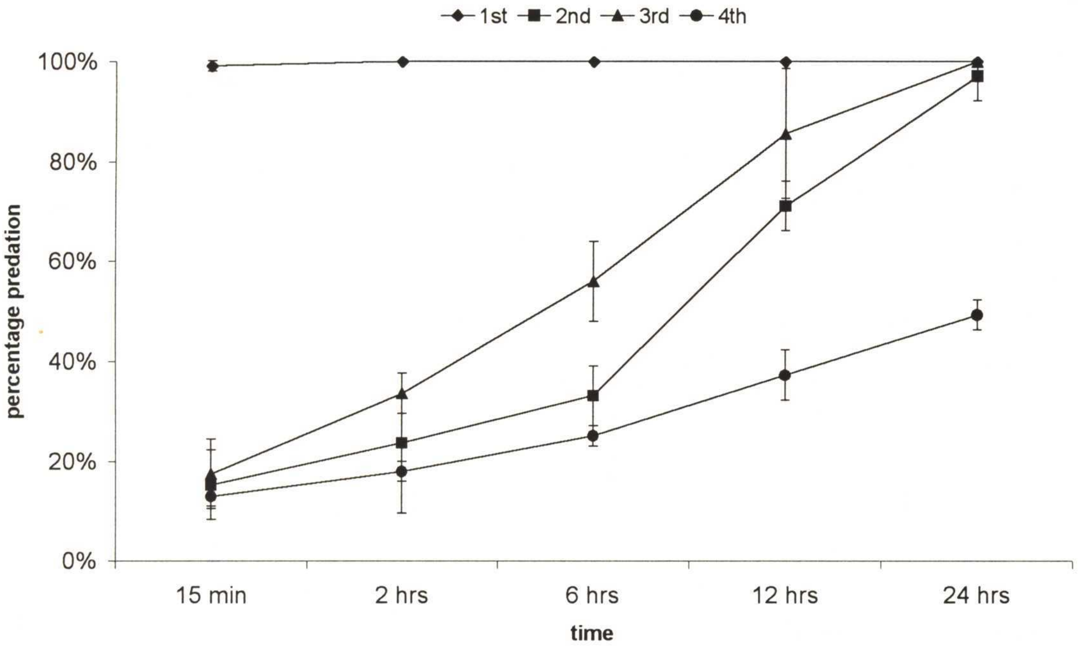
Since both species did not consume all the fourth instar larvae at 24 hours, data were analysed separately to determine significant differences between the species. FIGURE 5.5 shows the percentage of fourth instar larvae consumed at 24 hours by each species for the three larval densities. As larval density increased, fish ate more larvae, but this increase was not proportional to the change in density. Both fish species and larval density were independent predictors of the proportion of larvae consumed. The odds of a larva being eaten by *P. signifer* were 1.51 times higher (TABLE 5.3) than the odds of a larva being consumed by *G. holbrooki* ($P<0.001$), after adjusting for larval density. For every doubling in larval density, the odds that a larva was eaten by *G. holbrooki* (TABLE 5.4) decreased by a factor of 0.51 ($P<0.001$). For every doubling in larval density, the odds that a larvae was eaten by *P. signifer* (TABLE 5.5) decreased by a factor of 0.57 ($P<0.001$).

FIGURE 5.3 Percentage predation by a) *Gambusia holbrooki*, and b) *Pseudomugil signifer* over time for first, second, third and fourth instar *Culex annulirostris* larvae at the highest density (200 larvae). Error bars represent standard deviation.

a) *Gambusia holbrooki*



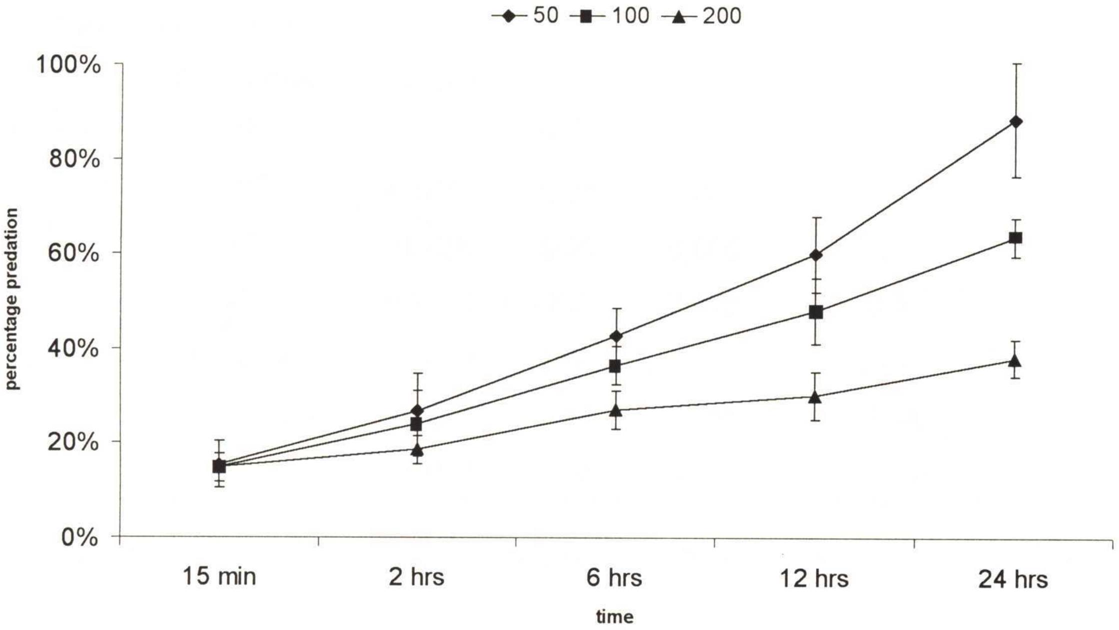
b) *Pseudomugil signifer*



1st=first instar, 2nd=second instar, 3rd=third instar, 4th=fourth instar

FIGURE 5.4 Percentage predation of fourth instar *Culex annulirostris* larvae over time by a) *Gambusia holbrooki*, and b) *Pseudomugil signifer* at three larval densities. Error bars represent standard deviation.

a) *Gambusia holbrooki*



b) *Pseudomugil signifer*

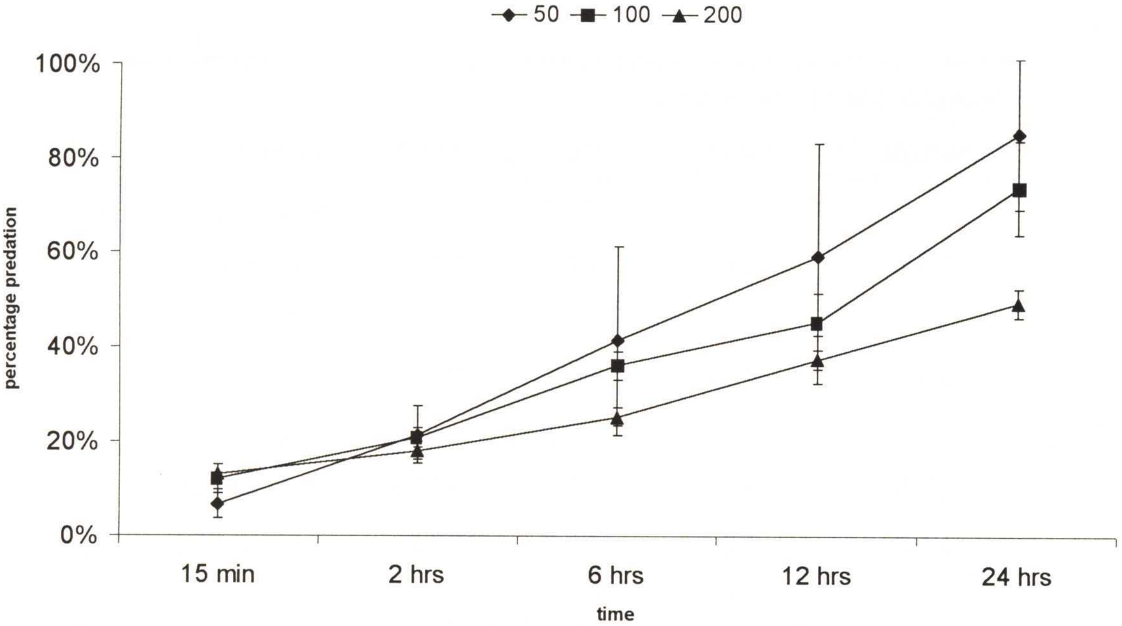


TABLE 5.1 Summary of the logistic regression model for the number of larvae eaten at 15 minutes by *Gambusia holbrooki* and *Pseudomugil signifer*.

