THE UNIVERSITY OF
WESTERN AUSTRALIA
Achieving International Excellence

The University of Western Australia Arbovirus Surveillance and Research Laboratory Annual Report: 2012-2013

Cheryl Johansen, Jay Nicholson, Sarah Power, Shani Wong, Michael Burley, Margaret Wallace, David Smith† and Geoffrey Shellam

Arbovirus Surveillance and Research Laboratory, School of Pathology and Laboratory Medicine, Faculty of Medicine, Dentistry and Health Sciences, The University of Western Australia, Nedlands, WA, 6009

and

†PathWest Laboratory Medicine WA, Western Australian Department of Health, QEII Medical Centre, Nedlands, WA, 6009

The Arbovirus Surveillance and Research Laboratory at The University of Western Australia is funded by the Western Australian Department of Health (DOH). The authors acknowledge and thank the DOH for its continued support of this important public health initiative.

The program involves a close association and collaboration between the Arbovirus Surveillance and Research Laboratory and the Mosquito Borne Disease Control Branch (MBDC) in the DOH. In particular, we would like to thank Dr Michael Lindsay, Dr Peter Neville, Mrs Amber Douglas, Dr Andrew Jardine and Mr Ryan Janes of the MBDC, who have had regular input and involvement in many aspects of the studies and surveillance programs described in this report.

Section 1: The University of Western Australia Arbovirus Surveillance and Research Program, 2012/13, Executive Overview

Objectives

1. To monitor mosquito populations in coastal regions of the southwest of Western Australia (WA) at risk of Ross River virus (RRV) and other arbovirus activity by routine (prospective) sampling; to identify the major pest and vector species for each region and monitor population fluctuations of important species;
2. To rapidly identify arbovirus activity (in mosquito populations sampled as part of Objective 1) by processing mosquito samples for virus isolation using cell culture and identifying isolated viruses using serological and molecular techniques; to determine infection rates of mosquito populations and associated risks to human health;
3. To provide forewarning of flavivirus activity in WA by deployment and serological testing of flocks of sentinel chickens at up to 30 locations in the northern half of WA;
4. To carry out an annual audit of late wet season mosquito fauna and arbovirus activity at key locations in the Kimberley region;
5. To determine the major vector and pest mosquito species in different regions of WA under different environmental conditions by opportunistic sampling following flood rains, high tides or during outbreaks of disease;
6. To analyse environmental (climate and weather) data relevant to arbovirus, vector mosquito and vertebrate host ecology;
7. To investigate the epidemiology and risk factors of human arboviral disease throughout WA, particularly in relation to arbovirus activity in mosquitoes and sentinel chickens;
8. To provide confirmatory diagnosis for suspected human infections with Murray Valley encephalitis virus (MVEV), Kunjin virus (KUNV) or other flaviviruses for WA PathWest Laboratory Medicine;
9. To develop and refine new field and laboratory techniques for surveillance of mosquito-borne diseases in WA; and
10. To carry out applied research into the molecular epidemiology, seroprevalence, impact of human activities upon and other aspects of arbovirus activity throughout WA.

Goals

1. To provide early warning of arbovirus and vector mosquito activity for the public health of Western Australians;
2. To increase understanding of the ecology and epidemiology of mosquito-borne viruses of public health importance and their inter-relationships with vectors, vertebrate hosts, the environment and human activities, in order to predict and be prepared for future outbreaks of human disease; and
3. To increase speed, accuracy and sensitivity of the WA arbovirus surveillance program.

Benefits

1. The Western Australian Department of Health (DOH) and Local Government Authorities (LGAs) will have early warning of the need to undertake vector control measures and issue media releases warning the public to take self-protective measures;
2. The DOH and LGAs will have accurate information required to maximise the effect of current mosquito control programs or assist with the creation of new control plans, as well as directing strategies for public education and management of natural, rural and urban environments; and
3. Overall, in conjunction with DOH and LGA initiatives, the risk and incidence of mosquito-borne diseases in WA will be reduced.

Summary of results from the 2012/13 program

Monitoring of mosquito fauna and arbovirus activity continued at key locations on the Swan Coastal Plain. Surveillance of mosquito fauna and arbovirus activity was also carried out following seasonal rains in the Kimberley region. Year-round flavivirus surveillance continued in northern WA using sentinel chicken flocks. Environmental conditions and other predisposing factors were also monitored.

Southwest

- Rainfall was generally below average in the southwest of WA, with the exception of November and December when rainfall was higher than usual. Warm conditions prevailed for most of the year. Tides regularly inundated vector breeding saltmarsh during the warm spring and summer.
- Vector abundance was high, particularly *Aedes camptorhynchus* in winter and spring, and *Ae. vigilax* during summer in the Peel and Leschenault regions. *Aedes camptorhynchus* dominated collections in Capel-Busselton, remaining high through to June in Capel.
- Like previous years, prolonged warm temperatures in the southwest of WA probably enabled continued breeding of *Ae. vigilax* through to June.
- The first arbovirus isolate for the season was Barmah Forest virus (BFV) from *Ae. camptorhynchus* collected at Capel on 11 September 2012. Eight further isolates of BFV were identified from Capel/Busselton, through to December, prompting the DOH to issue a media release about increased risk of vector-borne disease. The minimum infection rate (MIR) peaked at 3.6 per 1000 mosquitoes on 20 November.
- The first arbovirus isolations from the Peel region occurred when RRV was obtained from *Ae. camptorhynchus* collected on 4 December 2012 at the southern end of the Harvey Estuary, and the MIR peaked for the season at 19.1 per 1000 mosquitoes at that time. Between then and 29 January 2013 further isolations of RRV (1), Edge Hill virus (EHV; 3) and BFV (1) were obtained from *Ae. camptorhynchus* and *Ae. vigilax*. Most isolates were from Harvey Estuary sites.
- In the Leschenault region, BFV was first detected on 4 December 2012, when it was isolated from *Ae. camptorhynchus* collected at the Belvidere trapsite. Eight isolates of RRV were later obtained from *Ae. camptorhynchus*, *Ae. vigilax* and *Culex annulirostris* collected in January.
- All isolates of RRV from the southwest of WA were of the northern/eastern phenotype.
- A total of 1103 human cases of RRV disease were reported in WA in the 2012/13 season, including 338 cases from the southwest of WA, 355 cases from the Perth Metropolitan area and 211 cases from the Goldfields/Esperance. The incidence of RRV disease was lower than the 2011/12 season (1542), but still above average, particularly in the latter half of the year.
- The incidence of BFV disease was not provided by the WA DOH due to concerns related to false-positives in diagnostic assays at some private health laboratories.
- Three isolates of EHV were obtained from *Ae. camptorhynchus* and *Ae. vigilax*.
- One non alphavirus/non flavivirus was isolated from *Ae. camptorhynchus* collected in the Ludlow forest on 20 November 2012, and this virus is yet to be identified.
- 2012/13 outlook: The Bureau of Meteorology models suggest the chance of exceeding median rainfall between October and December is around 50% for most of WA. Warmer than usual day and night temperatures are more likely across most of WA.

Kimberley

2011/12 wet season

- Of the estimated 284166 mosquitoes collected in 2012, 63511 (22.3%) were processed for virus isolation in 4077 pools, yielding 65 arbovirus isolates: 47 RRV (northern/eastern phenotype), 13 new flaviviruses in the yellow fever group, three MVEV and two Kokobera virus (KOKV).
- Most isolates of RRV were from *Cx. annulirostris* and *Ae. normanensis* and it occurred across the Kimberley region.

- The new flavivirus was predominantly isolated from *Ae. normanensis*, and it was obtained from mosquitoes in the southeast and northeast Kimberley regions.
- MVEV was isolated from *Cx. annulirostris* collected at Fitzroy Crossing and Billiluna and the KOKV isolates were from *Cx. annulirostris* collected at Wyndham.
- The low number of isolates of MVEV and absence of KUNV in mosquitoes was surprising given the relatively high level and widespread activity of these viruses in sentinel chickens in the 2011/12 season (ASRL 2011/12 Annual Report; Appendix 1). It is possible that the timing of adult mosquito collections preceded increased flavivirus activity in the West Kimberley region but after the peak in flavivirus activity in the northeast Kimberley region in 2011/12.
- No *Aedes vexans* were collected, however low numbers of *Cx. gelidus* specimens were collected from Kununurra in 2012.

2012/13 wet season

- Above average rainfall was observed in northern parts of WA between October and December 2012. Between January and March 2013 conditions were average or drier than usual in the Kimberley region, with the exception of the west Kimberley. The west Kimberley and parts of the east Pilbara experienced above average rainfall and in some parts, highest on record rainfall during January to March. Tropical cyclones (TC) Narelle and Peta caused heavy rainfall in the western Gascoyne, Pilbara and northern Interior in January. An active monsoon and TC Rusty also caused heavy rain in the Kimberley and Pilbara regions, causing major flooding in the De Grey catchment area in February. Seasonal thunderstorms resulted in more rain in March. Between April and June above to very much above average rainfall was recorded in the Kimberley and most of the Pilbara regions, with highest on record rainfall being recorded in the East Pilbara and northern Interior. Above average rainfall continued into May and June 2013 in northern parts of WA.
- 4508 serum samples from 28 flocks were tested for antibodies to flaviviruses during 2012/13. Seroconversions at Beagle Bay (1 MVEV) in July and Kununurra (1 MVEV), Beagle Bay (1 KUNV) and Roebuck Plains Station (1 flavivirus infection) in August were associated with activity continuing from the 2011/12 season. The first activity associated with the 2012/13 wet season occurred in late May 2013 when a KUNV seroconversion was detected at Roebuck Plains. One KUNV infection was detected in the Harding Dam flock in June. This was a very late start to the flavivirus season and was the lowest level of activity observed since 1995/96 when just two seroconversions to KUNV were detected in March-April**.**
- There were no cases of MVEV or KUNV disease in WA during the 2012/13 season.

Opportunistic trapping

- Mosquito collections at Nullagine, Marble Bar and Port Hedland (in the east and northeast Pilbara regions) were prior to and following the mosquito and arbovirus survey in the Kimberley region in March and April 2013. Results of this work will be presented in the ASRL 2013/14 Annual Report.
- Additional sampling was conducted at Kalgoorlie/Boulder in May 2013 after very high rainfall occurred in the region in March and increased notifications of RRV disease. Low numbers of mosquitoes were collected (1296), however two isolates of RRV were obtained. One isolate of the northern/eastern phenotype was obtained from a pool of *Ae. bancroftianus*, whilst *Ae. notoscriptus* yielded an isolate of the southwestern phenotype. Detection of two different phenotypes of RRV in mosquitoes at Kalgoorlie/Boulder suggests multiple sources of RRV activity.

Cheryl A. Johansen, B.Sc., M.Sc., Ph.D.

Table of contents

Western Australian Arbovirus Surveillance and Research Program, 2012/13

Section 2: Introduction

Mosquitoes in Western Australia (WA) spread several serious communicable diseases. Ross River virus (RRV) and Barmah Forest virus (BFV) occur throughout WA and cause a non-fatal, but potentially debilitating, long-term polyarthritic disease lasting an average of 3-18 months. They impose a substantial burden of suffering and economic loss to the individual and their families as well as to affected communities. Murray Valley encephalitis virus (MVEV) is found in the northern half of WA and causes a rarer but sometimes fatal disease in humans. The disease is now referred to as Murray Valley encephalitis. Kunjin virus (KUNV) infections are generally milder, often non-encephalitic and the disease is referred to as Kunjin virus disease. All of these viruses are endemic in WA and exist in environmentally driven natural cycles, which are largely independent of human involvement. Currently none of these mosquito-borne diseases are curable, nor are they preventable using vaccines. Reduction of disease in our community therefore depends upon prevention of exposure to infected mosquitoes.

A program to monitor and predict high levels of activity of these viruses has been carried out by The University of Western Australia (UWA) on behalf of the Commonwealth and State Health Departments since 1972. In addition, the program aims to detect incursions of medically important exotic mosquito-borne viruses, such as Japanese encephalitis virus (JEV), into WA. JEV causes thousands of cases of potentially fatal encephalitis in Southeast Asia each year. The virus was detected in the Torres Strait in all years between 1995 and 2006, with the exception of 1999, and in north Queensland in 1998 and 2004. The public health implications are immense should JEV become established in northern Australia.

The major goal of this program is to provide an understanding of the basic ecology and epidemiology of mosquito-borne viruses of public health importance, necessary to devise appropriate mosquito control and public education strategies, thereby minimising the health impact of RRV, BFV, MVEV and KUNV in WA. The key to the success of these surveillance programs is the compilation and analysis of data from successive years. Variations in environmental conditions, human activities and many other factors are known to greatly influence arbovirus transmission cycles. Thus, long-term surveillance programs are essential to provide a basis for determining the mechanisms that lead to seasonal and annual fluctuations in arbovirus activity. Results from these programs are instrumental in predicting the potential for future outbreaks of human disease. The information is also the basis for modification of current vector control plans and the creation of new ones, as well as directing strategies for public education and management of natural, rural and urban environments in order to minimise the impact of arboviruses on Western Australians.

Time frame of this report

This report describes surveillance and research into arboviruses and mosquito fauna carried out by this laboratory between 1 July 2012 and 30 June 2013. Results of routine and opportunistic mosquito collections from the north of WA (Kimberley region) during the first half of 2012 (2011/12 wet season) have also been included. This is done because processing of these samples was a substantial component of the laboratory work carried out (during the time frame of this report) in 2012/13. The results of this work are analysed and discussed in relation to results from the sentinel chicken program and human case data from the 2011/12 wet season.

Section 3: Human cases of arboviral disease in WA

Information on human cases of arboviral disease reported in WA between 1 July 2012 and 30 June 2013 were obtained from the following sources:

1. Alphavirus (RRV) human case data from the Mosquito-Borne Disease Control (MBDC) Branch, DOH; and
2. Flavivirus (MVEV and KUNV) human cases diagnosed at PathWest Laboratory Medicine QEII site.

Investigations to identify the location and timing of human cases of RRV and BFV diseases in WA are undertaken by the MBDC of DOH, in collaboration with Local Government Environmental Health Officers (EHOs) and DOHs Communicable Diseases Control Directorate (CDCD). The data are compiled from laboratory-reported and doctor-notified cases of RRV and BFV diseases that meet the definition of a recently acquired case. This information is provided to MBDC by the CDCD and Regional Population Health Units via the Western Australian Notifiable Infectious Diseases Database (WANIDD). Copies of notifications are also provided to EHOs in the Local Government of residence of all confirmed cases. EHOs then administer a short survey to the patient to determine the likely place of exposure and timing of onset of symptoms. Completed survey forms are sent to MBDC for interpretation. MBDC uses the data to compile an enhanced surveillance database, which provides a more precise picture of the incidence, timing and location of RRV and BFV activity than the raw notification and laboratory data. Summaries of the monthly incidence of RRV and BFV in each major region of WA from MBDCs enhanced surveillance data are provided below. MBDC can provide details about the incidence and timing of RRV and BFV disease cases by suburb, locality, local government area or region upon request. In 2012/13 MBDC did not provide BFV disease human case data due to concerns related to large numbers of potentially false-positive BFV results obtained in serological tests performed at private diagnostic laboratories.

Ross River virus

The number of cases of RRV disease reported in WA in the 2012/13 season (Table 1) was lower than the previous year (ASRL 2011/12 Annual Report), but nonetheless greater than average, particularly in the latter half of the year. Overall, 1103 cases of RRV disease were serologically confirmed in WA. The majority of cases occurred in the southwest of WA (388), the Perth Metropolitan Area (355) and the Goldfields/Esperance region (211). In the southwest of WA, the greatest proportion of cases occurred in the Peel (254) and Leschenault (103) regions. The number of cases peaked in February in the Perth Metropolitan Area and the southwest of WA, whilst the incidence of RRV disease in the Goldfields/Esperance region peaked in April. Cases of RRV disease were also reported in the Kimberley (54), Pilbara (20), Gascoyne (3), Midwest (18), Wheatbelt (38) and Great Southern (16) regions. In the Kimberley and Pilbara regions the number of RRV cases peaked in April.

Table 1. Serologically confirmed doctor-notified and laboratory reported cases of Ross River virus disease each month in WA, July 2012 - June 2013#

#Compiled by the Mosquito-Borne Disease Control Branch, WA Department of Health

REGION			Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
KIMBERLEY			0	0	1	1	3	2	5	4	3	31	4	0	54
PILBARA			0	1	1	1	1	1	1	3	0	6	4	1	20
GASCOYNE			1	0	0	0	0	0	0	0	0	0	1	1	3
MIDWEST			0	0	0	2	2	0	1	4	2	1	3	3	18
WHEATBELT			1	0	0	1	0	0	0	14	6	7	7	2	38
PERTH METRO			11	11	11	18	18	15	73	99	45	28	16	10	355
	SOUTHWEST	*PEEL*	*4*	*4*	*6*	*5*	*13*	*16*	*86*	*71*	*24*	*10*	*8*	*7*	*254*
		LESCHENAULT	*4*	*0*	*1*	*2*	*3*	*4*	*21*	*42*	*19*	*4*	*3*	*0*	*103*
		CAPEL	*0*	*2*	*0*	*0*	*1*	*0*	*0*	*2*	*0*	*0*	*1*	*1*	*7*
		BUSSELTON	*0*	*0*	*1*	*2*	*1*	*2*	*2*	*3*	*2*	*0*	*0*	*0*	*13*
		ELSEWHERE SW	*1*	*0*	*0*	*1*	*1*	*2*	*4*	*1*	*0*	*1*	*0*	*0*	*11*
SOUTHWEST (region total)			9	6	8	10	19	24	113	119	45	15	12	8	388
GREAT SOUTHERN			0	1	1	1	2	0	3	2	2	0	2	2	16
GOLDFIELDS-ESPERANCE			1	0	1	0	1	0	2	4	12	122	55	13	211
WA UNDETERMINED			0	0	0	0	0	0	0	0	0	0	0	0	0
INTERSTATE			0	0	0	0	0	0	2	1	3	0	0	0	6
WA TOTAL (does not include interstate)			**23**	**19**	**23**	**34**	**46**	**42**	**198**	**249**	**115**	**210**	**104**	**40**	**1103**

1) Data current as at 08/08/2013 - table may vary from previous or future versions due to inclusion of additional enhanced surveillance data

2) Source of data: Western Australian Notifiable Infectious Diseases Database (comprising Doctor's notifications to Public Health Units & Communicable Disease Control Directorate; Laboratory reports to Communicable Disease Control Directorate from participating pathology laboratories); Enhanced Surveillance Data (comprising case follow-ups from Environmental Health Officers; patient interviews; Doctor's comments on notification forms)

3) Month of onset and suburb/town of exposure determined from Enhanced Surveillance Data where available, and from Doctor's notifications or laboratory reports where not available

4) Data varies from official Western Australian Notifiable Infectious Diseases Database records due to inclusion of Enhanced Surveillance Data

5) Where it is not clearly defined if a case occurred in a particular suburb or a local Government (e.g. Mandurah suburb or the City of Mandurah the case has been entered as a "local government case - unknown suburb" - (e.g. City of Mandurah unknown)

6) Where a place of exposure occurs in a suburb that carries over 2 Local Governments and it is not clearly defined which local government it occurred in, the case has been entered in the Local Government where the largest portion of the suburb occurs

7) This information is the intellectual property of the Mosquito-Borne Disease Control Branch of the WA Department of Health and may not be used for any purpose without prior permission

Flavivirus testing & cases

The Arbovirus Surveillance and Research Laboratory (ASRL) tests human sera for antibodies to flaviviruses on behalf of PathWest Laboratory Medicine WA. Such tests are conducted to support diagnostic testing conducted by PathWest in response to suspected clinical cases. Independent tests are used to improve the specificity and sensitivity of the assays used by both organisations. Sera are tested by blocking ELISA following Hall *et al*. (1995). In 2012/13, seven samples were tested by the ASRL with one sample positive for MVEV, one sample positive for JEV, and five samples positive to a flavivirus. None of these were recent locally acquired infections. No MVEV or KUNV cases were reported in WA during 2012/13 (Table 2).

Table 2. Cases of disease caused by Murray Valley encephalitis and Kunjin viruses in Australia since 1974.[†]

Year	WA		NT		QLD[##]		NSW		VIC		SA[#]		Total/
	MVE	KUN	MVE	KUN	MVE	KUN	MVE	KUN	MVE	KUN	MVE	KUN	year
1974*	1		5		10		5		27		10		**58**
1978	4	1											**5**
1979	1												**1**
1981	7		1		2								**10**
1984	2									1			**3**
1986	1												**1**
1987			1										**1**
1988			3										**3**
1989	1												**1**
1990	1												**1**
1991	1	1	2		2			1		1			**8**
1992						1							**1**
1993	9		7										**16**
1994					1								**1**
1995		1											**1**
1997	2	2	1	3									**8**
1998	1	1											**2**
1999		1											**1**
2000	11	2	4	1							1		**19**
2001		1	3	2	1								**7**
2002**	2												**2**
2004			1			2							**3**
2005			1		1	1							**3**
2006	1	2											**3**
2008	1						1						**2**
2009	2		2										**4**
2010			1			1							**2**
2011	9		4	1			1				2		**17**
2012***							1	1					**2**
TOTAL	**57**	**12**	**36**	**7**	**17**	**5**	**8**	**2**	**27**	**2**	**13**	**0**	**186**

[†]Some interstate data supplied by Stephen Doggett (Medical Entomology, University of Sydney), Peter Whelan (Medical Entomology, Northern Territory Department of Health), Dr Alyssa Pyke (Queensland Health Forensic and Scientific Services) and Conan Liu (National Notifiable Diseases Surveillance System, Australian Government Department of Health and Ageing).

[#]Case in 2000 was undifferentiated MVEV/KUNV infection.

[##]Additional KUN cases may have occurred in Queensland but these have not been published.

* Cases in 1974 were all reported as MVE.

** One MVE case occurred in December 2001 but was associated with the 2001/2002 wet season.

***One MVE case and one KUNV disease case in NSW occurred in December 2011 but was associated with the 2011/12 wet season.

Section 4: Meteorological data

Source(s) of data

Weekly and monthly weather reviews were supplied by The Western Australian Bureau of Meteorology (BOM). These were used to compile summaries of significant weather events, temperature records and other meteorological records for WA. Tidal forecasting data for 2012/13, used to predict likely timing and extent of inundation of coastal and estuarine mosquito breeding sites, were obtained from The National Tidal Facility, Flinders University, South Australia.

Significant events relevant to this program

The Southern Oscillation Index (SOI) provides a measure of the state of the El Niño-Southern Oscillation (ENSO) cycle, which affects much of the long-term climatic patterns in Australasia. Negative values of the SOI usually mean that eastern and northern Australia will be drier than normal. This is referred to as an El Niño episode. These conditions are reversed in the opposite phase known as La Niña. Positive values of the SOI are associated with stronger Pacific trade winds and warmer sea temperatures in northern Australia. Together these give a high probability that eastern and northern Australia will be wetter than normal. In addition, saltmarshes and tidal flats along the southwest coast of Australia are inundated more frequently in years when the SOI is neutral or positive. The SOI values are useful predictors for rainfall in the eastern states of Australia but are not accurate predictors in WA and the Northern Territory. The SOI was largely neutral (between -8 and +8) for most of the 2012/13 season (Figure 1). Rainfall was generally above average in the southwest of WA between October 2012 and March 2013, and otherwise it was drier than usual (Figure 2A-D). In the Kimberley and Pilbara regions rainfall was above or well above average between October to December 2012 and April to June 2013. BOM models suggest the chance of exceeding median rainfall between October and December is around 50% for most of WA. Warmer than usual day and night temperatures are more likely across most of WA, particularly northern WA.

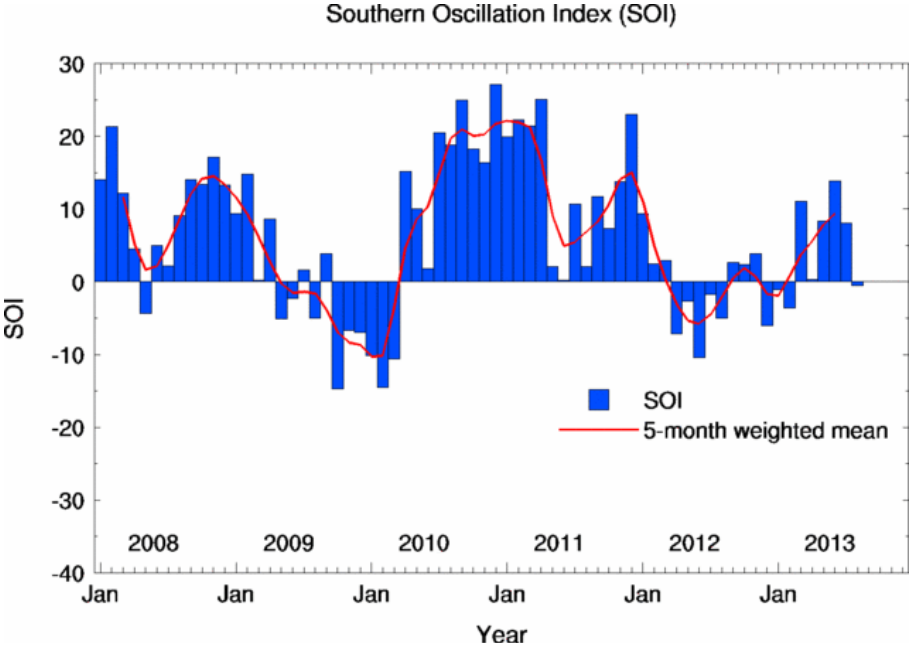

Figure 1. Southern Oscillation Index (SOI) 2008-2013 (source: Commonwealth Bureau of Meteorology http://www.bom.gov.au).

Weather data

Three-monthly summaries of rainfall trends throughout WA for the period July 2012 to June 2013 are shown in Figures 2A-D. A summary of the rainfall patterns throughout the State is given below. Monthly rainfall at Mandurah, Bunbury, Capel (North) and Busselton are shown in Figure 3A-D. Detailed summaries of temperature trends throughout this period can be found in Western Australian weather reviews (http://www.bom.gov.au/climate/mwr/) and are not shown here.

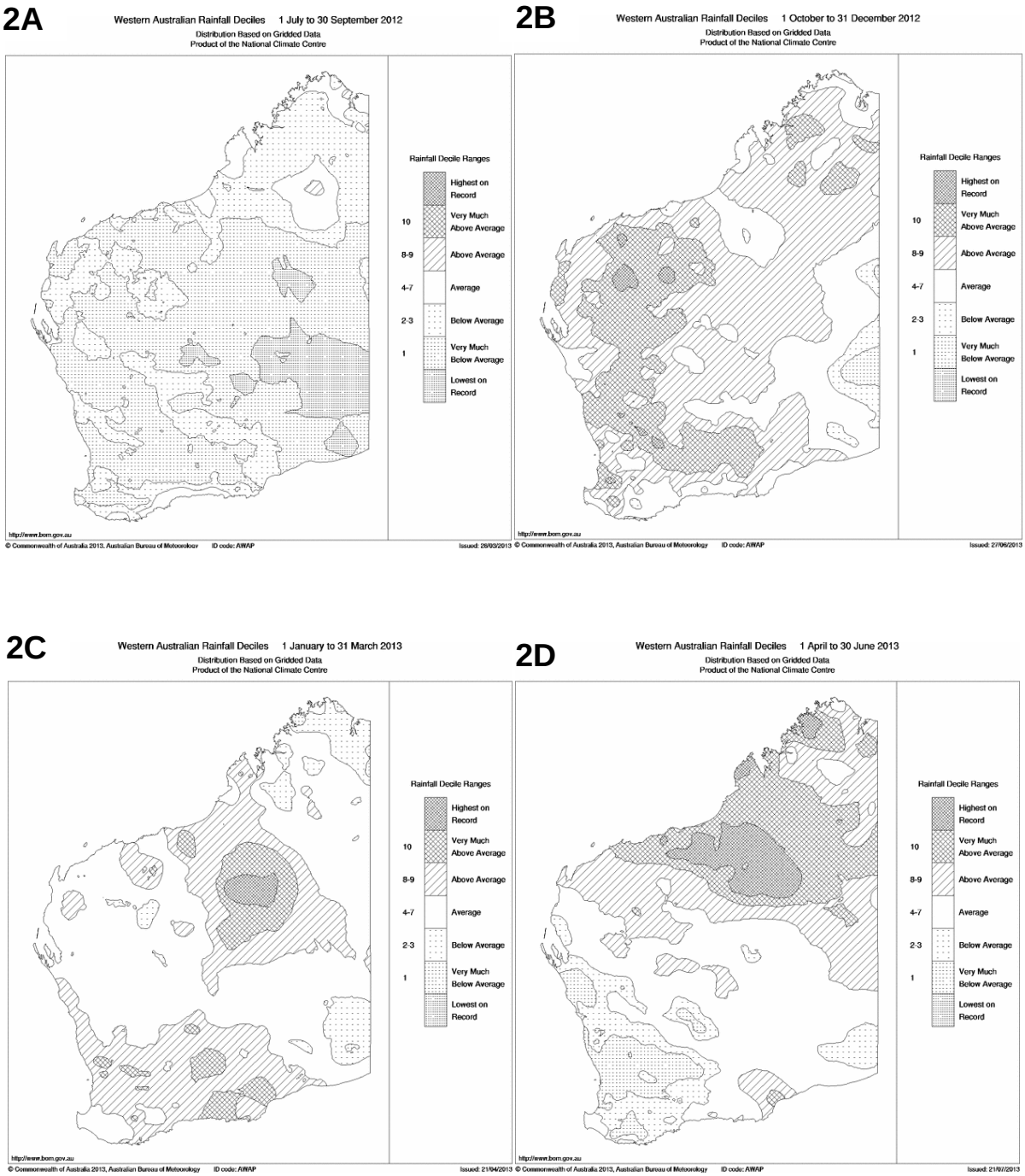

Figure 2A-D. Three-monthly summary of Western Australian rainfall deciles. A: July-September 2012; B: October-December 2012; C: January-March 2013; D: April-June 2013 (source: Commonwealth Bureau of Meteorology).

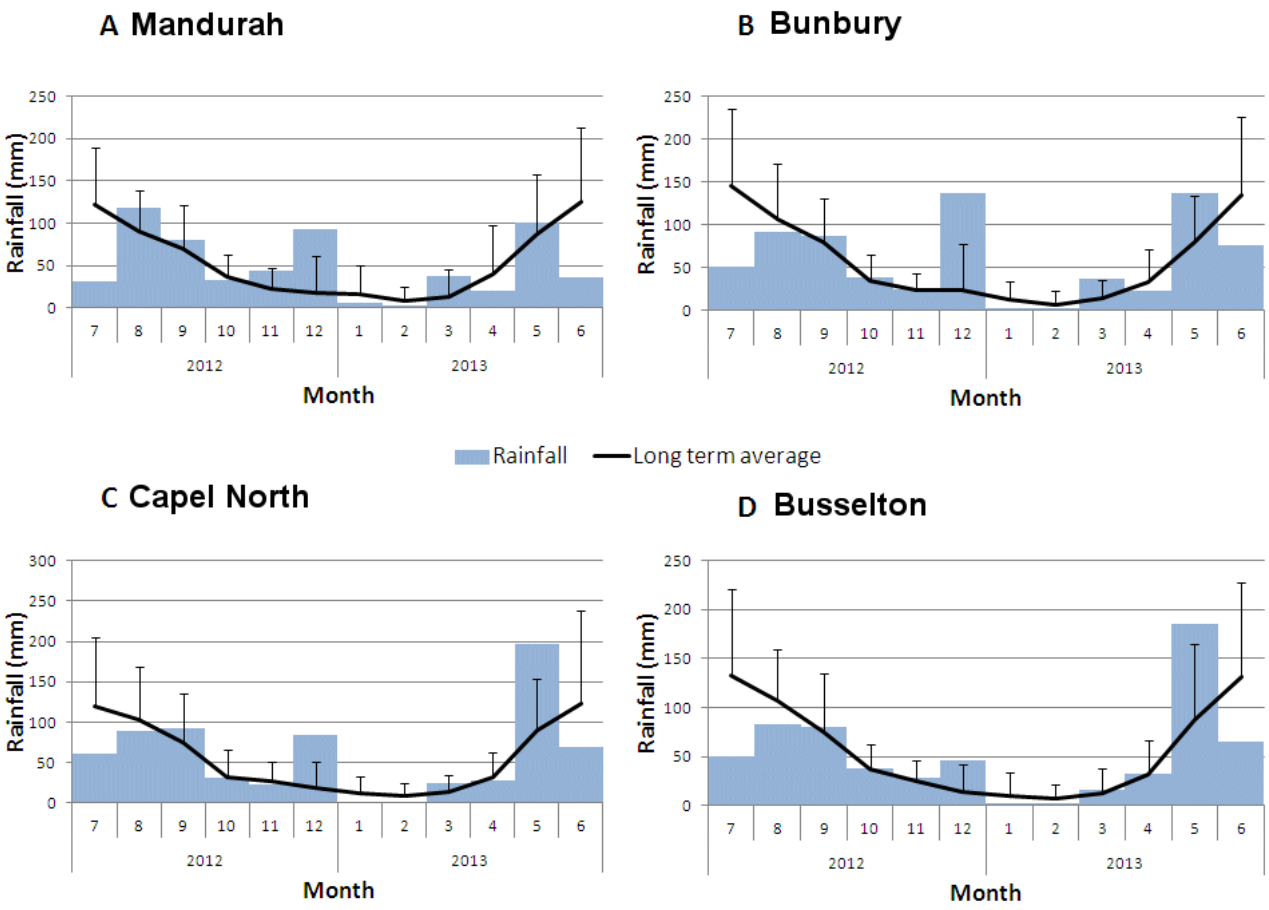

Figure 3A-D: Monthly rainfall compared to long term average at Mandurah, Bunbury, Capel North and Busselton, July 2012 to June 2013 (graphs produced with the assistance of Dr Andrew Jardine, MBDC; bars represent upper confidence intervals).

