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The University of Western Australia Arbovirus Surveillance and Research Laboratory Annual Report: 2013-2014

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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, Dr Andrew Jardine, Ms Amber Douglas, Mr Ryan Janes and Dr Abbey Potter of the MBDC, who have had regular input and involvement in many aspects of the studies and surveillance programs described in this report. We are also grateful for the expertise and assistance of Dr Glenys Chidlow (PathWest Laboratory Medicine WA) with molecular testing of mosquito homogenates for arbovirus detection.

Section 1: The University of Western Australia Arbovirus Surveillance and Research Program, 2013/14, 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 2013/14 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 above average at the start of the season, and then declined to below/very much below average from October 2013 to April 2014, particularly during summer. Temperatures were generally warmer than average for most of the season. Tides impacted saltmarsh breeding sites with the exception of summer months when they had less impact than predicted.
- A total of 143589 adult mosquitoes were collected, of which 59541 (41.5%) were processed for virus isolation in 4339 pools yielding 27 isolates of Ross River virus (RRV) and/or Barmah Forest virus (BFV).
- Vector abundance was initially high and then declined to very low abundance in summer and autumn, likely due to reduced impact of high tides and very low rainfall. Like previous years, prolonged warm temperatures in the southwest of WA enabled continued breeding of *Aedes vigilax* through to June.
- Polymerase chain reaction (PCR) was used in addition to virus isolation for detection of RRV and BFV in mosquitoes from the southwest of WA in 2013/14. The first arbovirus detection for the season was RRV by PCR only from *Ae. clelandi* collected at Mandurah and eight RRV detections by virus isolation and PCR in mosquitoes from Capel on 1 October 2013, prompting the WA DOH to issue a media release advising residents and travellers of the increased risk of mosquito-borne disease. RRV was also detected in the Leschenault region and Busselton later in the season.
- BFV was first detected in the Peel region on 29 October 2013, and was subsequently also detected in the Leschenault region and Capel.
- Most arbovirus detections were from *Ae. camptorhynchus* (20/27), although other detections occurred in *Ae. clelandi*, *Ae. vigilax, Ae. alboannulatus*, *Culex globocoxitus* and *Anopheles annulipes*.
- RRV was detected through to 18 February 2014. The highest minimum infection rate (MIR) for RRV was 7.7 per 1000 mosquitoes on 10 October 2013 at Capel. All isolates of RRV were of the northern/eastern phenotype. BFV was detected through to 4 February 2014 in the Leschenault region, when the MIR peaked at 8.0 per 1000 mosquitoes.
- A total of 1,471 cases of RRV disease were reported in WA, mostly from the Perth Metropolitan Area (615), the southwest of WA (388, including 208 from the Peel region), the Pilbara (115) and the Goldfields/Esperance region (92). The incidence of BFV was low, although human case data was only provided to the ASRL from January 2014.
- 2012/13 outlook: The Bureau of Meteorology models suggest equal odds of the chance of exceeding median rainfall between December 2014 and January 2015. Milder day and night temperatures are more likely.

Kimberley

2012/13 wet season

- Adult mosquito collections were restricted to the west Kimberley region between 18 and 21 March 2013. Of the estimated 104135 mosquitoes collected in 2013, 15847 (15.2%) were processed for virus isolation in 872 pools, yielding 26 arbovirus isolates: 16 RRV (northern/eastern phenotype), eight BFV and two Kokobera virus (KOKV). Most isolates of RRV were from *Ae. vigilax* and *Cx. annulirostris.* BFV was also detected in *Ae. phaecasiatus*, the first arbovirus isolate from this species in WA. The KOKV isolates were from *Ae. vigilax*.
- RRV, BFV and KOKV were detected in mosquitoes collected at Broome and Derby.

- No introduced mosquito species (eg. *Ae. vexans*, *Cx. gelidus*) were detected in 2013, although sampling did not occur in areas where these species were previously recorded in WA.
- There were no isolations of Murray Valley encephalitis virus (MVEV) or Kunjin virus (KUNV) from mosquitoes collected in 2013. The absence of MVEV and KUNV in mosquitoes from the west Kimberley region reflects the low levels of flavivirus activity seen in sentinel chickens in the 2012/13 season, with just one KUNV seroconversion detected at Roebuck Plains in May 2013 (ASRL 2012/13 Annual Report; Appendix 1).

2013/14 wet season

- Above average rainfall was observed in northern parts of WA between October 2013 and February 2014. Monsoonal activity was weaker than usual in northern WA in March, however typical rainfall patterns returned in April and May.
- 4798 serum samples from 27 flocks were tested for antibodies to flaviviruses during 2013/14. Just 15 seroconversions (0.3%) were detected.
- Low level flavivirus activity associated with the end of the 2012/13 season was detected in the west Kimberley region, continuing to October 2013.
- 2013/14 flavivirus activity commenced late in the season, when a KUNV seroconversion was detected in the Derby 2 flock in May, and three KUNV, two MVEV and an unknown flavivirus infection were detected at Roebuck Plains. Further south, a KUNV infection was detected in May at Ophthalmia Dam in the Pilbara region, where an additional two KUNV and one unknown flavivirus infection were detected in June 2014. This is the second consecutive late start to flavivirus activity and low-level flavivirus activity in northern WA.
- The WA DOH issued a media release in early May based after heavy rainfall and late season flooding in the Pilbara and Gascoyne regions. A second media release was issued in mid-June after detections of KUNV and MVEV seroconversions in sentinel chickens.
- No cases of MVE or KUNV disease were recorded in WA during the 2013/14 season.
- Mosquito collections were only undertaken in the northeast Kimberley region between 15 and 17 April 2014.

Opportunistic trapping

- **2012/13:** Mosquito collections at Nullagine, Marble Bar and Port Hedland (in the east and northeast Pilbara regions) in March and April 2013 yielded low mosquito abundance, with the exception of Port Hedland collections, where *Cx. annulirostris* was the most abundant species.
- Two non alphavirus/non flavivirus isolates were obtained, one from *Ae. normanensis* collected at Nullagine on 13 March 2013 and the other from damaged *Anopheles* sp. mosquitoes collected at Marble Bar on 14-15 March 2013. No arboviruses were detected in mosquitoes from Port Hedland.
- **2013/14:** The ASRL assisted DOH with mosquito collections at Point Samson and nearby towns in October 2013 and January 2014 after diagnosis of a case of dengue. Mosquito abundance was very low during both visits to the area.
- No known vectors were detected in EVS/CO_2 and BG traps, although evidence of other container-breeding mosquito species was found; no arboviruses were detected in mosquitoes from these sites.
- The ASRL processed mosquitoes collected by the WA DOH at Mt Magnet and Cue in March 2014. Mosquito abundance was very low, and no arboviruses were isolated.

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Western Australian Arbovirus Surveillance and Research Program, 2013/14

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. Japanese encephalitis virus 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 2013 and 30 June 2014. Results of routine and opportunistic mosquito collections from the north of WA (Kimberley region) during the first half of 2013 (2012/13 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 2013/14. The results of this work are analysed and discussed in relation to results from the sentinel chicken program and human case data from the 2012/13 wet season.