				95% Confidence interval	
Variable	**Values taken**	**P-value**	**Odds**	**Lower**	**Upper**
Species	***G. holbrooki***	-	1.00	-	-
	P. signifer	<0.001	0.59	0.52	0.67
Instar	**1^{st}**	-	1.00	-	-
	2^{nd}	<0.001	0.01	0.005	0.01
	3^{rd}	<0.001	0.01	0.005	0.01
	4^{th}	<0.001	<0.01	0.002	0.004
Density	**50 larvae**	-	1.00	-	-
	100 larvae	<0.001	0.33	0.28	0.40
	200 larvae	<0.001	0.26	0.22	0.30

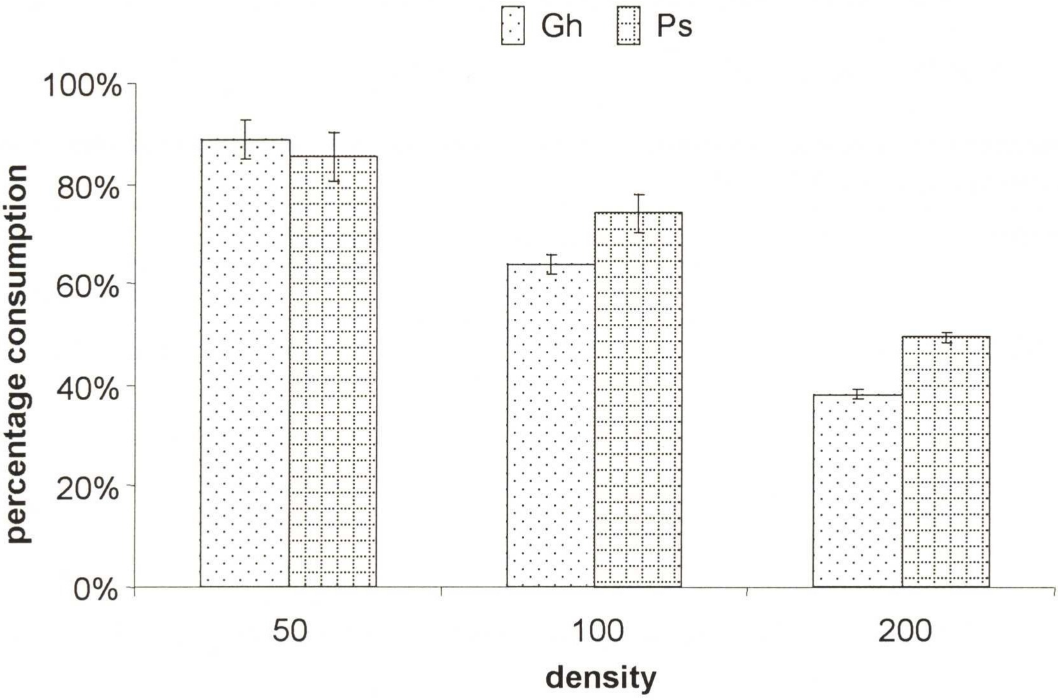
1^{st}=first instar; 2^{nd}=second instar; 3^{rd}=third instar; 4^{th}=fourth instar

TABLE 5.2 Summary of the logistic regression model for the number of larvae eaten at 24 hours by *Gambusia holbrooki* and *Pseudomugil signifer*.

				95% Confidence interval	
Variable	**Values taken**	**P-value**	**Odds**	**Lower**	**Upper**
Species	***G. holbrooki***	-	1.00	-	-
	P. signifer	<0.001	1.75	1.47	2.08
Instar	**1^{st}, 2^{nd}, 3^{rd}**	-	1.00	-	-
	4^{th}	<0.001	0.02	0.01	0.02
Density	**50 larvae**	-	1.00	-	-
	100 larvae	<0.001	0.34	0.23	0.49
	200 larvae	<0.001	0.10	0.07	0.14

1^{st}=first instar; 2^{nd}=second instar; 3^{rd}=third instar; 4^{th}=fourth instar

FIGURE 5.5 The percentage of fourth instar *Culex annulirostris* larvae consumed at 24 hours by each fish species for the three larval densities. Error bars show standard deviation.



Gh = *Gambusia holbrooki*, Ps = *Pseudomugil signifer*

TABLE 5.3 Summary of the logistic regression model for consumption of fourth instar *Culex annulirostris* larvae by *Pseudomugil signifer* compared to *Gambusia holbrooki* at 24 hours, adjusted for larval density.

			95% Confidence interval	
	P-value	**Odds**	**Lower**	**Upper**
G. holbrooki	-	1.00	-	-
P. signifer	<0.001	1.51	1.26	1.82

TABLE 5.4 Summary of regression model for consumption of fourth instar *Culex annulirostris* larvae by *Gambusia holbrooki* at 24 hours, adjusted for larval density.

			95% Confidence interval	
	P-value	**Odds**	**Lower**	**Upper**
Larval density	-	1.00	-	-
G. holbrooki	<0.001	0.51	0.46	0.58

TABLE 5.5 Summary of regression model for consumption of fourth instar *Culex annulirostris* larvae by *Pseudomugil signifer* at 24 hours, adjusted for larval density.

			95% Confidence interval	
	P-value	**Odds**	**Lower**	**Upper**
Larval density	-	1.00	-	-
P. signifer	<0.001	0.57	0.51	0.64

5.1.4 Discussion

This investigation set out to compare the feeding efficiencies of *G. holbrooki* and *P. signifer* using mosquito larvae in controlled laboratory conditions. The results showed that the two species performed similarly, although *P. signifer* consumed slightly more larvae than *G. holbrooki* in the experimental period.

Earlier investigations by Booth (1980) to determine the extent of prey-size selection by *G. holbrooki* and *P. signifer* found similarities in the relationship of body length to weight and the digestive tract histology over the range of each species examined. According to Booth (1980), these similarities indicate that the two species have comparable metabolic requirements, and the findings in this investigation support this proposition.

Investigating the diet of *P. signifer* from Moreton Bay, within a subtropical Australian saltmarsh, Morton *et al.* (1988) found that in the summer, mosquito larvae comprised 89% of their daytime diet. *Culex annulirostris*, the major freshwater pest mosquito at Homebush Bay, reaches peak abundance during the summer months (Chapter 3). If *P. signifer* responded similarly in the field as in the laboratory, a school of five would have no difficulty in consuming 200 first instar larvae upon first encounter and over a 24 hour period they could potentially consume nearly 50 fourth instar *Cx. annulirostris* larvae. While such predictions can be made based on findings of this laboratory investigation, further testing in the field is necessary to understand how the species will respond in natural conditions. Experiments investigating interactions between *P. signifer* and *G. holbrooki* were not undertaken since *P. signifer* would suffer adverse impacts from *G. holbrooki* (Lloyd 1990a) and, they were rarely found together in Homebush Bay habitats.