Summary of rainfall events in WA, 2012/13

Northern WA rainfall (July 2012 to June 2013)

In August, nights were cooler than usual across the Kimberley, adjacent Interior and coastal East Pilbara. Some parts of the Pilbara and west Kimberley had above average maximum temperatures and rainfall was generally below average. Below average minima were recorded in the northern Kimberley in September. Much of the Kimberley and northeastern Interior experienced below to very much below average minima and average daily maxima, and most of northern WA experienced above to very much above average rainfall in October. Overall rainfall in November was very much above average and above average mean daily maxima and minima were recorded. Parts of the Kimberley had their wettest November on record. In contrast, some parts of the central Pilbara, inland Gascoyne and eastern Interior received below average rain. Rainfall was similarly higher than usual through much of northern WA in December. Parts of the Pilbara and Gascoyne recorded their wettest December on record, although the far north Kimberley experienced below average rainfall. Maxima were above average in most areas, although inland areas experienced below average maximum temperatures. Minima were generally above average in the Pilbara, and below average in parts of the Kimberley and northern Interior. Tropical Cyclone (TC) Mitchell developed off the WA coast on 29 December but caused minimal impact and did not cross the coast. Local thunderstorms associated with TC Narelle (8-15 January) caused above average rainfall

in western parts of the Gascoyne in January. TC Peta crossed the coast near Roebourne on 23 January and quickly weakened to a tropical low, causing local thunderstorms with heavy rainfall in the Pilbara and northern Interior. The Kimberley and Interior experienced below average rainfall in January. Much of northern WA experienced above average maxima, although parts of the Pilbara experienced below average daytime temperatures due to cloudy conditions. An active monsoon and tropical low pressure system caused heavy rainfall in the Kimberley and Pilbara regions from 22-28 February. TC Rusty (24-28 February) developed off the northwest of WA and crossed the Pilbara coast near Pardoo Station on 27 February. It rapidly decreased in intensity as it moved south, but caused prolonged gale force winds at Port Hedland and heavy rainfall in affected areas. Major flooding occurred in the De Grey River catchment area due to prolonged heavy rain. In March, seasonal thunderstorm activity resulted in heavy rainfall in the Kimberley region on 11 March, and thunderstorms developed in the Gascoyne region on 16 March. In April, TC Victoria developed in the Indian Ocean, however the cyclone weakened quickly and had no direct impact on WA. Rainfall was mostly near average, although the northeast Kimberley recorded very much above average rainfall. Maxima were near average in northern WA and warmer than average in the western Pilbara and Gascoyne. Minima were cooler than usual in the Kimberley region and warmer than average elsewhere. Average maxima and above average maxima were recorded in most northern regions in May. Above average rainfall was recorded in the Kimberley and Interior, however elsewhere the rainfall was average or close to average. Unseasonal high rainfall continued in June in northern WA. Parts of the central and east Pilbara and west Kimberley recorded their wettest June on record, average maxima and above average minima were also recorded.

Southwest WA rainfall (July 2012 to June 2013)

In July, Record low rainfall was observed for most of the southwest land division (SWLD), particularly the western districts. Many sites experienced their driest July on record due to a high pressure system over southern WA. The lower southwest experienced above to very much above average maxima and below average minima. In August, the lower southwest experienced below average rainfall in August, and had its 5th driest January to August period on record. Maxima and minima were above average. A cold front caused strong winds and heavy rainfall in the early part of September. Rainfall was near average to above average in western parts of the SWLD. Some parts of the South Coastal and Great Southern observed their wettest September on record. Other parts of the southwest (central, southern Interior and Eucla) had lower than usual rainfall. Most of the southern areas experienced warmer days and nights. It was the 5th driest October on record for the SWLD, and conditions were warmer than average. In contrast, conditions in November and December were wetter than usual. A tornado at Wonnerup on 3 November caused damage in the tuart forest, and a strong cold front caused severe winds on 28-29 November in the Central West, Lower West, Southwest, Southeast Coastal and Eucla districts. Widespread showers and thunderstorms were recorded from 10-13 December, particularly in the northern parts of the Southwest district, including Bunbury, Harvey and Collie. Conditions in December were warmer than average. Ex-TC Narelle caused above average rainfall in northern parts of the SWLD in January. Very hot conditions were observed in the first half of the month. Some regions in the southwest experienced their warmest January on record. In February below average rainfall and above average maxima and minima were recorded in the southwest. Wetter than usual conditions occurred in the southwest of WA in March, and days were cooler than usual. Ex-TC Rusty caused heavy rainfall in the Goldfields and Southeast Coastal district in early March. Thunderstorm activity and heavy rainfall was reported in parts of the SWLD on 11 and 15 March. In April, maxima and minima were above average and rainfall was lower than usual. The passage of several strong cold fronts affected a large part of the SWLD from 7-9 May. The heaviest falls were recorded in the Lower West and Southwest forecast districts. Overall, rainfall was near average in the southwest, and maxima and minima were average. Extremely dry conditions and warm days prevailed in June.

Tidal data

Fifteen minute tide height readings from Fremantle Fishing Boat Harbour, the Peel Inlet and Bunbury Inner Harbour (Figure 4A-C) were obtained from Ms Siobain Mulligan of the Department of Transport (Coastal Infrastructure). Graphs were prepared by Dr Cheryl Johansen. Given that tide heights at Fremantle (Fishing Boat Harbour) of approx. 1.1m are typically considered sufficiently high enough to inundate a substantial area of salt marsh in the Peel region (Mr Scott Severn, City of Mandurah, personal communication), tides were high enough to inundate large areas of salt marsh in the Peel region for most of the year (Figures 4A). High tides of approx. 0.9m above sea level at Bunbury Inner Harbour are sufficient to inundate salt marsh areas in the Leschenault region (Mr Scott Dandridge, Shire of Harvey, personal communication). During 2012/13, regular peak tides were sufficiently high to flood mosquito breeding sites in the area most of the year (Figure 4C).

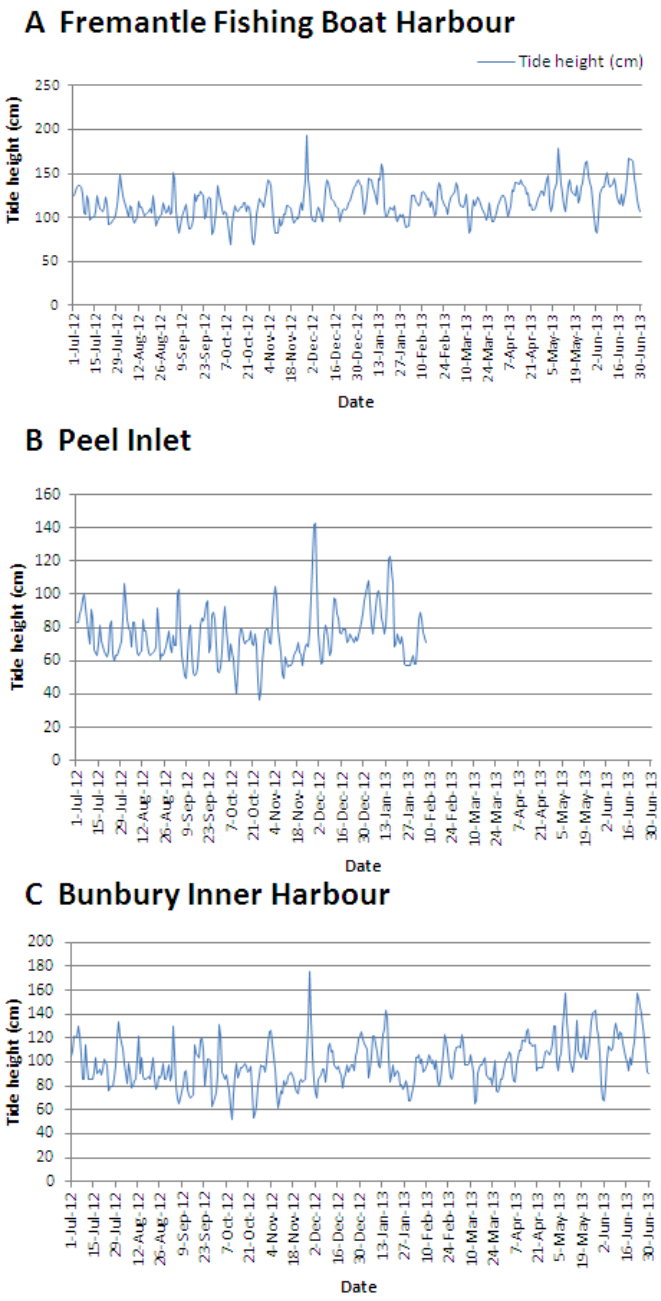

Figure 4A-C. Observed maximum daily tide heights (cm) at the Fremantle Fishing Boat Harbour, Peel Inlet and Bunbury Inner Harbour during 2012/13 (the Peel Inlet tide guage did not function from 7 February 2013 to 30 June 2013).

Section 5: Mosquito Surveillance

Mosquito and arbovirus monitoring

RRV and BFV activity in the southwest of WA is monitored through surveillance of adult mosquito populations and their infection rates with these viruses. In the Kimberley region (tropics), the sentinel chicken program (Section 6) is supplemented by annual monitoring of adult mosquitoes and arbovirus activity at major population centres and previously identified foci of virus activity. Opportunistic collections of adult mosquitoes with subsequent determination of their infection rates are made in other populated regions of WA whenever extreme meteorological conditions occur.

Methods and Materials

Location and timing of mosquito trapping

Southwest mosquito surveillance

The Peel, Leschenault and Capel-Busselton regions are located on the Swan Coastal Plain, south of Perth, in the South Coastal meteorological district (Figure 5). Priority is given to these regions as RRV and BFV appear to be endemic, high case attack rates (compared to other southwest regions) are frequently reported and virus activity appears to commence in these regions and then spread to other areas of the southwest during major outbreaks (Lindsay, 1995). The geography of these regions and features pertaining to arbovirus ecology has been described previously (Lindsay, 1995). Trap sites, chosen for their high level of mosquito breeding, their proximity to bushland that may harbour potential vertebrate hosts of RRV or their proximity to human habitation, have also been previously described (Jasinska *et al*., 1997, Lindsay, 1995).

Mosquito collections (trapping runs) in the Swan Coastal Plain were carried out on average once a fortnight during late spring, summer and early autumn and once a month during the remainder of the year. Each trapping run was timed to coincide, as closely as possible, with peak numbers of blood-seeking adult females following high tides or rain in each locality. This was determined using predicted tide heights supplied by The Western Australian Department of Transport and meteorological records supplied by The Western Australian BOM. Fine-tuning of timing for each collection was done in collaboration with DOH personnel, local government Environmental Health Officers (EHOs) and Mosquito Management Officers who carried out regular monitoring of known breeding sites. Whenever possible, trapping runs were carried out when weather conditions were considered optimal (for the time of year) for mosquito host-seeking activity. Nights with low winds, moderate temperatures and high humidity were given preference whereas nights with extremes of temperature, high winds or low humidity were avoided where possible.

In 2004/05, following discussions with MBDC personnel and positive feedback from southwest LGAs, it was decided that mosquitoes collected during low-risk winter months would be collected and identified, but would not be processed for virus isolation. A total of only eight isolates of RRV have been obtained from mosquitoes collected in winter months, the last in 2004/05. This has provided important evidence that RRV is enzootic (permanently present) in the southwest of WA. However there is no significant relationship between detection of RRV in late-autumn-winter mosquitoes and subsequent outbreaks of RRV disease in the southwest of WA (Fisher exact test, $p = 0.584$). That is, since the RRV surveillance program commenced in the southwest of WA in 1987, large outbreaks of RRV disease in the southwest of WA only occurred in two years that RRV was isolated from winter mosquitoes, and there were two years of average or below average incidence of RRV when winter RRV isolates were obtained prior to human disease. During the 2012/13 season, mosquitoes collected from May to June 2012 were not processed for virus isolation. Mosquitoes collected in April 2013 were processed due to continued large numbers of cases of BFV disease notified from private diagnostic laboratories.

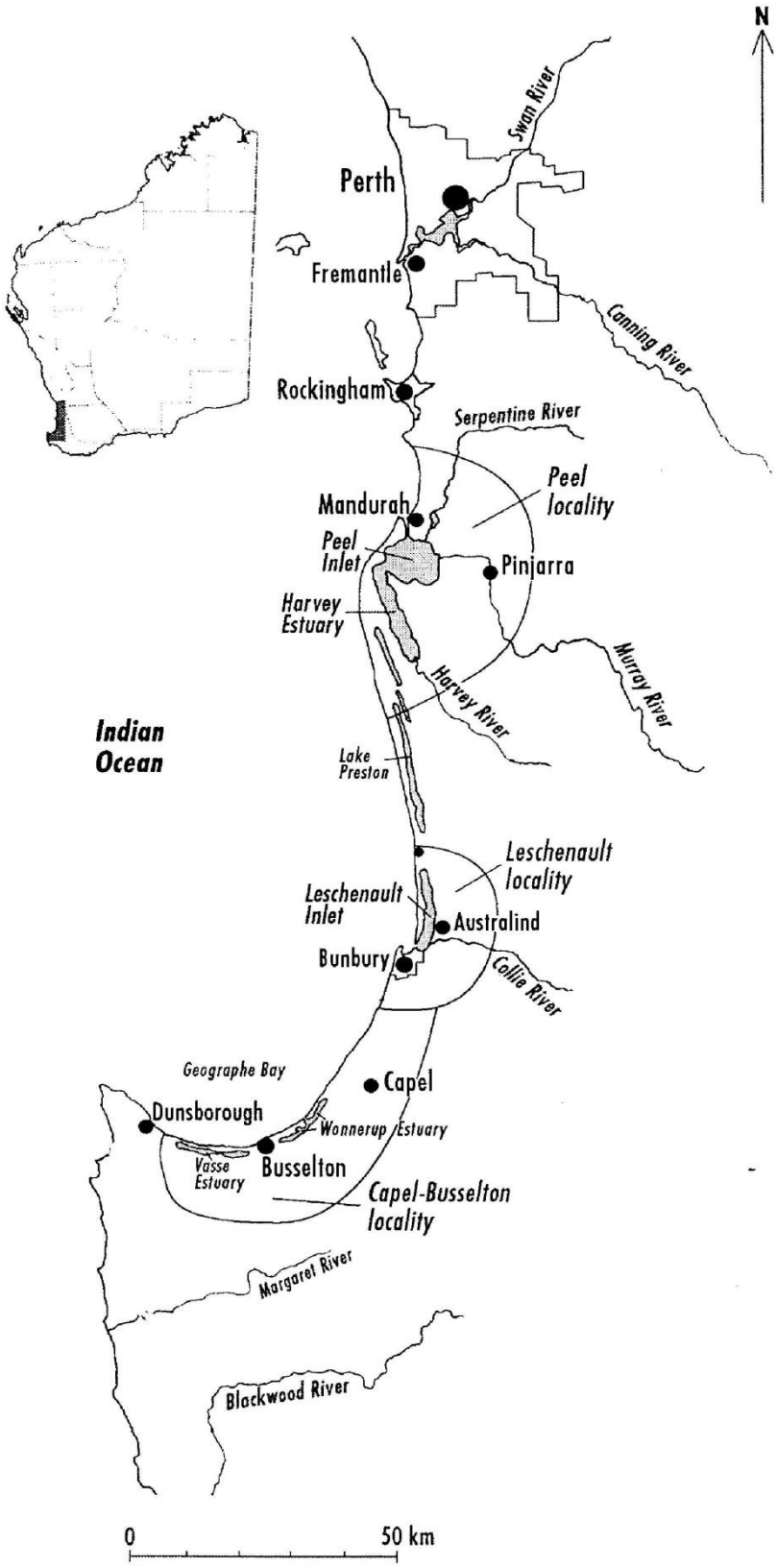

Figure 5. Location of the Peel, Leschenault, Capel and Busselton regions where prospective surveillance of mosquito populations and arbovirus activity is conducted in the southwest of WA (Lindsay, 1995).

Kimberley mosquito surveillance

Mosquito collecting trips to the Kimberley region are carried out at least once every wet season and are timed to coincide with peak flavivirus activity in the region. Previous studies have shown that MVEV and KUNV activity peaks late in the wet season, usually between March and May. Individual trap sites around each town or community were selected using similar criteria described for the southwest mosquito monitoring program. Detailed maps showing the major mosquito trapping locations around each town or community were presented in the 1997/98 Annual Report. Mosquito monitoring was carried out in and around all major Kimberley towns and the Aboriginal Community of Billiluna in 2012 (Figure 6). The 2013 field plan was modified in consultation with staff from MBDC DOH, and involved concentration of the sampling effort in the west Kimberley region to facilitate development of a mosquito management plan for the Shire of West Kimberley. In addition, opportunistic sampling of adult mosquitoes was undertaken in the Pilbara region (Newman, Nullagine, Marble Bar and Port Hedland) following heavy flooding in the area.

Mosquito trapping

Adult mosquito populations were sampled using EVS/CO_2 traps (Rohe & Fall, 1979), manufactured by D & D Technical Models (Perth, Australia) or by Mr Paul Bonella (private model manufacturer, 425 Abernethy Road, Cloverdale, Perth, Australia). Traps were modified as described by Broom *et al*., (1989) to suit local meteorological conditions. Traps were set mid-afternoon, run overnight and collected after sunrise the following day in order to collect day-biting, crepuscular (dawn and dusk) and nocturnal species. Trap failure, generally due to motor failure, was relatively rare but was recorded if it occurred. Mosquitoes were frozen on dry ice before being transported back to Perth on dry ice, where they were transferred to –90^{o}C freezers prior to processing. Estimates of mosquito abundance and dominant mosquito fauna in mosquito traps from the Swan Coastal Plain were reported directly back to local health authorities on return to the laboratory.

Mosquito identification and pooling

Adult mosquitoes were identified to species on refrigerated (frozen) tables illuminated with a cold light source using stereoscopic microscopes. Species were designated based on descriptions and keys in Liehne (1991), Lee *et al*. (1980) and Russell (1996). Once identified, mosquitoes were separated into pools of $\leq$ 20 for samples from the southwest or $\leq$ 25 from elsewhere in WA, according to collection site and date, species and sex. Blood-fed specimens were stored at –20^{o}C for host blood-meal analysis. All specimens were processed for virus isolation unless more than 500 mosquitoes were obtained in a single trap, in which case the first 500 specimens were identified and processed for virus isolation. In instances when many traps from the Swan Coastal Plain contained more than 500 mosquitoes, the first 350 specimens were processed for virus isolation. The number of remaining mosquitoes was estimated by extrapolation by weight. All female mosquitoes that could not be identified or needed confirmation were examined by Mr Peter Whelan (Northern Territory Health) or Professor Richard Russell (Westmead Hospital, New South Wales). Male mosquitoes were identified when possible, however unknown males were not usually sent away for further identification.

Virus isolation

Mosquitoes were ground and processed as described previously (WA Arbovirus Laboratory Annual Report 1997/98). The presence of virus in mosquito homogenates was detected using a cell culture assay system (Figure 7). Ninety-six well plates containing the 2nd passage on C6/36 cells of samples from the Kimberley, Pilbara or Goldfields regions were acetone-fixed after the supernatants had been passaged onto Vero and PSEK cell lines. The plates were then screened with a Sindbis virus (SINV) specific monoclonal antibody (2F2) and a flavivirus specific monoclonal antibody (4G2) to enable detection of flaviviruses that do not necessarily cause cytopathic effect (CPE) on indicator cell lines (e.g. Edge Hill or Stratford viruses). Similarly, 96 well plates containing the 2nd passage of

C6/36 cells of samples from the southwest of WA were screened using 9E8 (specific for BFV) and 4G2 monoclonal antibodies.
All medically important Australian alpha and flavivirus isolates were identified using a tissue culture ELISA with virus-specific monoclonal antibodies. The reagents and methods used have been described elsewhere (Broom *et al*., 1998). If isolates could not be identified as alpha or flaviviruses by ELISA they were initially regrown for an extended period on C6/36 cells before passaging onto indicator cell lines (PSEK/Vero) and re-tested. Isolates unable to be identified will be tested further at PathWest Laboratory Medicine WA. Three suspected Edge Hill virus isolates were confirmed by PCR by Dr Glenys Chidlow at PathWest Laboratory Medicine WA.
Results of preliminary identification and final confirmation of virus isolates from mosquitoes collected on the Swan Coastal Plain were reported directly to the relevant health authorities on completion of the serological assays. This information, combined with ASRL and DOH interpretation of environmental conditions and RRV disease patterns in preceding seasons, facilitated timely media releases by the DOH warning local residents of increased risk of RRV transmission, and ensured local government authorities were aware of the risk of virus transmission in their areas.

Microneutralisation test

This test is occasionally used to identify rare arboviruses (including some Bunya and Orbiviruses) that are not recognised in either the antigen capture or fixed cell ELISAs. The reagents and methodology used are described in detail elsewhere (Lindsay, 1995).

Determination of mosquito infection rates

Infection rates of southwest mosquito populations with arboviruses are calculated from the number of confirmed isolates, number of mosquitoes tested and pool size used for processing. This is done using the statistical model of Chiang & Reeves (1962) that allows for the probability that >1 mosquito in each pool may be infected, particularly when the infection rates in mosquitoes are high. The minimum infection rate (MIR) used to compare virus infection rates in *Cx. annulirostris* mosquitoes collected in different areas of the Kimberley was calculated as:

$$\frac{\text{Number of viruses detected in } \textit{Cx. annulirostris}}{\text{Total number of } \textit{Cx. annulirostris} \text{ processed}} \times 1000$$

This formula assumes only one infected mosquito per pool processed. Infection rates are expressed as MIR per 1000 mosquitoes.

Typing of virus strain

A fixed cell ELISA capable of distinguishing between geographical variants of RRV (Lindsay *et al*., 1993, Oliveira *et al*., 1997, Sammels *et al*., 1995) is used to screen all RRV isolates obtained from throughout WA. This enables determination of the strain of virus involved during outbreaks in different regions or at different times in the same region. This is important following the recognition of a ‘new’ strain of RRV in the southwest during a major outbreak of RRV disease in 1995 (Lindsay *et al*., 1997) with possible differences in pathogenicity (Prow, 2006).

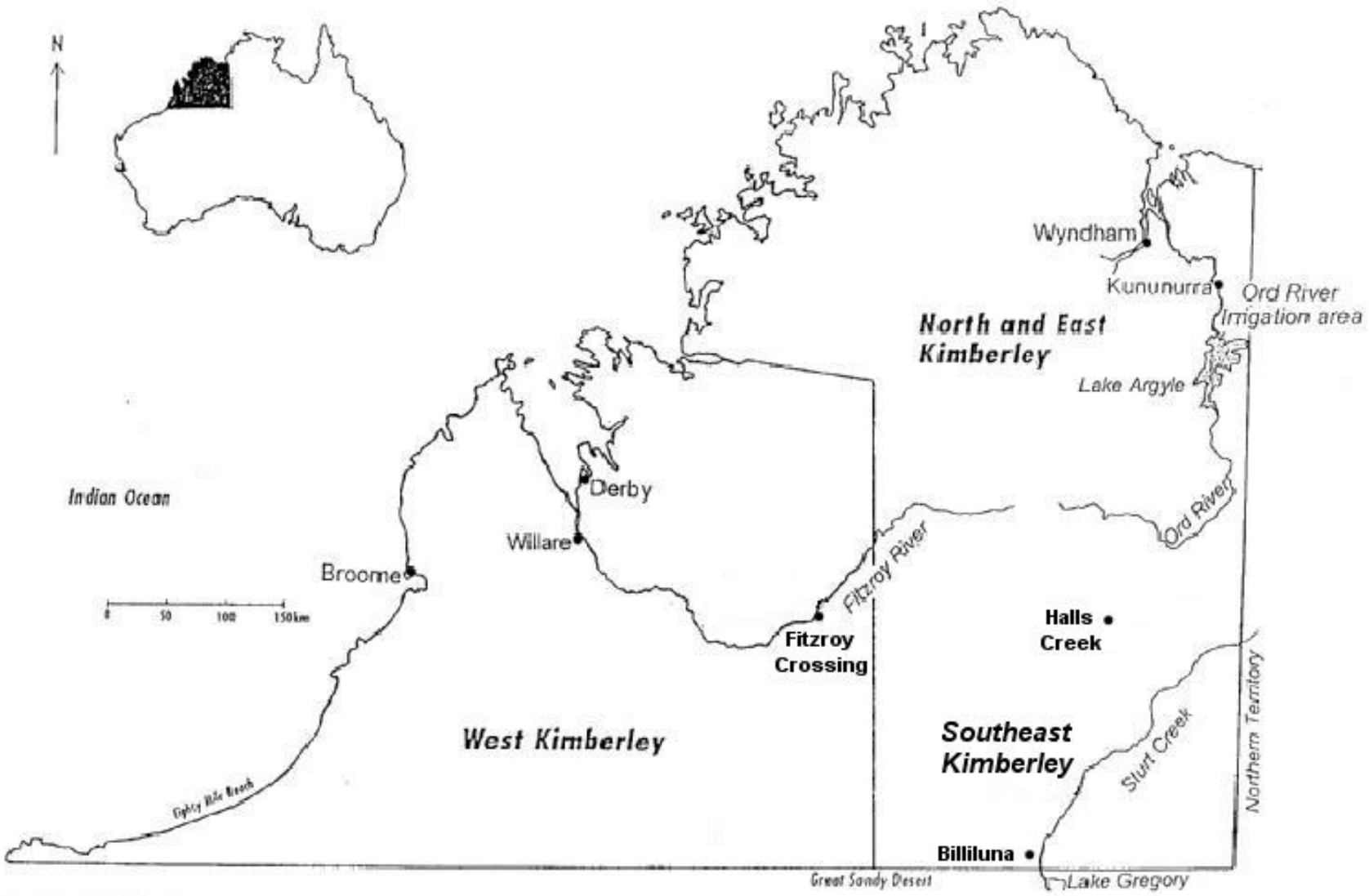

Figure 6. The locations where adult mosquito sampling was conducted in the Kimberley region (northern WA) (adapted from Lindsay, 1995)**.**

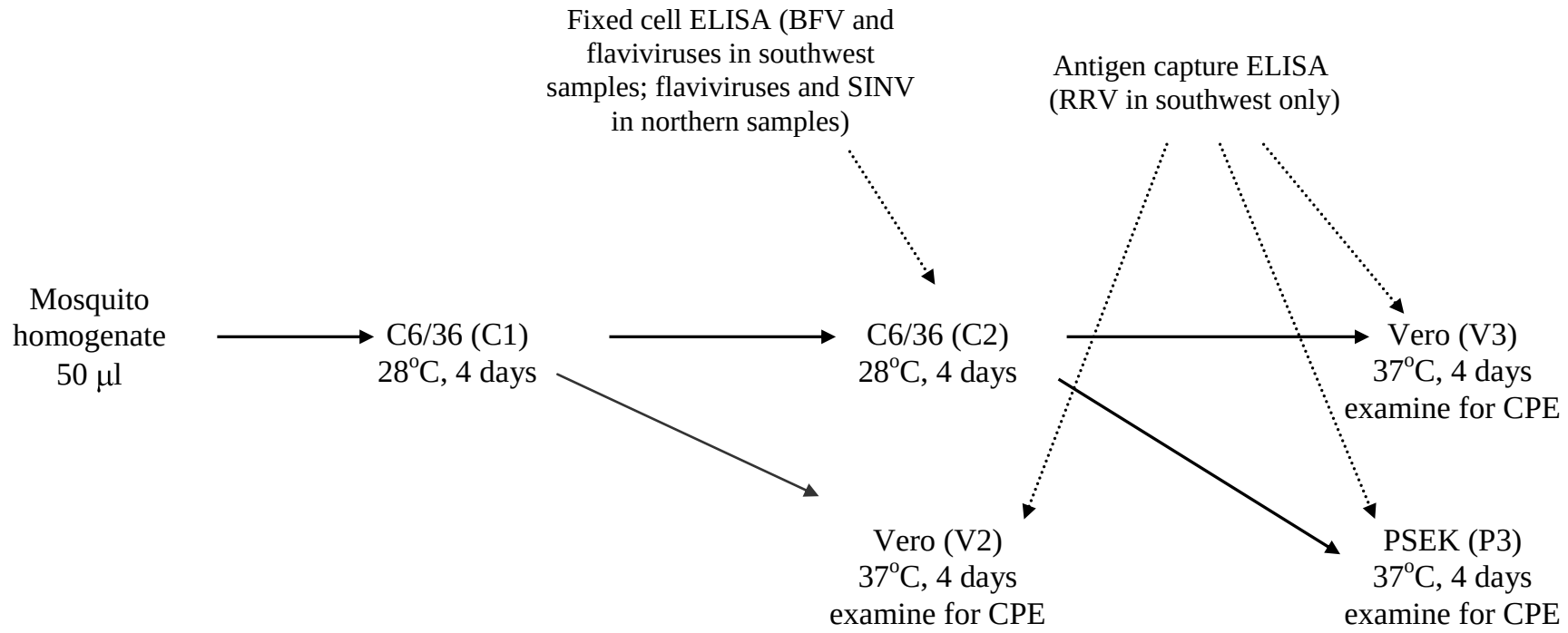

Figure 7. The cell culture virus isolation protocol (in 96 well cell culture plates) used to isolate virus from mosquitoes in the Arbovirus Surveillance and Research Laboratory. The volume of initial inoculum (sample) and passage volume from C6/36 cells onto vertebrate cell lines is always 50 µl.

Results and Discussion

Southwest

Results of mosquito population monitoring and arbovirus surveillance from the southwest component of the program are presented and discussed by region. Mosquitoes collected from April to June 2012 were not processed for virus isolation. Results from the Peel region are separated into Peel Inlet sites and Harvey Estuary sites. This was done as these major water bodies differ substantially in their tidal patterns, surrounding human population, degree of habitat modification by humans, amount and type of vegetation/mosquito harbourage and potential vertebrate host population and distribution. Results from all Leschenault region sites were analysed and presented together because most sampling sites in this region monitor salt marsh and brackish wetland mosquitoes breeding around and dispersing away from the Leschenault Inlet and its associated rivers and waterways. Sampling sites in the Capel and Busselton regions were classified as 'forest' and 'wetland' environments, respectively, in recognition of their distance from known brackish or freshwater wetland breeding sites and the amount of forest/vegetation at each location. This was done in an attempt to examine the effect of mosquito harbourage and vertebrate host habitat on mosquito survival and dispersal and arbovirus activity.

Peel region (Dawesville monitoring program in the Peel Inlet and Harvey Estuary)

Twenty three adult mosquito surveillance collections (trap runs) were conducted in the Peel Inlet and Harvey Estuary regions between July 2012 and June 2013 (Table 3). A total of 207 traps were set with a 96.1% success rate.

A total of 60636 mosquitoes comprising 14 species were collected from Peel Inlet sites, 30633 (50.5%) of which were processed for virus isolation (Table 4). The most abundant species collected were *Ae. vigilax* (53.4%) and *Ae. camptorhynchus* (40.6%). Vector mosquito numbers were similar to the previous season (Figure 8). There were large peaks in abundance of *Ae. vigilax* in January and February 2013, and this species was collected through to June 2013. A single isolate of RRV (northern/eastern phenotype) was obtained from *Ae. vigilax* collected at the Falcon sampling site on 29 January 2013 (Tables 4 and 5), with a MIR of 0.4 per 1000 mosquitoes (Figure 8).

Of the 10001 adult mosquitoes (comprising 14 species) collected from Harvey Estuary sites in 2012/13, 7002 (70.0%) were processed for virus isolation (Table 6). The most abundant species were *Ae. vigilax* (43.4%) and *Ae. camptorhynchus* (42.7%). Overall mosquito abundance was slightly less than the previous (2011/12) season (Figure 9). *Aedes vigilax* populations were collected through to June 2013, with one relatively large peak in late January 2013 (Figure 9). Eight arboviruses were isolated from mosquitoes caught at the Harvey Estuary sites in 2012/13, with *Ae. vigilax* yielding three RRV isolates, one BFV isolate and one EHV isolate (Tables 5 and 6). Isolates of RRV (two) and EHV (one) were also obtained from *Ae. camptorhynchus*. All isolates of RRV were of the northern/eastern phenotype. The MIR of mosquitoes with RRV reached 19.1 per 1000 mosquitoes on 14 December 2012, whilst the infection rate for BFV reached 2.6 per 1000 mosquitoes on 29 January 2013 (Figure 9). The large MIR in Harvey Estuary mosquitoes preceded the peak in human cases in the Peel region. Although the first arbovirus isolates were not obtained from mosquitoes collected in the Peel region until early December 2012, media releases advising of increased risk of mosquito-borne disease was issued by the WA DOH on 6 November 2012 following detection of BFV in mosquitoes in the Capel-Busselton region. A second media warning was released on 27 December 2012 after continued detections of BFV in mosquitoes and the first detection of RRV from the Peel region. The isolation of BFV from mosquitoes collected on 29 January was the last detection of BFV along the Swan Coastal Plain for the season.

A very high tide in late November and a regular series of high tides through the summer months (Figure 4), coupled with well above average rainfall in the Peel region in December (Figure 3) is likely to have facilitated mosquito breeding and increased adult mosquito abundance through the warmer summer months. The large population of vector mosquitoes during these months is likely to have contributed towards increased incidence of RRV disease and BFV disease in this region.

Table 3. Summary of the adult mosquito trapping effort (including successful and failed traps) for the mosquito and arbovirus surveillance program in the Peel region, July 2012 to June 2013.[1]

Region	Locality	Date	Successful	Failed	Total
Peel	Peel Inlet	Tuesday, July 17, 2012	7		7
"	"	Tuesday, July 31, 2012	7		7
"	"	Tuesday, August 14, 2012	7		7
"	"	Tuesday, August 28, 2012	7		7
"	"	Tuesday, September 11, 2012	6	1	7
"	"	Tuesday, September 25, 2012	6	1	7
"	"	Tuesday, October 09, 2012	7		7
"	"	Tuesday, October 23, 2012	6	1	7
"	"	Tuesday, November 06, 2012	7		7
"	"	Tuesday, November 20, 2012	7		7
"	"	Tuesday, December 04, 2012	7		7
"	"	Tuesday, December 18, 2012	7		7
"	"	Wednesday, January 02, 2013	7		7
"	"	Tuesday, January 15, 2013	7		7
"	"	Tuesday, January 29, 2013	7		7
"	"	Tuesday, February 12, 2013	7		7
"	"	Tuesday, February 26, 2013	7		7
"	"	Tuesday, March 12, 2013	7		7
"	"	Tuesday, March 26, 2013	7		7
"	"	Tuesday, April 09, 2013	7		7
"	"	Tuesday, April 23, 2013	7		7
"	"	Monday, May 20, 2013	6	1	7
"	"	Tuesday, June 18, 2013	7		7
"	Harvey Estuary	Tuesday, July 17, 2012	2		2
"	"	Tuesday, July 31, 2012	2		2
"	"	Tuesday, August 14, 2012	2		2
"	"	Tuesday, August 28, 2012	2		2
"	"	Tuesday, September 11, 2012	2		2
"	"	Tuesday, September 25, 2012	1	1	2
"	"	Tuesday, October 09, 2012	2		2
"	"	Tuesday, October 23, 2012	2		2
"	"	Tuesday, November 06, 2012	2		2
"	"	Tuesday, November 20, 2012	2		2
"	"	Tuesday, December 04, 2012	2		2
"	"	Tuesday, December 18, 2012	2		2
"	"	Wednesday, January 02, 2013	2		2
"	"	Tuesday, January 15, 2013	2		2
"	"	Tuesday, January 29, 2013	1	1	2
"	"	Tuesday, February 12, 2013	2		2
"	"	Tuesday, February 26, 2013	2		2
"	"	Tuesday, March 12, 2013	2		2
"	"	Tuesday, March 26, 2013	2		2
"	"	Tuesday, April 09, 2013	2		2
"	"	Tuesday, April 23, 2013	0	2	2
"	"	Monday, May 20, 2013	2		2
"	"	Tuesday, June 18, 2013	2		2
Total			**199**	**8**	**207**
		% successful =	**96.1**		

[1]Only results from successful traps were used to calculate mean mosquito populations/trap/night.
Minimum infection rates were calculated from pools processed from successful traps as well as failed traps that yielded a sample.
There was no access to the two Harvey Estuary sites on 23 April 2013.