Section 3: Human cases of arboviral disease in WA

Information on human cases of arboviral disease reported in WA between 1 July 2013 and 30 June 2014 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. An increase in the number of winter, or 'off season' cases of RRV disease between from 2006 to 2009 in the Greater Perth Metropolitan region was recently investigated, and recommendations were made to change the case definition to not include single IgM positive/IgG negative results as laboratory evidence of confirmation of BFV infection (Selvey et al. 2014). Similarly, false positive BFV diagnosis has been reported as a possible cause of increased notifications of BFV disease in winter months (Knope et al. 2014). In 2013/14 MBDC provided BFV disease human case data from January 2014 onwards only, due to concerns related to large numbers of potentially false-positive BFV results obtained in serological tests performed at private diagnostic laboratories in 2013.

Ross River virus disease

The number of cases of RRV disease reported in WA in the 2013/14 season (Table 1) was greater than the previous year (ASRL 2012/13 Annual Report). A total of 1471 cases of RRV disease were serologically confirmed in WA. The majority of cases occurred in the Perth Metropolitan Area (615), the southwest of WA (388), the Pilbara (115) and the Goldfields/Esperance region (92). In the southwest of WA, the greatest proportion of cases occurred in the Peel (208) region. The number of cases peaked in February in the Perth Metropolitan Area and in January in the southwest of WA. In the Pilbara and Goldfields/Esperance regions the majority of cases occurred in February and March. Cases of RRV disease were also reported in the Kimberley (86), Gascoyne (4), Midwest (57), Wheatbelt (60) and Great Southern (53) regions.

Barmah Forest virus disease

Just 36 cases of BFV disease were reported in WA from January to July 2014 (Table 2). The majority of cases were from the southwest of WA (15) and most of these were in the Peel region (8). No more than eight cases of BFV disease were reported from other regions of the state.

Table 1. Serologically confirmed doctor-notified and laboratory reported cases of Ross River virus disease each month in WA, July 2013 - June 2014#

#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			2	2	3	1	1	0	7	16	32	10	8	4	86
PILBARA			1	1	1	2	4	2	5	39	41	8	5	6	115
GASCOYNE			0	0	0	0	0	1	0	1	1	0	1	0	4
MIDWEST			3	1	0	2	3	5	4	19	15	1	2	2	57
WHEATBELT			1	0	0	5	11	11	10	5	6	4	4	3	60
PERTH METRO			18	16	15	39	19	58	94	122	103	55	35	41	615
	SOUTHWEST	*PEEL*	15	12	11	15	14	11	29	34	24	17	17	9	208
		LESCHENAULT	3	2	2	5	5	8	16	4	7	1	3	0	56
		CAPEL	0	0	2	2	17	12	5	3	2	2	2	0	47
		BUSSELTON	0	0	1	1	6	7	8	9	3	1	3	0	39
		ELSEWHERE SW	1	0	0	0	2	3	6	12	11	2	1	0	38
SOUTHWEST			19	14	16	23	44	41	64	62	47	23	26	9	388
GREAT SOUTHERN			2	1	2	2	1	6	6	14	13	0	4	2	53
GOLDFIELDS-ESPERANCE			3	4	7	15	8	7	5	17	17	5	1	3	92
WA UNDETERMINED			0	0	0	0	0	0	0	0	1	0	0	0	1
INTERSTATE			2	1	0	0	1	1	2	4	7	2	3	1	24
WA TOTAL (does not include interstate)			**49**	**39**	**44**	**89**	**91**	**131**	**195**	**295**	**276**	**106**	**86**	**70**	**1471**

1) Data current as at 09/07/2014 - 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

Table 2. Serologically confirmed doctor-notified and laboratory reported cases of Barmah Forest virus disease each month in WA, July 2013 - June 2014#

#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									1	2	1	1	1	0	6
PILBARA									0	0	0	1	0	0	1
GASCOYNE									0	0	0	0	0	0	0
MIDWEST									0	1	0	0	0	1	2
WHEATBELT									1	1	0	0	0	1	3
PERTH METRO									1	3	0	2	0	1	7
	SOUTHWEST	*PEEL*							3	0	1	3	0	1	8
		LESCHENAULT							2	0	1	0	0	0	3
		CAPEL							0	0	0	1	0	0	1
		BUSSELTON							0	0	0	0	0	0	0
		ELSEWHERE SW							0	2	0	1	0	0	3
SOUTHWEST									5	2	2	5	0	1	15
GREAT SOUTHERN									1	0	1	0	0	0	2
GOLDFIELDS-ESPERANCE									0	0	0	0	0	0	0
WA UNDETERMINED									0	0	0	0	0	0	0
INTERSTATE									0	1	0	0	0	0	1
WA TOTAL (does not include interstate)									**9**	**9**	**4**	**9**	**1**	**4**	**36**

1) Data current as at 09/07/2014 - 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 2013/14, one human sera tested KUNV antibody-positive. One possible non-encephalitis case of KUNV in a resident near Derby in June 2014 was investigated, however it did not meet the case definition due to declining antibody titres between the first and second serum samples (Dr David Speers, PathWest Laboratory Medicine WA, personal communication). No MVE cases were reported in WA during 2013/14 (Table 3).

Table 3. 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, Queensland Health 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 2013/14, 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 2013/14 season (Figure 1). Rainfall was generally above average in the southwest of WA between July and September 2013, and otherwise it was drier than usual (Figure 2A-D). In the Kimberley and Pilbara regions rainfall was below average between July and September 2013. Generally above average rainfall occurred from October 2013 to March 2014. BOM models suggest the chance of exceeding median rainfall between November 2014 and January 2015 is around 55% for the southwest of WA but drier than normal conditions are expected in the north. Warmer than usual day and night temperatures are likely across most of WA.

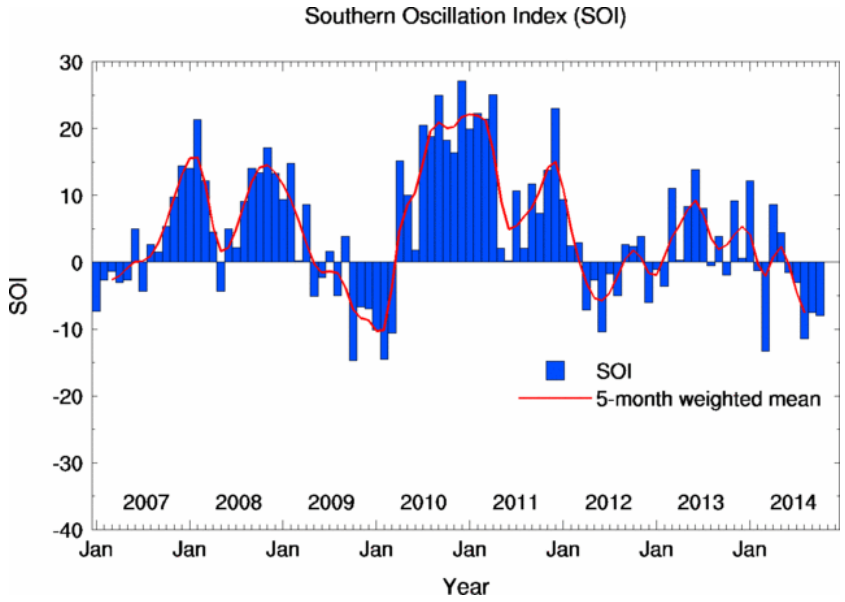

Figure 1. Southern Oscillation Index (SOI) 2007-2014 (source: Commonwealth Bureau of Meteorology http://www.bom.gov.au).

Weather data

Three-monthly summaries of rainfall trends throughout WA for the period July 2013 to June 2014 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.