5.2 The effects of (simulated) vegetation density on the feeding efficiency of *Pseudomugil signifer* Kner and *Gambusia holbrooki* (Girard)

5.2.1 Introduction

Larval abundance of freshwater mosquitoes has been shown to be positively associated with moderate to dense vegetative growth (Walton and Mulla 1990, Walton *et al.* 1990, Kettle *et al.* 1991, Workman and Walton 2000). Dense macrophyte stands provide favourable habitat for mosquito larvae by influencing oviposition, providing nutrients for developing larvae and refuge from predators and physical disturbance (Berkelhamer and Bradley 1989, Walton and Workman 1998). Several studies have also shown that dense vegetation reduces a predator's efficiency in consuming prey, either by physically excluding the predator, reducing visual acuity or increasing prey search and 'handling' time (Savino and Stein 1982, Anderson 1984, Cook and Streams 1984, Diehl 1988). This laboratory investigation was designed to determine the effects of vegetation density on *G. holbrooki* and *P. signifer* within an artificial environment and therefore contribute to a greater understanding of the feeding efficiency of the native species *P. signifer*.

5.2.2 Materials and methods

Experimental fish and maintenance were identical to the previous investigation (Section 5.1.2). Fish were tested in groups of five to reduce stress. Experimental trays contained approximately 21 litres of conditioned water and had a surface area of 55 cm x 37.5 cm. Gravel was shaped to a slope of 1:5 to simulate the bank of a pond along one side of the tray with a maximum depth of 10 cm. The stems of the weed

Juncus acutus, collected from Homebush Bay, were placed along the side of the tray in a grid pattern, covering approximately one third of the surface area to represent emergent vegetation occurring in the wetlands at Homebush Bay. Three stem densities were tested: 100 stems (low), 200 stems (medium), 400 stems (high), and nil stems used as a control. The least distance between stalks decreased from approximately 1.8 cm at the low stem density to 0.90 cm at the high stem density.

One hundred and fifty *Cx. annulirostris* fourth instar larvae were placed in each tray 30 minutes prior to introduction of the fish. The mean standard length of *G. holbrooki* and *P. signifer* was 20.58 ± 1.41 mm and 21.08 ± 1.53 mm, respectively. There were four replicates per species of fish at each density. The number of larvae remaining after 24 hours were counted and recorded. The larvae could not be counted over time since they could not be located without disturbing the vegetation. Logistic regression analyses (see APPENDIX 1 for an explanation of logistic regression analysis) were used to determine the independent predictors of the proportion of larvae consumed. The software program used to perform the analyses was SPSS for Windows, Version 10.0.

5.2.3 Results

5.2.3.1 Larval behaviour

Larvae were observed casually, prior to introduction of the fish, to determine their behaviour at each density. In trays with nil stems, larvae were distributed evenly around the perimeter, sometimes aggregating in the corners. In low density trays, approximately half of the larvae were observed to distribute within the vegetated area.

At medium and high density, the majority of larvae were distributed well within the vegetation with larvae only occasionally swimming into open water.

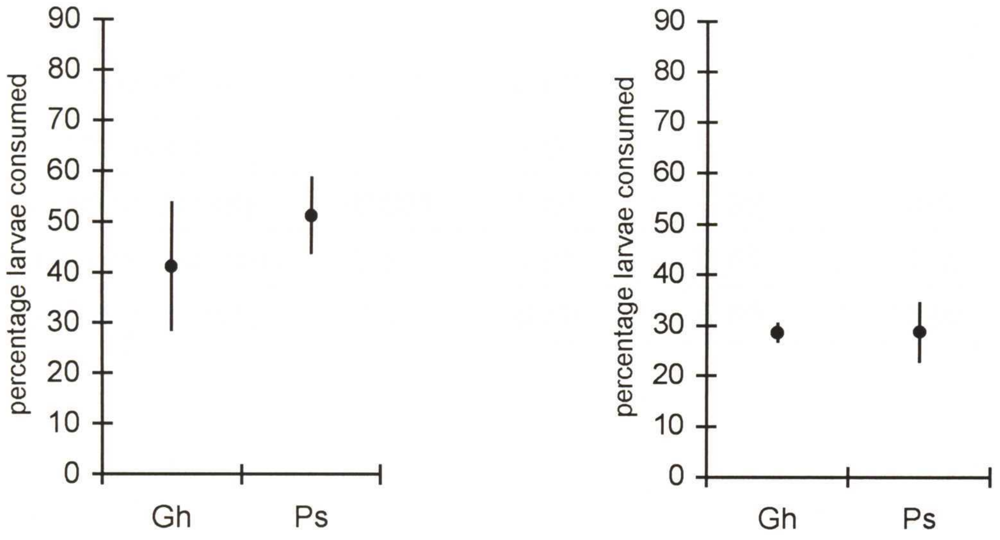
5.2.3.2 Effects of simulated vegetation density on fish

Using logistic regression analysis, the number of larvae consumed was examined separately for each fish species because of the significant interaction between the species variable and vegetation density.

Gambusia holbrooki responded similarly ($P>0.05$) at the medium and high densities of stems and in trays with no stems, consuming approximately 45% of the larvae (FIGURE 5.4 and TABLE 5.6). Significantly fewer larvae ($P<0.001$) were consumed at the low stem density compared to nil stems (FIGURE 5.6 and TABLE 5.6).

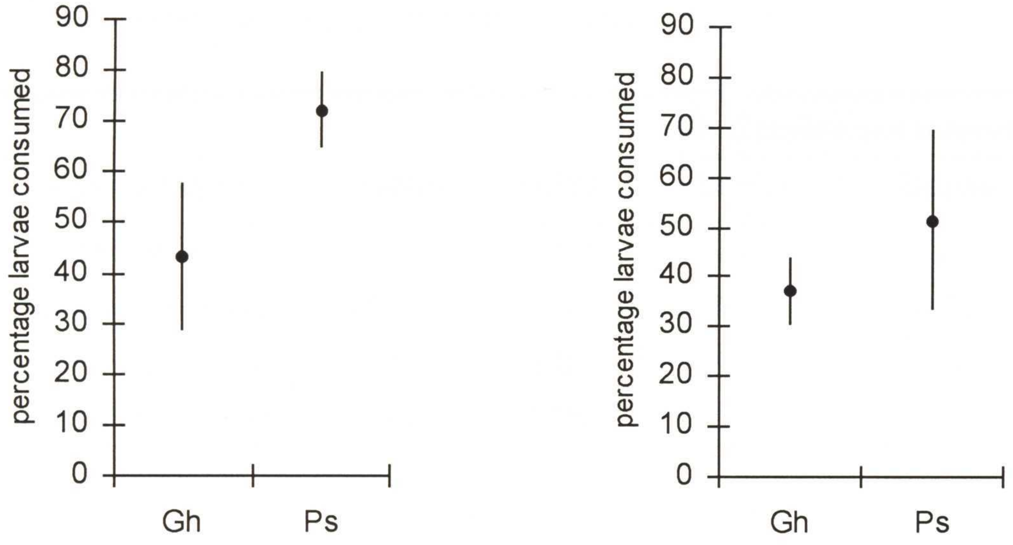
Pseudomugil signifer was most effective in the medium stem density (FIGURE 5.4 and TABLE 5.7), consuming 70% of larvae followed by similar consumption rates (50%) at the high stem density and nil stems ($P>0.05$). Significantly fewer larvae ($P<0.001$) were consumed at the low stem density than with nil stems.

FIGURE 5.6 Percentage (%) of *Culex annulirostris* larvae consumed by *Gambusia holbrooki* and *Pseudomugil signifer* at a) Nil stems, b) Low density stems, c) Medium density stems, and d) High density stems with 95% confidence intervals.



a) Nil stems

b) Low density stems



c) Medium density stems

d) High density stems

Gh = *Gambusia holbrooki;* Ps = *Pseudomugil signifier.*

TABLE 5.6 Summary of logistic regression analysis for the effects of simulated vegetation density on larval predation by *Gambusia holbrooki.*

			95% Confidence interval	
Values taken	**P-value**	**Odds**	**Lower**	**Upper**
Nil stems	-	1.00	-	-
Low stem density	<0.001	0.51	0.38	0.69
Medium stem density	0.67	1.06	0.81	1.40
High stem density	0.11	0.79	0.60	1.06

TABLE 5.7 Summary of logistic regression analysis for the effects of simulated vegetation density on larval predation by *Pseudomugil signifer*

			95% Confidence interval	
Values taken	**P-value**	**Odds**	**Lower**	**Upper**
Nil stems	-	1.00	-	-
Low stem density	<0.001	0.47	0.35	0.63
Medium stem density	<0.001	1.86	1.42	2.42
High stem density	=0.17	1.21	0.92	1.58

5.2.4 Discussion

A range of factors is known to influence the foraging success of fish in habitats that provide refuge for prey organisms. Vegetation density can have significant effects on the swimming speed of fish during foraging as well as affecting manoeuvrability (Winfield 1986). Search and 'handling' time may be significantly increased within vegetation (Anderson 1984), while activity declines due to a reduction in visual contact (Savino and Stein 1982).