Table 4. Details of mosquitoes collected and processed for virus isolation, Peel Inlet sites, southwest of Western Australia, 1 July 2012 to 30 June 2013.[1]

Species	Class	Total	(%)	Processed	Pools	RRV[2]	(MIR)[3]
Ae. (Finlaya) alboannulatus	Bloodfed	1	(<0.1)	0	0		
Ae. (Finlaya) alboannulatus	Female	203	(0.3)	146	59		
Ae. (Finlaya) notoscriptus	Bloodfed	2	(<0.1)	0	0		
Ae. (Finlaya) notoscriptus	Female	449	(0.7)	353	75		
Ae. (Ochlerotatus) camptorhynchus	Bloodfed	43	(0.1)	0	0		
Ae. (Ochlerotatus) camptorhynchus	Female	24610	(40.6)	14839	805		
Ae. (Ochlerotatus) clelandi	Bloodfed	1	(<0.1)	0	0		
Ae. (Ochlerotatus) clelandi	Female	40	(0.1)	32	9		
Ae. (Ochlerotatus) hesperonotius	Female	55	(0.1)	43	12		
Ae. (Ochlerotatus) turneri	Female	2	(<0.1)	2	2		
Ae. (Ochlerotatus) vigilax	Bloodfed	29	(<0.1)	0	0		
Ae. (Ochlerotatus) vigilax	Female	32367	(53.4)	12897	678	1	(0.08)
Ae. species (unidentified) - new or difficult to ID species	Male	249	(0.4)	224	29		
An. (Cellia) annulipes s.l.	Female	154	(0.3)	146	36		
Cq. (Coquillettidia) species near *linealis*	Female	1	(<0.1)	1	1		
Cs. (Culicella) atra	Female	44	(0.1)	33	15		
Cx. (Culex) annulirostris	Female	185	(0.3)	113	37		
Cx. (Culex) australicus	Female	95	(0.2)	75	29		
Cx. (Culex) globocoxitus	Bloodfed	8	(<0.1)	0	0		
Cx. (Culex) globocoxitus	Female	1720	(2.8)	1398	140		
Cx. (Culex) quinquefasciatus	Bloodfed	2	(<0.1)	0	0		
Cx. (Culex) quinquefasciatus	Female	290	(0.5)	249	57		
Cx. species (unidentified) - new or difficult to ID species	Male	80	(0.1)	76	25		
Unidentifiable (too damaged/features missing)	Female	1	(<0.1)	1	1		
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	5	(<0.1)	5	1		
Total		**60636**	**(100.0)**	**30633**	**2011**	**1**	**(0.03)**

[1]No mosquitoes were pinned. [2]RRV is Ross River virus; [3]MIR is the minimum infection rate per 1000 mosquitoes (Chiang and Reeves, 1962).

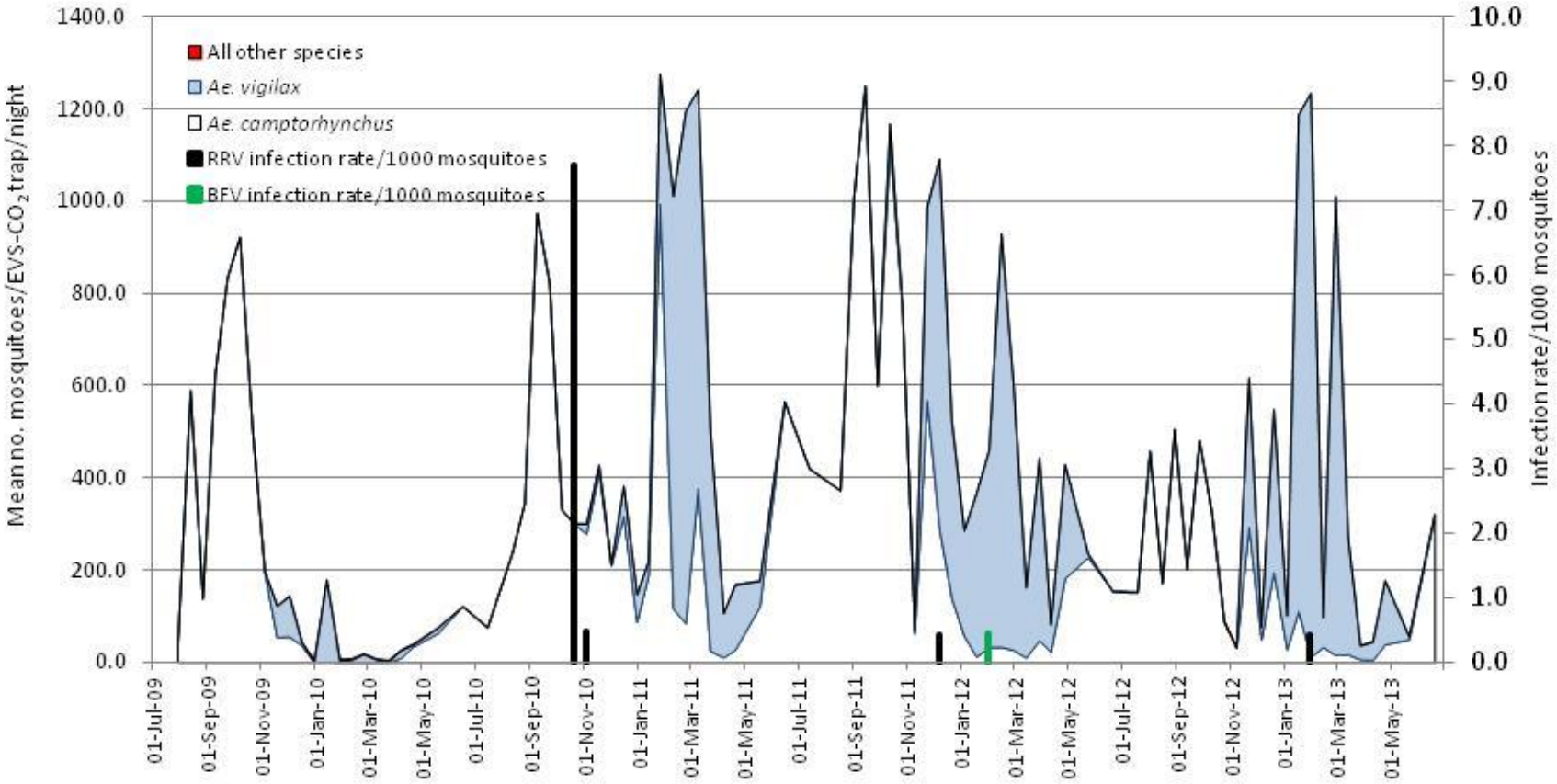

Figure 8. Abundance of adult mosquitoes and their infection rates (all species) with RRV and BFV, Peel Inlet sites, 1 July 2009 to 30 June 2013.

Table 5. Details of viruses isolated from mosquitoes collected during the mosquito/arbovirus surveillance program on the Swan Coastal Plain, southwest of Western Australia, 1 July 2012 to 30 June 2013.[1]

Region	Isolate no.	Trap ID	Date	Locality	Trap location	Species name	Class	No in pool	Virus ID
Peel									
	DC55591	12DC197	04-Dec-12	Waroona	Southern Estuary Road, Harvey Estuary	*Ae. camptorhynchus*	F	21	NE RRV
	DC55597	"	"	"	"	*Ae. vigilax*	F	20	NE RRV
	DC55598	"	"	"	"	"	F	6	NE RRV
	DC55607	12DC198	"	"	400 m north of NFS Research Station, Harvey Estuary	*Ae. camptorhynchus*	F	20	NE RRV
	DC55611	"	"	"	"	*Ae. vigilax*	F	12	NE RRV
	DC55747	12DC206	18-Dec-12	"	Southern Estuary Road, Harvey Estuary	*Ae. camptorhynchus*	F	9	EHV
	DC56041	13DC018	15-Jan-13	"	400 m north of NFS Research Station, Harvey Estuary	*Ae. vigilax*	F	20	EHV
	DC56184	13DC025	29-Jan-13	Mandurah	Falcon, Peel Inlet	"	F	20	NE RRV
	DC56192	13DC026	"	Waroona	Southern Estuary Road, Harvey Estuary	"	F	20	BFV
Leschenault									
	SW94457	12SW253	04-Dec-12	Harvey	Belvidere, Buffalo Rd, west side of Leschenault Inlet	*Ae. camptorhynchus*	F	20	BFV
	SW94566	"	"	"	"	"	F	20	BFV
	SW94735	13SW001	02-Jan-13	"	"	"	F	11	NE RRV
	SW94741	"	"	"	"	*Ae. vigilax*	F	15	NE RRV
	SW94862	13SW013	15-Jan-13	"	"	"	F	20	NE RRV
	SW94871	13SW015	"	"	Crimp Crescent, North Australind, Shire of Harvey	*Ae. camptorhynchus*	F	20	NE RRV
	SW94872	"	"	"	"	"	F	12	NE RRV
	SW94873	"	"	"	"	*Ae. vigilax*	F	20	NE RRV
	SW94874	"	"	"	"	"	F	4	NE RRV
	SW94876	"	"	"	"	*Cx. annulirostris*	F	9	NE RRV
Capel-Busselton									
	SW93518	12SW189	11-Sep-12	Capel	CALM Village, Ludlow Road North	*Ae. camptorhynchus*	F	20	BFV
	SW94093	12SW224	23-Oct-12	"	Intersection of Stirling and Higgins Rds, Ludlow Forest	"	F	20	BFV
	SW94096	"	"	"	"	"	F	20	BFV
	SW94245	12SW236	06-Nov-12	"	"	"	F	20	BFV
	SW94273	12SW239	"	Busselton	Busselton Radio Tower, Siesta Park	"	F	20	BFV
	SW94393	12SW248	20-Nov-12	Capel	Intersection of Stirling and Higgins Rds, Ludlow Forest	"	F	20	BFV
	SW94401	12SW249	"	"	CALM Village, Ludlow Road North	"	F	20	BFV
	SW94407	"	"	"	"	"	F	20	Non A/F
	SW94429	12SW251	"	Busselton	Busselton Radio Tower, Siesta Park	"	F	14	BFV
	SW94589	12SW263	04-Dec-12	"	"	"	F	20	BFV

[1]ID is identity; F is female; NE RRV is northern/eastern phenotype of RRV; EHV is Edge Hill virus; BFV is Barmah Forest virus, Non A/F is not an alphavirus or flavivirus.

Table 6. Details of mosquitoes collected and processed for virus isolation, Harvey Estuary sites, southwest of Western Australia, 1 July 2012 to 30 June 2013.

Species	Class	Total	(%)	Processed	Pools	Pinned	RRV[1]	(MIR)[2]	BFV[3]	(MIR)	EHV[4]	(MIR)
Ae. (Finlaya) alboannulatus	Bloodfed	2	(<0.1)	0	0	0						
Ae. (Finlaya) alboannulatus	Female	70	(0.7)	70	16	0						
Ae. (Finlaya) notoscriptus	Bloodfed	1	(<0.1)	0	0	0						
Ae. (Finlaya) notoscriptus	Female	278	(2.8)	265	23	0						
Ae. (Ochlerotatus) camptorhynchus	Bloodfed	46	(0.5)	0	0	0						
Ae. (Ochlerotatus) camptorhynchus	Female	4268	(42.7)	3123	182	1	2	(0.64)			1	(0.32)
Ae. (Ochlerotatus) clelandi	Female	1	(<0.1)	1	1	0						
Ae. (Ochlerotatus) ratcliffei	Female	7	(0.1)	7	3	0						
Ae. (Ochlerotatus) vigilax	Bloodfed	10	(0.1)	0	0	0						
Ae. (Ochlerotatus) vigilax	Female	4343	(43.4)	2636	141	0	3	(1.15)	1	(0.38)	1	(0.38)
Ae. species (unidentified) - new or difficult to ID species	Male	17	(0.2)	13	2	0						
An. (Cellia) annulipes s.l.	Female	520	(5.2)	507	36	0						
An. (Cellia) annulipes s.l.	Male	1	(<0.1)	1	1	0						
An. (Cellia) annulipes species A	Female	17	(0.2)	17	1	0						
An. (Cellia) annulipes species D	Female	13	(0.1)	13	2	0						
Cq. (Coquillettidia) species near *linealis*	Female	6	(0.1)	6	6	0						
Cs. (Culicella) atra	Female	2	(<0.1)	2	1	0						
Cx. (Culex) annulirostris	Bloodfed	2	(<0.1)	0	0	0						
Cx. (Culex) annulirostris	Female	156	(1.6)	121	16	0						
Cx. (Culex) australicus	Female	19	(0.2)	16	10	0						
Cx. (Culex) globocoxitus	Female	156	(1.6)	143	23	0						
Cx. (Culex) quinquefasciatus	Female	5	(<0.1)	4	4	0						
Cx. species (unidentified) - new or difficult to ID species	Male	61	(0.6)	57	11	0						
Total		**10001**	**(100.0)**	**7002**	**479**	**1**	**5**	**(0.72)**	**1**	**(0.14)**	**2**	**(0.29)**

[1]RRV is Ross River virus; [2]MIR is the minimum infection rate per 1000 mosquitoes (Chiang and Reeves, 1962); [3]BFV is Barmah Forest virus; [4]EHV is Edge Hill virus.

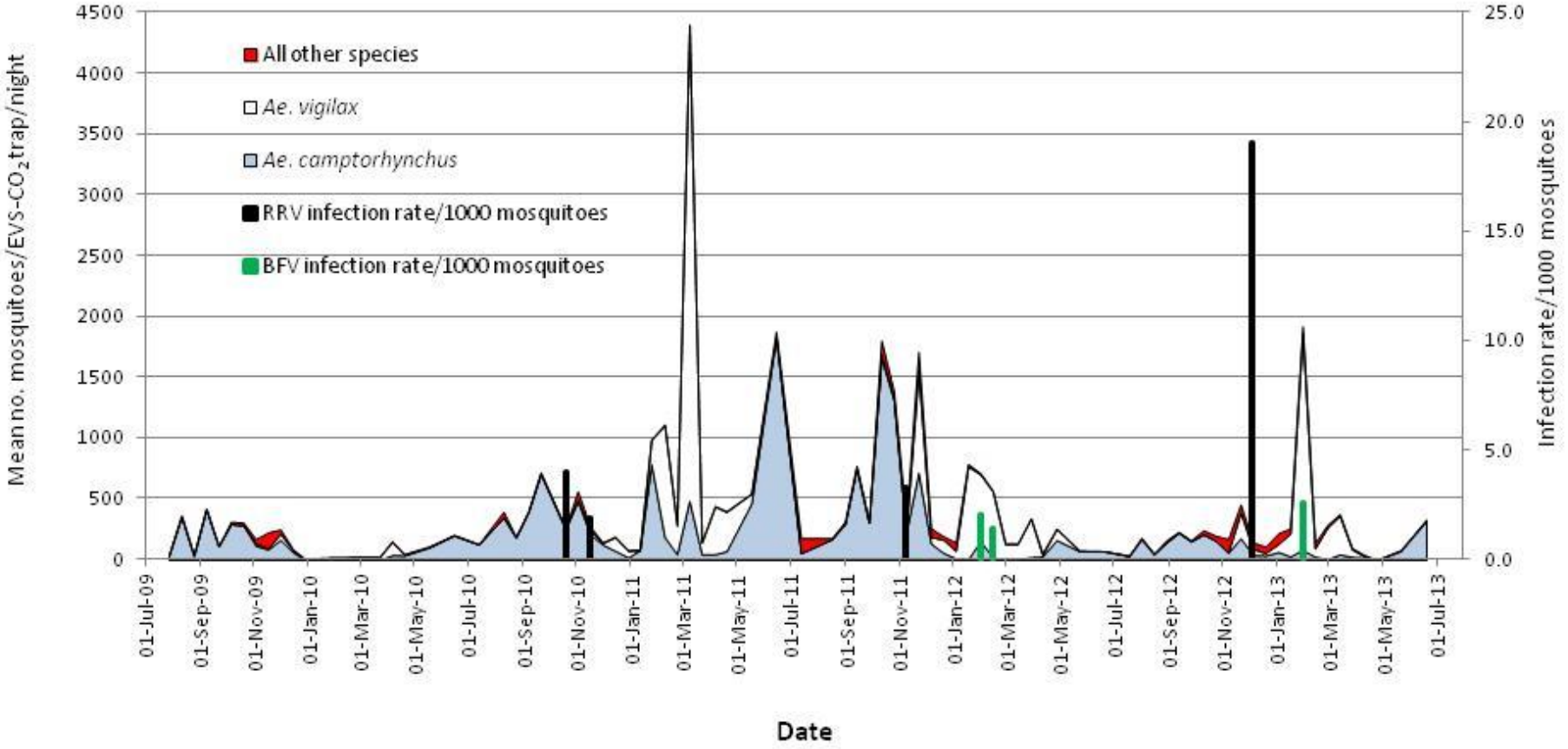

Figure 9. Abundance of adult mosquitoes and their infection rates (all species) with RRV and BFV, Harvey Estuary sites, 1 July 2009 - 30 June 2013.

Leschenault region

A total of 137 traps were set during 23 adult mosquito collection trips in the Leschenault region between July 2012 and June 2013, with successful outcomes for 95.7% of all traps set (Table 7). A total of 37982 mosquitoes belonging to 19 species were collected during the year, 22116 (58.2%) of which were processed for virus isolation (Table 8). The most abundant species collected were *Ae. camptorhynchus* (60.8%) and *Ae. vigilax* (27.7%). Mosquito abundance was high between August and October 2012, and increased again in January and February 2013 (Figure 10). *Aedes camptorhynchus* populations peaked in August 2012. *Ae. vigilax* populations peaked in January and February, and continued to be collected in mosquito traps through to June 2013. There were ten virus isolates from the Leschenault region during the 2012/13 season, including eight isolates of RRV (northern/eastern phenotype) and two isolates of BFV. *Aedes vigilax* yielded four isolates of RRV, *Ae. camptorhynchus* yielded three RRV isolates and one isolate of RRV was obtained from a pool of *Cx. annulirostris*. Both BFV isolates were from *Ae. camptorhynchus*. The MIR of mosquitoes for BFV reached 2.6 per 1000 mosquitoes on 4 December 2012, whilst the MIR of mosquitoes for RRV (2.6 per 1000 mosquitoes) peaked on 15 January 2013. All RRV and BFV isolates were from mosquitoes collected along the western and eastern side of the Leschenault Inlet (Belvidere and Crimp Crescent) (Table 5). The number of cases of RRV disease (Table 1) in the Leschenault region in 2012/13 (103) was higher than the previous season (78) (ASRL 2011/12 Annual Report).

Table 7. Summary of the adult mosquito trapping effort (including successful and failed traps) for the mosquito and arbovirus surveillance program in the Leschenault region, July 2012 to June 2013.[1]

Region	Date	Successful	Failed	Total
Leschenault	Tuesday, July 17, 2012	5	1	6
"	Tuesday, July 31, 2012	6		6
"	Tuesday, August 14, 2012	6		6
"	Tuesday, August 28, 2012	4	2	6
"	Tuesday, September 11, 2012	5	1	6
"	Tuesday, September 25, 2012	5	1	6
"	Tuesday, October 09, 2012	6		6
"	Tuesday, October 23, 2012	6		6
"	Tuesday, November 06, 2012	6		6
"	Tuesday, November 20, 2012	6		6
"	Tuesday, December 04, 2012	6		6
"	Tuesday, December 18, 2012	6		6
"	Wednesday, January 02, 2013	6		6
"	Tuesday, January 15, 2013	6		6
"	Tuesday, January 29, 2013	6		6
"	Tuesday, February 12, 2013	6		6
"	Tuesday, February 26, 2013	6		6
"	Tuesday, March 12, 2013	6		6
"	Tuesday, March 26, 2013	5	1	6
"	Tuesday, April 09, 2013	6		6
"	Tuesday, April 23, 2013	6		6
"	Monday, May 20, 2013	6		6
"	Tuesday, June 18, 2013	6		6
Total		**132**	**6**	**138**
	% successful =	95.7		

[1]Only results from successful traps were used to calculate mean mosquito populations/trap/night.
Minimum infection rates were calculated from pools processed from successful traps as well as failed traps that yielded a sample.

Table 8. Details of mosquitoes collected and processed for virus isolation, Leschenault Inlet sites, southwest of Western Australia, 1 July 2012 to 30 June 2013.

Species	Class	Total	(%)	Processed	Pools	Pinned	RRV[1]	(MIR)[2]	BFV[3]	(MIR)
Ae. (Finlaya) alboannulatus	Female	527	(1.4)	444	76	0				
Ae. (Finlaya) mallochi	Female	5	(<0.1)	4	3	0				
Ae. (Finlaya) notoscriptus	Female	545	(1.4)	524	55	0				
Ae. (Finlaya) notoscriptus	Male	1	(<0.1)	1	1	0				
Ae. (Ochlerotatus) camptorhynchus	Bloodfed	149	(0.4)	0	0	0				
Ae. (Ochlerotatus) camptorhynchus	Female	23087	(60.8)	11986	645	0	3	(0.25)	2	(0.17)
Ae. (Ochlerotatus) camptorhynchus	Male	1	(<0.1)	1	1	0				
Ae. (Ochlerotatus) camptorhynchus, bands join	Female	1	(<0.1)	0	0	1				
Ae. (Ochlerotatus) clelandi	Female	52	(0.1)	38	14	0				
Ae. (Ochlerotatus) hesperonotius	Bloodfed	1	(<0.1)	0	0	0				
Ae. (Ochlerotatus) hesperonotius	Female	372	(1.0)	372	29	0				
Ae. (Ochlerotatus) mackintoshi	Female	2	(<0.1)	0	0	2				
Ae. (Ochlerotatus) nigrithorax	Female	1	(<0.1)	0	0	1				
Ae. (Ochlerotatus) ratcliffei	Female	20	(0.1)	20	5	0				
Ae. (Ochlerotatus) turneri	Female	2	(<0.1)	2	2	0				
Ae. (Ochlerotatus) vigilax	Bloodfed	35	(0.1)	0	0	0				
Ae. (Ochlerotatus) vigilax	Female	10504	(27.7)	6694	359	0	4	(0.60)		
Ae. species (unidentified) - new or difficult to ID species	Male	378	(1.0)	237	23	0				
An. (Cellia) annulipes s.l.	Female	183	(0.5)	162	41	0				
An. (Cellia) annulipes s.l.	Male	1	(<0.1)	1	1	0				
An. (Cellia) annulipes species D	Female	1	(<0.1)	1	1	0				
An. species (unidentified) - new or difficult to ID species	Male	1	(<0.1)	1	1	0				
Cq. (Coquillettidia) species near *linealis*	Bloodfed	5	(<0.1)	0	0	0				
Cq. (Coquillettidia) species near *linealis*	Female	600	(1.6)	480	49	0				
Cs. (Culicella) atra	Bloodfed	1	(<0.1)	0	0	0				
Cs. (Culicella) atra	Female	85	(0.2)	76	16	0				
Cx. (Culex) annulirostris	Bloodfed	5	(<0.1)	0	0	0				
Cx. (Culex) annulirostris	Female	454	(1.2)	308	40	0	1	(3.28)		
Cx. (Culex) australicus	Female	105	(0.3)	93	32	0				
Cx. (Culex) globocoxitus	Bloodfed	4	(<0.1)	0	0	0				
Cx. (Culex) globocoxitus	Female	749	(2.0)	586	83	0				
Cx. (Culex) quinquefasciatus	Female	81	(0.2)	64	26	0				
Cx. species (unidentified) - new or difficult to ID species	Male	23	(0.1)	21	10	0				
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	1	(<0.1)	0	0	0				
Total		**37982**	**(100.0)**	**22116**	**1513**	**4**	**8**	**(0.36)**	**2**	**(0.09)**

[1]RRV is Ross River virus; [2]MIR is the minimum infection rate per 1000 mosquitoes (Chiang and Reeves, 1962); [3]BFV is Barmah Forest virus.

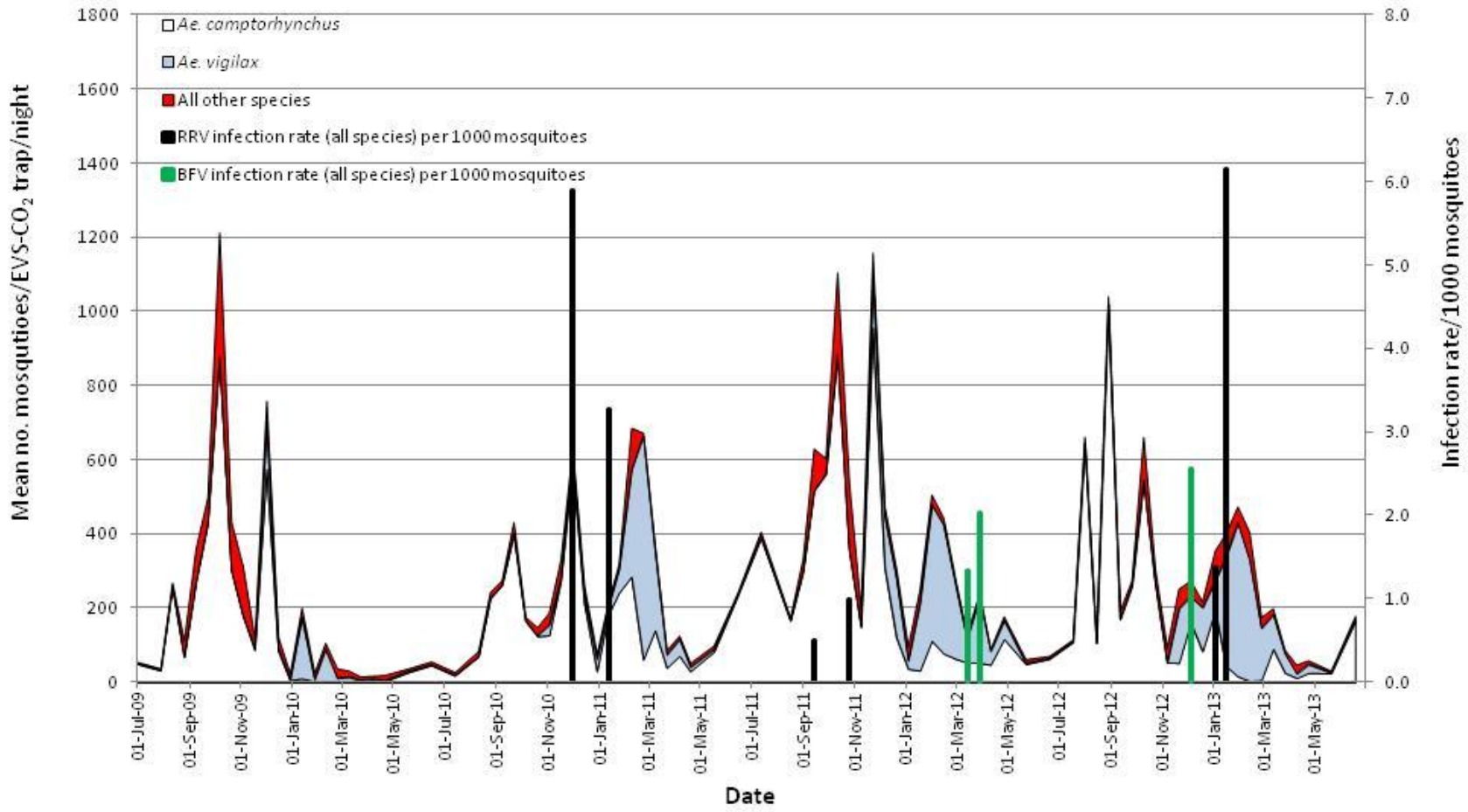

Figure 10. Abundance of adult mosquitoes and their infection rates (all species) with RRV and BFV, Leschenault Inlet sites, 1 July 209 - 30 June 2013.

Capel-Busselton region

Of 134 traps set during 23 trap runs to collect adult mosquitoes at Capel forest sites and Busselton wetlands sites, 97.1% were successful (Table 9). A total of 16122 adult mosquitoes were collected from Capel forest sites between July 2012 and June 2013, 7673 (47.6%) of which were processed for virus isolation (Table 10). The most abundant mosquito species collected was *Ae. camptorhynchus* (91.9%). *Aedes camptorhynchus* populations were similar to 2011/12 (ASRL 2011/12 Annual Report), with the exception of a very large peak in abundance of more than 1800 per trap on 18 June 2013 (Figure 11). Six BFV isolates and one non-alphavirus/non-flavivirus were obtained from *Ae. camptorhynchus* (Tables 5 and 10). The isolation of BFV from mosquitoes collected at the CALM Village trap site on 11 September 2012 was the first arbovirus isolation for the season, and detections continued through to 4 December 2012. A media release was issued by the WA DOH on 25 November 2012, warning of increased risk of mosquito-borne disease in the Geographe area. It also advised people in other parts of the southwest, including the Perth Metropolitan area, of the risk of BFV disease. A second media release was issued on 27 December 2012 after continued detections of BFV in mosquitoes, as well as the detection of RRV in mosquitoes in the southwest of WA. The MIR of mosquitoes for BFV ranged from 1.4 per 1000 mosquitoes on 11 September 2012 up to 3.6 per 1000 mosquitoes on 20 November 2012 (Figure 11).

A total of 11585 adult mosquitoes were collected at Busselton wetlands sites, 5726 (49.4%) of which were processed for virus isolation (Table 11). *Aedes camptorhynchus* (67.0%), *Cx. globocoxitus* (8.43%) and *Ae. hesperonotius* (5.6%) were the most abundant mosquitoes collected. Mosquito abundance was similar to the previous season (ASRL 2011/12 Annual Report), however abundance of the vector *Ae. camptorhynchus* was greater (Figure 12). There was a large peak in vector abundance in January 2013, probably due to well above average rainfall in the Capel-Busselton region in December 2012 (Figure 3). Three isolates of BFV were obtained from *Ae. camptorynchus* collected at wetlands sites on 6 and 20 November and 4 December 2012 (Tables 5 and 11). The MIR of mosquitoes for BFV was sustained between 2.5 and 3.2 per 1000 mosquitoes between 6 November and 4 December 2012 (Figure 12). One non-alphavirus/non-flavivirus isolate was obtained from *Ae. camptorhynchus* collected at the CALM Village trap site on 20 November 2012, and is the subject of further identification using molecular techniques.

A total of 13 cases of RRV disease were reported from Busselton and seven cases were reported from Capel in 2012/13 (Table 1). The number of cases in Capel and Busselton were substantially lower than the previous season (ASRL 2011/12 Annual Report).

Table 9. Summary of the adult mosquito trapping effort (including successful and failed traps) for the mosquito and arbovirus surveillance program in the Capel-Busselton region, July 2012 to June 2013.[1]

Region	Locality	Date	Successful	Failed	Total
Capel-Busselton	Capel (forest)	Tuesday, July 17, 2012	3		3
"	"	Tuesday, July 31, 2012	3		3
"	"	Tuesday, August 14, 2012	3		3
"	"	Tuesday, August 28, 2012	3		3
"	"	Tuesday, September 11, 2012	3		3
"	"	Tuesday, September 25, 2012	3		3
"	"	Tuesday, October 09, 2012	3		3
"	"	Tuesday, October 23, 2012	3		3
"	"	Tuesday, November 06, 2012	3		3
"	"	Tuesday, November 20, 2012	3		3
"	"	Tuesday, December 04, 2012	3		3
"	"	Tuesday, December 18, 2012	3		3
"	"	Wednesday, January 02, 2013	2	1	3
"	"	Tuesday, January 15, 2013	2	1	3
"	"	Tuesday, January 29, 2013	3		3
"	"	Tuesday, February 12, 2013	3		3
"	"	Tuesday, February 26, 2013	3		3
"	"	Tuesday, March 12, 2013	3		3
"	"	Tuesday, March 26, 2013	3		3
"	"	Tuesday, April 09, 2013	3		3
"	"	Tuesday, April 23, 2013	3		3
"	"	Monday, May 20, 2013	3		3
"	"	Tuesday, June 18, 2013	3		3
"	Busselton (wetlands)	Tuesday, July 17, 2012	3		3
"	"	Tuesday, July 31, 2012	3		3
"	"	Tuesday, August 14, 2012	3		3
"	"	Tuesday, August 28, 2012	3		3
"	"	Tuesday, September 11, 2012	3		3
"	"	Tuesday, September 25, 2012	2	1	3
"	"	Tuesday, October 09, 2012	3		3
"	"	Tuesday, October 23, 2012	3		3
"	"	Tuesday, November 06, 2012	3		3
"	"	Tuesday, November 20, 2012	3		3
"	"	Tuesday, December 04, 2012	3		3
"	"	Tuesday, December 18, 2012	3		3
"	"	Wednesday, January 02, 2013	3		3
"	"	Tuesday, January 15, 2013	3		3
"	"	Tuesday, January 29, 2013	3		3
"	"	Tuesday, February 12, 2013	3		3
"	"	Tuesday, February 26, 2013	3		3
"	"	Tuesday, March 12, 2013	3		3
"	"	Tuesday, March 26, 2013	3		3
"	"	Tuesday, April 09, 2013[2]	2	1	3
"	"	Tuesday, April 23, 2013	3		3
"	"	Monday, May 20, 2013	3		3
"	"	Tuesday, June 18, 2013	3		3
Total			**134**	**4**	**138**
		% successful =	97.1		

[1]Only results from successful traps were used to calculate mean mosquito populations/trap/night. Minimum infection rates were calculated from pools processed from successful traps as well as failed traps that yielded a sample; [2]Busselton trap stolen/vandalised on 9 April 2013.