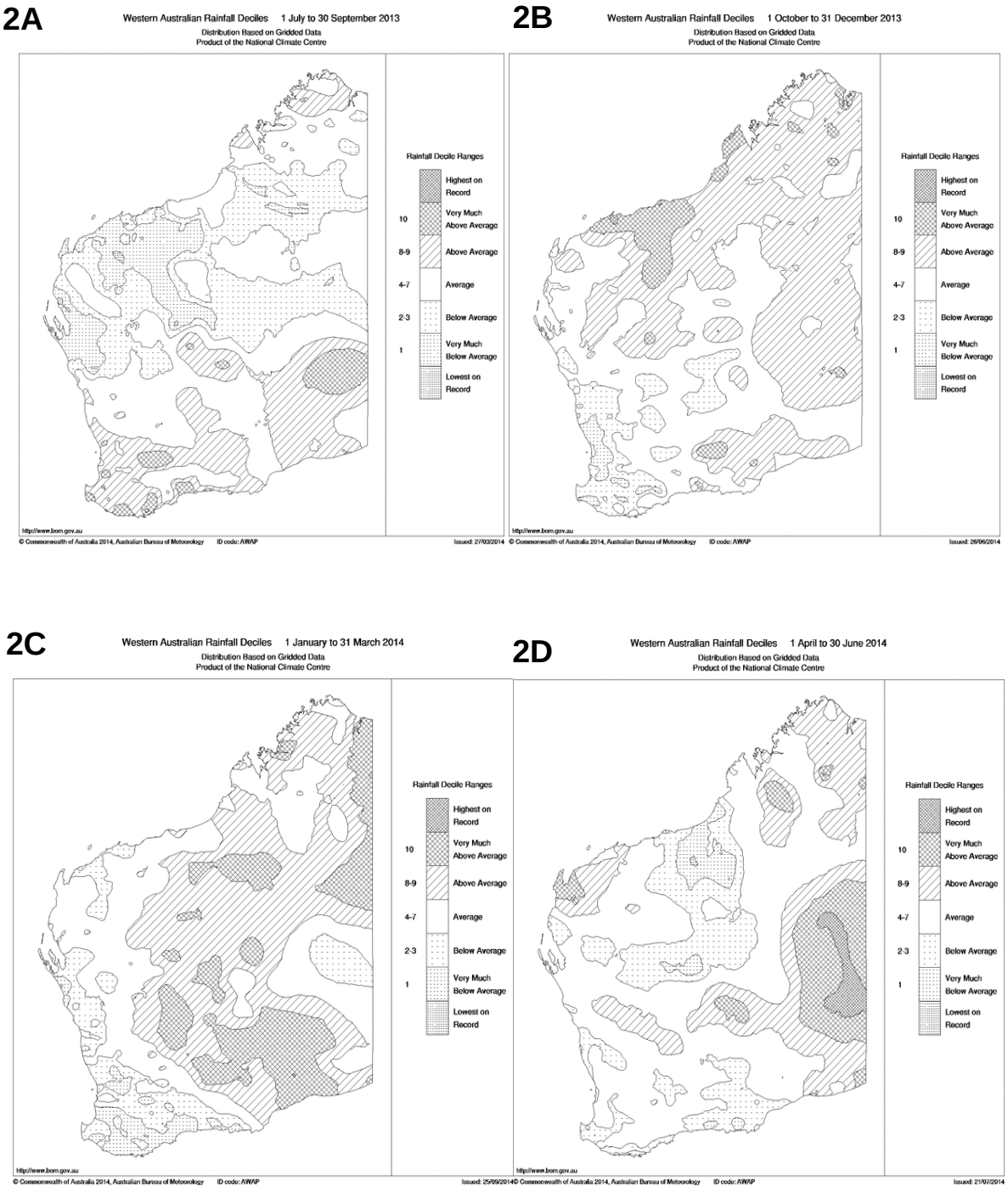

Figure 2A-D. Three-monthly summary of Western Australian rainfall deciles. A: July-September 2013; B: October-December 2013; C: January-March 2014; D: April-June 2014 (source: Commonwealth Bureau of Meteorology).

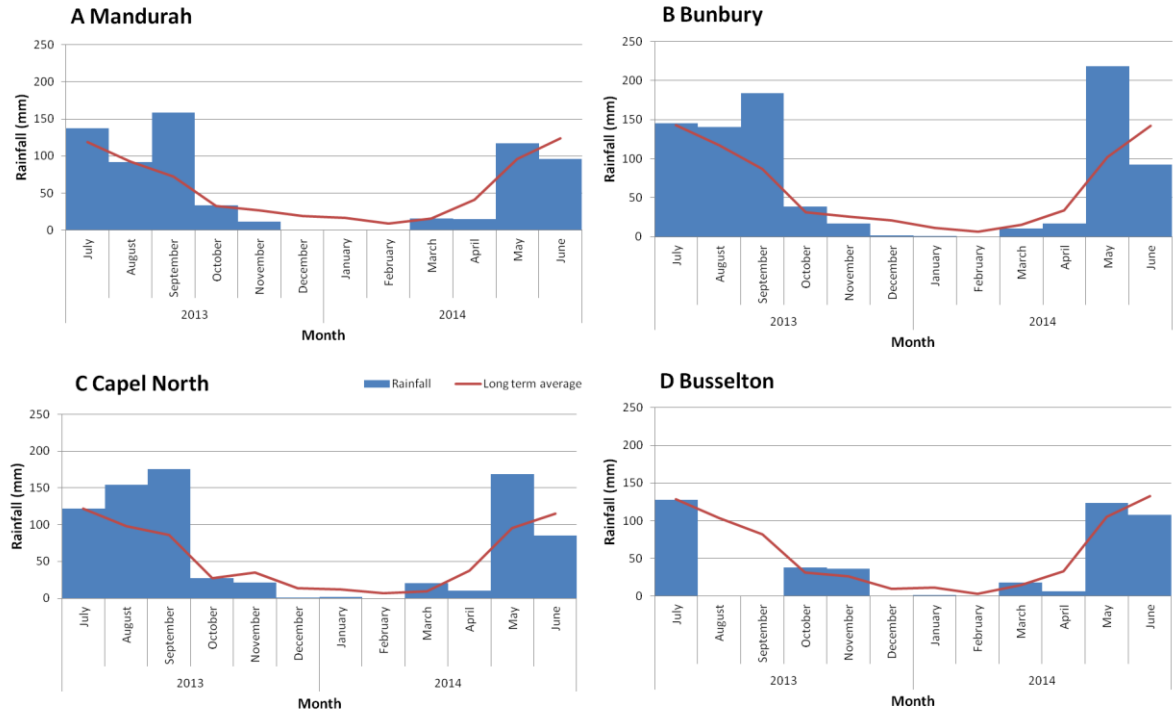

Figure 3A-D: Monthly rainfall (bars) compared to long term average (line) at Mandurah, Bunbury, Capel North and Busselton, July 2013 to June 2014. No data was available in August and September 2013 for Busselton (data sourced from the Bureau of Meteology).