Fish also respond to changes in prey behaviour. Predators will aggregate in the areas of highest prey density, increase their search efficiency and remain in the area until the 'reward rate' becomes low (Hassell and May 1974).

During this experiment, when larvae were clustered in the corners and distributed around the edges of the trays without 'vegetation', they were easily detected and consumed. At the lowest stem density, approximately half of the larvae were distributed within the 'vegetated' area and half in the open water. A reduction in swimming speed, manoeuvrability, activity and visual acuity may have contributed to the decrease in consumption rate for both fish at this density. At the medium and high stem density, almost all of the larvae were seeking refuge within the 'vegetated' area. The distribution of larvae within the 'vegetation' may have elicited an aggregative response in the fish so that the fish spent more time searching in the area of highest larval density. Whether the consumption rate would therefore increase and override the effects of other factors that decrease consumption such as a reduction in swimming speed, manoeuvrability, activity and visual activity, was not measured in this experiment, but it is reasonable to assume that other factors were at work to influence the levels of predation at each stem density.

The aim of this experiment was to determine the effects of increasing stem densities on the percentage of larvae consumed by the native species *P. signifer* and the exotic species *G. holbrooki*. At its best performance (with nil stems, and at the medium and high stem densities), *G. holbrooki* only consumed 45% of the larvae. Early literature has reported on the poor foraging ability of this species in emergent weed habitats (Legner and Medved 1974, Walters and Legner 1980), supported by the results of this experiment. *Pseudomugil signifer*, on the other hand, achieved 70% consumption at the medium stem density and 50% with nil stems and the high stem density. Within the confines of this experiment, *P. signifer* performed marginally better than *G. holbrooki* in 'non-vegetated' water and with medium to high density vegetation. While these results are interesting, it is important to understand that to predict the performance of *P. signifer* in the field, further studies in natural habitats are required.

However, while *P. signifer* is thus potentially useful in densely vegetated habitats that have no fish, it would suffer deleterious impact if introduced to habitats with *G. holbrooki* (Lloyd *et al.* 1986, Howe *et al.* 1997). Mosquito management decisions, based on the desire for use of native predator species, may need to determine whether removal of *G. holbrooki* before introduction of *P. signifer* is feasible. Sites that *G. holbrooki* has colonised downstream of drainage systems are most likely subject to reintroduction of the fish after removal. These sites may well be left as they are and may not produce significant amounts of larvae if the vegetation density is managed. In isolated or newly constructed ponds needing mosquito management, *P. signifer* may flourish and effect substantial larval control. In addition, *P. signifer* is not adversely affected by *B.t.i.* (Brown *et al.* 1998) so the bacterial control agent can be used in habitats once the fish predator has been introduced if supplemental control is required.

CHAPTER 6

INVESTIGATION OF PREDATION OF IMMATURE *CULEX ANNULIROSTRIS* LARVAE BY FOUR ENDEMIC LARVAL ANURANS FOR MANAGEMENT OF MOSQUITOES IN FROG PONDS

6.1 Introduction

Evidence for predation by the North American tadpole *Bufo viridus* and the Australian tadpole *Lechriodus fletcheri* on mosquito larvae has been documented by Blaustein and Margalit (1996) and Pimm and Kitching (1987), respectively. The aim of this investigation was to determine if the larvae of the endemic anurans *Limnodynastes peronii*, *Limnodynastes tasmaniensis*, *Litoria aurea* and *Litoria peronii* consume mosquito larvae under experimental conditions. If so, what were the levels of predation between fed and starved tadpoles, and what factors were significant predictors of predation? If any of the tadpoles proved to be efficient at consuming larvae, they may be useful as biological control agents for small to medium sized ponds needing mosquito management.

To avoid confusion between *Limnodynastes peronii* and *Litoria peronii* in the following sections, the genus *Limnodynastes* will be abbreviated as *Lim.* and the genus *Litoria* will be abbreviated as *Lit.*

6.2 Materials and methods

Limnodynastes peronii, *Lim. tasmaniensis*, *Lit. aurea* and *Lit. peronii* were collected by scoop net from a single egg mass and held by a Class A licensed collector (by authority of the NSW National Parks and Wildlife) until they reached Stage 25 of embryonic development (Gosner, 1960). A brief description of the experimental species is presented below.

6.2.1 Experimental tadpoles

6.2.1.1 *Limnodynastes peronii* (Duméril and Bibron), the Striped Marsh Frog

Cogger (1986), Tyler (1994) and Barker *et al.* (1995) have provided descriptions and details of natural history. The species is distributed throughout the coast and ranges of eastern Australia, including Tasmania, and is found in permanent, slow moving water usually hiding under logs, stones or leaf litter.

The dorsal surface is light brown or grey-brown with a series of dark and light brown stripes and spots and frequently a pale mid-dorsal stripe. The ventral surface is white with a few brown flecks. The iris is golden above and dark brown below, the snout is pointed and the tympanum is indistinct. The fingers are without webbing, the first finger is slightly shorter than the second and there is one large inner and two small outer metacarpal tubercles. The toes are free from webbing and long with a small inner metatarsal tubercle. Males average 48-69 mm and females range from 46-73 mm in length.

Breeding takes place from August to March. Approximately 700-1,000 eggs are deposited in a foam mass at the water's edge among vegetation. The tadpoles (FIGURE 6.1) are pale brown in colour, reaching a maximum length of 65 mm. The larval life is 11-12 months.

6.2.1.2 *Limnodynastes tasmaniensis* Günther, the Spotted Grass Frog

Descriptions and natural history of the species are given by Cogger (1986), Tyler (1994), Barker *et al.* (1995). The species occurs throughout eastern QLD, NSW, VIC and TAS. A localised, introduced population has been recorded from Kununurra, WA. Habitats range from marshy coastal areas to dry inland regions.

The dorsal surface is pale greyish-brown or dull green with a series of irregular olive-green or dark brown blotches usually with a pale mid-dorsal stripe. The ventral surface is white except for a dusky yellow throat of males in breeding condition. The snout is pointed, the iris is subcircular and the pupil is golden. The fingers have no webbing, usually two metatarsal tubercles are present and the first finger is shorter than the second. Toes have basal webbing only and a small metatarsal tubercle. Males range from 31-42 mm and females from 32-47 mm in length.

Breeding can occur at any time of year with enough rainfall, however the main breeding season occurs between August and March. Eggs are deposited in a foam mass of 900-1,350 eggs, attached to emergent vegetation. Tadpoles are dark brown in colour and can reach a length of 46 mm. The larval life is 3-5 months.

6.2.1.3 *Litoria aurea* (Lesson), the Green and Golden Bell Frog

Previously, the species was regarded as common throughout southeastern NSW and far eastern VIC, however there has been a marked decline in its distribution and abundance (White and Pyke 1996). The species has disappeared from the Southern Tablelands of NSW and from the Australian Capital Territory (Osborne *et al.* 1996). Habitat investigations by Pyke and White (1996) demonstrated that the species preferred water bodies that were still, shallow, ephemeral, unpolluted, unshaded and free from predatory fish. In addition, aquatic plants, especially *Typha* sp., and diurnal shelter sites such as vegetation and rocks are preferred by the species.