Table 10. Details of mosquitoes collected and processed for virus isolation, Capel forest sites, southwest of Western Australia, 1 July 2012 to 30 June 2013.[1]

Species	Class	Total	(%)	Processed	Pools	BFV[2]	(MIR)[3]	Non A/F[4]	(MIR)
Ae. (Finlaya) alboannulatus	Bloodfed	1	(<0.1)	0	0				
Ae. (Finlaya) alboannulatus	Female	116	(0.7)	87	30				
Ae. (Finlaya) mallochi	Female	6	(<0.1)	6	2				
Ae. (Finlaya) notoscriptus	Female	57	(0.4)	43	17				
Ae. (Ochlerotatus) camptorhynchus	Bloodfed	145	(0.9)	0	0				
Ae. (Ochlerotatus) camptorhynchus	Female	14822	(91.9)	6774	361	6	(0.89)	1	(0.15)
Ae. (Ochlerotatus) clelandi	Female	6	(<0.1)	4	3				
Ae. (Ochlerotatus) hesperonotius	Female	1	(<0.1)	1	1				
Ae. (Ochlerotatus) ratcliffei	Female	110	(0.7)	56	7				
Ae. (Ochlerotatus) vigilax	Female	5	(<0.1)	5	3				
Ae. species (unidentified) - new or difficult to ID species	Male	3	(<0.1)	2	2				
An. (Cellia) annulipes s.l.	Bloodfed	10	(0.1)	0	0				
An. (Cellia) annulipes s.l.	Female	300	(1.9)	251	31				
Cq. (Coquillettidia) species near *linealis*	Female	15	(0.1)	15	7				
Cs. (Culicella) atra	Female	2	(<0.1)	2	2				
Cx. (Culex) annulirostris	Female	168	(1.0)	168	23				
Cx. (Culex) australicus	Bloodfed	1	(<0.1)	0	0				
Cx. (Culex) australicus	Female	24	(0.1)	16	12				
Cx. (Culex) globocoxitus	Bloodfed	2	(<0.1)	0	0				
Cx. (Culex) globocoxitus	Female	294	(1.8)	219	35				
Cx. (Culex) quinquefasciatus	Female	7	(<0.1)	7	3				
Cx. species (unidentified) - new or difficult to ID species	Male	27	(0.2)	17	9				
Total		**16122**	**(100.0)**	**7673**	**548**	**6**	**(0.79)**	**1**	**(0.13)**

[1]No mosquitoes were pinned; [2]BFV is Barmah Forest virus, [3]MIR is the minimum infection rate per 1000 mosquitoes (Chiang and Reeves, 1962); [4]Non A/F is not an alphavirus and flavivirus and is yet to be identified by polymerase chain reaction.

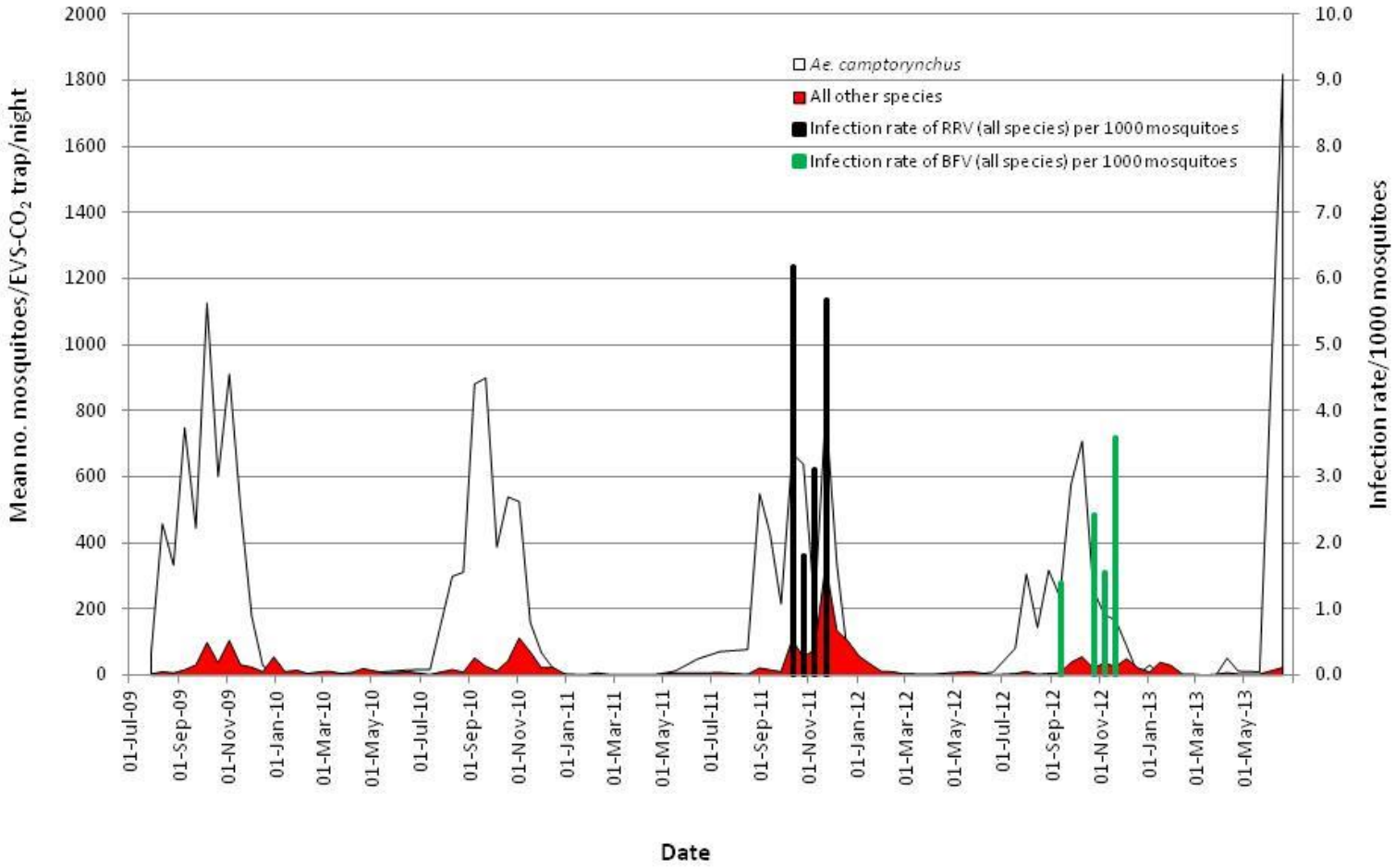

Figure 11. Abundance of adult mosquitoes and their infection rate (all species) with RRV and BFV, Capel forest sites, 01 July 2009 - 30 June 2013.

Table 11. Details of mosquitoes collected and processed for virus isolation, Busselton wetland sites, southwest of Western Australia, 1 July 2012 to 30 June 2013.

Species	Class	Total	(%)	Processed	Pools	Pinned	BFV[1]	(MIR)[2]
Ae. (Finlaya) alboannulatus	Bloodfed	7	(0.1)	0	0	0		
Ae. (Finlaya) alboannulatus	Female	264	(2.3)	199	34	0		
Ae. (Finlaya) notoscriptus	Female	59	(0.5)	51	13	0		
Ae. (Ochlerotatus) camptorhynchus	Bloodfed	84	(0.7)	0	0	0		
Ae. (Ochlerotatus) camptorhynchus	Female	7760	(67.0)	3326	189	0	3	(0.91)
Ae. (Ochlerotatus) clelandi	Female	260	(2.2)	204	16	0		
Ae. (Ochlerotatus) E.N. Marks' species "Koorda"	Female	3	(<0.1)	0	0	1		
Ae. (Ochlerotatus) hesperonotius	Bloodfed	7	(0.1)	0	0	0		
Ae. (Ochlerotatus) hesperonotius	Female	651	(5.6)	333	23	0		
Ae. (Ochlerotatus) ratcliffei	Female	72	(0.6)	41	6	0		
Ae. (Ochlerotatus) turneri	Female	8	(0.1)	5	3	0		
Ae. (Ochlerotatus) vigilax	Female	384	(3.3)	47	6	0		
Ae. species (unidentified) - new or difficult to ID species	Male	15	(0.1)	6	4	0		
An. (Cellia) annulipes s.l.	Bloodfed	2	(<0.1)	0	0	0		
An. (Cellia) annulipes s.l.	Female	328	(2.8)	263	37	0		
An. species (unidentified) - new or difficult to ID species	Male	1	(<0.1)	1	1	0		
Cq. (Coquillettidia) species near *linealis*	Female	9	(0.1)	1	1	0		
Cs. (Culicella) atra	Female	13	(0.1)	9	7	0		
Cx. (Culex) annulirostris	Female	138	(1.2)	113	17	0		
Cx. (Culex) australicus	Female	212	(1.8)	104	21	1		
Cx. (Culex) globocoxitus	Female	968	(8.4)	768	66	0		
Cx. (Culex) quinquefasciatus	Female	15	(0.1)	14	8	0		
Cx. species (unidentified) - new or difficult to ID species	Male	321	(2.8)	239	22	0		
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	1	(<0.1)	1	1	0		
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	3	(<0.1)	1	1	0		
Total		**11585**	**(100.0)**	**5726**	**476**	**2**	**3**	**(0.53)**

[1]BFV is Barmah Forest virus; [2]MIR is the minimum infection rate per 1000 mosquitoes (Chiang and Reeves, 1962).

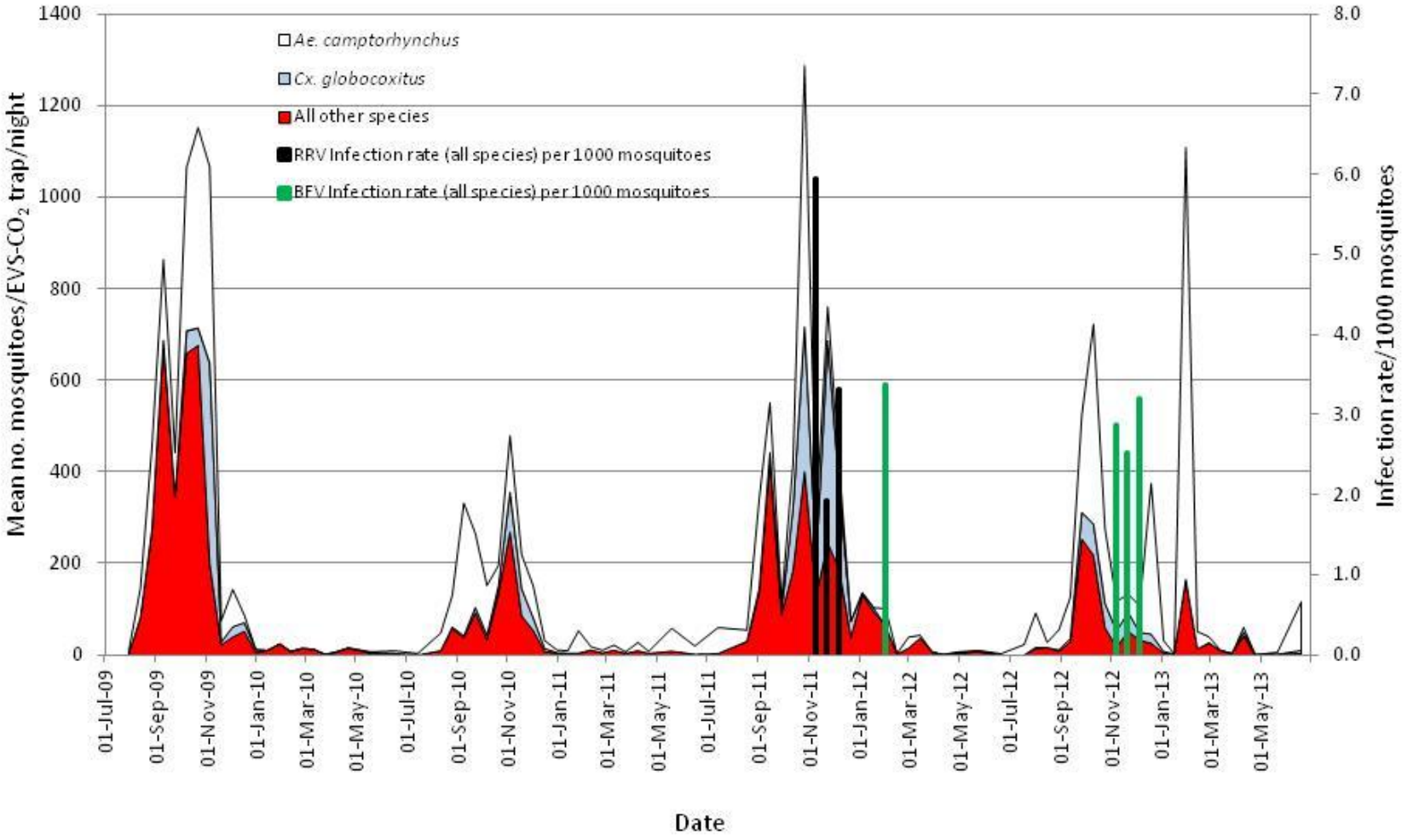

Figure 12. Abundance of adult mosquitoes and their infection rate (all species) with RRV and BFV, Busselton wetland sites, 01 July 2009 - 30 June 2013.

Kimberley

Results of mosquito trapping and virus isolations in 2012 for the Kimberley region are presented in this report, as well as details of mosquito trapping carried out in 2013. Mosquito identifications from the 2013 collections have been completed however are yet to be processed for virus isolation. This will be completed in 2014 and the results will be presented in the 2013/14 Annual Report.

2012 field and laboratory results

The annual collecting trip to investigate flavivirus activity throughout the Kimberley region was carried out between 29 March and 14 April 2012 (Table 12). A total of 173 traps were set with a 98.3% success rate. Of the traps set, 68 were set in the West Kimberley, 34 were set in the Southeast Kimberley and 71 were set in the northeast Kimberley. Mosquito species collected, their percentage composition of the overall total, processing details and viruses yielded at all localities within each town or community in 2012 are summarised in Tables 13A-W.

Table 12. Details of Kimberley meteorological regions, localities and suburbs where mosquito sampling was conducted in 2012.

				Trap outcomes		
Meteorological region	**Town or region**	**Locality**	**Date**	**Successful**	**Failed**	**Total**
West Kimberley	Roebuck Plain	Plain and environs	29-Mar-12	11		11
"	Dampier Peninsula	Willie Creek	"	4		4
"	Broome	Coconut Wells	"	2		2
"	"	Town and environs	"	3		3
"	Roebuck Plain	Plain and environs	30-Mar-12	2		2
"	Broome	Town and environs	"	9	1	10
"	Willare	Locality and environs	1-Apr-12	4		4
"	Derby	Town and environs	"	8	1	9
"	"	Gibb River road transect	"	4		4
"	Fitzroy Crossing	Town and environs	2-Apr-12	11		11
"	Geikie Gorge	Gorge and environs	"	8		8
Southeast Kimberley	Halls Creek	Town and environs	3-Apr-12	13		13
Northeast Kimberley	Kununurra	Point Springs/Keep River	5-Apr-12	12		12
"	"	Town and environs	6-Apr-12	9		9
"	"	Packsaddle Plain	"	5		5
"	"	"	7-Apr-12	1	1	2
"	"	Town and environs	"	6		6
"	"	Irrigation area	"	12		12
"	"	Lake Argyle	8-Apr-12	5		5
"	Wyndham	Parry's Creek region	10-Apr-12	12		12
"	"	Wyndham (Six Mile)	"	4		4
"	"	Wyndham Town (Three Mile)	"	3		3
"	"	Wyndham Port	"	1		1
Southeast Kimberley	Billiluna	Myarra Pool	13-Apr-12	5		5
"	"	Billiluna/Balgo Rd	"	4		4
"	"	Community and environs	"	4		4
"	"	Stretch Lagoon	14-Apr-12	8		8
Total				**170**	**3**	**173**
			% Successful =	98.3		

Eighteen mosquitoes were pinned to add to the reference collection, including possible albino *Anopheles* specimens from Kununurra in the northeast Kimberley region and one *Ae. phaecasiatus* from Willie Creek in the West Kimberley region. No *Ae. vexans* (Johansen et al. 2004a; Johansen et al. 2005) were collected in 2012.

Of the estimated 284166 mosquitoes collected in 2012, 63511 (22.3%) were processed for virus isolation in 4077 pools, yielding 65 arbovirus isolates: 47 RRV (northern/eastern phenotype), 13 new flaviviruses in the yellow fever group, 3 MVEV and 2 KOKV (Tables 14-16). Most virus isolates were from *Cx. annulirostris* (44.6%) and *Ae. normanensis* (26.2%), with low numbers of isolates from another ten species (Table 15). The RRV isolates were from a wide range of species, although most were from *Cx. annulirostris* and *Ae. normanensis*. All MVEV and KOKV isolates were from *Cx. annulirostris*, whilst the majority of new flavivirus isolates in the yellow fever group were from *Ae. normanensis* (84.6%) with single isolates from a pool of damaged unidentifiable *Aedes* mosquitoes and a pool of *Cx. annulirostris*. Most isolates were from Broome and Roebuck Plain (31), Kununurra (15) and Wyndham/Parry's Creek (12) (Table 16). Roebuck Plain, Kununurra (Town and environs) and Parry's Creek were the localities that yielded the greatest number of isolates (22, 11 and nine, respectively). The new flavivirus was isolated from mosquitoes collected at Kununurra, Wyndham and Billiluna. The infection rate of *Cx. annulirostris* in the Kimberley region with MVEV was low (Table 17). MVEV was only isolated from a pool of mosquitoes collected at Fitzroy Crossing (Geikie Gorge transect) (0.6 per 1000 mosquitoes) and two mosquito pools at Billiluna (0.6 per 1000 mosquitoes).

Central parts of WA experienced wetter than normal conditions prior to the commencement of the 2011/12 wet season. November 2011 was the second wettest on record in WA with thunderstorm activity creating heavy rainfall through the Pilbara, Gascoyne and northern Goldfields. The combination of an active monsoonal trough, TC Heidi crossing the Pilbara coast and ex-TC Iggy resulted in parts of the west Kimberley, Pilbara and north Gascoyne recording their wettest January on record. Monsoonal activity during the middle of March and TC Lua crossing the Pilbara coast resulted in much of the Kimberley and Pilbara experiencing above average rainfall during the month. A total of 4185 serum samples from 28 chicken flocks across WA were tested for antibodies to flaviviruses during 2011/12 (ASRL 2011/12 Annual Report, Appendix 1). Seroconversions to flaviviruses were detected in 225 (5.4%) samples. The first activity associated with the 2011/12 wet season occurred in November 2011, when MVEV and KUNV infections were detected in sentinel chickens at Kununurra in the northeast Kimberley region and KUNV infections were detected at Moora, in the Wheatbelt. This was the earliest start to the flavivirus season in more than ten years. High levels of flavivirus activity were subsequently detected throughout the Kimberley, Pilbara and Midwest regions in December, and the activity continued through until August 2012 (Table 21). Overall there were 192 seroconversions to MVEV, 28 to KUNV, three chickens developed antibody to both MVEV and KUNV and two chickens developed flavivirus antibody but not specifically to MVEV or KUNV. The overall level of flavivirus activity was slightly lower than the very high levels seen in the previous season, but still high (ASRL 2010/11 Annual Report). Despite the large number of seroconversions to MVEV and KUNV, no human cases of disease caused by infection with MVEV or KUNV were diagnosed in WA during 2011/12 (Table 2). The low number of isolations of MVEV (and absence of KUNV) in mosquitoes collected during the season is surprising given the relatively high level of activity in sentinel chickens during the season. However flavivirus infections in the northeast Kimberley peaked between November and February, whilst in the west Kimberley they did not occur until April/May. Mosquitoes were collected in late March through to mid-April, so it is possible the timing of mosquito collections preceded flavivirus activity in the West Kimberley region but occurred after the peak in flavivirus activity in the northeast Kimberley region.

West Kimberley

Mosquito abundance at Roebuck Plain trap sites in Broome was slightly higher than the previous season. On average, 8209 mosquitoes were collected per trap (Table 13A). *Culex annulirostris* was the most abundant species collected (59.1%), followed by *Cx. sitiens* (13.7%) and *Ae. vigilax* (10.2%). Twenty two isolates of RRV (northern/eastern phenotype) were obtained from five mosquito species. The majority (17) of isolates were from *Cx. annulirostris*. Elsewhere in Broome, mosquito abundance was lower than the previous season (see Appendix 2A for revised table for Broome Town and environs in 2011). At Willie Creek, 1752 mosquitoes were collected per trap, and the dominant species were *Ae. vigilax* (47.5%), *Cx. annulirostris* (20.6%) and *Cx. sitiens* (16.2%) (Table 13B). The collection included 65 *Ae. phaecasiatus*, a species only recently recognised in the West Kimberley region. No viruses were isolated from mosquitoes collected at Willie Creek. On average, 1168 mosquitoes were collected per trap at Coconut Wells (Table 13C). The most abundant species comprised *Ae. vigilax* (63.9%) and *Cx. annulirostris* (20.6%), and three isolates of RRV (northern/eastern phenotype) were obtained from these species. Six isolates of RRV (northern/eastern phenotype) were also obtained from mosquitoes collected at Broome town sites (Table 13D). On average 417 mosquitoes were collected per trap and *Cx. sitiens* (62.4%), *Cx. annulirostris* (9.0%) and *Ae. vigilax* (7.7%) were the most abundant species. Species yielding isolates of RRV were *Cx. sitiens* (2), *Ae. notoscriptus* (1), *Ae. alternans* (1), *Ae. normanensis* (1) and *Ae. vigilax* (1). *Aedes phaecasiatus* was also collected at Coconut Wells and Broome town collection sites.

Mosquito abundance was slightly higher than the previous season at Willare and Derby (Tables 13E-G) (see Appendix 2B for revised table for Derby Gibb River Road transect). At Willare (Table 13E), on average 4978 mosquitoes were collected per trap, the majority of which were *Ae. normanensis* (62.8%) and *Cx. annulirostris* (22.2%). A single isolate of RRV (northern/eastern phenotype) was obtained from *Ae. normanensis*. Within the town of Derby mosquito abundance was high (2419/trap) (Table 13F). *Aedes vigilax* (54.1%), *Cx. annulirostris* (20.5%) and *Ae. normanensis* (19.0%) were the main species collected. The same species dominated in the Gibb River Road transect trap sites, where mosquito abundance was very high (5332/trap) (Table 13G). No arboviruses were isolated from mosquitoes processed from Derby town and Gibb River Road transect trap sites.

The abundance of mosquitoes at Fitzroy Crossing and Geikie Gorge was similar to the previous season (see Appendix 2C-D for revised tables for Fitzroy Crossing and Geikie Gorge in 2011). On average 692 mosquitoes were collected per trap (Tables 13H-I). Dominant species at Fitzroy Crossing trap sites comprised *Ae. normanensis* (38.3%), *Cx. annulirostris* (22.6%), *An. annulipes* s.l. (12.8%) and *An. amictus* (10.2%). A single isolate of MVEV was obtained from *Cx. annulirostris*, and the infection rate was 0.6 per 1000 mosquitoes (Table 17). This was the only isolate of MVEV from the West Kimberley region. *Aedes normanensis*, *An. annulipes* s.l. and *Cx. annulirostris* also dominanted the Geikie Gorge traps (33.4%, 27.1% and 20.9%, respectively). A relatively large number of specimens of an undescribed *Ae. (Finlaya)* species (128) were collected in 2012, and this species is consistently collected in traps near Geikie Gorge. No viruses were obtained from mosquitoes collected at Geikie Gorge. Of note, the new flavivirus in the yellow fever group was not detected in mosquitoes from Fitzroy Crossing or Geikie Gorge trap sites, where as three isolates were obtained from mosquitoes in this vicinity in the previous year (ASRL 2011/12 Annual Report). However this virus was detected in the southeast and northeast Kimberley regions in mosquitoes collected in the 2011/12 wet season (summarised in Tables 14-16).

Southeast Kimberley

Mosquito abundance at Halls Creek (Table 13J) was relatively low and similar to the previous season. On average, 261 mosquitoes were collected per trap. *Aedes normanensis* (74.2%) was the most abundant species collected, and *Cx. annulirostris* comprised just 1.9% of the population

collected. *Aedes vittiger* has rarely been collected from this locality in the past, however in the 2012 wet season it comprised 2.1% of the population. No arboviruses were isolated from mosquitoes collected at Halls Creek.

Mosquito abundance at the Aboriginal community of Billiluna (Tables 13K-N) was substantially lower than the previous season. Abundance was higher in the Myarra Pool and Billiluna/Balgo Road trapsites than elsewhere. Along the Myarra Pool transect an average of 1204 mosquitoes were collected per trap, and the dominant species were *Cx. annulirostris* (44.9%) and *An. annulipes* s.l. (31.9%) (Table 13K). The most abundant species along the Billiluna/Balgo Road transect (Table 13L) were *An. annulipes* s.l. (35.8), *Cx. annulirostris* (34.8%) and *Ae. normanensis* (15.8%), and an average of 912 mosquitoes were collected per trap. Three flaviviruses were isolated, comprising one isolate of MVEV from *Cx. annulirostris* and two isolates of the new yellow fever group flavivirus from *Ae. normanensis*. Mosquito abundance in the community (Table 13M) was low (95/trap), and no viruses were isolated. The average mosquito abundance at Stretch Lagoon (Table 13N) was 362/trap. *Culex annulirostris* (50.5%), *Ae. normanensis* (19.9%) and *An. amictus* (9.3%) comprised the majority of mosquitoes collected. *Culex annulirostris* and *Ae. normanensis* yielded one isolate of MVEV and the new yellow fever group flavivirus, respectively. The infection rate of *Cx. annulirostris* in Billiluna for MVEV was 0.6 per 1000 mosquitoes (Table 17).

Northeast Kimberley

Average mosquito abundance was higher along the Point Springs/Keep River (PSKR) transect compared with the previous season, but generally lower elsewhere at Kununurra (Tables 13O-S). An average of 1986 mosquitoes were collected per trap in PSKR traps (Table 13O). *Aedes normanensis* (43.1%), *Cx. annulirostris* (17.5%), *Ae. lineatopennis* (11.3%) and *An. meraukensis* (10.0%) were the most abundant species collected, and two isolates of the new yellow fever group flavivirus were obtained from *Ae. normanensis*. One unusual *Anopheles* sp. mosquito was also collected and this will be examined by Mr Peter Whelan and Prof. Richard Russell. On average 275 mosquitoes were collected per trap site at Kununurra town and environs (Table 13P). The most abundant species included *Cx. annulirostris* (32.7%), *Ae. normanensis* (14.3%) and *Cq. xanthogaster* (12.0%). Three isolates of the new yellow fever group flavivirus were obtained from *Ae. normanensis*. The northern/eastern strain of RRV was detected in pools of *Ae. normanensis* (2), *Ma. uniformis* (2), *An. amictus* (1), *Cx. annulirostris* (1) and a pool of damaged *Culex* mosquitoes. Mosquito abundance at Packsaddle Plain (490/trap) was substantially lower than the previous season (1386/trap) (ASRL 2011/12 Annual Report). One isolate of the new yellow fever group flavivirus was obtained from *Ae. normanensis*, one of the dominant species (23.2%) collected (Table 13Q). Other dominant species included *Cx. annulirostris* (17.4%), *Cq. xanthogaster* (12.3%) and *Ae. lineatopennis* (11.4%). Mosquito abundance was lower than usual in the Irrigation area (Table 13R; 277/trap). The collections comprised predominantly *Cx. annulirostris* (41.6%) and *Ae. normanensis* (18.2%), and included low numbers of *Cx. gelidus* (two specimens). No arboviruses were isolated from mosquitoes collected in the Irrigation area. For the second consecutive year five adult mosquito traps were set at Lake Argyle and environs (Table 13S). Mosquito abundance (239/trap) was slightly lower than the previous year (440/trap), and major species included *Ae. normanensis* (31.2%), *An. annulipes* s.l. (27.0%) and *Cx. annulirostris* (23.9%). Another unusual *Anopheles* sp. mosquito was collected and will be examined by Mr Peter Whelan and Prof. Richard Russell. One isolate of the new yellow fever group flavivirus was isolated from *Ae. normanensis*.

Abundance of mosquitoes at Parry's Creek (2037/trap) was higher than the previous season (1480/trap) (Table 13T). A diverse range of species were collected, however *Cx. annulirostris* (72.5%) was the major species and it yielded four isolates of the northern/eastern phenotype of RRV and a single isolate of KOKV. Other isolations included single isolates of northern/eastern phenotype of RRV from *Ae. normanensis*, *An. bancroftii* and a pool of damaged *Culex* mosquitoes, and one isolate of the new yellow fever group flavivirus from a pool of damaged *Aedes* mosquitoes. Mosquito populations were also higher at Wyndham Six Mile and Wyndham Three Mile (1588/trap

and 341/trap, respectively) than the previous season (706/trap and 224/trap, respectively) (Tables 13U-V). *Culex annulirostris*, *An. amictus*, *An. annulipes* s.l. and *Ae. normanensis* were the most numerous species at Six Mile (44.4%, 14.6%, 10.5% and 9.1%, respectively). A pool of *Ae. normanensis* and another of *Cx. annulirostris* yielded isolates of the new yellow fever group flavivirus. One isolate of KOKV was obtained from *Cx. annulirostris* collected at Three Mile, and this was the dominant species collected, comprising 76.5% of the catch. Mosquito abundance at Wyndham Port was low (Table 13W) and no viruses were isolated from mosquitoes collected in the Wyndham Port trap.

Culex gelidus survey

Culex gelidus is an important vector of JEV in Asia and JEV has been isolated from this species in the Torres Strait (van den Hurk *et al*., 2001). Recent vector competence studies have demonstrated the capacity of *Cx. gelidus* to transmit JEV, KUNV, MVEV and RRV in the laboratory (Johnson et al. 2009). These findings suggest that, if the virus becomes established in Australia, *Cx. gelidus* could become an important vector species for a number of Australian arboviruses. *Culex gelidus* had previously been found to be breeding in areas of the NT, Queensland and the northeast Kimberley region (Kununurra) (Johansen *et al*., 2003, Johansen *et al*., 2004b, Muller *et al*., 2001, Ritchie *et al*., 2001, Whelan *et al*., 2000, M. Lindsay and A. Broom, unpublished data). Prolific breeding of this species has been shown to occur in the NT in many artificial sites with high levels of organic matter, including dairy wastewater, abattoirs and sewage works (Whelan *et al*., 2001). From 2002, additional adult mosquito traps were set at sites suitable for *Cx. gelidus* breeding. In the 2005/06 season this species was detected in Kununurra, Halls Creek and Fitzroy Crossing (ASRL 2006/07 Annual Report). However no *Cx. gelidus* mosquitoes were detected at Kununurra in the Kimberley region in the 2008/09 season, and only three were detected at Kununurra in 2007/08 (ASRL 2008/09 Annual Report). A single specimen was collected from Halls Creek in 2009/10 (ASRL 2010/11 Annual Report). Twenty *Cx. gelidus* specimens were collected from Kununurra in 2011 and it was also collected at Warmun in 2011 (ASRL 2011/12 Annual Report), providing continued evidence for a range that extends beyond Kununurra in WA. Two specimens were collected in the Kununurra Irrigation area in 2012 (Table 13R).

2013 field results

Field work was carried out in the Kimberley region from 18 to 21 March 2013 (Table 18). A total of 48 adult mosquito traps were set in the west Kimberley region, with a success rate of 97.9%. Results will be presented in the 2013/14 ASRL Annual Report.

Table 13A. Results of mosquito trapping and virus isolations from Roebuck Plain (Plain and environs), West Kimberley.[1]
Trap date: 29th and 30th March 2012 (13 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	Virus isolates[2]
Ae. (Finlaya) notoscriptus	Female	6	(<0.1)	2	1	
Ae. (Macleaya) species	Female	31	(<0.1)	10	1	
Ae. (Macleaya) species	Male	12	(<0.1)	4	1	
Ae. (Mucidus) alternans	Female	152	(0.1)	10	6	
Ae. (Neomellanoconion) lineatopennis	Female	982	(0.9)	45	8	1 NE RRV
Ae. (Ochlerotatus) normanensis	Bloodfed	26	(<0.1)	0	0	
Ae. (Ochlerotatus) normanensis	Female	867	(0.8)	70	10	1 NE RRV
Ae. (Ochlerotatus) vigilax	Bloodfed	121	(0.1)	0	0	
Ae. (Ochlerotatus) vigilax	Female	10847	(10.2)	550	27	
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	237	(0.2)	11	6	
An. (Cellia) amictus	Female	559	(0.5)	28	3	
An. (Cellia) annulipes s.l.	Female	16	(<0.1)	5	1	
An. (Cellia) hilli	Female	7414	(6.9)	504	25	1 NE RRV
An. species (unidentified) - new or difficult to ID species	Male	3	(<0.1)	1	1	
Cx. (Culex) annulirostris	Bloodfed	1378	(1.3)	0	0	
Cx. (Culex) annulirostris	Female	63017	(59.1)	4079	168	17 NE RRV
Cx. (Culex) crinicauda	Female	5	(<0.1)	3	1	
Cx. (Culex) palpalis	Female	26	(<0.1)	1	1	
Cx. (Culex) sitiens	Bloodfed	272	(0.3)	0	0	
Cx. (Culex) sitiens	Female	14657	(13.7)	1053	48	1 NE RRV
Cx. (Culex) squamosus	Female	5	(<0.1)	3	1	
Cx. (Culiciomyia) pullus	Female	9	(<0.1)	1	1	
Cx. species (unidentified) - new or difficult to ID species	Male	80	(0.1)	4	3	
Unidentifiable (too damaged/features missing)	Male	3	(<0.1)	1	1	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	162	(0.2)	8	5	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	2828	(2.7)	147	10	
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	115	(0.1)	0	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	2882	(2.7)	145	9	1 NE RRV
Total		**106712**	**(100.0)**	**6685**	**338**	**22 NE RRV**

[1]No mosquitoes were pinned. [2]NE RRV is Ross River virus of the northern/eastern phenotype.