Summary of rainfall events in WA, 2013/14

Northern WA rainfall (July 2013 to June 2014)

From July to September 2013 rainfall was below to very much below average throughout the Kimberley, Pilbara and Gascoyne regions. Mean maximum temperatures were above to very much above average through most of the region. A broad area extending from the Pilbara and adjacent Northern Interior saw above to very much above average mean minima in July 2013, as did parts of the east Kimberley. This trend continued through the west Gascoyne and west Pilbara in August 2013 however, below to very much below average mean minimum temperatures occurred in parts of the Kimberley. From October 2013 through until February 2014 the Kimberley and Pilbara regions experienced above to very much above average rainfall. The Gascoyne region experienced above average rainfall during November 2013 and January 2014. Very much above average temperatures were observed over northern WA throughout October, including some regions in the west Kimberley with highest on record observations. The Kimberley experienced typical thunderstorm activity during November and the passage of the first tropical cyclone (TC) for the season (TC *Alessia)* near the north Kimberley coast on the 23-24 November brought heavy falls to the region. The above average rainfall across northern WA in December was due to a weak low in the Timor Sea followed by TC *Christine* towards the end of month, which made landfall on the Pilbara coast between Whim Creek and Roebourne on 30 December. Some sites in the Pilbara received their highest daily rainfall in December on record on 31 December. Temperatures were below to very much below average throughout the Kimberley and Pilbara regions during December. A number of sites in the Kimberley, east Pilbara and east Gascoyne recorded their wettest January on record, mainly due to the passage of a deep tropical low and associated cloud band during the middle part of the month. Above to very much above average mean maxima were recorded in the west Kimberley, whilst below average mean maxima generally followed the passage of the tropical

low. An active monsoon in the first half of February brought some very heavy falls and below average temperatures. Kununurra Aerodrome, in the east Kimberley, recorded its wettest month in 41 years of records. Northern regions that were not affected by the monsoon, including parts of the Gascoyne and Pilbara, saw below to very much below average rainfall. Mean minimum temperatures were above to very much above average in the west Pilbara and Gascoyne. In March, monsoonal activity across northern parts of WA was below average, although TC *Gillian* reformed northwest of WA to bring some rainfall toward the end of the month. The general lack of rainfall across WA in March resulted in above to very much above average mean temperatures. Rainfall was above average in April over the west Pilbara, western and southern Gascoyne, and Kimberley regions. Sunny and warm weather was experienced for most of WA in the middle part of the month, but a middle level trough combined with tropical moisture associated with ex-TC *Jack* off the northwest coast produced an extensive cloud band with widespread rainfall over the west Pilbara and Gascoyne regions. Mean maxima were above to very much above across much of the northern half of WA. Rainfall was above average for large parts of northern WA in May. The east Kimberley and east Pilbara were exceptions to this, with average to below average rainfall experienced in these regions. Maximum temperatures were generally above average with the exception of western coastal areas, which experienced below average maxima. Rainfall in June was below to very much below average throughout northern WA.

Southwest WA rainfall (July 2013 to June 2014)

Rainfall and minimum temperatures were below to very much below average and above to very much above average mean maxima over the central Goldfields and much of the Southwest Land Division (SWLD) in July. A series of cold fronts caused heavy rain in the SWLD between 15-17 July. Flash flooding was reported in the southwest as rainfall totals in the 60-100 mm range were reported on 15 July. In August and September the SWLD recorded above to very much above average rainfall, with a number of locations in the Lower Southwest registering their wettest September on record. Very much above average mean maxima and minima were experienced across southern WA, with a few sites in the Lower West observing their warmest or equal warmest August on record, including the Perth Metropolitan area. Mean minima up to 3°C warmer than normal were recorded in the southern half of the SWLD. From October 2013 through to April 2014 rainfall was below to very much below average over much of southern WA. Mandurah had 105 consecutive rainless days from 8 December 2013 to 22 March 2014. Notable exceptions to the dry conditions included south-eastern WA in January and February 2014, when rainfall was above to very much above average. A number of sites in the Goldfields and Eucla regions recorded their wettest January on record, due to the passage of Ex-TC *Christine* early in the month and a deep tropical low during the latter part of the month bringing more heavy rainfall. A slow moving tropical low in February continued the above average rainfall in the region. Much of the SWLD experienced above to very much above average temperatures from October through until December 2013. A number of sites in the SWLD recorded their highest mean minima on record in November. During January and February 2014 mean minima were above to very much above average in the Central West, Central Wheat Belt, Lower West, and eastern Eucla. Below average mean maximum temperatures followed the passage of tropical lows in January and February in the Goldfields and Eucla regions. Tropical moisture associated with ex-TC *Jack* off the northwest coast produced widespread rainfall over the Goldfields and SWLD from 24-27 April, whilst a weak cold front brought light to moderate rainfall to western parts of the SWLD from 27-29 April. Despite this, the general lack of rainfall across southern WA in March and April meant that most of southern WA saw above to very much above average temperatures. Rainfall and temperatures were generally above average for much of southern WA. The monthly mean minima was in the highest 10% of records for May for the SWLD. Below to very much below average rainfall across the SWLD saw it record its sixth driest 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 Jay Nicholson. Tide heights at Fremantle (Fishing Boat Harbour) of approx. 110 cm 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). In 2013/14 tides were high enough to inundate large areas of salt marsh in the Peel region for most of the year except during summer (Figure 4A) (Severn and Ingle 2014). High tides of approx. 90 cm 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 2013/14, regular peak tides were sufficiently high to flood mosquito breeding sites in the area most of the year (Figure 4C).