The description and biology of the species are discussed by Cogger (1986) and Barker *et al.* (1995). The dorsal surface is usually green with irregular large blotches and gold stripes. A yellow or white stripe from the eye continues along the body as a dorso-lateral fold, with a thin, black stripe underlining the pale band. The ventral surface is white with groin and hind side of thighs bright blue or blue-green. Fingers have no webbing while toes are about three-quarters webbed with a moderate inner metatarsal tubercle. Finger and toe discs are large. Males range from 57-69 mm and females from 65-108 mm in length.

The breeding season begins in August and eggs are laid in a loose mass among vegetation. Spawn sizes may range from approximately 4,000-6,000 eggs (van de Mortel and Buttemer 1996). Tadpoles (FIGURE 6.2) are usually heavily pigmented and have a characteristically high tail fin.

FIGURE 6.1 Photograph of *Limnodynastes peronii* tadpole

FIGURE 6.2 Photograph of *Litoria aurea* tadpoles

6.2.1.4 *Litoria peronii* (Tschudi), Peron's Tree Frog

Descriptions and biology of the species are given by Cogger (1986) and Barker *et al.* (1995). It is distributed from the coast to the dry inland regions of southeastern QLD, NSW, VIC and southeastern SA. On the coast, it is found in trees and shrubs, and at night it forages in vegetation near the water's edge.

The dorsal surface is rich grey with irregular darker mottling and reticulations and numerous bright green spots. The ventral surface is cream or yellowish with mottled black and yellow colouring on the thighs. Finger and toe discs are large. Fingers are half-webbed with the second finger longer than the first. The toes are almost fully webbed. Males range from 44-53 mm and females 46-65 mm in length.

Spawn ranges from approximately 500-1,800 eggs (van de Mortel and Buttemer 1996). Tadpoles are pale golden yellow in colour with dark lateral stripes; they reach a maximum length of 44 mm.

6.2.2 Tadpole maintenance

Tadpoles were transferred to Westmead Hospital (under a Class B license) where they were maintained in the laboratory in aerated plastic aquaria (23 x 32 x 43 cm) with local habitat water. To maintain water quality, one third of the water and all faecal matter were removed and replaced with conditioned (chloramines removed) tap water once a week. Tadpoles were fed softened lettuce and fish flakes *ad libitum*. Lettuce was softened by freezing to break down the cell walls. Aquaria were maintained at a constant temperature of 19-21° C and illuminated by natural lighting. Tadpoles developing limb buds were transferred to a vivarium containing gravel, leaves, twigs and water, with springtails provided as food. Identification of the species was confirmed as the adult features became distinctive. Upon completion of experimentation, all tadpoles and froglets were returned to frog habitats within the Homebush Bay area by the Class A licensee.

6.2.3 Experimental procedures

Experimental procedures were approved by the Westmead Hospital Animal Ethics Committee (Protocol Nos. 805-07-98 and 805-06-99). Although actual experimental time per tadpole species took no more than two weeks, the investigation continued over two breeding seasons due to drought conditions in the field that restricted the supply of tadpoles for experimentation.

Tadpoles of a single species were placed in groups of three into experimental trays (18 cm x 12 cm x 6.5 cm) with 600 ml of water from their aquaria to reduce stress. The experiment was set up in a temperature-controlled environment (20-22° C) with natural lighting. In 12 experimental trays, tadpoles were starved for 24 hours prior to the introduction of ten first instar *Cx. annulirostris* larvae from the colony at Westmead Hospital, while in 12 other trays, tadpoles were fed 1 hour before introduction of the larvae. Control trays without tadpoles were set up to determine if the water or other conditions were detrimental to the mosquito larvae. After 24 hours, live and killed mosquito larvae were removed and counted, because preliminary testing had shown that tadpoles sometimes killed larvae by 'mouthing' them without ingesting. Predation was considered the number of larvae killed and/or ingested.

The same experimental protocol was repeated for second, third and fourth instar larvae, and for each species of tadpole. Logistic regression analyses (see APPENDIX 1 for an explanation of logistic regression analysis) were used to determine any significant differences between starved and fed tadpoles, between frog species, and between mosquito instars. The software program SPSS for Windows, Version 10.0, was used to analyse the data.

6.3 Results

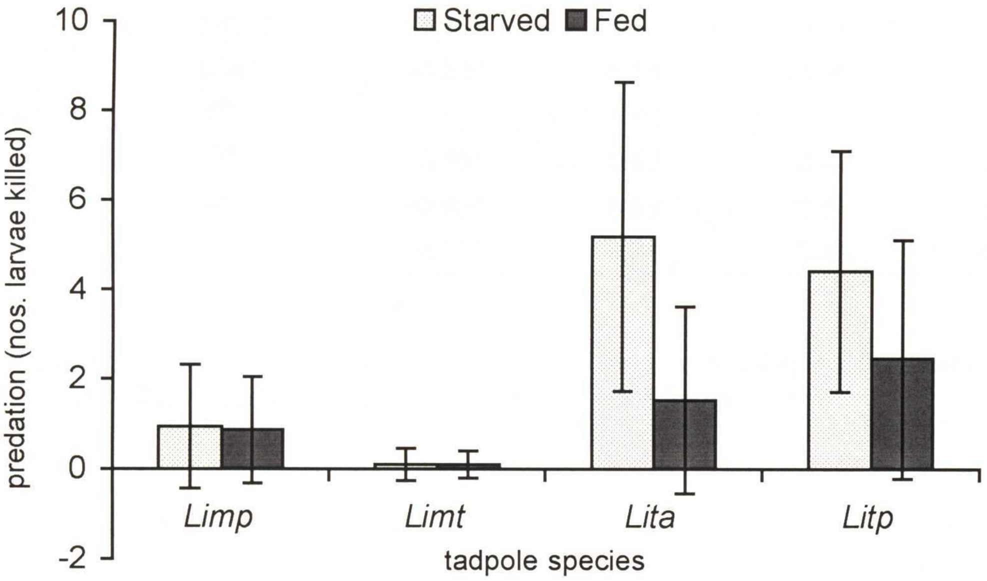
Although tadpoles were observed swimming, grazing along the walls of the experimental container and along the surface of the water, or resting motionless on the bottom of the container, each genus behaved distinctively. In the fed and starved tests, *Lim.* spp. spent the majority of time at the bottom either grazing or motionless. The *Lit.* spp., especially *Lit. aurea*, spent most of the time swimming and foraging at the surface.

Starving increased the mean level of larval predation of the *Lit.* spp. but not the *Lim.* spp (FIGURE 6.3). The mean level of predation was low and similar for fed and starved tadpoles of the *Lim.* species, as if the starvation had made no difference. Fed and starved groups of *Lim. peronii* consumed less than one out of 10 larvae while *Lim. tasmaniensis* consumed approximately one tenth of a larva in 24 hours. In contrast, *Lit.* spp. consumed more larvae, and predation by *Lit. aurea* over 24 hours increased from 1.5 to 5 larvae with starved tadpoles, and increased from 2.5 to 4.5 larvae with starved *Lit. peronii* (FIGURE 6.3).

The summary results of logistic regression analysis for predation of larvae between fed and starved tadpoles are shown in TABLE 6.1. The odds of a larva being eaten by a starved tadpole were approximately three times higher than those of being eaten by a fed tadpole ($P<0.001$) after adjusting for species and instar. All (starved and fed) *Lit. aurea* and *Lit. peronii* preyed upon significantly more larvae ($P<0.001$) than the two *Lim.* spp. after adjusting for starvation and instar.

Since *Lit. aurea* and *Lit. peronii* preyed upon more larvae than the other tadpoles, they were analysed separately (TABLE 6.2). After adjusting for species and instar, starved *Litoria* spp. tadpoles preyed upon significantly more larvae ($P<0.001$) than fed *Litoria* spp. tadpoles (odds ratio of 3.79). There was no significant difference ($P=0.78$) in the level of predation between all *Lit. aurea* and *Lit. peronii* after adjusting for starvation and instar, although *Lit. peronii* preyed upon slightly more larvae (odds ratio of 1.03). FIGURE 6.4 shows the mean predation of first, second, third and fourth instar larvae by *Lit. aurea* and *Lit. peronii* between fed and starved groups. The fed tadpoles appeared to be interested in the fourth instar larvae while starved tadpoles ate more of everything. Interestingly, *Lit. aurea* ate less ($P<0.001$) of second instar larvae than all other instars, which were consumed in similar amounts.