Table 13B. Results of mosquito trapping and virus isolations from Dampier Peninsula (Willie Creek), West Kimberley.[1]
Trap date: 29th March 2012 (4 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Macleaya) E.N. Marks' species No. 76	Female	21	(0.3)	5	2
Ae. (Macleaya) species	Female	9	(0.1)	4	2
Ae. (Macleaya) species	Male	25	(0.4)	8	2
Ae. (Macleaya) tremulus	Female	59	(0.8)	17	2
Ae. (Mucidus) alternans	Bloodfed	2	(<0.1)	0	0
Ae. (Mucidus) alternans	Female	10	(0.1)	4	3
Ae. (Neomellanoconion) lineatopennis	Female	13	(0.2)	5	2
Ae. (Ochlerotatus) normanensis	Female	31	(0.4)	13	2
Ae. (Ochlerotatus) phaecasiatus	Female	65	(0.9)	25	3
Ae. (Ochlerotatus) vigilax	Bloodfed	18	(0.3)	0	0
Ae. (Ochlerotatus) vigilax	Female	3326	(47.5)	963	40
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	29	(0.4)	8	3
Ae. (Subgenus *Nov.*) *daliensis*	Female	32	(0.5)	7	3
An. (Anopheles) bancroftii	Female	11	(0.2)	4	2
An. (Cellia) hilli	Female	136	(1.9)	33	4
Cx. (Culex) annulirostris	Bloodfed	14	(0.2)	0	0
Cx. (Culex) annulirostris	Female	1440	(20.6)	508	22
Cx. (Culex) crinicauda	Female	2	(<0.1)	1	1
Cx. (Culex) palpalis	Female	2	(<0.1)	1	1
Cx. (Culex) quinquefasciatus	Female	13	(0.2)	4	2
Cx. (Culex) sitiens	Female	1136	(16.2)	328	15
Cx. species (unidentified) - new or difficult to ID species	Male	4	(0.1)	2	1
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	147	(2.1)	55	5
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	13	(0.2)	4	2
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	4	(0.1)	0	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	438	(6.3)	176	9
Ur. (Uranotaenia) albescens	Female	2	(<0.1)	1	1
Ur. (Uranotaenia) lateralis	Female	5	(0.1)	2	1
Total		**7007**	**(100.0)**	**2178**	**130**

[1]No mosquitoes were pinned; no viruses were isolated.

Table 13C. Results of mosquito trapping and virus isolations from Broome (Coconut Wells), West Kimberley.[1]
Trap date: 29th March 2012 (2 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. of pools	Virus isolates[2]
Ae. (Finlaya) pecuniosus	Female	4	(0.2)	2	2	
Ae. (Macleaya) species	Female	2	(0.1)	1	1	
Ae. (Mucidus) alternans	Female	2	(0.1)	1	1	
Ae. (Neomellanoconion) lineatopennis	Female	9	(0.4)	4	2	
Ae. (Ochlerotatus) normanensis	Female	41	(1.8)	18	2	
Ae. (Ochlerotatus) phaecasiatus	Female	9	(0.4)	4	1	
Ae. (Ochlerotatus) vigilax	Bloodfed	7	(0.3)	0	0	
Ae. (Ochlerotatus) vigilax	Female	1492	(63.9)	650	27	2 NE RRV
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	7	(0.3)	3	1	
An. (Cellia) hilli	Female	11	(0.5)	5	2	
Cx. (Culex) annulirostris	Bloodfed	2	(0.1)	0	0	
Cx. (Culex) annulirostris	Female	481	(20.6)	208	9	1 NE RRV
Cx. (Culex) sitiens	Bloodfed	2	(0.1)	0	0	
Cx. (Culex) sitiens	Female	138	(5.9)	61	3	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	56	(2.4)	25	1	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	2	(0.1)	1	1	
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	5	(0.2)	0	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	65	(2.8)	29	2	
Total		**2335**	**(100.0)**	**1012**	**55**	**3 NE RRV**

[1]No mosquitoes were pinned. [2]NE RRV is Ross River virus of the northern/eastern phenotype.

Table 13D. Results of mosquito trapping and virus isolations from Broome (Town and environs), West Kimberley.[1]
Trap date: 29th and 30th March 2012 (12 traps, 1 failure)

Species	Class	No. collected	(%)	No. processed	No. pools	Virus isolates[2]
Ae. (Finlaya) notoscriptus	Bloodfed	1	(<0.1)	0	0	
Ae. (Finlaya) notoscriptus	Female	414	(7.6)	315	19	1 NE RRV
Ae. (Macleaya) species	Female	66	(1.2)	55	6	
Ae. (Macleaya) species	Male	78	(1.4)	74	9	
Ae. (Macleaya) tremulus	Female	142	(2.6)	133	10	
Ae. (Mucidus) alternans	Female	25	(0.5)	17	7	1 NE RRV
Ae. (Neomellanoconion) lineatopennis	Female	3	(0.1)	2	2	
Ae. (Ochlerotatus) normanensis	Female	90	(1.7)	84	13	1 NE RRV
Ae. (Ochlerotatus) phaecasiatus	Female	9	(0.2)	5	2	
Ae. (Ochlerotatus) vigilax	Bloodfed	6	(0.1)	0	0	
Ae. (Ochlerotatus) vigilax	Female	417	(7.7)	303	18	1 NE RRV
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	4	(0.1)	3	3	
An. (Cellia) amictus	Female	10	(0.2)	10	2	
An. (Cellia) hilli	Female	6	(0.1)	6	2	
Cx. (Culex) annulirostris	Bloodfed	10	(0.2)	0	0	
Cx. (Culex) annulirostris	Female	486	(9.0)	350	23	
Cx. (Culex) quinquefasciatus	Female	27	(0.5)	26	9	
Cx. (Culex) sitiens	Bloodfed	35	(0.6)	0	0	
Cx. (Culex) sitiens	Female	3380	(62.4)	2050	90	2 NE RRV
Cx. (Culiciomyia) pullus	Female	4	(0.1)	2	2	
Cx. species (unidentified) - new or difficult to ID species	Male	2	(<0.1)	2	2	
Tripteroides (Polylepidomyia) punctolateralis	Female	5	(0.1)	5	3	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	5	(0.1)	5	1	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	1	(<0.1)	1	1	
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	3	(0.1)	0	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	187	(3.5)	135	12	
Total		**5416**	**(100.0)**	**3583**	**236**	**6 NE RRV**

[1]No mosquitoes were pinned. [2]NE RRV is Ross River virus of the northern/eastern phenotype.

Table 13E. Results of mosquito trapping and virus isolations from Willare (Town and environs), West Kimberley.[1]
Trap date: 1st April 2012 (4 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. of pools	Virus isolates[2]
Ae. (Macleaya) E.N. Marks' species No. 121	Female	24	(0.1)	2	1	
Ae. (Macleaya) tremulus	Female	36	(0.2)	3	1	
Ae. (Mucidus) alternans	Bloodfed	5	(<0.1)	0	0	
Ae. (Mucidus) alternans	Female	99	(0.5)	14	4	
Ae. (Ochlerotatus) normanensis	Bloodfed	122	(0.6)	0	0	
Ae. (Ochlerotatus) normanensis	Female	12504	(62.8)	1303	54	1 NE RRV
Ae. (Ochlerotatus) vigilax	Female	475	(2.4)	38	4	
An. (Cellia) amictus	Female	69	(0.3)	8	3	
An. (Cellia) annulipes s.l.	Bloodfed	12	(0.1)	0	0	
An. (Cellia) annulipes s.l.	Female	1317	(6.6)	242	12	
An. (Cellia) hilli	Female	5	(<0.1)	1	1	
An. species (unidentified) - new or difficult to ID species	Male	12	(0.1)	1	1	
Cx. (Culex) annulirostris	Bloodfed	33	(0.2)	0	0	
Cx. (Culex) annulirostris	Female	4421	(22.2)	370	17	
Cx. (Culex) palpalis	Female	4	(<0.1)	1	1	
Cx. (Culex) starckeae	Female	5	(<0.1)	1	1	
Cx. (Culiciomyia) pullus	Female	4	(<0.1)	1	1	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Bloodfed	12	(0.1)	0	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	374	(1.9)	50	4	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	39	(0.2)	3	2	
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	11	(0.1)	0	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	329	(1.7)	32	3	
Total		**19912**	**(100.0)**	**2070**	**110**	**1 NE RRV**

[1]No mosquitoes were pinned. [2]NE RRV is Ross River virus of the northern/eastern phenotype.

Table 13F. Results of mosquito trapping and virus isolations from Derby (Town and environs), West Kimberley.[1]
Trap date: 1st April 2012 (9 traps, 1 failure)

Species	Class	No. collected	(%)	No. processed	No. of pools
Ae. (Finlaya) notoscriptus	Female	71	(0.3)	29	2
Ae. (Macleaya) species	Female	9	(<0.1)	2	2
Ae. (Macleaya) species	Male	4	(<0.1)	1	1
Ae. (Macleaya) tremulus	Bloodfed	3	(<0.1)	0	0
Ae. (Macleaya) tremulus	Female	8	(<0.1)	4	3
Ae. (Mucidus) alternans	Female	181	(0.7)	47	8
Ae. (Neomellanoconion) lineatopennis	Female	77	(0.3)	17	5
Ae. (Ochlerotatus) normanensis	Bloodfed	27	(0.1)	0	0
Ae. (Ochlerotatus) normanensis	Female	4605	(19.0)	727	34
Ae. (Ochlerotatus) vigilax	Bloodfed	78	(0.3)	0	0
Ae. (Ochlerotatus) vigilax	Female	13096	(54.1)	2198	95
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	132	(0.5)	38	5
Ae. species (unidentified) - new or difficult to ID species	Male	2	(<0.1)	1	1
An. (Cellia) amictus	Female	18	(0.1)	4	3
An. (Cellia) annulipes s.l.	Female	38	(0.2)	6	4
An. (Cellia) hilli	Female	17	(0.1)	4	3
Cx. (Culex) annulirostris	Bloodfed	146	(0.6)	0	0
Cx. (Culex) annulirostris	Female	4964	(20.5)	1039	48
Cx. (Culex) palpalis	Female	11	(<0.1)	3	2
Cx. (Culex) quinquefasciatus	Female	60	(0.2)	23	2
Cx. (Culex) sitiens	Female	62	(0.3)	22	6
Cx. (Culiciomyia) pullus	Female	11	(<0.1)	3	3
Cx. species (unidentified) - new or difficult to ID species	Male	2	(<0.1)	1	1
Tripteroides (Polylepidomyia) punctolateralis	Female	2	(<0.1)	1	1
Unidentifiable (too damaged/features missing)	Female	12	(<0.1)	4	1
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	134	(0.6)	50	5
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	67	(0.3)	7	4
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	12	(<0.1)	0	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	338	(1.4)	104	8
Total		**24187**	**(100.0)**	**4335**	**247**

[1]No mosquitoes were pinned; no viruses were isolated.

Table 13G. Results of mosquito trapping and virus isolations from Derby (Gibb River Road transect), West Kimberley.[1]
Trap date: 1st April 2012 (4 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Finlaya) notoscriptus	Female	2	(<0.1)	1	1
Ae. (Macleaya) species	Female	2	(<0.1)	1	1
Ae. (Mucidus) alternans	Female	91	(0.4)	19	4
Ae. (Mucidus) alternans	Male	21	(0.1)	1	1
Ae. (Neomellanoconion) lineatopennis	Female	63	(0.3)	7	3
Ae. (Ochlerotatus) normanensis	Bloodfed	40	(0.2)	0	0
Ae. (Ochlerotatus) normanensis	Female	6710	(31.5)	593	24
Ae. (Ochlerotatus) vigilax	Bloodfed	23	(0.1)	0	0
Ae. (Ochlerotatus) vigilax	Female	4073	(19.1)	311	14
An. (Cellia) amictus	Female	104	(0.5)	11	3
An. (Cellia) annulipes s.l.	Female	354	(1.7)	28	4
Cx. (Culex) annulirostris	Bloodfed	156	(0.7)	0	0
Cx. (Culex) annulirostris	Female	8931	(41.9)	1021	42
Cx. (Culex) palpalis	Female	2	(<0.1)	1	1
Cx. (Culex) sitiens	Female	10	(<0.1)	3	2
Cx. (Culiciomyia) pullus	Female	4	(<0.1)	2	1
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	135	(0.6)	9	4
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	13	(0.1)	0	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	595	(2.8)	54	4
Total		**21329**	**(100.0)**	**2062**	**109**

[1]No mosquitoes were pinned; no viruses were isolated.

Table 13H. Results of mosquito trapping and virus isolations from Fitzroy Crossing (Town and environs), West Kimberley.
Trap date: 2nd April 2012 (11 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	Virus isolates[1]
Ae. (Finlaya) notoscriptus	Female	9	(0.1)	7	3	0	
Ae. (Finlaya) pecuniosus	Female	3	(<0.1)	1	1	1	
Ae. (Macleaya) species	Female	26	(0.4)	19	4	0	
Ae. (Macleaya) species	Male	5	(0.1)	4	1	0	
Ae. (Macleaya) tremulus	Female	3	(<0.1)	2	2	0	
Ae. (Mucidus) alternans	Female	402	(6.2)	223	26	0	
Ae. (Mucidus) alternans	Male	11	(0.2)	5	3	0	
Ae. (Neomellanoconion) lineatopennis	Female	290	(4.5)	212	14	0	
Ae. (Ochlerotatus) normanensis	Bloodfed	12	(0.2)	0	0	0	
Ae. (Ochlerotatus) normanensis	Female	2492	(38.3)	1283	56	0	
Ae. (Ochlerotatus) pseudonormanensis	Female	1	(<0.1)	1	1	0	
Ae. (Ochlerotatus) vigilax	Female	5	(0.1)	4	3	0	
Ae. species (unidentified) - new or difficult to ID species	Male	2	(<0.1)	2	1	0	
An. (Anopheles) bancroftii	Female	8	(0.1)	5	4	0	
An. (Cellia) amictus	Bloodfed	2	(<0.1)	0	0	0	
An. (Cellia) amictus	Female	664	(10.2)	302	19	0	
An. (Cellia) annulipes s.l.	Bloodfed	4	(0.1)	0	0	0	
An. (Cellia) annulipes s.l.	Female	835	(12.8)	544	25	0	
An. (Cellia) meraukensis	Female	4	(0.1)	2	2	0	
Cx. (Culex) annulirostris	Bloodfed	24	(0.4)	0	0	0	
Cx. (Culex) annulirostris	Female	1472	(22.6)	912	42	0	1 MVEV
Cx. (Culex) palpalis	Female	3	(<0.1)	2	2	0	
Cx. (Culex) quinquefasciatus	Bloodfed	2	(<0.1)	0	0	0	
Cx. (Culex) quinquefasciatus	Female	24	(0.4)	21	3	0	
Cx. (Culex) quinquefasciatus	Male	2	(<0.1)	2	1	0	
Cx. (Culex) starckeae	Female	11	(0.2)	7	4	0	
Cx. (Culiciomyia) pullus	Female	3	(<0.1)	2	1	0	
Cx. (Culiciomyia) pullus	Male	1	(<0.1)	1	1	0	
Cx. species (unidentified) - new or difficult to ID species	Male	1	(<0.1)	1	1	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	2	(<0.1)	1	1	0	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Bloodfed	1	(<0.1)	0	0	0	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	110	(1.7)	49	7	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	77	(1.2)	56	8	0	
Unidentifiable *Uranotaenia* sp. (too damaged/features missing)	Bloodfed	1	(<0.1)	0	0	0	
Ve. (Verrallina) reesi	Female	1	(<0.1)	1	1	0	
Total		**6513**	**(100.0)**	**3671**	**237**	**1**	**1 MVEV**

[1]Murray Valley encephalitis virus.

Table 13I. Results of mosquito trapping and virus isolations from Geikie Gorge (Gorge and environs), West Kimberley.[1]
Trap date: 2nd April 2012 (8 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned
Ae. (Finlaya) mallochi	Female	2	(<0.1)	2	1	0
Ae. (Finlaya) new undescribed species[2]	Bloodfed	1	(<0.1)	0	0	0
Ae. (Finlaya) new undescribed species[2]	Female	128	(1.9)	124	6	4
Ae. (Finlaya) notoscriptus	Female	2	(<0.1)	2	1	0
Ae. (Finlaya) pecuniosus	Female	5	(0.1)	1	1	0
Ae. (Macleaya) species	Female	73	(1.1)	69	6	0
Ae. (Macleaya) species	Male	42	(0.6)	38	3	0
Ae. (Mucidus) alternans	Bloodfed	1	(<0.1)	0	0	0
Ae. (Mucidus) alternans	Female	206	(3.1)	115	14	0
Ae. (Neomellanoconion) lineatopennis	Bloodfed	2	(<0.1)	0	0	0
Ae. (Neomellanoconion) lineatopennis	Female	454	(6.8)	256	15	0
Ae. (Ochlerotatus) normanensis	Bloodfed	89	(1.3)	0	0	0
Ae. (Ochlerotatus) normanensis	Female	2219	(33.4)	840	36	0
Ae. (Ochlerotatus) vigilax	Female	1	(<0.1)	1	1	0
Ae. (Ochlerotatus) vittiger	Female	5	(0.1)	1	1	0
An. (Anopheles) bancroftii	Female	3	(<0.1)	2	2	0
An. (Cellia) amictus	Female	104	(1.6)	30	7	0
An. (Cellia) annulipes s.l.	Bloodfed	5	(0.1)	0	0	0
An. (Cellia) annulipes s.l.	Female	1797	(27.1)	1095	45	0
An. (Cellia) meraukensis	Female	5	(0.1)	1	1	0
An. species (unidentified) - new or difficult to ID species	Male	11	(0.2)	6	4	0
Cq. (Coquillettidia) xanthogaster	Female	2	(<0.1)	1	1	0
Cx. (Culex) annulirostris	Bloodfed	16	(0.2)	0	0	0
Cx. (Culex) annulirostris	Female	1390	(20.9)	769	33	0
Cx. (Culex) bitaeniorhynchus	Female	2	(<0.1)	2	1	0
Cx. (Culiciomyia) pullus	Female	15	(0.2)	10	4	0
Cx. (Lophoceraomyia) hilli	Female	1	(<0.1)	1	1	0
Cx. species (unidentified) - new or difficult to ID species	Male	3	(<0.1)	2	2	0
Tripteroides (Polylepidomyia) punctolateralis	Female	1	(<0.1)	1	1	0
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	2	(<0.1)	1	1	0
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	12	(0.2)	9	3	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	2	(<0.1)	0	0	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	36	(0.5)	26	2	0
Ve. (Verrallina) reesi	Female	4	(0.1)	3	3	0
Total		**6641**	**(100.0)**	**3408**	**196**	**4**

[1]No viruses were isolated. [2]This species has been collected previously and examined by Mr Peter Whelan and Prof. Richard Russell.

Table 13J. Results of mosquito trapping and virus isolations from Halls Creek (Town and environs), Southeast Kimberley.[1]
Trap date: 3rd April 2012 (13 traps, 1 failure)

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned
Ad. (Aedeomyia) catasticta	Female	71	(1.9)	71	8	0
Ae. (Finlaya) notoscriptus	Female	10	(0.3)	10	6	0
Ae. (Macleaya) species	Female	1	(<0.1)	1	1	0
Ae. (Macleaya) species	Male	31	(0.8)	29	4	0
Ae. (Macleaya) tremulus	Female	30	(0.8)	30	5	0
Ae. (Mucidus) alternans	Bloodfed	2	(0.1)	0	0	0
Ae. (Mucidus) alternans	Female	94	(2.6)	89	15	0
Ae. (Neomellanoconion) lineatopennis	Female	257	(7.0)	203	16	0
Ae. (Ochlerotatus) normanensis	Bloodfed	104	(2.8)	0	0	0
Ae. (Ochlerotatus) normanensis	Female	2712	(74.2)	1552	66	0
Ae. (Ochlerotatus) vigilax	Female	4	(0.1)	4	2	0
Ae. (Ochlerotatus) vittiger	Bloodfed	10	(0.3)	0	0	0
Ae. (Ochlerotatus) vittiger	Female	78	(2.1)	49	6	0
Ae. (Pseudoskusea) bancroftianus	Bloodfed	1	(<0.1)	0	0	0
Ae. (Pseudoskusea) bancroftianus	Female	20	(0.5)	13	6	2
An. (Cellia) amictus	Female	24	(0.7)	17	8	0
An. (Cellia) annulipes s.l.	Bloodfed	1	(<0.1)	0	0	0
An. (Cellia) annulipes s.l.	Female	37	(1.0)	37	7	0
An. (Cellia) meraukensis	Female	5	(0.1)	5	1	0
Cx. (Culex) annulirostris	Female	70	(1.9)	58	12	0
Cx. (Culex) bitaeniorhynchus	Female	1	(<0.1)	1	1	0
Cx. (Culex) quinquefasciatus	Bloodfed	2	(0.1)	0	0	0
Cx. (Culex) quinquefasciatus	Female	28	(0.8)	28	8	0
Tripteroides (Polylepidomyia) punctolateralis	Female	1	(<0.1)	1	1	0
Unidentifiable (too damaged/features missing)	Female	33	(0.9)	33	2	0
Unidentifiable (too damaged/features missing)	Male	1	(<0.1)	1	1	0
Unidentifiable *Aedes* sp. (too damaged/features missing)	Bloodfed	1	(<0.1)	0	0	0
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	23	(0.6)	23	7	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	1	(<0.1)	0	0	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	2	(0.1)	2	1	0
Ve. (Verrallina) reesi	Female	1	(<0.1)	1	1	0
Total		**3656**	**(100.0)**	**2258**	**185**	**2**

[1]No viruses were isolated.

Table 13K. Results of mosquito trapping and virus isolations from Billiluna (Myarra Pool), Southeast Kimberley.[1]
Trap date: 13th April 2012 (5 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools
Ad. (Aedeomyia) catasticta	Female	8	(0.1)	3	2
Ae. (Macleaya) species	Female	4	(0.1)	1	1
Ae. (Macleaya) species	Male	10	(0.2)	5	4
Ae. (Macleaya) tremulus	Bloodfed	3	(<0.1)	0	0
Ae. (Macleaya) tremulus	Female	189	(3.1)	100	6
Ae. (Mucidus) alternans	Female	14	(0.2)	7	4
Ae. (Ochlerotatus) eidsvoldensis	Female	12	(0.2)	4	2
Ae. (Ochlerotatus) normanensis	Bloodfed	9	(0.1)	0	0
Ae. (Ochlerotatus) normanensis	Female	448	(7.4)	232	11
Ae. (Pseudoskusea) bancroftianus	Female	22	(0.4)	9	3
Ae. (Subgen. *Nov.*) E.N. Marks' species No. 160	Female	17	(0.3)	9	3
An. (Cellia) amictus	Bloodfed	11	(0.2)	0	0
An. (Cellia) amictus	Female	211	(3.5)	101	7
An. (Cellia) annulipes s.l.	Bloodfed	29	(0.5)	0	0
An. (Cellia) annulipes s.l.	Female	1922	(31.9)	768	33
An. (Cellia) novaguinensis	Female	158	(2.6)	78	5
An. species (unidentified) - new or difficult to ID species	Male	1	(<0.1)	1	1
Cx. (Culex) annulirostris	Bloodfed	35	(0.6)	0	0
Cx. (Culex) annulirostris	Female	2702	(44.9)	1087	46
Cx. (Culex) palpalis	Female	20	(0.3)	10	4
Cx. (Culex) squamosus	Female	1	(<0.1)	1	1
Cx. (Culex) starckeae	Female	3	(<0.1)	1	1
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	4	(0.1)	2	2
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	70	(1.2)	30	4
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	118	(2.0)	51	5
Total		**6021**	**(100.0)**	**2500**	**145**

[1]No mosquitoes were pinned, no viruses were isolated.

Table 13L. Results of mosquito trapping and virus isolations from Billiluna (Billiluna/Balgo Road), Southeast Kimberley.[1]
Trap date: 13th April 2012 (4 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	Virus isolates[2]
Ad. (Aedeomyia) catasticta	Female	1	(<0.1)	1	1	
Ae. (Macleaya) E.N. Marks' species No. 121	Female	46	(1.3)	15	2	
Ae. (Macleaya) species	Female	2	(0.1)	2	2	
Ae. (Macleaya) species	Male	9	(0.2)	4	2	
Ae. (Macleaya) tremulus	Bloodfed	2	(0.1)	0	0	
Ae. (Macleaya) tremulus	Female	41	(1.1)	13	2	
Ae. (Mucidus) alternans	Female	37	(1.0)	29	5	
Ae. (Ochlerotatus) E.N. Marks' species No. 159	Female	2	(0.1)	2	1	
Ae. (Ochlerotatus) eidsvoldensis	Female	23	(0.6)	13	4	
Ae. (Ochlerotatus) normanensis	Bloodfed	21	(0.6)	0	0	
Ae. (Ochlerotatus) normanensis	Female	578	(15.8)	374	16	2 Flavi
Ae. (Pseudoskusea) bancroftianus	Female	1	(<0.1)	1	1	
An. (Cellia) amictus	Bloodfed	1	(<0.1)	0	0	
An. (Cellia) amictus	Female	102	(2.8)	74	6	
An. (Cellia) annulipes s.l.	Bloodfed	42	(1.2)	0	0	
An. (Cellia) annulipes s.l.	Female	1305	(35.8)	560	26	
An. (Cellia) novaguinensis	Female	49	(1.3)	35	4	
Cx. (Culex) annulirostris	Bloodfed	39	(1.1)	0	0	
Cx. (Culex) annulirostris	Female	1268	(34.8)	748	32	1 MVEV
Cx. (Culex) palpalis	Female	9	(0.2)	8	2	
Cx. (Culex) starckeae	Female	1	(<0.1)	1	1	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	7	(0.2)	4	2	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	16	(0.4)	8	3	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	45	(1.2)	20	3	
Total		**3647**	**(100.0)**	**1912**	**115**	**1 MVEV, 2 Flavi**

[1]No mosquitoes were pinned. [2]Flavi is a new flavivirus in the yellow fever group, MVEV is Murray Valley encephalitis virus.

Table 13M. Results of mosquito trapping and virus isolations from Billiluna (Community), Southeast Kimberley.[1]
Trap date: 13th April 2012 (4 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned
Ae. (Macleaya) E.N. Marks' species No. 126 (orange thorax)	Male	1	(0.3)	0	0	1
Ae. (Macleaya) species	Female	5	(1.3)	5	1	0
Ae. (Macleaya) species	Male	10	(2.6)	10	4	0
Ae. (Macleaya) tremulus	Female	14	(3.7)	14	2	0
Ae. (Mucidus) alternans	Female	2	(0.5)	2	1	0
Ae. (Ochlerotatus) normanensis	Bloodfed	1	(0.3)	1	0	0
Ae. (Ochlerotatus) normanensis	Female	76	(20.0)	76	5	0
An. (Cellia) amictus	Female	68	(17.9)	68	5	0
An. (Cellia) annulipes s.l.	Female	1	(0.3)	1	1	0
An. (Cellia) annulipes species B	Female	1	(0.3)	1	1	0
An. (Cellia) meraukensis	Female	1	(0.3)	1	1	0
An. (Cellia) novaguinensis	Female	17	(4.5)	17	3	0
Cx. (Culex) annulirostris	Bloodfed	3	(0.8)	0	0	0
Cx. (Culex) annulirostris	Female	175	(46.1)	175	9	0
Cx. (Culex) crinicauda	Female	1	(0.3)	1	1	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	4	(1.1)	4	1	0
Total		**380**	**(100.0)**	**376**	**35**	**1**

[1]No viruses were isolated.

Table 13N. Results of mosquito trapping and virus isolations from Billiluna (Stretch Lagoon), Southeast Kimberley.[1]
Trap date: 14th April 2012 (8 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	Virus isolates[2]
Ae. (Macleaya) species	Female	3	(0.1)	3	3	
Ae. (Macleaya) species	Male	4	(0.1)	4	4	
Ae. (Macleaya) tremulus	Female	8	(0.3)	8	4	
Ae. (Mucidus) alternans	Bloodfed	1	(<0.1)	0	0	
Ae. (Mucidus) alternans	Female	6	(0.2)	6	3	
Ae. (Ochlerotatus) normanensis	Bloodfed	19	(0.7)	0	0	
Ae. (Ochlerotatus) normanensis	Female	577	(19.9)	562	26	1 Flavi
Ae. (Subgen. *Nov.*) E.N. Marks' species No. 160	Female	4	(0.1)	4	3	
Ae. species (unidentified) - new or difficult to ID species	Male	1	(<0.1)	1	1	
An. (Cellia) amictus	Bloodfed	1	(<0.1)	0	0	
An. (Cellia) amictus	Female	269	(9.3)	265	24	
An. (Cellia) annulipes s.l.	Bloodfed	10	(0.3)	0	0	
An. (Cellia) annulipes s.l.	Female	228	(7.9)	226	14	
An. (Cellia) novaguinensis	Female	23	(0.8)	22	6	
An. species (unidentified) - new or difficult to ID species	Male	6	(0.2)	6	2	
Cx. (Culex) annulirostris	Bloodfed	151	(5.2)	0	0	
Cx. (Culex) annulirostris	Female	1463	(50.5)	1414	61	1 MVEV
Cx. (Culex) palpalis	Female	10	(0.3)	5	3	
Cx. (Culex) starckeae	Female	1	(<0.1)	1	1	
Cx. (Lophoceraomyia) cylindricus	Female	1	(<0.1)	1	1	
Cx. species (unidentified) - new or difficult to ID species	Male	2	(0.1)	2	1	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	13	(0.4)	13	2	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Bloodfed	1	(<0.1)	0	0	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	45	(1.6)	45	7	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	51	(1.8)	36	6	
Total		**2898**	**(100.0)**	**2624**	**172**	**1 Flavi, 1 MVEV**

[1]No mosquitoes were pinned. [2]Flavi is a new flavivirus in the yellow fever group, MVEV is Murray Valley encephalitis virus.

Table 13O. Results of mosquito trapping and virus isolations from Kununurra (Point Sprints/Keep River transect), Northeast Kimberley.

Trap date: 5th April 2012 (12 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	Virus isolates[1]
Ae. (Aedimorphus) alboscutellatus	Female	1	(<0.1)	0	0	1	
Ae. (Chaetocruiomyia) elchoensis	Female	10	(<0.1)	5	4	0	
Ae. (Finlaya) britteni	Bloodfed	1	(<0.1)	0	0	0	
Ae. (Finlaya) kochi	Female	3	(<0.1)	1	1	0	
Ae. (Finlaya) notoscriptus	Female	1	(<0.1)	1	1	0	
Ae. (Macleaya) species	Female	3	(<0.1)	1	1	0	
Ae. (Mucidus) alternans	Female	35	(0.1)	11	5	0	
Ae. (Neomellanoconion) lineatopennis	Bloodfed	5	(<0.1)	0	0	0	
Ae. (Neomellanoconion) lineatopennis	Female	2691	(11.3)	932	40	0	
Ae. (Ochlerotatus) normanensis	Bloodfed	299	(1.3)	0	0	0	
Ae. (Ochlerotatus) normanensis	Female	10277	(43.1)	2856	118	0	2 Flavi
Ae. (Ochlerotatus) vigilax	Female	3	(<0.1)	1	1	0	
An. (Anopheles) bancroftii	Female	2022	(8.5)	376	21	0	
An. (Anopheles) bancroftii	Male	7	(<0.1)	1	1	0	
An. (Cellia) amictus	Female	134	(0.6)	45	9	0	
An. (Cellia) annulipes s.l.	Bloodfed	7	(<0.1)	0	0	0	
An. (Cellia) annulipes s.l.	Female	903	(3.8)	232	14	0	
An. (Cellia) farauti	Bloodfed	3	(<0.1)	0	0	0	
An. (Cellia) farauti	Female	59	(0.2)	17	5	0	
An. (Cellia) hilli	Bloodfed	7	(<0.1)	0	0	0	
An. (Cellia) meraukensis	Bloodfed	12	(0.1)	0	0	0	
An. (Cellia) meraukensis	Female	2383	(10.0)	584	28	0	
An. (Cellia) novaguinensis	Female	15	(0.1)	4	3	0	
An. species (unidentified) - new or difficult to ID species[2]	Female	3	(<0.1)	0	0	1	
Cq. (Coquillettidia) xanthogaster	Female	1	(<0.1)	1	1	0	
Cx. (Culex) annulirostris	Bloodfed	119	(0.5)	0	0	0	
Cx. (Culex) annulirostris	Female	4172	(17.5)	959	44	0	
Cx. (Culex) bitaeniorhynchus	Female	21	(0.1)	5	3	0	
Cx. (Culex) crinicauda	Female	73	(0.3)	35	6	0	
Cx. (Culex) palpalis	Female	23	(0.1)	6	3	0	
Cx. (Culex) starckeae	Female	18	(0.1)	3	2	0	
Cx. (Culex) vicinus	Female	7	(<0.1)	1	1	0	
Cx. (Culiciomyia) pullus	Bloodfed	5	(<0.1)	0	0	0	
Cx. (Culiciomyia) pullus	Female	50	(0.2)	16	5	0	
Ma. (Mansonioides) uniformis	Female	1	(<0.1)	1	1	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	46	(0.2)	18	6	0	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	125	(0.5)	25	7	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	107	(0.4)	23	6	0	
Ve. (Verrallina) reesi	Female	184	(0.8)	53	9	0	
Total		**23836**	**(100.0)**	**6213**	**346**	**2**	**2 Flavi**

[1]Flavi is a new flavivirus in the yellow fever group; [2]This mosquito appears to be an albino *Anopheles* and will be sent to Mr Peter Whelan for identification.