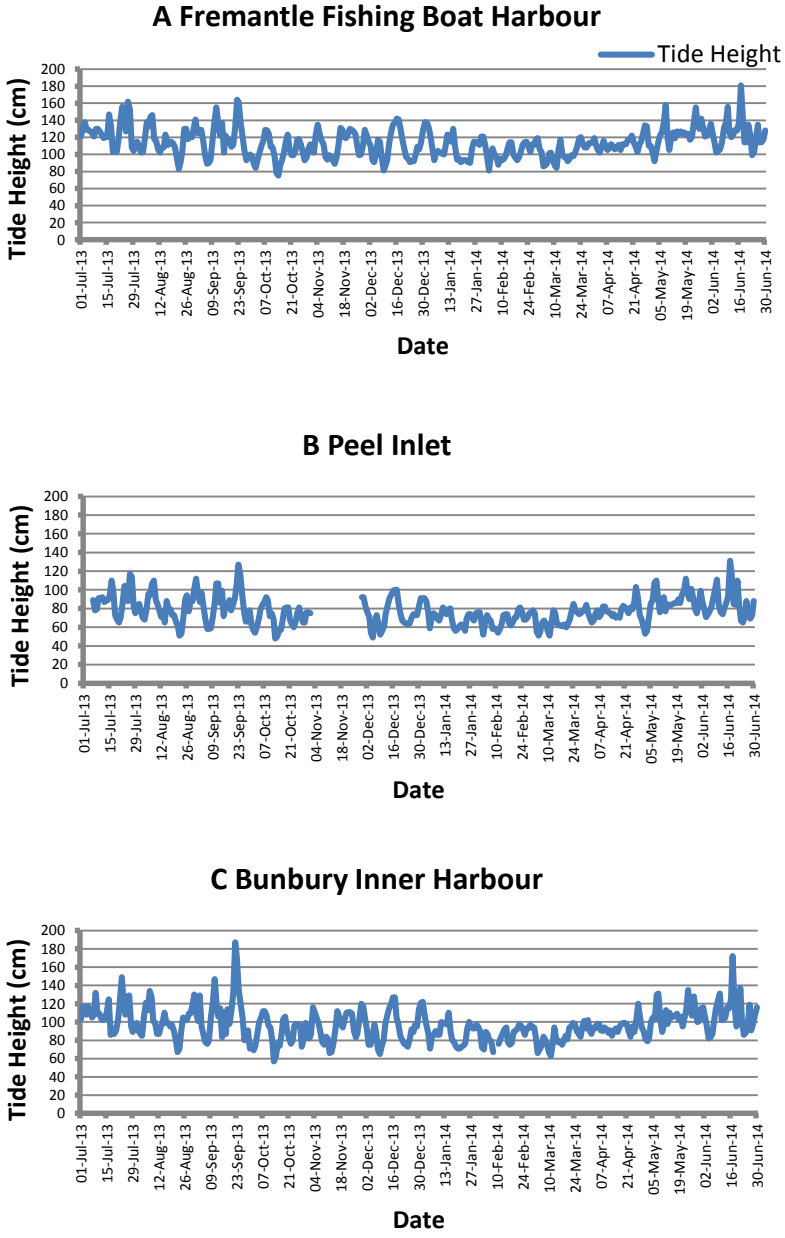

Figure 4A-C. Observed maximum daily tide heights (cm) at the Fremantle Fishing Boat Harbour, Peel Inlet and Bunbury Inner Harbour during 2013/14 (the Peel Inlet tide guage did not function from 1 July 2013 to 5 July 2013 and 2 November 2013 to 28 November 2013).

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 2013/14 season, mosquitoes collected from May to June 2014 were not processed for virus isolation. Mosquitoes collected in April 2014 were processed due to continued large numbers of cases of BFV disease notified from private diagnostic laboratories. In addition, mosquitoes collected between September 2013 and April 2014 were tested by virus isolation and molecular methods for arbovirus detection.

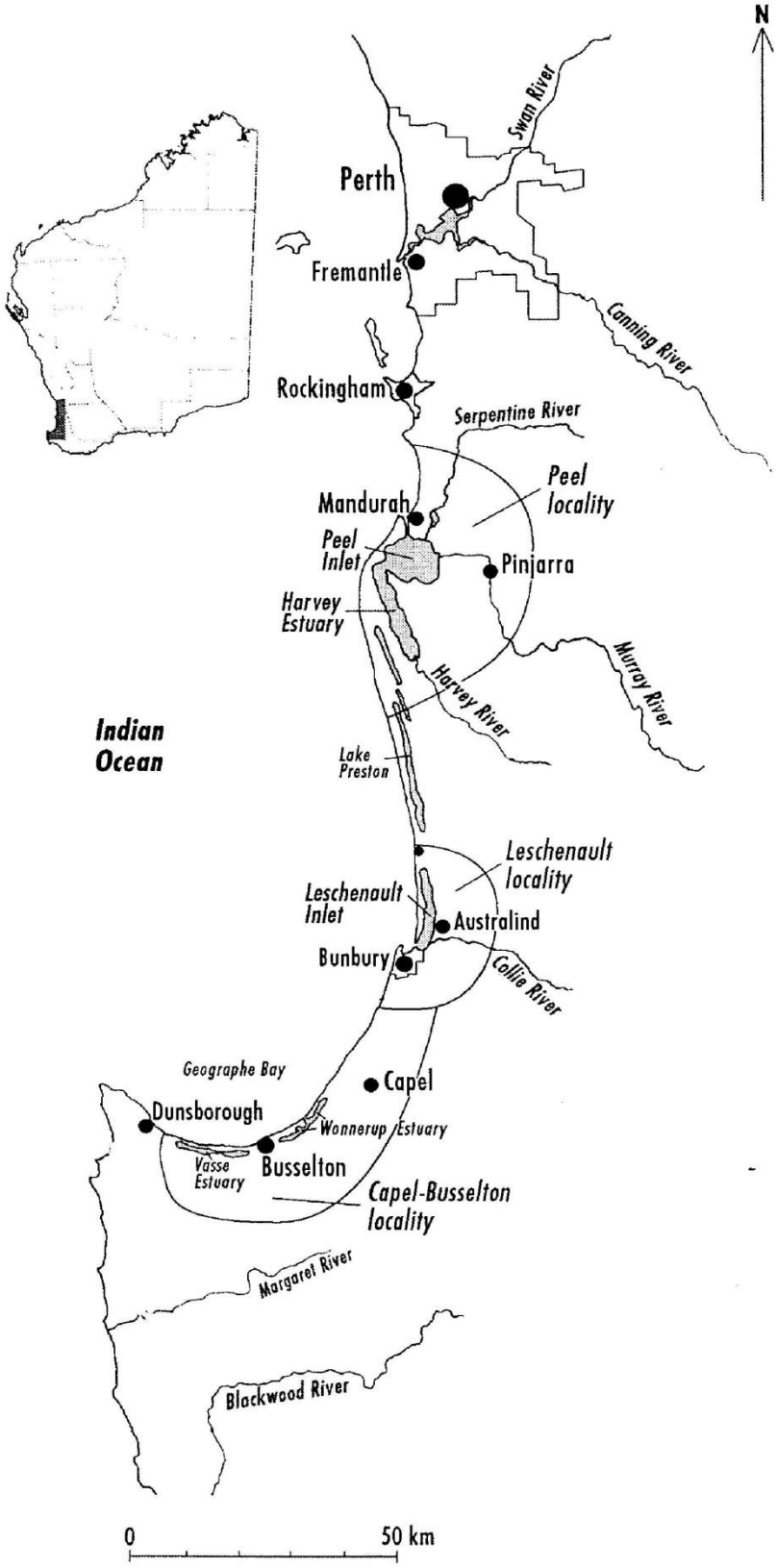

Figure 5. Location of the Peel, Leschenault, Capel and Busselton regions where prospective surveillance of mosquito populations and arbovirus activity is conducted in the southwest of WA (Lindsay, 1995).

Northern WA 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 ASRL 1997/98 Annual Report. 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. Mosquito collections were only conducted in the northeast Kimberley region (comprising Kununurra and Wyndham) in April 2014. Opportunistic sampling of adult mosquitoes was undertaken in the Pilbara region (Nullagine, Marble Bar and Port Hedland) following heavy flooding in the area in 2013. In 2013/14, the ASRL assisted with WA DOH mosquito surveys conducted around Point Samson and Wickham in the Pilbara region after a human case of dengue was diagnosed in a resident, and additional surveys were conducted by MBDC at Mt Magnet in 2014.

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. BG-Sentinel traps and ovitraps were also used at Point Samson and Wickham in 2013/14.

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 ≤ 20 for samples from the southwest or ≤ 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 detection unless more than 500 mosquitoes were obtained in a single trap, in which case the first 500 specimens were identified and processed for virus detection. In instances when many traps from the Swan Coastal Plain contained more than 500 mosquitoes, the first 350 specimens were processed. 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 they were not usually sent away for further identification.

Virus isolation and identification

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 cause obvious 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 tested using 9E8 (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 (Broom *et al*., 1998). This ELISA is capable of distinguishing between geographical variants of RRV (Lindsay *et al*., 1993, Oliveira *et al*., 1997, Sammels *et al*., 1995) and is used to screen all RRV isolates obtained from throughout WA, enabling determination of the virus strain involved in 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). If isolates could not be identified as alpha or flaviviruses by ELISA they were regrown for an extended period on C6/36 cells before passaging onto indicator cell lines (PSEK/Vero) and being re-tested. Isolates unable to be identified will be tested further 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 advising of increased risk of RRV transmission, and ensured local government authorities were aware of the risk of virus transmission in their areas.