FIGURE 6.3 Predation of all stages of *Culex annulirostris* larvae by each species between starved and fed tadpoles with error bars showing standard deviation.



Limnodynastes peronii =Limp, Lim. tasmaniensis = Limt, Litoria aurea = Lita, Lit. peronii = Litp

TABLE 6.1 Summary of the logistic regression model to determine independent predictors of the amount of larvae consumed by fed and starved tadpoles of the four species.

Variable	Values taken	P-value	Odds	95% Confidence interval	
				Lower	Upper
Diet	Fed	-	1.00	-	-
	Starved	<0.001	2.94	2.44	3.55
Species	*Limp*	-	1.00	-	-
	Limt	<0.001	0.10	0.05	0.20
	Lita	<0.001	5.52	4.24	7.19
	Litp	<0.001	5.83	4.46	7.61
Instar	1st	-	1.00	-	-
	2nd	<0.001	0.61	0.47	0.79
	3rd	=0.004	0.69	0.53	0.88
	4th	<0.001	0.56	0.43	0.72

Limp = *Lim. peronii*; Limt = *Lim. tasmaniensis*; Lita = *Litoria aurea*; Litp = *Lit. peronii*
1st = first instar; 2nd = second instar; 3rd = third instar; 4th = fourth instar

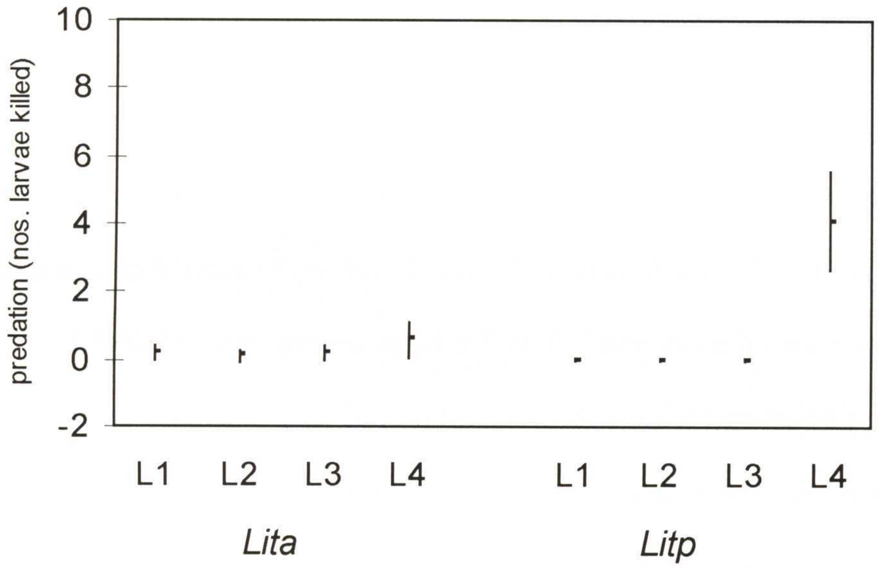
TABLE 6.2 Summary of the logistic regression model to determine independent predictors of the amount of larvae consumed by *Litoria aurea* and *Lit. peronii*.

Variable	Values taken	P-value	Odds	95% Confidence interval	
				Lower	Upper
Diet	Fed	-	1.00	-	-
	Starved	<0.001	3.79	3.07	4.68
Species	*Lita*	-	1.00	-	-
	Litp	=0.78	1.03	0.84	1.26
Instar	1st	-	1.00	-	-
	2nd	<0.001	0.55	0.41	0.74
	3rd	=0.97	0.99	0.75	1.32
	4th	=0.14	0.81	0.60	1.08

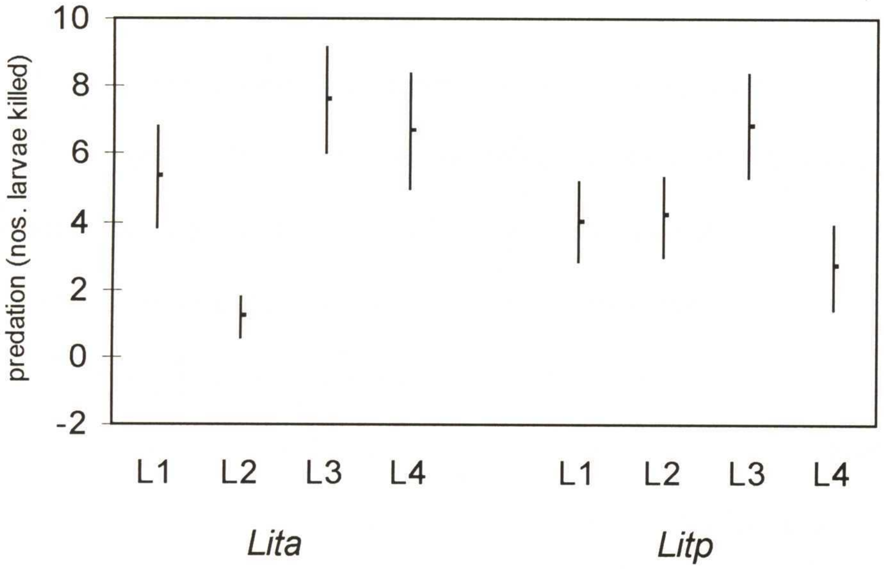
Lita = *Lit. aurea*, Litp = *Lit. peronii*
1st = first instar; 2nd = second instar; 3rd = third instar; 4th = fourth instar

FIGURE 6.4 Predation of first, second, third and fourth instar *Culex annulirostris* larvae by *Litoria aurea* and *Lit. peronii* tadpoles between a) fed and b) starved groups with error bars showing 95% confidence intervals.

a) Fed group



b) Starved group



Litoria aurea = Lita, *Litoria peronii = Litp*
1st = first instar, 2nd = second instar, 3rd = third instar, 4th = fourth instar

6.4 Discussion

This study was designed to determine if *Lim. peronii*, *Lim. tasmaniensis*, *Lit. aurea* and *Lit. peronii* tadpoles consumed mosquito larvae. The results show that they did, and that the species of tadpole and larval instar of mosquito are significant predictors of predation.

However, the consumption levels over a 24 hour period were very low compared to the predatory capabilities of the fish, *P. signifer* and *G. holbrooki* (CHAPTER 5). All (starved and fed) *Lit.* spp. preyed upon 5.5 to 6 times more larvae than *Lim.* spp., although tadpole behaviour may have influenced the results (see below). All *Lit.* spp. and *Lim.* spp. tadpoles chose more first, third, and fourth instar larvae than second. These results were influenced primarily by the *Lit.* spp. Larval instar was a significant predictor of larval predation.

Many tadpoles are herbivores and suspension feeders. Tadpoles feed by filtering their food and by browsing (Tyler 1994). In filter-feeding, mucus cords secreted within the internal gills act as a trap to collect filterable food. As water passes across the gill arches, various microscopic organisms are trapped in the mucus and are directed toward the oesophagus by ciliary currents (Roberts 1993, Tyler 1994). Feeding by browsing involves scraping the surface of food matter such as vegetation, dead animals and the pond substrate, using the labial teeth (Davies and Withers 1993, Roberts 1993, Tyler 1994). The majority of tadpoles have a generalised morphology with labial teeth, keratinised beaks, buccopharyngeal gill filters and other structures associated with suspension feeding (Petranka and Kennedy 1999). Notwithstanding these feeding behaviours, this study demonstrated that *Lit. aurea*, *Lit. peronii*, and to a lesser extent *Lim. peronii* and *Lim. tasmaniensis* tadpoles can prey upon mosquito larvae opportunistically.