Table 13P. Results of mosquito trapping and virus isolations from Kununurra (Town and environs), Northeast Kimberley.[1]
Trap date: 5th April 2012 (12 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	Virus isolates[2]
Ad. (Aedeomyia) catasticta	Female	11	(0.3)	11	7	1 NE RRV
Ae. (Chaetocruiomyia) elchoensis	Female	3	(0.1)	3	2	
Ae. (Finlaya) britteni	Female	1	(<0.1)	1	1	
Ae. (Finlaya) notoscriptus	Bloodfed	2	(0.1)	0	0	
Ae. (Finlaya) notoscriptus	Female	116	(3.5)	116	12	
Ae. (Macleaya) species	Female	2	(0.1)	2	2	
Ae. (Macleaya) species	Male	8	(0.2)	8	4	
Ae. (Macleaya) tremulus	Female	12	(0.4)	12	2	
Ae. (Mucidus) alternans	Female	8	(0.2)	8	6	
Ae. (Neomellanoconion) lineatopennis	Female	30	(0.9)	30	6	
Ae. (Ochlerotatus) normanensis	Bloodfed	51	(1.5)	0	0	
Ae. (Ochlerotatus) normanensis	Female	470	(14.3)	470	26	3 Flavi, 2 NE RRV
Ae. species (unidentified) - new or difficult to ID species	Male	1	(<0.1)	1	1	
An. (Anopheles) bancroftii	Female	105	(3.2)	105	12	
An. (Cellia) amictus	Female	38	(1.2)	38	7	1 NE RRV
An. (Cellia) annulipes s.l.	Bloodfed	2	(0.1)	0	0	
An. (Cellia) annulipes s.l.	Female	154	(4.7)	154	14	
An. (Cellia) farauti	Female	9	(0.3)	9	4	
An. (Cellia) hilli	Female	1	(<0.1)	1	1	
An. (Cellia) meraukensis	Female	100	(3.0)	100	10	
An. species (unidentified) - new or difficult to ID species	Male	1	(<0.1)	1	1	
Cq. (Coquillettidia) xanthogaster	Bloodfed	5	(0.2)	0	0	
Cq. (Coquillettidia) xanthogaster	Female	397	(12.0)	397	25	
Cq. (Coquillettidia) xanthogaster	Male	10	(0.3)	10	3	
Cx. (Culex) annulirostris	Bloodfed	19	(0.6)	0	0	
Cx. (Culex) annulirostris	Female	1078	(32.7)	1078	50	1 NE RRV
Cx. (Culex) bitaeniorhynchus	Bloodfed	1	(<0.1)	0	0	
Cx. (Culex) bitaeniorhynchus	Female	17	(0.5)	17	6	
Cx. (Culex) crinicauda	Female	40	(1.2)	40	10	
Cx. (Culex) E.N. Marks' species No. 92	Female	45	(1.4)	45	4	
Cx. (Culex) palpalis	Female	3	(0.1)	3	2	
Cx. (Culex) quinquefasciatus	Female	13	(0.4)	13	4	
Cx. (Culex) sitiens	Female	1	(<0.1)	1	1	
Cx. (Culex) squamosus	Female	3	(0.1)	3	1	
Cx. (Culex) starckeae	Female	19	(0.6)	19	7	
Cx. (Culiciomyia) pullus	Female	113	(3.4)	113	10	
Cx. (Lophoceraomyia) E.N.Marks' species No. 167	Female	32	(1.0)	32	4	
Cx. (Lophoceraomyia) species	Female	30	(0.9)	30	4	
Ma. (Mansonioides) uniformis	Bloodfed	2	(0.1)	0	0	
Ma. (Mansonioides) uniformis	Female	175	(5.3)	175	13	2 NE RRV
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	12	(0.4)	12	5	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	9	(0.3)	9	5	
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	1	(<0.1)	0	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	113	(3.4)	113	9	1 NE RRV
Ur. (Uranotaenia) albescens	Female	2	(0.1)	2	1	
Ve. (Verrallina) funerea	Female	13	(0.4)	13	2	
Ve. (Verrallina) reesi	Female	18	(0.5)	18	9	
Total		**3296**	**(100.0)**	**3213**	**293**	**3 Flavi, 8 NE RRV**

[1]No mosquitoes were pinned. [2]Flavi is a new flavivirus in the yellow fever group, NE RRV is the northern/eastern phenotype of RRV.

Table 13Q. Results of mosquito trapping and virus isolations from Kununurra (Packsaddle Plain), Northeast Kimberley.[1]
Trap date: 6th and 7th April 2012 (7 traps, 1 failure)

Species	Class	No. collected	(%)	No. processed	No. pools	Virus isolates[2]
Ad. (Aedeomyia) catasticta	Female	1	(<0.1)	1	1	
Ae. (Finlaya) britteni	Female	2	(0.1)	1	1	
Ae. (Finlaya) notoscriptus	Female	6	(0.2)	6	1	
Ae. (Finlaya) pecuniosus	Female	2	(0.1)	1	1	
Ae. (Macleaya) species	Female	4	(0.1)	4	1	
Ae. (Macleaya) species	Male	1	(<0.1)	1	1	
Ae. (Mucidus) alternans	Female	6	(0.2)	4	3	
Ae. (Neomellanoconion) lineatopennis	Bloodfed	2	(0.1)	0	0	
Ae. (Neomellanoconion) lineatopennis	Female	446	(11.4)	318	17	
Ae. (Ochlerotatus) normanensis	Bloodfed	20	(0.5)	0	0	
Ae. (Ochlerotatus) normanensis	Female	907	(23.2)	620	27	1 Flavi
Ae. (Ochlerotatus) phaecasiatus	Female	4	(0.1)	2	1	
Ae. (Ochlerotatus) vigilax	Female	3	(0.1)	2	2	
An. (Anopheles) bancroftii	Female	279	(7.1)	221	11	
An. (Cellia) amictus	Female	13	(0.3)	8	2	
An. (Cellia) annulipes s.l.	Bloodfed	3	(0.1)	0	0	
An. (Cellia) annulipes s.l.	Female	199	(5.1)	139	9	
An. (Cellia) farauti	Female	38	(1.0)	28	6	
An. (Cellia) hilli	Female	2	(0.1)	2	1	
An. (Cellia) meraukensis	Female	159	(4.1)	105	8	
An. (Cellia) novaguinensis	Female	8	(0.2)	6	3	
Cq. (Coquillettidia) xanthogaster	Bloodfed	4	(0.1)	0	0	
Cq. (Coquillettidia) xanthogaster	Female	482	(12.3)	348	19	
Cq. (Coquillettidia) xanthogaster	Male	13	(0.3)	13	3	
Cx. (Culex) annulirostris	Bloodfed	8	(0.2)	0	0	
Cx. (Culex) annulirostris	Female	683	(17.4)	524	24	
Cx. (Culex) bitaeniorhynchus	Female	3	(0.1)	2	2	
Cx. (Culex) crinicauda	Female	96	(2.5)	60	4	
Cx. (Culex) E.N. Marks' species No. 92	Female	3	(0.1)	3	2	
Cx. (Culex) starckeae	Female	4	(0.1)	2	2	
Cx. (Culiciomyia) pullus	Female	31	(0.8)	19	6	
Cx. (Lophoceraomyia) species	Female	98	(2.5)	79	6	
Ma. (Mansonioides) uniformis	Female	87	(2.2)	72	6	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	13	(0.3)	7	3	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	34	(0.9)	22	5	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	30	(0.8)	17	4	
Ur. (Uranotaenia) albescens	Female	2	(0.1)	1	1	
Ur. (Uranotaenia) nivipes	Female	2	(0.1)	1	1	
Ur. (Uranotaenia) nivipes	Male	2	(0.1)	1	1	
Ve. (Verrallina) reesi	Female	217	(5.5)	168	10	
Total		**3917**	**(100.0)**	**2808**	**195**	**1 Flavi**

[1]No mosquitoes were pinned. [2]Flavi is a new flavivirus in the yellow fever group.

Table 13R. Results of mosquito trapping and virus isolations from Kununurra (Irrigation area), Northeast Kimberley.[1]
Trap date: 7th April 2012 (12 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools
Ad. (Aedeomyia) catasticta	Female	1	(<0.1)	1	1
Ae. (Aedimorphus) alboscutellatus	Female	1	(<0.1)	1	1
Ae. (Chaetocruiomyia) elchoensis	Female	1	(<0.1)	1	1
Ae. (Chaetocruiomyia) elchoensis	Male	1	(<0.1)	1	1
Ae. (Finlaya) notoscriptus	Female	329	(9.9)	255	16
Ae. (Macleaya) tremulus	Female	3	(0.1)	3	2
Ae. (Mucidus) alternans	Female	19	(0.6)	17	6
Ae. (Neomellanoconion) lineatopennis	Bloodfed	1	(<0.1)	0	0
Ae. (Neomellanoconion) lineatopennis	Female	287	(8.6)	257	15
Ae. (Ochlerotatus) normanensis	Bloodfed	20	(0.6)	0	0
Ae. (Ochlerotatus) normanensis	Female	605	(18.2)	598	30
Ae. (Ochlerotatus) vigilax	Female	5	(0.2)	5	4
Ae. (Stegomyia) katherinensis	Female	4	(0.1)	3	1
An. (Anopheles) bancroftii	Female	19	(0.6)	18	7
An. (Cellia) amictus	Female	47	(1.4)	47	9
An. (Cellia) annulipes s.l.	Bloodfed	3	(0.1)	0	0
An. (Cellia) annulipes s.l.	Female	180	(5.4)	178	15
An. (Cellia) meraukensis	Female	33	(1.0)	32	9
An. (Cellia) novaguinensis	Female	1	(<0.1)	1	1
Cq. (Coquillettidia) xanthogaster	Bloodfed	3	(0.1)	0	0
Cq. (Coquillettidia) xanthogaster	Female	51	(1.5)	50	10
Cx. (Culex) annulirostris	Bloodfed	36	(1.1)	0	0
Cx. (Culex) annulirostris	Female	1382	(41.6)	1349	61
Cx. (Culex) bitaeniorhynchus	Female	4	(0.1)	4	2
Cx. (Culex) crinicauda	Female	79	(2.4)	78	10
Cx. (Culex) E.N. Marks' species No. 92	Female	12	(0.4)	10	4
Cx. (Culex) gelidus	Female	2	(0.1)	2	1
Cx. (Culex) palpalis	Female	3	(0.1)	3	3
Cx. (Culex) quinquefasciatus	Female	14	(0.4)	13	5
Cx. (Culex) starckeae	Female	9	(0.3)	9	6
Cx. (Culiciomyia) pullus	Female	31	(0.9)	30	11
Cx. (Lophoceraomyia) species	Female	2	(0.1)	2	2
Cx. species (unidentified) - new or difficult to ID species	Male	1	(<0.1)	1	1
Ma. (Mansonioides) uniformis	Female	31	(0.9)	30	6
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	4	(0.1)	4	3
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Bloodfed	1	(<0.1)	0	0
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	7	(0.2)	7	4
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	58	(1.7)	58	7
Ur. (Uranotaenia) albescens	Female	3	(0.1)	3	2
Ve. (Verrallina) funerea	Female	2	(0.1)	2	2
Ve. (Verrallina) reesi	Female	29	(0.9)	28	3
Total		**3324**	**(100.0)**	**3101**	**262**

[1]No mosquitoes were pinned; no viruses were isolated.

Table 13S. Results of mosquito trapping and virus isolations from Kununurra (Lake Argyle and environs), Northeast Kimberley.
Trap date: 8th April 2012 (5 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	Virus isolates[1]
Ad. (Aedeomyia) catasticta	Female	1	(0.1)	1	1	0	
Ae. (Chaetocruiomyia) elchoensis	Female	1	(0.1)	1	1	0	
Ae. (Finlaya) britteni	Female	1	(0.1)	1	1	0	
Ae. (Finlaya) britteni	Male	1	(0.1)	1	1	0	
Ae. (Finlaya) notoscriptus	Female	3	(0.3)	3	1	0	
Ae. (Macleaya) species	Female	2	(0.2)	2	2	0	
Ae. (Macleaya) species	Male	1	(0.1)	1	1	0	
Ae. (Neomellanoconion) lineatopennis	Female	3	(0.3)	3	2	0	
Ae. (Ochlerotatus) normanensis	Bloodfed	3	(0.3)	0	0	0	
Ae. (Ochlerotatus) normanensis	Female	373	(31.2)	373	18	0	1 Flavi
Ae. (Subgen. *Nov*.) E.N. Marks' species No. 160	Female	2	(0.2)	1	1	0	
An. (Anopheles) bancroftii	Female	3	(0.3)	3	1	0	
An. (Cellia) amictus	Female	4	(0.3)	4	2	0	
An. (Cellia) annulipes s.l.	Bloodfed	11	(0.9)	0	0	0	
An. (Cellia) annulipes s.l.	Female	322	(27.0)	322	15	0	
An. (Cellia) farauti	Female	1	(0.1)	1	1	0	
An. (Cellia) meraukensis	Female	42	(3.5)	42	5	0	
An. (Cellia) novaguinensis	Bloodfed	1	(0.1)	0	0	0	
An. (Cellia) novaguinensis	Female	2	(0.2)	2	2	0	
An. species (unidentified) - new or difficult to ID species[2]	Female	1	(0.1)	0	0	1	
Cx. (Culex) annulirostris	Bloodfed	3	(0.3)	0	0	0	
Cx. (Culex) annulirostris	Female	285	(23.9)	285	14	0	
Cx. (Culex) bitaeniorhynchus	Female	1	(0.1)	1	1	0	
Cx. (Culex) crinicauda	Female	12	(1.0)	12	4	0	
Cx. (Culex) palpalis	Female	3	(0.3)	3	2	0	
Cx. (Culex) starckeae	Female	3	(0.3)	2	1	1	
Cx. (Culex) vicinus	Female	1	(0.1)	1	1	0	
Ma. (Mansonioides) uniformis	Female	1	(0.1)	1	1	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	17	(1.4)	17	3	0	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	28	(2.3)	28	5	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	42	(3.5)	42	4	0	
Ve. (Verrallina) reesi	Female	20	(1.7)	20	2	0	
Total		**1194**	**(100.0)**	**1173**	**93**	**2**	**1 Flavi**

[1]Flavi is a new flavivirus in the yellow fever group. [2]This mosquito appears to be an albino *Anopheles* and will be sent to Mr Peter Whelan for identification.

Table 13T. Results of mosquito trapping and virus isolations from Wyndham (Parry's Creek region), Northeast Kimberley.
Trap date: 10th April 2012 (12 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	Virus isolates[1]
Ad. (Aedeomyia) catasticta	Female	1	(<0.1)	1	1	0	
Ae. (Finlaya) notoscriptus	Female	7	(<0.1)	6	2	0	
Ae. (Finlaya) pecuniosus	Female	2	(<0.1)	1	1	0	
Ae. (Macleaya) species	Female	11	(<0.1)	7	3	0	
Ae. (Macleaya) species	Male	7	(<0.1)	5	3	0	
Ae. (Macleaya) tremulus	Female	5	(<0.1)	5	2	0	
Ae. (Mucidus) alternans	Female	53	(0.2)	18	7	0	
Ae. (Neomellanoconion) lineatopennis	Bloodfed	2	(<0.1)	0	0	0	
Ae. (Neomellanoconion) lineatopennis	Female	32	(0.1)	16	8	0	
Ae. (Ochlerotatus) normanensis	Bloodfed	10	(<0.1)	0	0	0	
Ae. (Ochlerotatus) normanensis	Female	1063	(4.3)	369	19	0	1 NE RRV
Ae. (Stegomyia) katherinensis	Female	1	(<0.1)	1	1	0	
An. (Anopheles) bancroftii	Female	476	(1.9)	105	8	0	1 NE RRV
An. (Cellia) amictus	Bloodfed	1	(<0.1)	0	0	0	
An. (Cellia) amictus	Female	635	(2.6)	100	9	0	
An. (Cellia) annulipes s.l.	Bloodfed	1	(<0.1)	0	0	0	
An. (Cellia) annulipes s.l.	Female	306	(1.3)	143	11	0	
An. (Cellia) annulipes species B	Bloodfed	1	(<0.1)	0	0	0	
An. (Cellia) annulipes species D	Female	35	(0.1)	33	2	2	
An. (Cellia) farauti	Female	2	(<0.1)	1	1	0	
An. (Cellia) hilli	Female	29	(0.1)	2	1	0	
An. (Cellia) meraukensis	Bloodfed	5	(<0.1)	0	0	0	
An. (Cellia) meraukensis	Female	1167	(4.8)	354	19	0	
An. (Cellia) novaguinensis	Female	15	(0.1)	14	3	0	
An. species (unidentified) - new or difficult to ID species	Male	4	(<0.1)	4	2	0	
Cq. (Coquillettidia) xanthogaster	Bloodfed	5	(<0.1)	0	0	0	
Cq. (Coquillettidia) xanthogaster	Female	203	(0.8)	103	12	0	
Cq. (Coquillettidia) xanthogaster	Male	12	(<0.1)	5	4	0	
Cx. (Culex) annulirostris	Bloodfed	902	(3.7)	0	0	0	
Cx. (Culex) annulirostris	Female	17710	(72.5)	3500	147	0	4 NE RRV, 1 KOKV
Cx. (Culex) bitaeniorhynchus	Female	145	(0.6)	25	9	0	
Cx. (Culex) crinicauda	Female	217	(0.9)	41	9	0	
Cx. (Culex) E.N. Marks' species No. 92	Bloodfed	1	(<0.1)	0	0	0	
Cx. (Culex) E.N. Marks' species No. 92	Female	24	(0.1)	10	4	0	
Cx. (Culex) palpalis	Bloodfed	1	(<0.1)	0	0	0	
Cx. (Culex) palpalis	Female	215	(0.9)	79	11	0	
Cx. (Culex) sitiens	Bloodfed	14	(0.1)	0	0	0	
Cx. (Culex) sitiens	Female	82	(0.3)	16	6	0	
Cx. (Culex) squamosus	Female	21	(0.1)	6	3	0	
Cx. (Culex) starckeae	Female	44	(0.2)	17	9	0	
Cx. (Culex) vicinus	Female	1	(<0.1)	1	1	0	
Cx. (Culiciomyia) pullus	Female	37	(0.2)	15	6	0	
Cx. (Culiciomyia) pullus	Male	1	(<0.1)	1	1	0	
Cx. (Lophoceraomyia) species	Female	2	(<0.1)	1	1	0	
Cx. (Lophoceraomyia) species	Male	1	(<0.1)	1	1	0	
Cx. species (unidentified) - new or difficult to ID species	Male	7	(<0.1)	6	2	0	
Ma. (Mansonioides) uniformis	Female	104	(0.4)	20	4	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	2	(<0.1)	2	2	0	1 Flavi
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	33	(0.1)	14	7	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	2	(<0.1)	0	0	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	785	(3.2)	186	12	0	1 NE RRV
Ur. (Uranotaenia) nivipes	Female	2	(<0.1)	1	1	0	
Total		**24444**	**(100.0)**	**5235**	**355**	**2**	**7 NE RRV, 1 Flavi, 1 KOKV**

[1]NE RRV is the northern/eastern phenotype of RRV, Flavi is a new flavivirus in the yellow fever group, KOKV is Kokobera virus.

Table 13U. Results of mosquito trapping and virus isolations from Wyndham (Six mile), Northeast Kimberley.
Trap date: 10th April 2012 (4 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	Virus isolates[1]
Ad. (Aedeomyia) catasticta	Female	15	(0.2)	2	1	0	
Ae. (Finlaya) notoscriptus	Female	17	(0.3)	4	2	0	
Ae. (Macleaya) species	Male	12	(0.2)	7	1	0	
Ae. (Macleaya) tremulus	Bloodfed	2	(<0.1)	0	0	0	
Ae. (Macleaya) tremulus	Female	73	(1.1)	44	3	0	
Ae. (Mucidus) alternans	Female	48	(0.8)	25	5	0	
Ae. (Neomellanoconion) lineatopennis	Female	4	(0.1)	2	1	0	
Ae. (Ochlerotatus) normanensis	Bloodfed	3	(<0.1)	0	0	0	
Ae. (Ochlerotatus) normanensis	Female	578	(9.1)	162	9	0	1 Flavi
Ae. (Ochlerotatus) vigilax	Female	81	(1.3)	65	4	0	
Ae. (Ochlerotatus) vigilax variant	Female	2	(<0.1)	2	1	0	
An. (Anopheles) bancroftii	Female	54	(0.9)	15	4	0	
An. (Cellia) amictus	Bloodfed	4	(0.1)	0	0	0	
An. (Cellia) amictus	Female	929	(14.6)	255	13	0	
An. (Cellia) annulipes s.l.	Bloodfed	10	(0.2)	0	0	0	
An. (Cellia) annulipes s.l.	Female	668	(10.5)	111	7	0	
An. (Cellia) hilli	Female	20	(0.3)	9	3	0	
An. (Cellia) meraukensis	Female	508	(8.0)	94	7	0	
An. (Cellia) novaguinensis	Female	23	(0.4)	3	1	0	
An. species (unidentified) - new or difficult to ID species	Male	13	(0.2)	6	2	0	
Cq. (Coquillettidia) xanthogaster	Female	59	(0.9)	16	4	0	
Cq. (Coquillettidia) xanthogaster	Male	8	(0.1)	1	1	0	
Cx. (Culex) annulirostris	Bloodfed	28	(0.4)	0	0	0	
Cx. (Culex) annulirostris	Female	2818	(44.4)	997	41	0	1 Flavi
Cx. (Culex) annulirostris variant	Female	8	(0.1)	0	0	1	
Cx. (Culex) bitaeniorhynchus	Female	10	(0.2)	2	2	0	
Cx. (Culex) crinicauda	Female	111	(1.7)	19	4	0	
Cx. (Culex) palpalis	Female	44	(0.7)	17	4	0	
Cx. (Culex) quinquefasciatus	Female	2	(<0.1)	1	1	0	
Cx. (Culex) sitiens	Female	12	(0.2)	12	1	0	
Cx. (Culex) starckeae	Female	35	(0.6)	13	3	0	
Cx. (Culex) vicinus	Female	1	(<0.1)	1	1	0	
Cx. species (unidentified) - new or difficult to ID species	Male	24	(0.4)	4	2	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	2	(<0.1)	1	1	0	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	8	(0.1)	4	2	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	2	(<0.1)	0	0	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	114	(1.8)	53	4	0	
Ur. (Uranotaenia) lateralis	Female	1	(<0.1)	1	1	0	
Total		**6351**	**(100.0)**	**1948**	**136**	**1**	**2 Flavi**

[1]Flavi is a new flavivirus in the yellow fever group.

Table 13V. Results of mosquito trapping and virus isolations from Wyndham town (Three mile), Northeast Kimberley.[1]
Trap date: 10th April 2012 (3 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	Virus isolates[2]
Ae. (Finlaya) notoscriptus	Female	3	(0.3)	3	1	
Ae. (Macleaya) species	Female	3	(0.3)	3	2	
Ae. (Macleaya) species	Male	3	(0.3)	3	3	
Ae. (Mucidus) alternans	Female	3	(0.3)	3	2	
Ae. (Neomellanoconion) lineatopennis	Female	1	(0.1)	1	1	
Ae. (Ochlerotatus) normanensis	Female	20	(2.0)	20	2	
Ae. (Ochlerotatus) vigilax	Female	70	(6.8)	70	4	
An. (Anopheles) bancroftii	Female	2	(0.2)	2	2	
An. (Cellia) amictus	Female	23	(2.2)	23	3	
An. (Cellia) annulipes s.l.	Female	8	(0.8)	8	2	
An. (Cellia) hilli	Female	13	(1.3)	13	2	
An. (Cellia) meraukensis	Female	2	(0.2)	2	1	
Cq. (Coquillettidia) xanthogaster	Female	12	(1.2)	12	3	
Cq. (Coquillettidia) xanthogaster	Male	1	(0.1)	1	1	
Cx. (Culex) annulirostris	Bloodfed	2	(0.2)	0	0	
Cx. (Culex) annulirostris	Female	783	(76.5)	783	33	1 KOKV
Cx. (Culex) crinicauda	Female	2	(0.2)	2	1	
Cx. (Culex) quinquefasciatus	Female	12	(1.2)	12	2	
Cx. (Culex) sitiens	Bloodfed	1	(0.1)	0	0	
Cx. (Culex) sitiens	Female	40	(3.9)	40	5	
Cx. (Culex) sitiens	Male	1	(0.1)	1	1	
Cx. (Culex) starckeae	Female	9	(0.9)	9	3	
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	1	(0.1)	0	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	8	(0.8)	8	1	
Total		**1023**	**(100.0)**	**1019**	**75**	**1 KOKV**

[1]No mosquitoes were pinned. [2]KOKV is Kokobera virus.

Table 13W. Results of mosquito trapping and virus isolations from Wyndham (Port), Northeast Kimberley.[1]
Trap date: 10th April 2012 (1 trap, no failure)

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Macleaya) species	Male	1	(0.8)	1	1
Ae. (Mucidus) alternans	Female	1	(0.8)	1	1
Ae. (Ochlerotatus) vigilax	Female	2	(1.6)	2	1
An. (Cellia) amictus	Female	2	(1.6)	2	1
Cq. (Coquillettidia) xanthogaster	Female	2	(1.6)	2	1
Cx. (Culex) annulirostris	Female	77	(60.6)	77	3
Cx. (Culex) sitiens	Female	39	(30.7)	39	2
Cx. (Lophoceraomyia) species	Male	1	(0.8)	1	1
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	2	(1.6)	2	1
Total		**127**	**(100.0)**	**127**	**12**

[1]No mosquitoes were pinned, no viruses were isolated.

Table 14. Details of virus isolations from mosquitoes collected in the Kimberley region during the 2012 wet season.

Locality	Code	Species	Collection site	Date	Virus[1]
Roebuck Plain (Plain and environs)	K75952	*Ae. lineatopennis*	Roebuck Plains, 13.8 km N of S edge	29-Mar-12	NE RRV
"	K75958	*Cx. annulirostris*	"	"	NE RRV
"	K75960	*Cx. annulirostris*	"	"	NE RRV
"	K75961	*Cx. annulirostris*	"	"	NE RRV
"	K75962	*Cx. annulirostris*	"	"	NE RRV
"	K75963	*Cx. annulirostris*	"	"	NE RRV
"	K75964	*Cx. annulirostris*	"	"	NE RRV
"	K75965	*Cx. annulirostris*	"	"	NE RRV
"	K75967	*Cx. annulirostris*	"	"	NE RRV
"	K75968	*Cx. annulirostris*	"	"	NE RRV
"	K75969	*Cx. annulirostris*	"	"	NE RRV
"	K75970	*Cx. annulirostris*	"	"	NE RRV
"	K75971	*Cx. annulirostris*	"	"	NE RRV
"	K75972	*Cx. annulirostris*	"	"	NE RRV
"	K75973	*Cx. annulirostris*	"	"	NE RRV
"	K75974	*Cx. annulirostris*	"	"	NE RRV
"	K75976	*Cx. sitiens*	"	"	NE RRV
"	K75977	*Cx*. species (damaged)	"	"	NE RRV
"	K76008	*An. hilli*	Roebuck Plains Station homestead	"	NE RRV
"	K76021	*Cx. annulirostris*	"	"	NE RRV
"	K76145	*Ae. normanensis*	Roebuck Plains station near cattle grid	"	NE RRV
"	K76181	*Cx. annulirostris*	Windmill on Roebuck Plains station road	"	NE RRV
Broome (Coconut Wells)	K76320	*Ae. vigilax*	Steins property	"	NE RRV
"	K76321	*Ae. vigilax*	"	"	NE RRV
"	K76352	*Cx. annulirostris*	Williams Road gully	"	NE RRV
Broome (Town and environs)	K76378	*Ae. alternans*	Broome Rodeo ground	"	NE RRV
"	K76380	*Ae. normanensis*	"	"	NE RRV
"	K76397	*Cx. sitiens*	"	"	NE RRV
"	K76400	*Cx. sitiens*	"	"	NE RRV
"	K76528	*Ae. notoscriptus*	New Roebuck Estate	30-Mar-12	NE RRV
"	K76529	*Ae. vigilax*	"	"	NE RRV
Willare (Town and environs)	K76713	*Ae. normanensis*	East end of Ski Lake	1-Apr-12	NE RRV
Fitzroy Crossing (Town and environs)	K77160	*Cx. annulirostris*	100m northwest of main Fitzroy River Crossing	2-Apr-12	MVEV
Kununurra (Point Springs/Keep River)	K77993	*Ae. normanensis*	32.0km from start of gravel Weaber Plains Rd.	5-Apr-12	Flavi
"	K78040	*Ae. normanensis*	38.3km from start of gravel Weaber Plains Rd.	"	Flavi
Kununurra (Town and environs)	K78071	*Ae. normanensis*	Ord Pistol Club	6-Apr-12	Flavi
"	K78096	*Ae. normanensis*	Quarantine Yards	"	Flavi
"	K78098	*Ae. normanensis*	"	"	NE RRV
"	K78099	*Ae. normanensis*	"	"	Flavi
"	K78100	*Ae. normanensis*	"	"	NE RRV
"	K78104	*Ad. catasticta*	"	"	NE RRV
"	K78105	*An. amictus*	"	"	NE RRV
"	K78111	*Cx. annulirostris*	"	"	NE RRV
"	K78116	*Cx*. species (damaged)	"	"	NE RRV
"	K78117	*Ma. uniformis*	"	"	NE RRV
"	K78118	*Ma. uniformis*	"	"	NE RRV
Kununurra (Packsaddle Plain)	K78344	*Ae. normanensis*	3.3km along Swamp Rd.	"	Flavi
Kununurra (Lake Argyle)	K78766	*Ae. normanensis*	35.1km along Victoria Hwy, after t/off to Lake Argyle.	8-Apr-12	Flavi
Wyndham (Parry's Creek)	K78870	*Ae*. species (damaged)	Crocodile Hole	10-Apr-12	Flavi
"	K78894	*Ae. normanensis*	Blue Lily Creek	"	NE RRV
Wyndham (Six Mile)	K79011	*Ae. normanensis*	Wyndham Golf Club	"	Flavi
"	K79034	*Cx. annulirostris*	"	"	Flavi
Wyndham (Three Mile)	K79160	*Cx. annulirostris*	Wyndham sewage lagoon	"	KOKV
Wyndham (Parry's Creek)	K79288	*An. bancroftii*	Jogalong Billabong	"	NE RRV
"	K79301	*Cx. annulirostris*	"	"	NE RRV
"	K79351	*Cx. annulirostris*	20 Mile Lagoon	"	NE RRV
"	K79352	*Cx. annulirostris*	"	"	NE RRV
"	K79355	*Cx. annulirostris*	"	"	KOKV
"	K79390	*Cx. annulirostris*	Parry's Creek Crossing	"	NE RRV
"	K79395	*Cx*. species (damaged)	"	"	NE RRV

Table 14 (continued). Details of virus isolations from mosquitoes collected in the Kimberley region during the 2012 wet season.

Locality	Code	Species	Collection site	Date	Virus[1]
Billiluna (Billiluna/Balgo Rd)	K79534	*Ae. normanensis*	0.7 km SE towards Balgo on rd from Sturt Creek crossing	13-Apr-12	Flavi
"	K79538	*Ae. normanensis*	"	"	Flavi
"	K79630	*Cx. annulirostris*	0.6 km W towards Billiluna on rd from Sturt Creek crossing	"	MVEV
Billiluna (Stretch Lagoon)	K79689	*Cx. annulirostris*	2.0 km S of Stretch Lagoon, west bank of creek	14-Apr-12	MVEV
"	K79752	*Ae. normanensis*	150 m N of south end of Stretch Lagoon	"	Flavi

[1]NE RRV is northern/eastern phenotype of RRV, MVEV is Murray Valley encephalitis virus, Flavi is a new flavivirus in the yellow fever group, KOKV is Kokobera virus.

Table 15. Mosquito species yielding arboviruses in the Kimberley region in March/April 2012.

	Virus[1]				
Species	**NE RRV**	**MVEV**	**KOKV**	**Flavi**	**Total**
Ad. catasticta	1				**1**
Ae. alternans	1				**1**
Ae. lineatopennis	1				**1**
Ae. normanensis	6			11	**17**
Ae. notoscriptus	1				**1**
Ae. vigilax	3				**3**
Aedes sp. (damaged)				1	**1**
An. amictus	1				**1**
An. bancroftii	1				**1**
An. hilli	1				**1**
Cx. annulirostris	23	3	2	1	**29**
Cx. sitiens	3				**3**
Culex sp. (damaged)	3				**3**
Ma. uniformis	2				**2**
Total	**47**	**3**	**2**	**13**	**65**

[1]NE RRV is northern/eastern phenotype of RRV, MVEV is Murray Valley encephalitis virus, Flavi is a new flavivirus in the yellow fever group, KOKV is Kokobera virus.

Table 16. Virus isolations from localities in the Kimberley region in March/April 2012.

	Virus[1]				
Locality	**NE RRV**	**MVEV**	**KOKV**	**Flavi**	**Total**
Roebuck Plain (Plain and environs)	22				**22**
Broome (Coconut Wells)	3				**3**
Broome (Town and environs)	6				**6**
Willare (Town and environs)	1				**1**
Fitzroy Crossing (Town and environs)		1			**1**
Kununurra (Town and environs)	8			3	**11**
Kununurra (Packsaddle Plain)				1	**1**
Kununurra (Point Springs/Keep River)				2	**2**
Kununurra (Lake Argyle)				1	**1**
Wyndham (Parry's Creek)	7		1	1	**9**
Wyndham (Six Mile)				2	**2**
Wyndham (Three Mile)			1		**1**
Billiluna (Billiluna/Balgo Road)		1		2	**3**
Billiluna (Stretch Lagoon)		1		1	**2**
Total	**47**	**3**	**2**	**13**	**65**

[1]NE RRV is northern/eastern phenotype of RRV, MVEV is Murray Valley encephalitis virus, Flavi is a new flavivirus in the yellow fever group, KOKV is Kokobera virus.

Table 17. Infection rates of *Cx. annulirostris* with MVEV at major study sites in the Kimberley region during March and April 2012.[1]

Location	*Cx. annulirostris* No. collected	No. processed	No. pools	No. MVEV isolates (MIR[1])
Roebuck Plain	63017	4079	168	
Broome (Willie Creek)	1440	508	22	
Broome (Coconut Wells)	481	208	9	
Broome (Town and environs)	486	350	23	
Willare (Town and environs)	4421	370	17	
Derby (Town and environs)	4964	1039	48	
Derby (Gibb River Road transect)	8931	1021	42	
Fitzroy Crossing/Geikie Gorge	2862	1681	75	1 (0.6)
Halls Creek (Town and environs)	70	58	12	
Kununurra (Point Springs/Keep River, Town, Packsaddle and Irrigation)	7315	3909	171	
Kununurra (Lake Argyle and environs)	285	285	14	
Wyndham (Parry's Creek)	17710	3500	147	
Wyndham (Six Mile, Three Mile and Port)	3678	1857	77	
Billuna (Myarra Pool, Billiluna/Balgo Rd, Community and Stretch Lagoon)	5608	3424	148	2 (0.6)
Total	**121268**	**22289**	**973**	**3 (0.1)**

[1]Calculated per 1000 mosquitoes; MVEV is Murray Valley encephalitis virus; no isolates of KUNV were obtained from mosquitoes collected in the Kimberley region in 2012.

Table 18. Details of Kimberley meteorological regions, localities and suburbs where mosquito sampling was conducted in 2013.

Meteorological region	Town or region	LGA/Locality	Suburb	Date	Trap outcomes Successful	Failed	Total
West Kimberley	Broome	Shire of Broome	Town and environs	18-Mar-13	13	0	13
"	Roebuck plain	"	Plain and environs	19-Mar-13	11	0	11
"	Dampier Peninsular	"	Willie Creek	20-Mar-13	4	0	4
"	Coconut Wells	"	Locality and environs	"	2	0	2
"	Roebuck plain	"	Plain and environs	"	1	0	1
"	Willare	Shire of Derby-West Kimberley	Town and environs	21-Mar-13	4	0	4
"	Derby	"	"	"	8	1	9
"	Derby	"	Gibb River road transect	"	4	0	4
Total					47	1	48
		% Successful =	97.9				

Opportunistic adult mosquito trapping carried out in WA, 2012/2013

A total of 92 adult mosquito traps were set in the East Pilbara towns of Nullagine, Marble Bar and Port Hedland in March and April 2013, with a 97.8% trap success rate (Table 19, Figure 13). This mosquito sampling was conducted following heavy rainfall and flooding associated with the passage of TC Rusty in February 2013. The fieldwork was conducted by MBDC (Drs Michael Lindsay and Peter Neville) and ASRL (Dr Jay Nicholson and Mr Michael Burley) prior to and following the survey of adult mosquitoes and viruse in the Kimberley region. These mosquitoes have been identified to species, however they are yet to be processed for virus isolation. Results of mosquito collections in the Pilbara region in 2013 will be presented in the 2013/14 Annual Report.