One-step real-time reverse-transcriptase polymerase chain reaction

RNA extractions and one-step real-time RT-PCRs were conducted at PathWest Laboratory Medicine WA. RNA was purified from 200 µl of mosquito homogenates using a magnetic bead viral RNA isolation kit on an automated MagMAX Express-96. MS2 RNA coliphage was added to the lysis buffer to monitor extraction and reverse transcription efficiency and PCR inhibitors. Viral RNA was amplified using in-house primers and probes specific for RRV, BFV, MVEV and KUNV and MS2 using optimised PCR reagents in Rotorgene Q real-time thermocyclers and probe emission signals were acquired during the extension step of the program. Threshold cycle (Ct) scores <40 with good exponential curves indicated the presence of specific target sequence.

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.

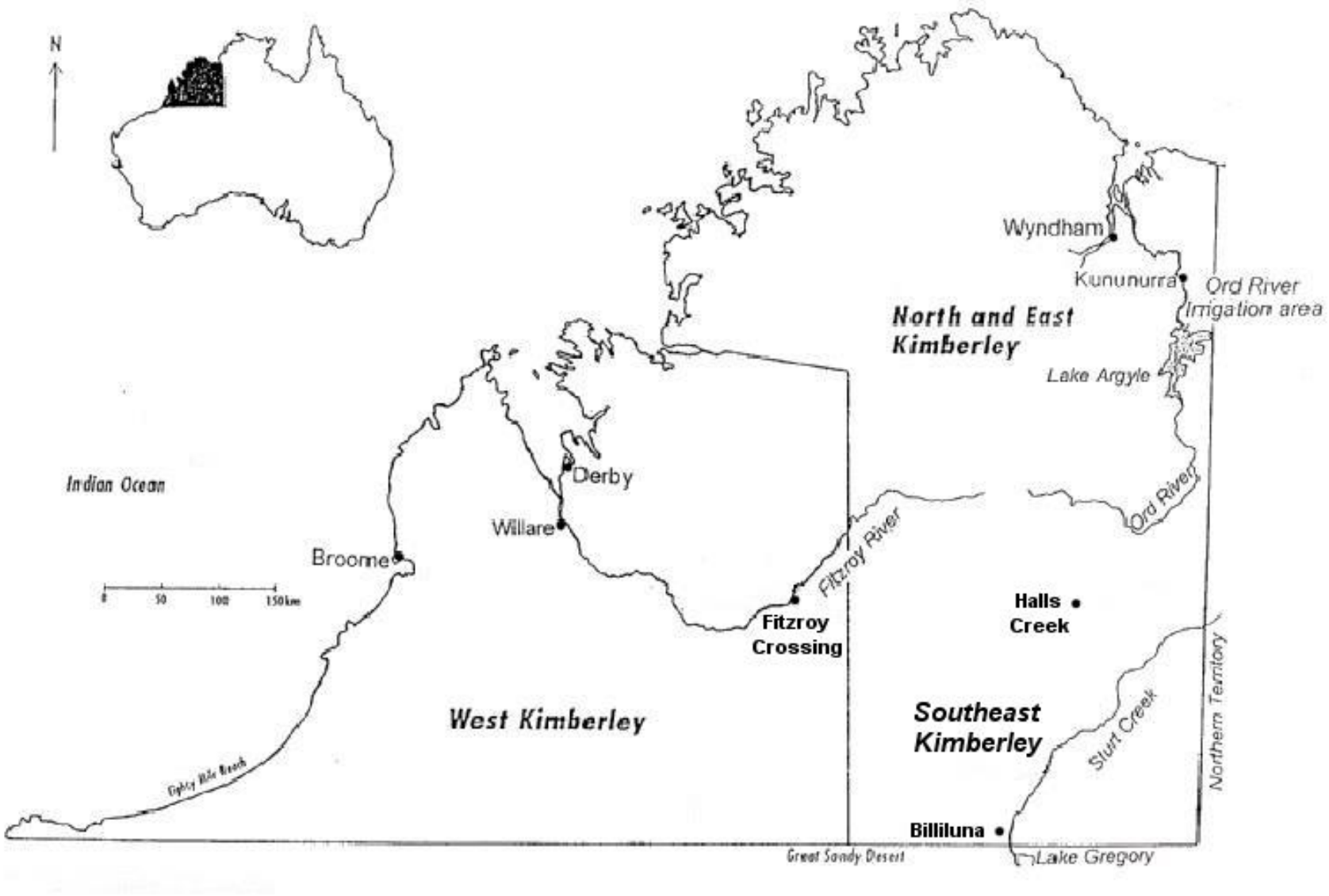

Figure 6. The locations where adult mosquito sampling was conducted in the Kimberley region (northern WA) (adapted from Lindsay, 1995)**.**

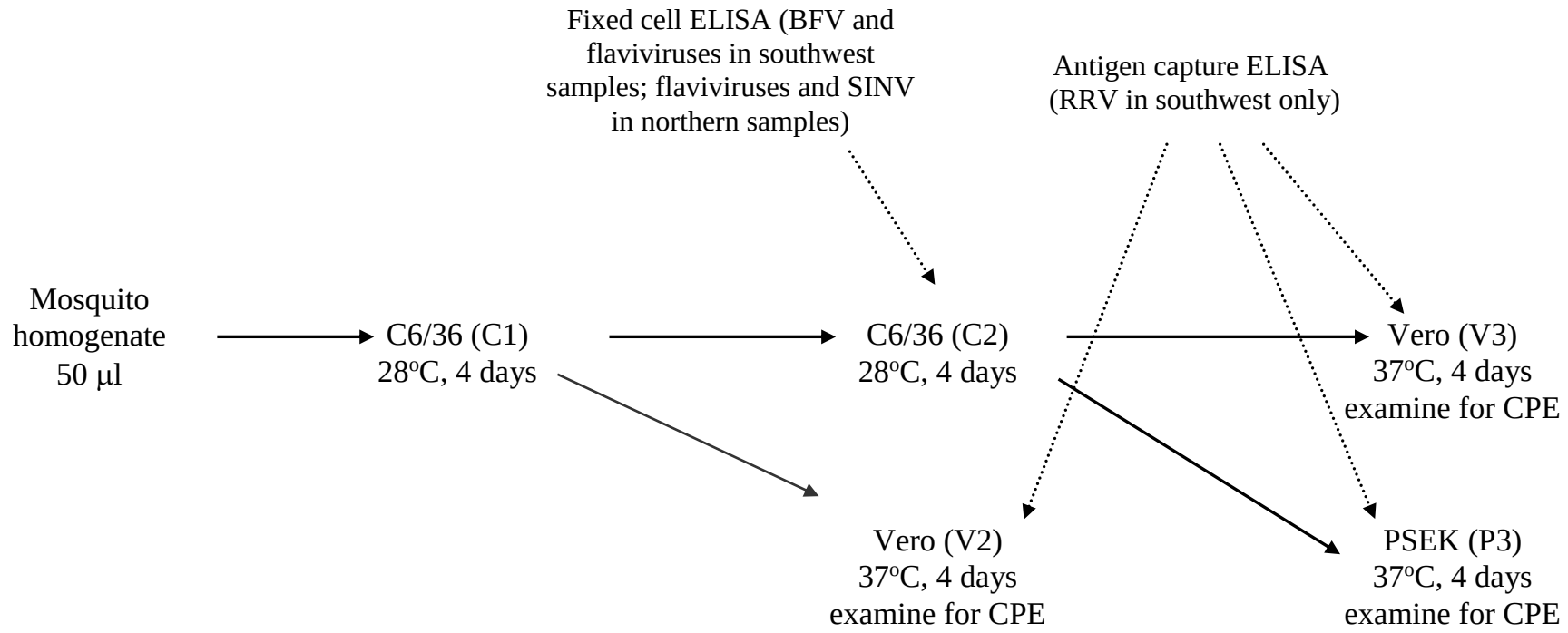

Figure 7. The cell culture virus isolation protocol (in 96 well cell culture plates) used to isolate virus from mosquitoes in the Arbovirus Surveillance and Research Laboratory. The volume of initial inoculum (sample) and passage volume from C6/36 cells onto vertebrate cell lines is always 50 µl.