A few authors have investigated the interactions between tadpoles and mosquito larvae. Blaustein and Kotler (1993) found in Israel that *Culiseta longiareolata* Macquart tended to avoid ponds containing *Bufo viridus* tadpoles for oviposition and their oviposition rate in these ponds was reduced by 50%. Later, Blaustein and Margalit (1994) demonstrated that *Cs. longiareolata* mosquito larvae and *B. viridus* tadpoles have mutual negative effects on each other when placed into artificial ponds at the same time. Interspecific competition occurs since they compete for the same food resources. The length of developmental time from L1 to pupae in *Cs. longiareolata* was lengthened and the pupal size reduced in the presence of *B. viridis* tadpoles. However, in the presence of *Cs. longiareolata* larvae, *B. viridis* tadpoles weighed less and the proportion reaching metamorphosis was lowered. In a follow-up study by Blaustein and Margalit (1996), *B. viridis* tadpoles were introduced 12 days prior to *Cs. longiareolata* larvae. After introduction of the larvae, tadpoles consumed larvae during the first few days but not after they reached a certain size. The developmental time of the surviving larvae was slower in the presence of tadpoles.

Pest mosquito species such as *Oc. alboannulatus*, *An. annulipes* s.l., *Cx. annulirostris* and *Cx. quinquefasciatus* colonise the small frog ponds at Homebush Bay (Chapter 3). A small group of tadpoles, as in this experiment, will not consume great numbers of larvae in a 24-hour period and are therefore not ideal as a short-term biological control agent for large numbers of mosquitoes. However, whether large populations of tadpoles are able to suppress populations of the mosquitoes colonising the small ponds or whether the mosquitoes might avoid choosing the small ponds for oviposition in the presence of tadpoles requires further investigation. The colonisation of small ponds by *Lit. aurea* especially, should be encouraged since they were the most successful tadpole predator. The *Lit.* spp. were observed to forage at the surface and to be more active than the *Lim.* spp. Tadpole high activity is a phenotypic trait that gives temporary pond dwellers a competitive advantage, whereas low relative activity decreases the chances of being detected by predators for those tadpoles living in more permanent habitats (Skelly 1997). A few authors in Australia have documented species specific tadpole activity. The inactivity of *Lim. peronii* has been noted by Webb (1994), and the surface swimming behaviour of *Lit. ewingi,* was noted by Peterson *et al.* (1992). The natural habitats of the tadpoles in this study reveal the reason for varying activity levels observed. Recent investigations of *Lit. aurea* have shown that the species prefers shallow, ephemeral pools (Pyke and White 1996). *Litoria peronii* is able to breed in permanent ponds and temporary low-lying areas inundated by summer rains (Cogger 1986, Barker *et al.* 1995). *Limnodynastes peronii* is found in slow moving water and *Lim. tasmaniensis* is usually found in marshy country (Cogger 1986, Barker *et al.* 1995). Thus, the degree of tadpole activity exhibited by the *Lit.* spp. and *Lim.* spp. in this experiment is perhaps a response to the requirements for survival in their preferred habitats, and the reason for the higher consumption levels by *Lit.* spp. (5.5 to 6 times more larvae than the *Lim.* spp).

In addition to phenotypic traits of tadpoles, the behaviour of the *Cx. annulirostris* prey larvae may have made them more susceptible to predation by the *Lit.* spp. Laboratory studies by McDonald (1978) found that the larvae spend more than 87% of the time resting/feeding at the surface. Therefore, the behaviour of larvae and tadpoles in this instance would allow for increased contact between the *Lit.* spp. and *Cx. annulirostris* larvae. The same may be found with *Anopheles* larvae, e.g. the local *An. annulipes* s.l., that feed predominantly while resting at the water surface. *Ochlerotatus* mosquitoes, however, typically feed on bottom substrate and local species may be affected more by foraging activity of the *Lim.* spp. Further research is needed to elucidate this possibility.

In general, the tadpole species in this experiment appear to be opportunistic predators of mosquito larvae. Mosquito larvae were the only food source for tadpoles in this study with the exception of microscopic organisms in the habitat water. In a freshwater pond, tadpoles typically would have an abundant supply of periphyton, dead animals and microscopic organisms to feed upon and are not likely to have a major impact on mosquito larvae unless starved. Even so, this study indicates that tadpoles would not be effective in quickly reducing a pest mosquito population within a pond environment. However, further studies are needed to investigate the effects of interspecific competition between tadpoles and mosquitoes in a natural system.

CHAPTER 7

CONCLUDING DISCUSSION

This study has investigated a) the major freshwater mosquitoes emanating from the freshwater wetlands at Homebush Bay, significant for their pest and vector status and their seasonal activity and relative abundance; b) the mosquito productivity of various habitats and, the characteristics of habitat associated with increased larval densities; c) the stadial duration of immature *Cx. annulirostris* in the field; f) the relative efficiency of a native fish, *Pseudomugil signifer* as a predator on mosquito larvae, and g) the potential of four endemic larval anurans as biological control agents.

7.1 Major freshwater species: seasonal activity and relative abundance

Culex annulirostris was the most abundant of the freshwater mosquitoes and represented approximately 10% of the total number of mosquitoes collected over the four-year study period, followed by *Cx. quinquefasciatus* (9.7%), *Cx. australicus* (5.7%), *Oc. notoscriptus* (2.2%), *An. annulipes* s.l. (2.0%), *Cq. linealis* (1.0%) and *Oc. alboannulatus* (0.2%).

Adults of *Cq. linealis*, which was as abundant as *Cx. annulirostris* in the first year of the program, progressively decreased over the study period as substantial areas of reed habitat (with which they were associated) were removed for redevelopment. If reed vegetation is allowed to spread in the new wetlands, *Cq. linealis* populations are likely to increase accordingly. Mosquito management strategies and practices incorporating vegetation management will be required for *Cq. linealis* populations to be minimised.

A model of *Cx. annulirostris* abundance was developed that emphasised the importance of rainfall to the temporal abundance of the species. The establishment of a population abundance threshold level would be useful in planning for population management through pesticide treatments.

7.2 Productivity of various habitats and habitat characteristics associated with increased larval densities

Investigations of habitat characteristics in relation to larval abundance from the first year and the last year of surveillance, with logistic regression analysis, showed that dense (> 70 %) vegetation produced statistically greater larval densities of mosquitoes compared to other habitats. Although not statistically significant, there was also a tendency for deep habitats (> 35 cm) to produce lower densities of larvae than shallow habitats.

Although *Cx. annulirostris* represented only 10% of the total trap catch during the four-year surveillance period, the predominant saltmarsh mosquito populations are being targeted for control and relatively large populations of *Cx. annulirostris* may occur in the future as a result of the establishment of dense vegetation in wetland habitat as mentioned above. Ross River virus and other arbovirus activity are driven by large increases in mosquito populations (Russell 1998), and such was the case in February 1999 when Ross River virus was isolated from *Oc. vigilax*, the major saltmarsh species at Homebush Bay. As a result of tidal flushing in NHB and BP, and treatment with *B.t.i.*, pest populations of *Oc. vigilax* are being reduced (Webb and Russell 1999). Unless management practices are utilised for the freshwater habitats, *Cx. annulirostris* and *Cq. linealis* have the potential to become major pest species locally, thereby increasing the risk for arbovirus activity.

7.3 Stadial duration of immature *Culex annulirostris* in the field

Appropriate timing of larvicide treatment requires the correct identification of larval instars and knowledge of the stadial duration of immatures in the field. This study showed that head capsule width of *Cx. annulirostris* larvae remains discrete with each moult although the width of some instars vary between the months as a result of changing field conditions. Siphon length however, was too variable to identify instars. Therefore, head capsule width is the most reliable method for identifying larval instars of *Cx. annulirostris* at Homebush Bay, regardless of changing field conditions.