Table 19. Details of Pilbara meteorological regions, localities and suburbs where mosquito sampling was conducted in 2013.

					Trap outcomes		
Meteorological region	**Town or region**	**LGA/Locality**	**Suburb**	**Date**	**Successful**	**Failed**	**Total**
East Pilbara	Nullagine	Shire of East Pilbara	Town and environs	13-Mar-13	14	0	14
"	Marble Bar	"	"	14-Mar-13	13	1	14
"	"	"	"	15-Mar-13	2	0	2
North east Pilbara	Port Hedland	Town of Port Hedland	East of town	"	1	1	2
"	De Grey River catchment	"	Bush	"	7	0	7
"	Port Hedland	"	Port	16-Mar-13	1	0	1
"	"	"	Spinifex Hill	"	1	0	1
"	"	"	Pretty Pool	"	2	0	2
"	"	"	Redbank	"	1	0	1
"	"	"	East of town	"	2	0	2
"	"	"	South Hedland	"	2	0	2
"	"	"	Wedgefield	"	2	0	2
"	"	"	Port Hedland	"	1	0	1
"	"	"	Port	24-Mar-13	2	0	2
"	"	"	East of town	"	2	0	2
"	"	"	Port Hedland	"	1	0	1
"	"	"	Spinifex Hill	"	1	0	1
"	"	"	Pretty Pool	"	2	0	2
"	"	"	Redbank	"	1	0	1
"	"	"	South Hedland	"	2	0	2
"	"	"	Wedgefield	"	2	0	2
East Pilbara	Marble Bar	Shire of East Pilbara	Town and environs	25-Mar-13	14	0	14
"	Nullagine	"	"	26-Mar-13	14	0	14
Total					**90**	**2**	**92**
			% Successful =	97.8			

Opportunistic adult mosquito collections were also conducted at Kalgoorlie town and environs (Figure 13) in the South East meteorological district following very high rainfall in March 2013 (Figure 14) and an unusually large number of cases of RRV disease in the locality. In total 223 cases of RRV disease were notified during the 2012/13 season, with most cases occurring in April and May 2013 (Table 1). A total of 26 adult mosquito traps (with a 100% success rate) were set on 3 and 4 May 2013 by Mr Ryan Janes (MBDC) and Dr Jay Nicholson. Mosquito abundance was relatively low by the time adult mosquitoes were collected. A total of 1296 mosquitoes representing 14 species were collected, and of these 99.2% were processed for virus isolation (Table 20). Two isolates of RRV were obtained. One isolate of the northern/eastern phenotype of RRV was obtained from a pool of nine *Ae. bancroftianus* mosquitoes collected at the Kalgoorlie sewage overflow. The other RRV isolate belonged to the southwestern phenotype of RRV, and was from a pool of 25 *Ae.*

notoscriptus, a container breeding mosquito and confirmed urban vector of RRV (Doggett and Russell, 1997; Watson and Kay, 1998). Detection of two different phenotypes suggests multiple origins of RRV activity in the area. This is the first time that the southwestern phenotype has been detected in WA since 2004. High MIRs were observed in these species (48.5 and 7.9 per 1000 mosquitoes, respectively) (Chiang and Reeves, 1962).

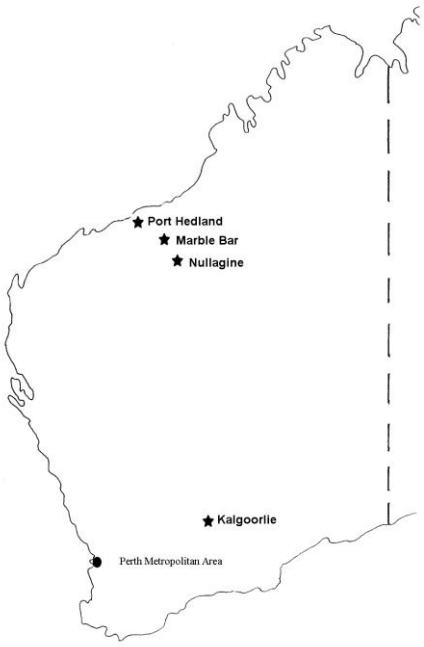

Figure 13. The locations (star) where opportunistic studies of mosquito and arbovirus activity were conducted in 2012/13.

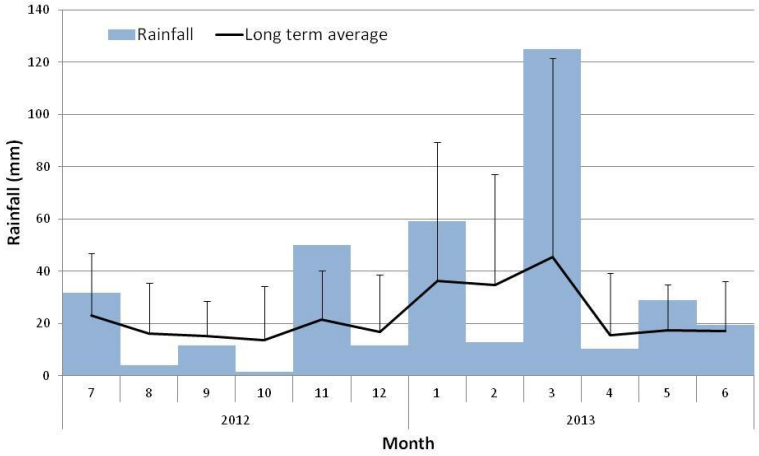

Figure 14. Monthly rainfall compared to long-term average rainfall at Kalgoorlie-Boulder, July 2012 to June 2013 (graph prepared with the assistance of Dr Andrew Jardine, MBDC).

Table 20. Results of mosquito collections from Kalgoorlie and environs, South East meteorological region, 2013.
Trap dates: 3 and 4 May 2013 (26 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	RRV[1]	(MIR)[2]
Ae. (Finlaya) mallochi	Female	1	(0.1)	1	1	0		
Ae. (Finlaya) notoscriptus	Bloodfed	2	(0.2)	0	0	0		
Ae. (Finlaya) notoscriptus	Female	129	(10.0)	129	21	0	1	(7.9)
Ae. (Macleaya) E.N. Marks' species No. 147	Female	2	(0.2)	2	1	0		
Ae. (Macleaya) species	Female	7	(0.5)	7	6	0		
Ae. (Mucidus) alternans	Female	2	(0.2)	2	2	0		
Ae. (Ochlerotatus) camptorhynchus	Bloodfed	3	(0.2)	0	0	0		
Ae. (Ochlerotatus) camptorhynchus	Female	89	(6.9)	89	9	0		
Ae. (Ochlerotatus) eidsvoldensis	Female	1	(0.1)	1	1	0		
Ae. (Pseudoskusea) bancroftianus	Female	22	(1.7)	22	6	0	1	(48.5)
Ae. species (unidentified) - new or difficult to ID species	Male	2	(0.2)	2	1	0		
An. (Cellia) annulipes s.l.	Female	52	(4.0)	52	16	0		
An. (Cellia) annulipes species A	Female	5	(0.4)	5	3	0		
An. (Cellia) annulipes species B	Female	3	(0.2)	3	2	0		
Cx. (Culex) annulirostris	Female	57	(4.4)	57	13	0		
Cx. (Culex) australicus	Female	17	(1.3)	17	8	0		
Cx. (Culex) globocoxitus	Female	17	(1.3)	17	4	0		
Cx. (Culex) molestus	Bloodfed	1	(0.1)	0	0	0		
Cx. (Culex) molestus	Female	267	(20.6)	267	22	0		
Cx. (Culex) quinquefasciatus	Bloodfed	3	(0.2)	0	0	0		
Cx. (Culex) quinquefasciatus	Female	543	(41.9)	543	40	1		
Cx. species (unidentified) - new or difficult to ID species	Male	3	(0.2)	3	2	0		
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	1	(0.1)	1	1	0		
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	1	(0.1)	0	0	0		
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	64	(4.9)	64	10	0		
Unidentifiable *Culex* sp. (too damaged/features missing)	Male	2	(0.2)	2	1	0		
Total		**1296**	**(100.0)**	**1286**	**170**	**1**	**2**	**(1.6)**

[1]RRV is Ross River virus, the isolate from *Ae. notoscriptus* was of the southwestern phenotype and the isolate from *Ae. bancroftianus* was of the northern/eastern phenotype; [2]MIR is minimum infection rate per 1000 mosquitoes (Chiang and Reeves, 1962).

Section 6: Sentinel chicken surveillance

Materials and Methods

Flavivirus activity in the north of WA is monitored throughout the year by detecting antibodies to MVEV and KUNV in serum samples from sentinel chicken flocks located in a number of key locations in the Kimberley, Pilbara, Gascoyne, Midwest and Goldfields regions of WA. The sentinel chicken program relies on assistance and co-operation from a large number of people living in towns and remote communities throughout the northern half of WA. Regular bleeding of chickens, immediate testing of samples in the laboratory and prompt notification of results is essential for an effective surveillance program. The sentinel chicken flavivirus surveillance program is approved by the UWA Animal Ethics Committee (RA/3/100/1122).

Location and management of flocks

Figure 15 shows the locations of sentinel flocks in the 2012/13 wet season. Each flock of 12 chickens is replaced annually in September - October. Additional replacement chickens are sent to

locations where more than six chickens in one flock seroconvert to MVEV or KUNV during a wet season. This laboratory manages the overall program, including annual and wet-season replacement of chicken flocks. Individual flocks are managed and bled by local government authority and water authority personnel, Aboriginal environmental health workers and trained members of the public.

Sampling of chicken flocks

All chickens in each flock are bled fortnightly. Blood samples are stored in 4 mL serum tubes and transported via regional WA laboratories associated with PathWest Laboratory Medicine WA (PathWest) to the ASRL in Perth for testing.

Detection of flavivirus antibodies in chicken serum samples

Sera are tested by blocking ELISA modified from the method described by Hall *et al*. (1995). Inactivated virus antigens used for coating ELISA plates are prepared using a method supplied by Queensland Health Forensic and Scientific Services, Brisbane. This protocol involves the use of a specific monoclonal antibody (3H6) to screen sera for antibodies to flaviviruses. Sera that test positive in this initial screen are then tested in a similar assay using virus-specific monoclonal antibodies to MVEV (10C6) and KUNV (3.1112G) to distinguish between infections with these two viruses. If a serum has antibodies to MVEV and KUNV, it is titrated and deemed positive for MVEV or KUNV if there is a $\geq$ four-fold difference in antibody titre. If a serum is found to be positive for antibodies to a flavivirus other than MVEV or KUNV it is re-tested against a specific JEV monoclonal antibody (989) (supplied by Dr Roy Hall, University of Queensland) to ensure that JEV is not active in the north of WA.

Dissemination of results from the sentinel chicken program

The DOH and EHOs of relevant WA LGAs are notified immediately if MVEV, KUNV or other flavivirus antibodies are detected in the chicken sera. A follow-up blood sample to confirm the result is also requested at this time. ELISA results are made available to all LGAs, Agriculture WA, Regional Public Health Units and the DOH each fortnight.

Results and Discussion

Rainfall

Generally, above average rainfall was observed in northern parts of WA between October and December 2012 (Figure 2). Between January and March 2013 conditions were average or drier than usual in the Kimberley region, with the exception of the west Kimberley. The west Kimberley and parts of the east Pilbara experienced above average rainfall and in some parts, highest on record rainfall during January to March. TCs Narelle and Peta caused heavy rainfall in the western Gascoyne, Pilbara and northern Interior in January. An active monsoon and TC Rusty also caused heavy rain in the Kimberley and Pilbara regions, causing major flooding in the De Grey catchment area in February. Seasonal thunderstorms resulted in more rain in March. Between April and June above to very much above average rainfall was recorded in the Kimberley and most of the Pilbara regions, with highest on record rainfall being recorded in the East Pilbara and northern Interior. Above average rainfall continued into May and June 2013 in northern parts of WA.

Flavivirus activity

A total of 4508 serum samples from 28 flocks were tested for antibodies to flaviviruses during 2012/13 (Table 21). Seroconversions to flaviviruses were detected in just six (0.1%) samples. Seroconversions at Beagle Bay (1 MVEV) in July and Kununurra (1 MVEV), Beagle Bay (1 KUNV) and Roebuck Plains Station (1 Flavivirus infection) in August were associated with activity continuing from the 2011/12 season. The first activity associated with the 2012/13 wet season

occurred in late May 2013 when a KUNV seroconversion was detected at Roebuck Plains. Shortly afterwards, one KUNV infection was detected in the Harding Dam flock in June. This was a very late start to the flavivirus season, and was also the lowest level of activity observed since 1995/96 when just two seroconversions to KUNV were detected in March-April 1996**.**

The first media release for the season was issued by the WA DOH on 28 March 2013. This was a general media release issued prior to the holiday Easter season, and warned residents and travellers to the north of WA of the increased risk of mosquito-borne disease in northern WA. A second media release was issued on 25 June 2013 in response to the detections of KUNV in sentinel chickens in the Kimberley and Pilbara regions and heavy unseasonal rain.

Figure 15. Locations of towns, communities, dams and mining centres at which flocks of chickens were located for surveillance of flavivirus activity in WA in 2012/13 (numbers in brackets indicate the number of flocks at that locality).

Table 21. Summary of WA Flavivirus Surveillance program, 2012/13*
Sentinel chicken flocks are tested for infection with Murray Valley encephalitis and Kunjin viruses.

July - December 2012														
Month	July		Aug		Sept		Oct		Nov		Dec		TOTAL	
Location	n	+ve	n	+ve	n	+ve	n	+ve	n	+ve	n	+ve	Bled (n)	Positive(+ve)
KIMBERLEY														
Wyndham							No Flock						0	0
Kununurra			6	1 1M									6	1
Savannah Nickel mine	16	0	15	0	16	0	16	0			16	0	79	0
Halls Creek	13	0	14	0	7	0			12	0			46	0
Fitzroy Crossing	11	0	11	0	11	0	22	0			10	0	65	0
Derby site 1	20	0	20	0	10	0	20	0	27	0	7	0	104	0
Derby site 2	21	0	22	0	10	0	20	0	27	0	10	0	110	0
Lombadina	12	0	11	0					22	0			45	0
Beagle Bay	9	1 1M	8	1 1K					4	0			21	2
Roebuck Plains	5	0	9	1 1F	4	0	6	0	7	0	14	0	45	1
PILBARA														
Port Hedland					11	0	22	0	11	0	11	0	55	0
Karratha	24	0	23	0	24	0	36	0	24	0	11	0	142	0
Harding Dam 1	24	0	36	0			24	0	30	0	9	0	123	0
Harding Dam 2	22	0	33	0			22	0	27	0	8	0	112	0
Marble Bar			10	0	10	0			9	0	6	0	35	0
Pannawonica	24	0	22	0	22	0	33	0	22	0	11	0	134	0
Tom Price	20	0	20	0	8	0	8	0	16	0	8	0	80	0
Paraburdoo	24	0	24	0	11	0	11	0	20	0	10	0	100	0
Onslow	22	0	11	0	10	0	10	0	10	0	18	0	81	0
Ophthalmia	10	0	30	0	10	0	20	0	20	0	19	0	109	0
Newman Shire	12	0	36	0	12	0	23	0	20	0	20	0	123	0
Exmouth	36	0	24	0	24	0	24	0	24	0	24	0	156	0
GASCOYNE														
Carnarvon	18	0	17	0	16	0	16	0	12	0	12	0	91	0
MID-WEST/WHEATBELT														
Moora	9	0	9	0	9	0	9	0			18	0	54	0
Geraldton (Walkaway)	15	0	9	0	8	0	8	0	24	0	12	0	76	0
Dongara	20	0			10	0	10	0	12	0	12	0	64	0
York	18	0	9	0	9	0	12	0	24	0	12	0	84	0
GOLDFIELDS														
Leonora			11	0									11	0

Table 21 (continued). Summary of WA Flavivirus Surveillance program, 2012/13*
Sentinel chicken flocks are tested for infection with Murray Valley encephalitis and Kunjin viruses.

January - June 2013																
Month	Jan		Feb		Mar		Apr		May		Jun		TOTAL	
Location	n	+ve	n	+ve	n	+ve	n	+ve	n	+ve	n	+ve	Bled (n)	Positive(+ve)
KIMBERLEY														
Wyndham									12	0	12	0	24	0
Kununurra									12	0	12	0	24	0
Savannah Nickel mine			7	0	23	0			13	0	14	0	57	0
Halls Creek	24	0	24	0	22	0	22	0	21	0	21	0	134	0
Fitzroy Crossing	10	0	20	0	10	0	10	0	10	0	10	0	70	0
Derby site 1	19	0	6	0	32	0	24	0	36	0	22	0	139	0
Derby site 2	29	0	9	0	42	0	22	0	33	0	22	0	157	0
Lombadina			9	0	8	0							17	0
Broome					10	0	10	0			10	0	30	0
Roebuck Plains					9	0	24	0	31 (1K)	1	18	0	82	1
PILBARA														
Port Hedland	10	0	10	0	10	0	8	0					38	0
Karratha	22	0	20	0	20	0	19	0	29	0	18	0	128	0
Harding Dam 1	14	0	13	0	17	0	10	0	17	0	23 (1K)	1	94	1
Harding Dam 2	15	0	13	0	33	0	21	0	22	0	22	0	126	0
Marble Bar			5	0	11	0			13	0	13	0	42	0
Pannawonica	20	0	20	0	20	0	19	0	30	0	19	0	128	0
Tom Price	15	0	8	0	14	0	14	0	14	0	14	0	79	0
Paraburdoo	20	0	10	0	20	0	20	0	20	0	20	0	110	0
Onslow	18	0			17	0	16	0	15	0	16	0	82	0
Ophthalmia	27	0	8	0	18	0	25	0	16	0	16	0	110	0
Newman Shire	30	0	10	0	20	0	30	0	19	0	20	0	129	0
Exmouth	24	0	24	0	24	0	36	0	24	0	24	0	156	0
GASCOYNE														
Carnarvon	23	0	24	0	21	0	10	0	19	0	18	0	115	0
MID-WEST/WHEATBELT														
Moora	7	0	13	0	6	0	12	0	10	0			48	0
Geraldton (Walkaway)	12	0	24	0	12	0	12	0	12	0	12	0	84	0
Dongara	24	0	19	0	12	0	11	0	11	0	22	0	99	0
York	22	0			11	0			11	0	11	0	55	0
GOLDFIELDS														
Leonora													0	0

*Flocks sampled fortnightly. Previous (or repeat) positive chickens are not recorded on this summary. n = number of samples tested, +ve = no. of flavivirus positive samples, M = MVEV, K = KUNV, F = Flavivirus only (not MVEV, KUNV or Japanese encephalitis virus), MK is MVEV + KUNV antibodies.

Section 7: Other research projects

Investigation of alternative flavivirus-specific monoclonal antibodies for detection of flavivirus infections in sentinel chickens

The ASRL currently screens sentinel chicken sera with the monoclonal antibody 3H6 by epitope blocking ELISA (Hall et al. 1995), and follow-up testing is performed on samples with flavivirus antibodies by using the MVEV and KUNV-specific monoclonal antibodies 10C6 and 31112G, respectively. The laboratory has occasionally detected MVEV and/or KUNV-specific antibodies in sentinel chicken sera that did not react in the flavivirus (3H6) screening assay. This finding is unusual, and may indicate that the surveillance program is occasionally not detecting MVEV or KUNV infections. For this reason, we are investigating the use of several alternative flavivirus-reactive monoclonal antibodies for the detection of flavivirus antibodies in screening assays. Preliminary studies with 6B6C-1 (Blitvich et al. 2003a, b) and 4G2 (Henchal et al. 1982) have shown promising results. Analysis of a panel of sera collected from laboratory-infected chickens and samples from sentinel chickens showed both 6B6C-1 and 4G2 monoclonal antibodies detected all MVEV and/or KUNV-specific antibodies that were detected by 3H6. In addition, both monoclonal antibodies appear to be more sensitive. Some sera that were previously determined to have KUNV-specific antibodies but were not positive in the 3H6 screening assay were detected in the 6B6C-1 and 4G2 assays. The ASRL is in the process of testing a large number of sentinel chicken sera previously determined to be positive to MVEV, KUNV, MVEV and KUNV and negative for flavivirus antibodies. These sera will be tested in parallel by neutralisation assay. This project will be a MSc of Infectious Diseases project in July to November 2013.

Investigating the origin of an emerging virulent strain of West Nile virus in Australia

The mosquito-borne West Nile virus (WNV) is responsible for outbreaks of viral encephalitis in humans and horses, with particularly virulent strains causing recent outbreaks of disease in Eastern Europe, the Middle East and North America. In Australia, KUNV (a subtype of WNV) is enzootic in northern Australia and although KUNV can cause febrile illness and encephalitis, infection is generally asymptomatic. However in early 2011, following extensive flooding, an unprecedented outbreak of encephalitis in horses occurred in southeastern Australia, resulting in more than 1000 cases and a mortality rate of approximately 10-15% (Frost et al. 2012)**.** An isolate of KUNV (NSW 2011) was obtained and found to be most closely related to the indigenous Kunjin strains rather than WNV. However, at least two amino acid changes associated with increased virulence of the North American New York 99 strain compared to the prototype KUNV were present in the NSW 2011 amino acid sequence (Mann et al. 2013). To investigate the origins of the NSW 2011 KUNV strain, a panel of KUNV isolates from different regions of Australia between 1960 and 2012 were analysed. Neuroinvasiveness was examined in established mouse models and their genetic sequences were determined to investigate the presence of known virulence markers for WNV. Results showed that some strains of KUNV from eastern Australia had similar levels of virulence to the NSW 2011 isolate in a young adult mouse model. In contrast, recent isolates of KUNV from Western Australia were relatively attenuated. Genetic analysis identified two possible evolutionary markers in the 3' untranslated region and NS5 gene. This work involves collaboration between The University of Queensland (Dr Natalie Prow, Ms Judith Edmonds, Dr Yin Setoh, Dr Helle Bielefeldt-Ohmann, Mr Willy Suen, Dr Jody Hobson-Peters, Dr Fiona May, Prof. Alexander Khromykh and Prof. Roy Hall), Queensland Health Forensic and Scientific Services (Assoc. Prof. Andrew van den Hurk, Dr Alyssa Pyke), The University of Western Australia (Assoc. Prof. Cheryl Johansen), the Elizabeth Macarthur Agriculture Institute in New South Wales (Dr Peter D. Kirkland), and The University of Sydney (Prof. Richard Russell, Mr Stephen Doggett).

The prevalence and biodiversity of insect-specific flaviviruses in northern Australia.

Most flaviviruses are defined as arboviruses based on their requirement to infect an arthropod vector and vertebrate host. However a number of studies on several continents have reported the presence of insect-specific flaviviruses (ISFs) that replicate in mosquito cells but do not grow in vertebrate cells. Recently Palm Creek virus (PCV) was detected in Darwin (NT), indicating insect-specific flaviviruses also circulate in Australia (Hobson-Peters et al. 2013). This study investigated the presence and diversity of ISFs in mosquito homogenates from the Kimberley region in 2010 and the Gulf of Carpentaria (QLD) in 2001, using novel monoclonal antibodies that detect a broad spectrum of viruses in parallel with RT-PCRs. PCV-like viruses were detected in two pools of *Cq. xanthogaster* collected from Kununurra in 2010, with up to 5% nucleotide sequence difference to the prototype PCV from Darwin. Five isolates of a novel ISF were obtained from *An. meraukensis* collected near Karumba in 2001. The results showed that there may be a high infection rate in some populations of mosquitoes in northern Australia. This was a MSc project at The University of Queensland, and involved a collaboration between Mr T.H.D. Nguyen, Prof. Roy Hall, Prof. Ross Barnard, Dr Jody Hobson-Peters (The University of Queensland), The University of Western Australia (Assoc. Prof. Cheryl Johansen) and Queensland Health Forensic and Scientific Services (Assoc. Prof. Andrew van den Hurk, Dr Sonja Hall-Mendelin, Dr. David Warrilow).

Section 8: Student reports

The role of genetic diversity on the replication, pathogenicity and virulence of Murray Valley encephalitis virus in Australia (PhD project).

Aziz Niazi's PhD project is investigating the role of genetic diversity on the replication, pathogenicity and virulence of MVEV in Australia. Aziz performed nucleotide sequencing and phylogenetic analysis on the partial envelope gene of all (84) MVEV isolates from mosquitoes collected in WA from 2005 to 2009. In addition, full-length prM and E genes and highly variable 3' untranslated region (UTR) of representative isolates were sequenced. Viruses representing genotype 1 (G1) were most dominant, however four genotype 2 (G2) isolates were identified from mosquitoes collected at Fitzroy Crossing (2006 and 2009) and Broome (2006). These results demonstrated that G2 strains of MVEV continue to circulate in the Kimberley region, and beyond its previously recognised geographic range of Kununurra. Phenotypic studies of representative strains from G1 and G2 revealed that isolates within G1 are highly virulent whereas G2 contains isolates of low virulence. To characterise the depth of genetic diversity and the quasispecies phenomenon in the MVEV population, Aziz performed next generation sequencing (NGS) on un-passaged homogenates of mosquito pools that contained representative MVEV isolates from G1 and G2. G1 isolates were highly diverse whereas only minor genetic diversity was detected in G2. Aziz also developed a real-time TaqMan PCR for detection of all four known genotypes of MVEV in mosquito and clinical specimens. In addition, genetic and phenotypic changes were examined after a series of passages in mosquito or avian cells or alternating passages in each cell line. Virus fitness was assessed both *in vitro* and *in vivo* using a single-step growth curve assay and a mouse model of MVE. Phenotyopic changes including significant differences in replicative ability, decreased neuroinvasiveness, improved survival time and increased human dose (HD_{50}) values were observed for MVEV passaged through avian cells alone and MVEV passaged alternately in avian and mosquito cells, however there was no significant change in these parameters for MVEV passaged in mosquito cells alone. Aziz has completed the laboratory work component of his study and submitted his PhD thesis for examination in September 2013. This work was conducted at Curtin University. Aziz is supervised by Dr David Williams (AAHL, ex Curtin University), Dr Paul

Constantino (Curtin University), Dr Geng Hooi Chua (Curtin University), Prof. Ricardo Mancera (Curtin University) and Assoc. Prof. Cheryl Johansen.

Dengue transmission and virulence in Papua New Guinea (PhD project)

In 2010, Mr Dagwin Suarkia commenced a research project investigating dengue transmission and virulence in Papua New Guinea (PNG). Dengue is accepted to be endemic in PNG, but knowledge of its disease burden and epidemiology is limited. In a lead-up study conducted among acute febrile patients in Madang province between 2007 and 2008 dengue was determined to be highly prevalent (Senn et al. 2011). Dengue IgG was detected in more than 80% of children aged ten years and less, and its confirmation in sera from acute cases further suggests it accounts for 10% of acute fevers seen in coastal PNG hospitals. Samples were subjected to further testing in the current study. The results show all four dengue serotypes are present. The fact that three of dengue serotypes were detected in a single epidemic shows a hyperendemic distribution in the studied population. This contrasts its distribution in the other South Pacific island nations where successive epidemics are caused by a single virus. Hyperendemicity has been associated with increases in the incidence of potentially fatal dengue complications, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) in Indonesia and other Southeast Asian countries. Thus, absence of both DHF and DSS in PNG implicates virulence and host factors to be involved in modulating a non-pathologic outcome. Initial phylogeny results based on partial sequence data show PNG viruses cluster closely with those responsible for epidemics in the region during the same period, including Indonesia and Australia. Analyses of longer sequences, growth phenotypes, as well as history of dengue transmission are expected to shed further insights. Results of archived sera from 1960's have demonstrated that multiple serotypes (dengue 1, 2 and 3) have circulated in PNG since that time**.** Such findings are novel and provide the necessary base-line data for future studies and intervention. They further confirm the longstanding concerns that PNG could be an important source for incursions into Australia. Dagwin is supervised by Assoc. Prof. Allison Imrie, Prof. Geoffrey Shellam, Dr Paul Effler (WA DOH), Dr David Smith (PathWest Laboratory Medicine WA), Dr John McBride (James Cook University) and Assoc. Prof. Cheryl Johansen. The study is expected to conclude in February 2014.

Mosquito species abundance and distribution and implications for mosquito management in three inner metropolitan local governments of Perth, Western Australia.

Ryan Janes was a Masters of Infectious Diseases student who conducted a three month research project in the ASRL in semester 1, 2013. A study of urban mosquitoes was conducted during January 11-18, 2013 to determine whether mosquitoes breeding locally in the study area (in the City of South Perth, Town of Victoria Park and City of Canning) were impacting on local residents. Eleven mosquito species were collected comprising two saltmarsh species and nine freshwater species. Four species were dominant: *Ae. vigilax* (36%), *Cx. quinquefasciatus* (25%), *Cx. annulirostris* (21%) and *Ae. notoscriptus* (15%). The mean number of mosquitoes and mean number of species collected in traps decreased as the distance from breeding habitats increased. Statistical analyses indicated that as mean maximum temperature increased, the mean number of mosquitoes and species collected also increased. Approximately 28,178 mosquitoes were marked with fluorescent dye and released from sites at Heirisson Island (HI) and Canning River Regional Park (CRRP) on January 10th 2013. A total of 51,252 female mosquitoes were collected during the study, of which 10 were fluorescent marked specimens. One marked specimen released from CRRP was recaptured 3km away from the release point, and one marked specimen released from HI was recaptured 2.1km away from the release point. Bloodmeal ELISAs (Johansen et al. 2009) were performed on blood engorged mosquito specimens collected during the study. Specimens were tested against sera of the following animal groups: Cow, Sheep/Goat, Pig, Kangaroo, Horse,

Human, Cat, Dog, Bird, and Rabbit. Bloodmeal sources identified included humans (38%), birds (33%), dogs (16%) and cats (8%). This project was supervised by Dr Peter Neville (DOH), Dr Michael Lindsay (DOH) and Dr Jay Nicholson.

Phylogenetic analysis of isolates of Murray Valley encephalitis virus from mosquitoes collected in Western Australia in 2011.

Rabi'atul'adawiyah Binte Abdullah was a Masters of Infectious Diseases student who conducted a three month research project in the ASRL in semester 1, 2013. Adawiyah conducted a phylogenetic analysis of isolates of MVEV obtained from mosquitoes collected in northern WA during a large outbreak of MVE across Australia in 2011. The MVEV isolates were from mosquitoes collected across the Kimberley region, including Broome, Derby, Fitzroy Crossing, Halls Creek, Billiluna, Warmun and Kununurra, and they were from a range of mosquito species. Partial E gene sequences were obtained after amplification by RT-PCR and nucleotide sequencing. The sequences were aligned and compared to sequences obtained from GenBank representing all four known genotypes of MVEV. Phylogenetic analysis revealed the viruses were highly homologous and all clustered in lineage I, the dominant genotype of MVEV found in Australia. This project was supervised by Associate Professor Allison Imrie and Dr Jay Nicholson.

Using different techniques to detect arboviruses circulating in humans and mosquitoes in Malaysia and Australia

Sultan Alamer was a Masters of Infectious Disease student who undertook his three month research project in the ASRL in semester 1, 2013. Sultan's project was designed to assess the suitability of different laboratory techniques for screening sera of suspected DENV patients, and to investigate the molecular epidemiology of BFV isolated from mosquitoes in south-west WA in 2012/13. Although there were difficulties encountered with screening suspected DENV samples, Sultan was able to show that nine BFV isolates from the 2013 outbreak had a high level of sequence homology ($\geq$ 99.3%). There was no evidence to suggest that BFV isolates from WA were genetically distinct from those found on the east coast of Australia. Sultan's supervisors were Assoc. Prof. Allison Imrie, Dr. Jay Nicholson and Prof. David Smith (PathWest).

Section 9: Publications of the group in 2012/13[a]

1. Knope, K., Whelan, P., Smith, D., **Johansen, C.,** Moran, R., Doggett, S., Sly, A., Hobby, M., Kurucz, N., Wright, P., **Nicholson, J.** and the National Arbovirus and Malaria Advisory Committee. (2013). Arboviral diseases and malaria in Australia, 2010-11: Annual report of the National Arbovirus and Malaria Advisory Committee. Communicable Diseases Intelligence (Australia) 37: E1-E20.
2. Hall, R.A., Blitvich, B.J., **Johansen, C.A.** and Blacksell, S.D. (2012). Advances in arbovirus surveillance, detection and diagnosis. *Journal of Biomedicine and Biotechnology*, 2012:512969. Epub 2012 May 16.
3. van den Hurk, A.F., Hall-Mendelin, S., **Johansen, C.A.,** Warrilow, D. and Ritchie, S.A. (2012). Evolution of mosquito-based arbovirus surveillance systems in Australia. *Journal of Biomedicine and Biotechnology*, 2012:325659. Epub 2012 Mar 11.

[a] Publications 2 and 3 were not reported in the 2011/12 Annual Report and instead are reported here.

Section 10: Formal presentations by the group in 2012/13[a]

1. Assoc. Professor Cheryl Johansen presented a seminar entitled "Surveillance of medically important arboviruses in Western Australia" to the Swan Districts Rotary Club, South Guildford, August 2011.
2. Dr Jay Nicholson presented a paper entitled "Consequences of a Torres Strait *Aedes albopictus* incursion onto mainland Australia," at the 10th Mosquito Control Association of Australia/11th Arbovirus Research in Australia Symposium, Gold Coast, September 2012.
3. Assoc. Professor Cheryl Johansen presented lectures entitled "Vector-Borne viral diseases: Flaviviridae", "Vector-Borne viral diseases: Togaviridae, Bunyaviridae and Reoviridae"; and "Surveillance of vector-borne diseases" to Masters of Infectious Diseases students at The University of Western Australia, Nedlands, August and September 2011.
4. Dr Jay Nicholson presented lectures entitled "Mosquitoes - biology and ecology" and "Mosquito identification – adults and larvae" to Masters of Infectious Diseases students at The University of Western Australia, Nedlands, August 2012.