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 May to June 2014 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 2013 and June 2014 (Table 4). A total of 207 traps were set with a 94.2% success rate. A total of 40013 mosquitoes comprising 15 species were collected from Peel Inlet sites, 18122 (45.3%) of which were processed for virus isolation (Table 5). The most abundant species collected were *Ae. camptorhynchus* (87.1%) and *Cx. globocoxitus* (5.1%). *Ae. vigilax* comprised just 2.4% of the overall population, compared with 53.4% the previous season (ASRL 2012/13 Annual Report). Vector mosquito numbers were greater than the previous season in August to November, facilitated by warmer and wetter conditions in Spring. Mosquito abundance then declined to very low levels during the warmer months (Figure 8), largely due to minimal impact of high tides and rainfall on saltmarsh breeding sites. A single detection of RRV was recorded from *Ae. clelandi* collected at Lake Goegrup on 1 October 2013 (Table 6), and the overall MIR for this species was 144.7 per 1000 mosquitoes (Table 5). There were three detections of BFV from *Ae. camptorhynchus* collected at Riverside Gardens on 29 October (Table 5 and 6), when the MIR was 1.4 per 1000 mosquitoes (Figure 8). Of the 10907 adult mosquitoes (comprising 15 species) collected from Harvey Estuary sites in 2013/14, 6020 (55.2%) were processed for virus isolation (Table 7). The most abundant species were *Ae. camptorhynchus* (77.0%), *An. annulipes* s.l. (5.8%) and *Cx. globocoxitus* (5.7%), and there was a large decline in the *Ae. vigilax* population from 43.4% in 2012/13 to 2.3% in 2013/14. Overall mosquito abundance was greater than 2012/13 between June and December 2013, however abundance was very low during January to May 2014 (Figure 9). There were three detections of BFV from *Ae. camptorhynchus* collected in October, November and December 2013 (Table 6), and the MIR peaked at 2.2 per 1000 mosquitoes (Figure 9).

A large number of cases of RRV disease were notified to the WA DOH between July 2013 and June 2014, mostly occurring between January and March 2014 (Table 1). The incidence of RRV disease does not reflect the low levels of RRV activity observed in mosquitoes. Indeed, some concerns have been raised that the incidence of RRV disease may have been overestimated in some regions, possibly due to problems with the commercial enzyme immunoassays used to diagnose RRV infections in some private laboratories (Selvey et al. 2014). Just eight cases of BFV disease were notified to WA DOH between January and June 2014 (Table 2).

An additional nine possible arbovirus isolates were obtained from mosquitoes collected in the Peel region that were not a recognised alphavirus or flavivirus and additional studies are required to confirm the presence and identity of an unknown arbovirus (results not shown).

Table 4. Summary of the adult mosquito trapping effort (including successful and failed traps) for the mosquito and arbovirus surveillance program in the Peel region, July 2013 to June 2014.[1]

Region	Locality	Date	Successful	Failed	Total
Peel	Peel Inlet	Wednesday, July 17, 2013	5	2	7
"	"	Tuesday, August 06, 2013	7		7
"	"	Tuesday, August 20, 2013	7		7
"	"	Tuesday, September 03, 2013	7		7
"	"	Tuesday, September 17, 2013	7		7
"	"	Tuesday, October 01, 2013	7		7
"	"	Tuesday, October 15, 2013	7		7
"	"	Tuesday, October 29, 2013	7		7
"	"	Tuesday, November 12, 2013	7		7
"	"	Tuesday, November 26, 2013	6	1	7
"	"	Tuesday, December 10, 2013	5	2	7
"	"	Monday, December 23, 2013	7		7
"	"	Tuesday, January 07, 2014	7		7
"	"	Tuesday, January 21, 2014	7		7
"	"	Tuesday, February 04, 2014	6	1	7
"	"	Tuesday, February 18, 2014	6	1	7
"	"	Tuesday, March 04, 2014	6	1	7
"	"	Tuesday, March 18, 2014	7		7
"	"	Tuesday, April 01, 2014	7		7
"	"	Tuesday, April 15, 2014	6	1	7
"	"	Tuesday, April 29, 2014	7		7
"	"	Tuesday, May 27, 2014	7		7
"	"	Tuesday, June 24, 2014	6	1	7
"	Harvey Estuary	Wednesday, July 17, 2013	2		2
"	"	Tuesday, August 06, 2013	2		2
"	"	Tuesday, August 20, 2013	2		2
"	"	Tuesday, September 03, 2013	2		2
"	"	Tuesday, September 17, 2013	2		2
"	"	Tuesday, October 01, 2013	2		2
"	"	Tuesday, October 15, 2013	2		2
"	"	Tuesday, October 29, 2013	1	1	2
"	"	Tuesday, November 12, 2013	2		2
"	"	Tuesday, November 26, 2013	2		2
"	"	Tuesday, December 10, 2013	2		2
"	"	Monday, December 23, 2013	2		2
"	"	Tuesday, January 07, 2014	1	1	2
"	"	Tuesday, January 21, 2014	2		2
"	"	Tuesday, February 04, 2014	2		2
"	"	Tuesday, February 18, 2014	2		2
"	"	Tuesday, March 04, 2014	2		2
"	"	Tuesday, March 18, 2014	2		2
"	"	Tuesday, April 01, 2014	2		2
"	"	Tuesday, April 15, 2014	2		2
"	"	Tuesday, April 29, 2014	2		2
"	"	Tuesday, May 27, 2014	2		2
"	"	Tuesday, June 24, 2014	2		2
Total			**195**	**12**	**207**
		% Successful =	**94.2**		

[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 5. Details of mosquitoes collected and processed for virus detection, Peel Inlet sites, southwest of Western Australia, 1 July 2013 to 30 June 2014.[1]