Larval development from first to fourth instar in December, January, February and March was 12.0, 9.0, 9.3 and 17.0 days respectively. Multiple regression analysis of various environmental factors showed that the only independent predictor of the median number of days to reach the fourth instar was average minimum temperature, explaining 93% of the variability. Using projected minimum weekly temperatures based on long-term averages, an estimate of the length of the fourth instar may be achieved. Successful abatement of substantial larval populations using *B.t.i.* requires treatment before the fourth instar because late fourth instar immatures stop feeding before pupation.

7.4 Efficiency of the native fish, *Pseudomugil signifer* for biological control of mosquitoes

This investigation compared the efficiency of *P. signifer*, a native species abundant in the waterways of Homebush Bay with that of *G. holbrooki*, an introduced noxious species, previously used for mosquito control. A comparison of feeding efficiencies with increasing sizes (first to fourth instar) and increasing densities (50, 100 and 200) of *Cx. annulirostris* larvae, demonstrated that the two species were generally similar. Both species consumed the first three instars readily. However, the fourth instar was avoided by both species.

In simulated vegetation trials, *P. signifer* performed marginally better than *G. holbrooki* in nil vegetation and medium to high density 'vegetation'. If *P. signifer* performed similarly in the field, the species could be more effective than *G. holbrooki* in consuming larvae within wetland habitats with dense vegetation. However, further studies must be conducted in the field to confirm these laboratory results.

7.5 Investigation of four endemic larval anurans

The results of this experiment showed that tadpoles of four endemic anurans, *Lim. peronii*, *Lim. tasmaniensis*, *Lit. aurea* and *Lit. peronii* will consume mosquito larvae under experimental conditions. However, compared to the predatory capabilities of the fish *P. signifer* and *G. holbrooki* (Chapter 5), consumption levels were low. *Litoria aurea*, the best predator, consumed five out of 10 larvae in a 24 hour period.

Although all species consumed larvae opportunistically, the *Lit.* spp. preyed upon 5.5 to 6 times more larvae than the *Lim.* spp., suggesting that behaviour influenced the results. The behaviour exhibited by *Lit. aurea* may be advantageous for mosquito managers if their abundance is encouraged by the establishment of new frog habitat. However, further study in the field is needed to understand the dynamics of the interactions between the endemic tadpoles at Homebush Bay and mosquito larvae, and their potential for long term suppression of mosquito populations.

7.6 Closing statements

The development of integrated control strategies for the reduction of pest and vector mosquito species will be successful when all aspects of mosquito ecology and environmental requirements of the site are fully considered. Continued weekly adult and larval surveillance during the mosquito breeding season, and an increased understanding of influential habitat, meteorological, and other environmental factors provide essential baseline data for the success of mosquito control programs. The introduction of native fish as predators in wetlands, the encouragement of frog populations in small ponds, along with the active maintenance of water quality and wetland vegetation will combine to ensure that mosquito pest populations are minimised with little detrimental effect on the environment.

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APPENDIX 1

Logistic regression analysis (Anderson *et al.* 1980)

To understand odds ratios and logistic regression analysis, consider the following simple experiment. A known number of larvae are placed in a container with fed tadpoles and the same number in a different container with starved tadpoles.

The odds of being eaten by a fed tadpole:

$$= \frac{\text{Pr (eaten by fed tadpole)}}{1 - \text{Pr (eaten by fed tadpole)}} = \frac{\text{Pr (eaten by fed tadpole)}}{\text{Pr (left by fed tadpole)}}$$

The odds of being eaten by a starved tadpole:

$$= \frac{\text{Pr (eaten by starved tadpole)}}{1 - \text{Pr (eaten by starved tadpole)}} = \frac{\text{Pr (eaten by starved tadpole)}}{\text{Pr (left by starved tadpole)}}$$

Therefore, the odds ratio of being eaten by a starved tadpole compared to being eaten by a fed tadpole is ψ.

$$\psi = \frac{\text{odds of being eaten by a starved tadpole}}{\text{odds of being eaten by a fed tadpole}}$$

$$= \frac{\text{Pr (eaten by starved tadpole)}}{1 - \text{Pr (eaten by starved tadpole)}} \text{ x } \frac{\text{1- Pr (eaten by fed tadpole)}}{\text{Pr (eaten by fed tadpole)}}$$

Logistic regression analysis (or logit analysis) can be applied to comparative studies to estimate the effect of a risk factor R (starved vs. fed tadpoles) on a 2 level (or dichotomous) outcome factor Y (larva eaten, $Y = 1$; larva not eaten, $Y = 0$) as measured by the odds ratio. By using this method, it is possible to adjust for many confounding variables X simultaneously. In the fish and tadpole studies examined, these variables included species, larval instar and larval density.

Logistic regression analysis (LRA) is the most suitable means of analysing the data from such a study. It is analogous to the analysis of covariance. LRA accomplishes for comparative studies with dichotomous outcome variables (eaten or not eaten) what ANCOVA does for comparative studies with numerical outcome variables.

We will develop the theory of LRA for the simple case of comparing starved and fed tadpoles with no other variables. It is easy to extend the concept to several confounding variables which can either be numerical or categorical. With this assumption, the ANCOVA model states that:

$Y = \alpha_0 + \text{error}$, if tadpole is fed

$Y = \alpha_1 + \text{error}$, if tadpole is starved

$$\text{where } Y = \begin{cases} 0 & \text{if larva not eaten} \\ 1 & \text{if larva eaten} \end{cases}$$

We could rewrite these models as a single linear regression model by introducing a variable R such that $R = 1$ if the tadpole is starved and $R = 0$ if the tadpole is fed.

Thus,

$$Y = \alpha_0 + (\alpha_1 - \alpha_0) R + \text{error}$$

Provided Y is a numerical variable, ANCOVA is the correct approach for estimating the effect $(\alpha_1 - \alpha_0)$ of the risk factor R. However here Y is a dichotomous variable such that:

$Y = 1$ if larva eaten and $Y = 0$ if larva is uneaten.

Instead of working with Y, we therefore work with P (R), the probability that $Y = 1$ given the values of the risk variable R.

It is clear that:

P (R) = Expected value of Y for given R

By taking the logit transform of P (R) we can form the logistic regression model:

$$\ln\left\{\frac{P(R)}{1 - P(R)}\right\} = \text{'log odds'} = \alpha + \psi R$$

The logit transform of P (R) has desirable property that it can now take values from $-\infty$ to ∞.

Usually there is no theoretical justification for choosing the logit transform. However it has been found to be useful in many fields of application and is preferable to other transformations because the model parameters can so easily be estimated and interpreted as now shown.

The problem is to estimate the effect associated with a risk factor (starved vs. fed). The odds ratio is usually chosen as the measure of risk factor (or treatment) effect since it is simply related to the parameters of the logistic regression model. Recall that the odds ratio ψ is defined by:

$$\psi = \frac{\text{odds of `success' when risk factor present}}{\text{odds of `success' when risk factor absent}}$$

$$= \frac{P\,(1, X) / \{\, 1 - P\,(1, X)\,\}}{P\,(0, X) / \{\, 1 - P\,(1, X)\,\}}$$

And that $\psi = 1$ if the risk factor has no effect on the outcome.

Hence, by taking the logarithm of ψ and assuming the logistic regression model holds, we have:

$$\ln \psi = \ln \left\{ \frac{P\,(1, X)}{1 - P\,(1, X)} \right\} - \ln \left\{ \frac{P\,(0, X)}{1 - P\,(1, X)} \right\}$$

$$= (\alpha + \Upsilon \cdot 1) - (\alpha + \Upsilon \cdot 0) + \beta$$

$$= \Upsilon$$

Then substituting in

$$= \ln \left\{ \frac{P(R)}{1 - P(R)} \right\} = \text{'log odds'} = \alpha + \Upsilon R$$

$$= \Upsilon$$

In other words, logistic regression analysis provides an estimate $\hat{\Upsilon}$ of Υ and then estimates the odds ratio ψ by

$$\hat{\psi} = \exp(\hat{\Upsilon}) = e^{\hat{\Upsilon}}$$