Section 11: Acknowledgments

We wish to thank the DOH for their financial support, without which this work would not be possible. A major strength of the WA Arbovirus Surveillance and Research program is the inter-departmental and inter-agency collaboration in different components of the program. We would therefore like to thank and acknowledge the following institutions and people:

- The DOH, in particular Dr Andrew Robertson (Divisional Director, Health Protection Group) and Mr Jim Dodds (Director, Environmental Health Directorate) for continued financial and logistical support of this important public health program.
- Dr Michael Lindsay, Dr Peter Neville, Mrs Amber Douglas, Mr Ryan Janes and Dr Andrew Jardine of the MBDC, DOH, for their continuing logistical and technical assistance with our program and for many invaluable discussions about the program during 2012/13.
- The valuable contributions of the people involved in the sentinel chicken program for detection of flaviviruses in northern WA in 2012/13 are listed in Table 22.
- EHOs in Local Government Authorities and members of Contiguous Local Authority Groups (CLAGs) throughout WA who provide valuable information about and assistance with determining timing of mosquito breeding cycles. In particular:
  - Mr Scott Dandridge and Mr Haydn Jones (Shire of Harvey), Mr Neil Nicholson (Shire of Dardanup), Ms Sarah Upton and Ms Meredith Chidlow (City of Bunbury) and other members of the Leschenault CLAG;
  - Mr Brendan Ingle, Mr Scott Severn and Ms Paula Boaden (City of Mandurah), Mr Ross Rose, Ms Samantha Ledger (Shire of Murray) and other members of the Peel Mosquito Management Group;
  - Ms Alison Edmunds, Ms Lynsey Mas, Ms Christine McAtee and Ms Renata Fourie (Shire of Busselton); and
  - Mr Colin Dent, Ms Carla Webster, Mr Keith Reeves and Ms Jane Cook (Shire of Capel).
- Additional people who must be acknowledged for their assistance include:

[a] Presentations delivered by Assoc. Professor Cheryl Johansen in August and September 2011 were not reported in the 2011/12 Annual Report and instead are reported here.

- Ms Iris Prouse for help distributing warnings to Aboriginal communities in the Kimberley region;
- Regional Public Health Units (Kimberley, Pilbara, Gascoyne, Midwest, Coastal, Wheatbelt and Goldfields) for their help with the sentinel chicken program;
- Department of Environment and Conservation for permission to take biological samples from nature reserves and native fauna in the southwest and Kimberley regions;
- Associate Professor Roy Hall, Dr Natalie Prow and Dr Jody Peters from the Department of Microbiology and Parasitology, The University of Queensland, Brisbane, Qld, for supplying some monoclonal antibodies and virus strains, many useful discussions about aspects of the program and collaborative research on innovative methods for remote area mosquito trapping;

Table 22. Personnel involved in management and bleeding of sentinel chickens in WA.

Personnel	Institution/Location (town or community)
Ms Tahi Morton	Shire of Wyndham- East Kimberley, Kununurra
Mr Cameron Heavens, Mr Paul Gerrard, Mr Haydn van Locken, Mr Lyndell Dudley	Savannah Nickel Mines, East Kimberley
Mr Phil Denniston, Mr Kyle Cameron	Shire of Halls Creek, Halls Creek
Mr Ken McLeod, Mr Tim Stuckey, Mr Peter Parker, Ms Emma Catlin	Shire of Derby / West Kimberley, Derby
Mr Chris Kloss	Private resident, Derby
Roebuck Plains Station personnel, Ms Sarah Mason	Roebuck Plains Station
Ms Sarah Starkey, Mr Chicky Clements	Nirrumbuk Aboriginal Corporation, Broome
Father Daniel Kilala	Beagle Bay Mission
Dr Heather Lyttle, Ms Phillipa Rose, Ms Jodie Bennett	Pilbara Public Health Unit, Port Hedland
Mr Craig Watts	Shire of Roebourne, Karratha
Mr and Mrs Kevin and Debbie Cutmore	Water Corporation, Harding Dam
Ms Michelle Jordan, Mr Jay Gordon	Shire of Carnarvon
Ms Helen Mitchell, Ms Debbie Cook, Katie	Private residents, Marble Bar
Mr Bill Hardy, Mr Mick Dunne	Shire of Ashburton (Tom Price, Paraburdoo, Pannawonica and Onslow)
Dr Yvonne Olgiers	Shire of Newman, Newman
Mr Ken Cameron, Ms Jenni Mitchell	Shire of Exmouth, Exmouth
Mr Don McLeod	Private resident, Carnarvon
Ms Maurie Struwig	Shire of Greenough-Geraldton
Mr Felix Neuweiler, Ms Kim Senior	Shire of Irwin, Dongara
Mr Garry Agnew	Shire of Leonora
Mr Sean Harris, Mr Murray Matthews	Private resident, Moora
Mr James Fisher	Private resident, York
Mr Lee Jeavons	Leonora Shire Recreational Centre Manager

- Mr Stephen Doggett, Dr Cameron Webb, Mr John Clancy and Mr John Haniotis, Department of Medical Entomology, Westmead Hospital, Sydney, NSW for their entomological expertise and advice/discussions about aspects of the program and collaborative research;
- Dr David Williams (Australian Animal Health Laboratory, Geelong) and Dr Aziz Niazi (Curtin University of Technology) for assisting with identification of arbovirus isolates, participation in collaborative research and helpful discussions;

- Mr Peter Whelan (Medical Entomology Branch, Territory Health Services, Darwin, NT) for entomological advice and helpful discussions;
- Professor Richard Russell (Honourary Professor of Medical Entomology, The University of Sydney) for entomological advice and helpful discussions;
- Dr Andrew van den Hurk, Dr Alyssa Pyke and Dr Sonja Hall-Mendelin, Queensland Health Forensic and Scientific Services, for testing of some sentinel chicken sera and valuable discussions about serological techniques, and for collaborative research on innovative methods for remote area mosquito trapping;
- Professor Scott Ritchie (James Cook University) for collaborative research on innovative mosquito traps for arbovirus surveillance;
- Assoc. Professor Linda Hueston (Curtin University) for collaborative research on longitudinal analyses of flavivirus seroconversions in sentinel chickens, human cases of MVE and rainfall;
- Staff of the PathWest Laboratory Medicine (QEII Site) for providing clinical specimens, facilitating sentinel chicken blood sample transport, assistance with virus identification and for advice about aspects of the program;
- Staff and students of the School of Pathology and Laboratory Medicine, UWA, for providing infrastructure and laboratory space for the program as well as a supportive and enjoyable working environment;
- Ms Siobain Mulligan (WA Department of Transport) for providing tidal data; and
- Mr Cameron Bell, Ms Penny Borzecki and Mr Murray Boyd (Altona Hatchery, WA) for supplying chickens for the sentinel chicken program.

It is inevitable with such an active surveillance and research program that we will have failed to acknowledge some people by name. We would therefore like to take this opportunity to thank and acknowledge other contributors to the program during 2012/13.

Section 12: References cited in this report

1. Arbovirus Surveillance and Research Laboratory Annual Report, 1997-1998.

2. Arbovirus Surveillance and Research Laboratory Annual Report, 2006-2007.

3. Arbovirus Surveillance and Research Laboratory Annual Report, 2008-2009.

4. Arbovirus Surveillance and Research Laboratory Annual Report, 2010-2011.

5. Blitvich, B.J., Bowen, R.A., Marlenee, N.L., Hall, R.A., Bunning, M.L. and Beaty, B.J. (2003a). Epitope blocking enzyme-linked immunosorbent assays for detection of West Nile virus antibodies in domestic mammals. *Journal of Clinical Microbiology* 41: 2676-2679.

6. Blitvich, B.J., Marlenee, N.L., Hall, R.A., Calisher, C.H., Bowen, R.A., Roehrig, J.T., Komar, N., Langevin, S.A. and Beaty, B.J. (2003b). Epitope blocking enzyme-linked immunosorbent assays for the detection of serum antibodies to West Nile virus in multiple avian species. *Journal of Clinical Microbiology* 41: 1041-1047.

7. Broom, A. K., Hall, R. A., Johansen, C. A., Oliveira, N., Howard, M. A., Lindsay, M. D., Kay, B. H. and Mackenzie, J. S. (1998). Identification of Australian arboviruses in inoculated cell cultures using monoclonal antibodies in ELISA. *Pathology* 30: 286-288.

8. Broom, A. K., Wright, A. E., Mackenzie, J. S., Lindsay, M. D. and Robinson, D. (1989). Isolation of Murray Valley encephalitis and Ross River viruses from *Aedes normanensis* (Diptera: Culicidae) in Western Australia. *Journal of Medical Entomology* 26: 100-103.

9. Chiang, C. L. and Reeves, W. C. (1962). Statistical estimation of virus infection rates in mosquito vector populations. *American Journal of Hygiene* 75: 377-391.

10. Doggett, S.L. and Russell, R.C. (1997). *Aedes notoscriptus* can transmit inland and coastal isolates of Ross River virus and Barmah Forest virus from New South Wales! *Arbovirus Research in Australia* 7: 79-81.

11. Frost, M.J., Zhang, J., Edmonds, J.H., Prow, N.A., Gu, X., Davis, R., Hornitzky, C., Arzey, K.E., Finlaison, D., Hick, P., Read, A., Hobson-Peters, J., May, F.J., Doggett, S.L., Haniotis, J., Ruxxell, R.C., Hall, R.A., Khromykh, A.A. and Kirkland, P.D. (2012). Characterization of virulent West Nile virus Kunjin strain, Australia, 2011. *Emerging Infectious Diseases* 18: 792-800.

12. Hall, R. A., Broom, A. K., Hartnett, A. C., Howard, M. J. and Mackenzie, J. S. (1995). Immunodominant epitopes on the NS1 protein of MVE and KUN viruses serve as targets for a blocking ELISA to detect virus-specific antibodies in sentinel animal serum. *Journal of Virological Methods* 51: 201-210.

13. Henchal, E.A., Gentry, M.K., McCown, J.M. and Brandt, W.E. (1982). Dengue virus-specific and flavivirus group determinants identified with monoclonal antibodies by indirect immunofluorescence. *American Journal of Tropical Medicine and Hygiene* 31: 830-836.

14. Hobson-Peters, J., Yam, A.W.Y., Lu, J.W.F., Setoh, Y.X., May, F.J., Kurucz, N., Walsh, S., Prow, N.A., Davis, S.S., Weir, R., Melville, L., Hunt, N., Webb, R.I., Blitvich, B.J., Whelan, P. and Hall, R.A. (2013). A new insect-specific flavivirus from northern Australia suppresses replication of West Nile virus and Murray Valley encephalitis virus in co-infected mosquito cells. *Plos One* 8: e56534.

15. Jasinskia, E.M., Lindsay, M., Wright, A.E. and Smith, D.W. (1997). Changes in mosquito populations and arbovirus activity following construction of a channel to increase tidal flushing in ane stuaring system in south-western Australia. *Arbovirus Research in Australia* 7: 116-122.

16. Johansen, C. A., Lindsay, M. D. A., Harrington, S. A., Whelan, P. I., Russell, R. C. and Broom, A. K. (2004a). First record of the mosquito species *Aedes (Aedimorphus) nocturnus* (Theobald) (Diptera: Culicidae) in Australia. *Northern Territory Disease Control Bulletin* 11: 3-5.

17. Johansen, C. A., Lindsay, M. D. A., Harrington, S. A., Whelan, P. I., Russell, R. C. and Broom, A. K. (2005). First record of *Aedes (Aedimorphus) vexans vexans* (Meigen) (Diptera: Culicidae) in Australia. *Journal of the American Mosquito Control Association* 21: 222-224.

18. Johansen, C. A., Nisbet, D. J., Foley, P. N., van den Hurk, A. F., Hall, R. A., Mackenzie, J. S. and Ritchie, S. A. (2004b). Flavivirus isolations from mosquitoes collected from Saibai Island in the Torres Strait, Australia, during an incursion of Japanese encephalitis virus. *Medical and Veterinary Entomology* 18: 281-287.

19. Johansen, C. A., Nisbet, D. J., Zborowski, P., van den Hurk, A. F., Ritchie, S. A. and Mackenzie, J. S. (2003). Flavivirus isolations from mosquitoes collected from western Cape York Peninsula, Australia, 1999-2000. *Journal of the American Mosquito Control Association* 19: 392-396.

20. Johansen, C.A., Power, S.L., Broom, A.K. (2009). Determination of mosquito (Diptera: Culicidae) bloodmeal sources in Western Australia: Implications for arbovirus transmission. *Journal of Medical Entomology* 46: 1167-1175.

21. Johnson, P.H., Hall-Mendelin, S., Whelan, P.I., Frances, S.P., Jansen, C.C., Mackenzie, D.O., Northill, J.A. and van den Hurk, A.F. (2009). Vector competence of Australian *Culex gelidus* Theobald (Diptera: Culicidae) for endemic and exotic arboviruses. *Australian Journal of Entomology* 48: 234-240.

22. Lee, D. J., Hicks, M. M., Griffiths, M., Russell, R. C. and Marks, E. N. (1980). The Culicidae of the Australasian region. Canberra, Australia: Australian Government Printing Service.

23. Liehne, P. F. S. (1991). An atlas of the mosquitoes of Western Australia. Perth: Health Department of Western Australia.

24. Lindsay, M. D. (1995). Ecology and epidemiology of Ross River virus in Western Australia. PhD Thesis, Perth: The University of Western Australia.

25. Lindsay, M., Oliveira, N., Jasinska, E., Johansen, C., Harrington, S., Wright, A., Smith, D. & Shellam, G. (1997). Western Australia's largest recorded outbreak of Ross River virus disease. *Arbovirus Research in Australia* 7: 147-152.

26. Lindsay, M. D. A., Coelen, R. J. and Mackenzie, J. S. (1993). Genetic Heterogeneity among Isolates of Ross River virus from Different Geographical Regions. *Journal of Virology* 67: 3576-3585.

27. Mann, R.A., Fegan, M., O'Riley, K., Motha, J. And Warner, S. (2013). Molecular characterization and phylogenetic analysis of Murray Valley encephalitis virus and West Nile virus (Kunjin subtype) from an arbovirus disease outbreak in horses in Victoria, Australia, in 2011. *Journal of Veterinary Diagnostic Investigation* 25: 35-44.

28. Muller, M. J., Montgomery, B. L., Ingram, I. and Ritchie, S. A. (2001). First records of *Culex gelidus* from Australia. *Journal of the American Mosquito Control Association* 17: 79-80.

29. Oliveira, N. M., Hall, R., Broom, A., Lindsay, M., Weir, R., Kay, B. and Mackenzie, J. (1997). Epitope analysis of Ross River virus using monoclonal antibodies. *Arbovirus Research in Australia* 7: 201-207.

30. Prow, N. A. (2006). Epidemiology of Ross River virus in the south-west of Western Australia and an assessment of genotype involvement in Ross River virus pathogenesis. PhD Thesis, Perth: The University of Western Australia.

31. Ritchie, S., Haseler, B., Foley, P. & Montgomery, B. (2001). Exotic mosquitoes in north Queensland: the true millenium bug? *Arbovirus Research in Australia* 8: 303-307.

32. Rohe, D. and Fall, R. P. (1979). A miniature battery powered CO_2 baited light trap for mosquito borne encephalitis surveillance. *Bulletin of the Society of Vector Ecology* 4: 24-27.

33. Russell, R. C. (1996). A Colour Photo Atlas of Mosquitoes of South-eastern Australia: Department of Medical Entomology, University of Sydney, Australia.

34. Sammels, L. M., Coelen, R. J., Lindsay, M. D. and Mackenzie, J. S. (1995). Geographic distribution and evolution of Ross River virus in Australia and the Pacific Islands. *Virology* 212: 20-29.

35. Senn, N., Luang-Suarkia, D., Manong, D., Siba, P.M., McBride, W.J.H. (2011). Contribution of Dengue fever to the burden of acute febrile illnesses in Papua New Guinea: an age-specific prospective study. *American Journal of Tropical Medicine and Hygiene* 85:132-137.

36. van den Hurk, A. F., Nisbet, D. J., Johansen, C. A., Foley, P. N., Ritchie, S. A. and Mackenzie, J. S. (2001). Japanese encephalitis on Badu Island, Australia: the first isolation of Japanese encephalitis virus from *Culex gelidus* in the Australasian region and the role of mosquito host feeding patterns in virus transmission cycles. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 95: 595-600.

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

38. Whelan, P., Hayes, G., Carter, J., Wilson, A. and Haigh, B. (2000). Detection of the exotic mosquito *Culex gelidus* in the Northern Territory. *Communicable Diseases Intelligence (Australia)* 24: 74-75.

39. Whelan, P., Hayes, G., Tucker, G., Carter, J., Wilson, A. and Haigh, B. (2001). The detection of exotic mosquitoes in the Northern Territory of Australia. *Arbovirus Research in Australia* 8: 395-404.

Appendix 1: Sentinel Chicken Surveillance Results 2011/12

Summary of WA Flavivirus Surveillance program, 2011/12*
Sentinel chicken flocks are tested for infection with Murray Valley encephalitis and Kunjin viruses.

July - December 2011																				
Month	July		Aug		Sept		Oct		Nov		Dec		TOTAL	
Location	n	+ve	n	+ve	n	+ve	n	+ve	n	+ve	n	+ve	Bled (n)	Positive(+ve)
KIMBERLEY														
Wyndham	12	0	24	0	12	0	23	0	21	0	15	7 (4M, 2K, 1MK)	107	7
Kununurra	8	0	8	0	8	0	6	0	20	2 (1M 1K)	20	4 (4M)	70	6
Savannah Nickel mine	12	0			23	0	22	0	35	0	11	0	103	0
Halls Creek	22	0	24	0	23	0	12	0	12	0	12	0	105	0
Fitzroy Crossing	12	1 (1M)	22	0	11	0	11	0	11	0	11	4 (4M)	78	5
Derby site 1	29	0	21	0	22	0	22	0	22	0	19	0	135	0
Derby site 2	36	0	24	0	24	0	24	0	11	0	8	1 (1M)	127	1
Lombadina											12	0	12	0
Beagle Bay											1	0	1	0
One Arm Point													0	0
Roebuck Plains			6	1 (1M)	11	2 (2M)			10	0	11	0	38	3
Broome - Town													0	0
PILBARA														
Port Hedland					12	4 (4M)			20	0	10	0	42	4
Karratha	22	1 (1M)	30	0	10	0	19	0	20	0	20	0	121	1
Harding Dam 1	24	0	24	0	24	0	24	0	8	0	13	0	117	0
Harding Dam 2	24	0	24	0	24	0	24	0	11	0	22	0	129	0
Marble Bar					11	7 (7M)			7	0	7	4 (4M)	25	11
Pannawonica	24	0	21	0	36	0	24	0	12	0	33	0	150	0
Tom Price	24	0	24	0	12	0	24	0	24	0	12	0	120	0
Paraburdoo	21	3 (3M)	18	0	9	0	18	0	24	0	12	0	102	3
Onslow	12	0	8	0	16	0	13	0	24	0	10	0	83	0
Ophthalmia	12	0	23	1 (1M)			11	0	10	0			56	1
Newman Shire	12	0	24	0					12	0			48	0
Exmouth	36	0	24	0	31	0	20	0	30	0	20	0	161	0
GASCOYNE														
Carnarvon	12	0	36	0	24	0	24	0	24	0	12	0	132	0
MID-WEST/WHEATBELT														
Moora	11	0	32	0	11	0			18	2 (2K)			72	2
Geraldton (Walkaway)	19	0	10	0			10	0	12	0			51	0
Dongara	7	0	12	0	21	0	7	0	36	0	24	2 (2K)	107	2
York	11	0	11	0	22	0			11	0	10	0	65	0
GOLDFIELDS														
Leonora													0	0

Appendix 1 (continued). Summary of WA Flavivirus Surveillance program, 2011/12*
Sentinel chicken flocks tested for infection with Murray Valley encephalitis and Kunjin viruses.

January - June 2012																								
Month	Jan			Feb			Mar			Apr			May			Jun			TOTAL	
Location	n	+ve		n	+ve		n	+ve		n	+ve		n	+ve		n	+ve		Bled (n)	Positive(+ve)
KIMBERLEY																								
Wyndham	4	1	1 M	5	1	1M	2	0											11	2
Kununurra	8	4	4M				12	1	1M	11	2	1M 1K							31	7
Savannah Nickel mine	19	1	1M	16	3	3M	11	2	2M	8	0		11	0		16	0		81	6
Halls Creek	20	1	1M	20	0		9	2	2M	7	0		14	0					70	3
Fitzroy Crossing	8	4	3M 1K				10	0		10	4	4M	6	1	1K	5	0		39	9
Derby site 1	16	0		16	0		3	0		21	0		19	3	3M	19	0		94	3
Derby site 2	11	0		10	0		7	0		21	5	4M 1K	14	0		17	2	2M	80	7
Lombadina				12	0		11	0		21	4	1M 3K	9	3	2M 1K				53	7
Beagle Bay										24	0		22	2	2M	10	0		56	2
Roebuck Plains	7	0								15	3	3M	14	2	2M	12	2	2F	48	7
																			0	0
PILBARA																				
Port Hedland	19	0		10	0		10	1	1M	9	7	7M				11	0		59	8
Karratha	18	0		18	0		18	9	9M				12	0		24	0		90	9
Harding Dam 1	3	0		6	0		16	3	3M				17	5	5M	24	0		66	8
Harding Dam 2	9	0		29	0		18	11	10M 1K				11	0		16	0		83	11
Marble Bar				6	4	4M				7	2	2M	5	2	2M				18	8
Pannawonica	22	2	2K	14	1	1K	16	8	8M							36	0		88	11
Tom Price	12	3	3M	17	2	2M	7	7	7M				12	0		10	0		58	12
Paraburdoo	6	0		11	1	1M	11	4	4M	8	6	6M	12	0		12	0		60	11
Onslow				10	0		16	5	5M	6	0		11	0		11	0		54	5
Ophthalmia	11	11	11M				23	5	5M	6	4	2M 1K 1MK				22	0		62	20
Newman Shire	10	0		12	0		18	11	10M 1MK	1	1	1M				24	0		65	12
Exmouth	19	1	1K	18	0		25	1	1M	13	1	1M	18	0		36	0		129	3
GASCOYNE																				
Carnarvon	10	0		30	0		10	0		29	1	1M	18	0		9	0		106	1
MID-WEST/WHEATBELT																				
Moora	17	1	1K	5	0		9	0		18	0		18	0		9	0		76	1
Geraldton (Walkaway)	12	0		12	1	1K	21	1	1K				28	0		18	0		91	2
Dongara	18	0		10	0		18	0		10	0		10	3	3K	10	0		76	3
York	19	1	1K	18	0		18	0		9	0		9	0		9	0		82	1
GOLDFIELDS																				
Leonora													12	0					12	0

*Flocks sampled fortnightly. Previous (or repeat) positive chickens are not recorded on this summary. n = number of samples tested, +ve = no. of flavivirus positive samples, M = MVEV, K = KUNV, F = Flavivirus only (not MVEV, KUNV or Japanese encephalitis virus), MK is MVEV + KUNV antibodies.

Appendix 2: Revised tables of results from the Kimberley region in 2011.

Appendix 2A. Results of mosquito trapping and virus isolations from Broome (Town and environs), West Kimberley.
Trap dates: 17th and 18th March 2011 (12 traps, 1 failure)

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	Virus isolates[1]
Ae. (Finlaya) notoscriptus	Bloodfed	2	(<0.1)	0	0	0	
Ae. (Finlaya) notoscriptus	Female	347	(1.7)	167	11	1	
Ae. (Finlaya) pecuniosus	Female	8	(<0.1)	0	0	1	
Ae. (Macleaya) E.N. Marks' species No. 125	Female	2	(<0.1)	0	0	1	
Ae. (Macleaya) species	Bloodfed	5	(<0.1)	0	0	0	
Ae. (Macleaya) species	Female	231	(1.2)	139	11	0	
Ae. (Macleaya) species	Male	341	(1.7)	177	15	0	
Ae. (Macleaya) tremulus	Female	33	(0.2)	13	2	0	
Ae. (Neomellanoconion) lineatopennis	Female	4	(<0.1)	1	1	0	
Ae. (Ochlerotatus) normanensis	Female	5	(<0.1)	2	2	0	
Ae. (Ochlerotatus) phaecasiatus	Female	8	(<0.1)	0	0	5	
Ae. (Ochlerotatus) vigilax	Female	56	(0.3)	23	9	0	
An. (Cellia) amictus	Female	21	(0.1)	5	1	1	
An. (Cellia) annulipes s.l.	Female	1	(<0.1)	1	1	0	
An. (Cellia) hilli	Female	46	(0.2)	9	4	1	
Cx. (Culex) annulirostris	Bloodfed	409	(2.1)	0	0	0	
Cx. (Culex) annulirostris	Female	17057	(86.0)	5190	211	1	1 MVEV, 1 ALFV, 1 SINV
Cx. (Culex) quinquefasciatus	Female	20	(0.1)	12	4	0	
Cx. (Culex) sitiens	Female	61	(0.3)	37	6	1	
Cx. (Culiciomyia) pullus	Female	30	(0.2)	8	5	0	
Cx. species (unidentified) - new or difficult to ID species	Male	8	(<0.1)	1	1	0	
Tripteroides (Polylepidomyia) punctolateralis	Female	15	(0.1)	8	7	0	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	1	(<0.1)	1	1	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	8	(<0.1)	0	0	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	1117	(5.6)	307	18	0	
Total		**19836**	**(100.0)**	**6101**	**310**	**12**	**1 MVEV, 1 ALFV, 1 SINV**

[1]MVEV is Murray Valley encephalitis virus, ALFV is Alfuy virus, SINV is Sindbis virus.

Appendix 2B. Results of mosquito trapping and virus isolations from Derby (Gibb River Road transect), West Kimberley.
Trap dates: 31st March 2011 (3 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	Virus isolates[1]
Ae. (Finlaya) pecuniosus	Female	6	(<0.1)	1	1	0	
Ae. (Macleaya) species	Female	13	(0.1)	2	1	0	
Ae. (Macleaya) tremulus	Female	6	(<0.1)	1	1	0	
Ae. (Mucidus) alternans	Female	115	(0.7)	15	4	0	
Ae. (Ochlerotatus) normanensis	Bloodfed	81	(0.5)	0	0	0	
Ae. (Ochlerotatus) normanensis	Female	5857	(35.3)	765	32	0	
Ae. (Ochlerotatus) pseudonormanensis	Female	6	(<0.1)	0	0	1	
Ae. (Ochlerotatus) vigilax	Bloodfed	7	(<0.1)	0	0	0	
Ae. (Ochlerotatus) vigilax	Female	1820	(11.0)	245	12	0	
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	13	(0.1)	2	2	0	
Ae. (Ochlerotatus) vigilax variant	Female	11	(0.1)	1	1	0	
Ae. species (unidentified) - new or difficult to ID species	Female	6	(<0.1)	0	0	1	
An. (Cellia) amictus	Female	245	(1.5)	32	4	0	
An. (Cellia) annulipes s.l.	Bloodfed	21	(0.1)	0	0	0	
An. (Cellia) annulipes s.l.	Female	2436	(14.7)	338	15	0	
An. (Cellia) hilli	Female	22	(0.1)	3	2	0	
An. (Cellia) meraukensis	Female	7	(<0.1)	1	1	0	
Cx. (Culex) annulirostris	Bloodfed	104	(0.6)	0	0	0	
Cx. (Culex) annulirostris	Female	5069	(30.6)	657	27	0	3 MVEV
Cx. (Culex) bitaeniorhynchus	Female	11	(0.1)	1	1	0	
Cx. (Culex) palpalis	Female	37	(0.2)	3	2	1	
Cx. (Culex) sitiens	Female	321	(1.9)	48	4	0	
Cx. (Culiciomyia) pullus	Female	7	(<0.1)	1	1	0	
Cx. species (unidentified) - new or difficult to ID species	Male	31	(0.2)	4	2	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	117	(0.7)	14	3	0	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Bloodfed	7	(<0.1)	0	0	0	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	162	(1.0)	23	3	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	44	(0.3)	6	3	0	
Total		**16582**	**(100.0)**	**2163**	**122**	**3**	**3 MVEV**

[1]MVEV is Murray Valley encephalitis virus.

Appendix 2C. Results of mosquito trapping and virus isolations from Fitzroy Crossing (Town and environs), West Kimberley.[1]
Trap dates: 1st April 2011 (11 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	Virus isolates[2]
Ae. (Finlaya) notoscriptus	Female	2	(<0.1)	2	1	0	
Ae. (Macleaya) E.N. Marks' species No. 121	Female	1	(<0.1)	0	0	1	
Ae. (Macleaya) E.N. Marks' species No. 125	Female	1	(<0.1)	0	0	1	
Ae. (Macleaya) E.N. Marks' species No. 147	Female	9	(0.2)	9	4	0	
Ae. (Macleaya) species	Female	23	(0.4)	22	6	0	
Ae. (Macleaya) species	Male	16	(0.3)	16	4	0	
Ae. (Macleaya) tremulus	Female	5	(0.1)	4	1	1	
Ae. (Mucidus) alternans	Female	60	(1.1)	50	13	0	
Ae. (Neomellanoconion) lineatopennis	Female	11	(0.2)	8	4	0	
Ae. (Ochlerotatus) E.N. Marks' species No. 159	Female	3	(0.1)	1	1	0	
Ae. (Ochlerotatus) normanensis	Bloodfed	14	(0.3)	0	0	0	
Ae. (Ochlerotatus) normanensis	Female	2368	(43.4)	1459	65	0	1 Non A/F
Ae. (Ochlerotatus) pseudonormanensis	Female	2	(<0.1)	2	1	0	
Ae. (Ochlerotatus) vigilax	Female	2	(<0.1)	2	1	0	
An. (Cellia) amictus	Bloodfed	1	(<0.1)	0	0	0	
An. (Cellia) amictus	Female	607	(11.1)	421	22	0	4 Non A/F
An. (Cellia) annulipes s.l.	Bloodfed	9	(0.2)	0	0	0	
An. (Cellia) annulipes s.l.	Female	1450	(26.6)	1174	53	0	3 Non A/F
An. (Cellia) hilli	Female	1	(<0.1)	1	1	0	
An. (Cellia) meraukensis	Female	3	(0.1)	3	2	0	
An. species (unidentified) - new or difficult to ID species	Male	18	(0.3)	18	2	0	
Cx. (Culex) annulirostris	Bloodfed	3	(0.1)	0	0	0	
Cx. (Culex) annulirostris	Female	629	(11.5)	532	27	0	1 MVEV, 3 Non A/F, 1 SINV
Cx. (Culex) palpalis	Female	1	(<0.1)	1	1	0	
Cx. (Culex) quinquefasciatus	Female	4	(0.1)	4	2	0	
Cx. (Culex) squamosus	Female	1	(<0.1)	1	1	0	
Cx. (Culiciomyia) pullus	Female	3	(0.1)	3	2	0	
Cx. (Culiciomyia) pullus	Male	6	(0.1)	5	1	0	
Cx. species (unidentified) - new or difficult to ID species	Male	12	(0.2)	12	3	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	12	(0.2)	12	1	0	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	70	(1.3)	63	8	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	13	(0.2)	11	2	0	
Total		**5460**	**(100.0)**	**3936**	**230**	**3**	**1 MVEV, 11 Non A/F, 1 SINV**

[1]One pool of approximately 100 *Culicoides* was tested for arboviruses, no arboviruses were isolated.
[2]Non A/F is not an alphavirus or flavivirus, MVEV is Murray Valley encephalitis virus, SINV is Sindbis virus.

Appendix 2D. Results of mosquito trapping and virus isolations from Geikie Gorge (Gorge and environs), West Kimberley.
Trap dates: 1st and 13th April 2011 (6 traps, no failures)

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	Virus isolates[2]
Ae. (Finlaya) new undescribed species[1]	Female	2	(<0.1)	0	0	1	
Ae. (Macleaya) E.N. Marks' species No. 147	Female	1	(<0.1)	1	1	0	
Ae. (Macleaya) species	Female	2	(<0.1)	1	1	0	
Ae. (Macleaya) tremulus	Female	1	(<0.1)	1	1	0	
Ae. (Mucidus) alternans	Female	13	(0.2)	8	3	0	
Ae. (Neomellanoconion) lineatopennis	Female	77	(1.1)	38	6	0	1 Non A/F
Ae. (Ochlerotatus) normanensis	Bloodfed	36	(0.5)	0	0	0	
Ae. (Ochlerotatus) normanensis	Female	2289	(33.9)	1038	45	0	2 Flavi, 2 Non A/F
Ae. (Ochlerotatus) pseudonormanensis	Female	2	(<0.1)	1	1	0	
Ae. (Ochlerotatus) vigilax	Female	2	(<0.1)	1	1	0	
Ae. species (unidentified) - new or difficult to ID species	Male	2	(<0.1)	1	1	0	
An. (Cellia) amictus	Bloodfed	1	(<0.1)	0	0	0	
An. (Cellia) amictus	Female	143	(2.1)	83	7	0	1 Flavi
An. (Cellia) annulipes s.l.	Bloodfed	15	(0.2)	0	0	0	
An. (Cellia) annulipes s.l.	Female	1429	(21.1)	724	31	0	3 Non A/F
An. (Cellia) meraukensis	Female	2	(<0.1)	2	2	0	
An. (Cellia) novaguinensis	Female	2	(<0.1)	0	0	1	
Cx. (Culex) annulirostris	Bloodfed	17	(0.3)	0	0	0	
Cx. (Culex) annulirostris	Female	2573	(38.1)	1241	52	0	1 MVEV, 1 KUNV, 4 SINV, 3 Non A/F
Cx. (Culex) bitaeniorhynchus	Female	2	(<0.1)	1	1	0	
Cx. (Culex) starckeae	Female	2	(<0.1)	1	1	0	
Cx. (Culiciomyia) pullus	Female	13	(0.2)	7	4	0	
Cx. species (unidentified) - new or difficult to ID species	Male	7	(0.1)	3	3	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	7	(0.1)	2	1	0	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	61	(0.9)	33	5	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	56	(0.8)	30	4	0	
Ve. (Verrallina) reesi	Female	2	(<0.1)	1	1	0	
Total		**6759**	**(100.0)**	**3218**	**172**	**2**	**9 Non A/F, 3 Flavi, 1 MVEV, 1 KUNV, 4 SINV**

[1]This species has been collected previously and examined by Mr Peter Whelan and Prof. Richard Russell.
[2]Non A/F is not an alphavirus or flavivirus, Flavi is a new flavivirus in the yellow fever group, MVEV is Murray Valley encephalitis virus, KUNV is Kunjin virus, SINV is Sindbis virus.