Species	Class	Total	(%)	Processed	Pools	Pinned	RRV	(MIR)	BFV	(MIR)
Ae. (Finlaya) alboannulatus	Female	450	(1.1)	364	76	0				
Ae. (Finlaya) notoscriptus	Female	335	(0.8)	226	60	0				
Ae. (Ochlerotatus) camptorhynchus	Bloodfed	78	(0.2)	0	0	0				
Ae. (Ochlerotatus) camptorhynchus	Female	34832	(87.1)	14754	799	0			3	(0.2)
Ae. (Ochlerotatus) camptorhynchus	Hermaphrodite	2	(<0.1)	0	0	1				
Ae. (Ochlerotatus) camptorhynchus	Male	2	(<0.1)	2	2	0				
Ae. (Ochlerotatus) camptorhynchus variant	Female	2	(<0.1)	2	1	0				
Ae. (Ochlerotatus) clelandi	Female	20	(<0.1)	7	6	0	1	(144.7)		
Ae. (Ochlerotatus) hesperonotius	Female	68	(0.2)	20	8	0				
Ae. (Ochlerotatus) vigilax	Female	972	(2.4)	918	94	0				
Ae. (Ochlerotatus) vigilax	Male	7	(<0.1)	7	2	0				
Ae. species (unidentified) - new or difficult to ID species	Male	18	(<0.1)	12	6	0				
An. (Anopheles) atratipes	Female	1	(<0.1)	1	1	0				
An. (Cellia) annulipes s.l.	Female	101	(0.3)	83	28	0				
Cq. (Coquillettidia) species near *linealis*	Female	3	(<0.1)	3	2	0				
Cs. (Culicella) atra	Female	28	(0.1)	9	7	0				
Cx. (Culex) annulirostris	Female	23	(0.1)	20	14	0				
Cx. (Culex) australicus	Bloodfed	2	(<0.1)	0	0	0				
Cx. (Culex) australicus	Female	408	(1.0)	189	32	0				
Cx. (Culex) globocoxitus	Bloodfed	2	(<0.1)	0	0	0				
Cx. (Culex) globocoxitus	Female	2112	(5.3)	1142	113	0				
Cx. (Culex) globocoxitus	Male	2	(<0.1)	1	1	0				
Cx. (Culex) quinquefasciatus	Bloodfed	3	(<0.1)	0	0	0				
Cx. (Culex) quinquefasciatus	Female	432	(1.1)	317	66	0				
Cx. species (unidentified) - new or difficult to ID species	Male	31	(0.1)	27	12	0				
Tripteroides (Polypleidomyia) atripes	Female	2	(<0.1)	2	2	0				
Unidentifiable (too damaged/features missing)	Female	21	(0.1)	1	1	0				
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	53	(0.1)	12	3	0				
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	3	(<0.1)	3	3	0				
Total		**40013**	**(100.0)**	**18122**	**1339**	**1**	**1**	**(0.1)**	**3**	**(0.2)**

[1]Mosquitoes collected in May and June 2014 were not processed for virus detection; RRV is Ross River virus, MIR is minimum infection rate per 1000 mosquitoes (Chiang and Reeves 1962), BFV is Barmah Forest virus.

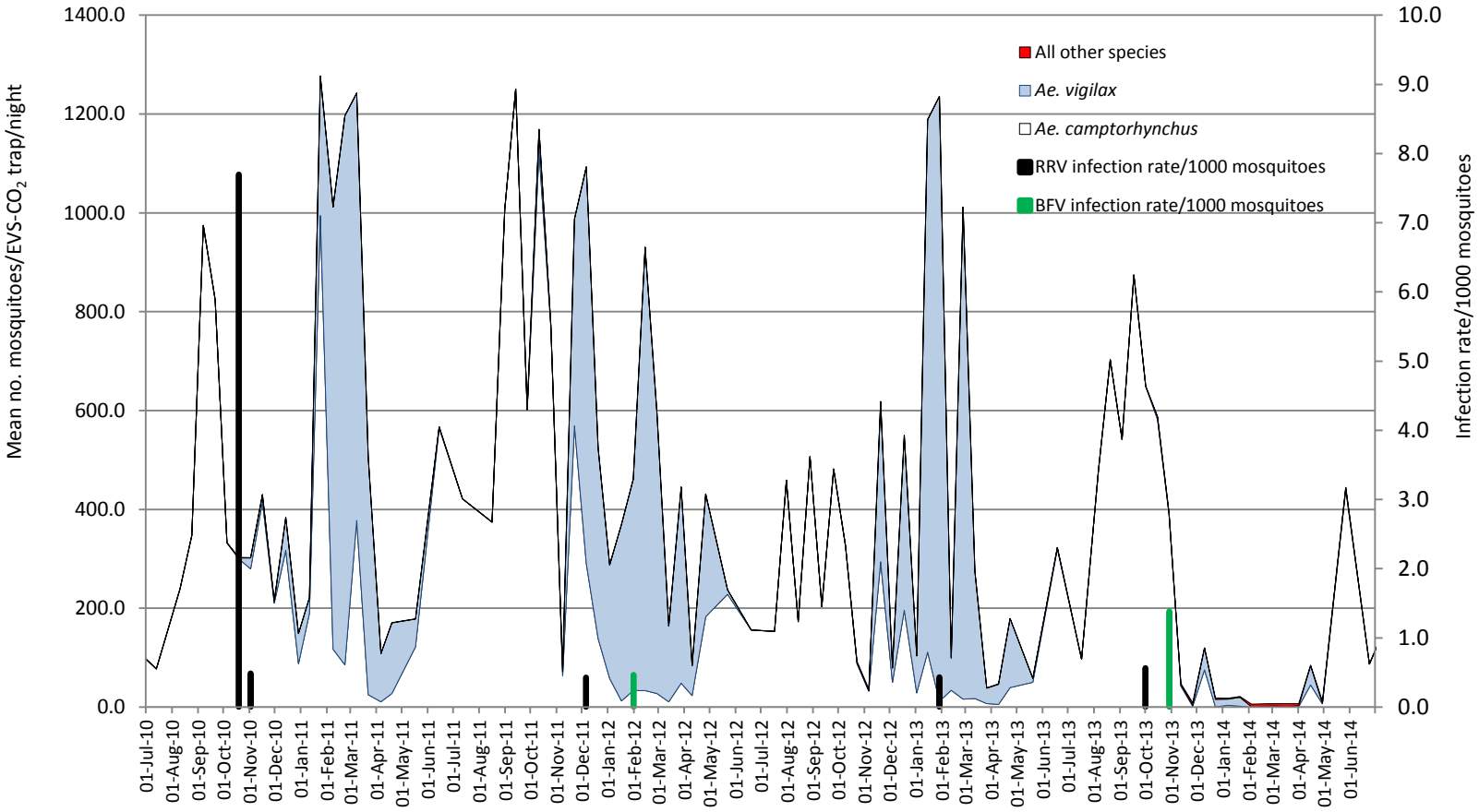

Figure 8. Abundance of adult mosquitoes and their infection rates (all species) with RRV and BFV, Peel Inlet sites, 1 July 2010 to 30 June 2014.

Table 6. Details of viruses detected in mosquitoes collected during the mosquito/arbovirus surveillance program on the Swan Coastal Plain, southwest of Western Australia, 1 July 2013 to 30 June 2014.[1]

Region	Isolate no.	Date	Locality	Trap location	Species	Class	No. in pool	Virus ID[3]
Peel								
	DC57568	1-Oct-13	Mandurah	Lake Goegrup, Mandurah	*Ae. clelandi*	F	1	RRV (PCR only)
	DC57908	29-Oct-13	"	Riverside Gardens, Mandurah	*Ae. camptorhynchus*	F	20	BFV
	DC57910	"	"	"	"	F	20	BFV
	DC57911	"	"	"	"	F	20	BFV
	DC58064	"	Waroona	400 m north of NFS Research Station, Harvey Estuary	"	F	20	BFV
	DC58220	26-Nov-13	"	"	"	F	20	BFV
	DC58315	10-Dec-13	"	"	"	F	16	BFV
Leschenault								
	SW97160	12-Nov-13	Harvey	Belvidere, Buffalo Rd, west side of Leschenault Inlet	*Ae. camptorhynchus*	F	20	NE RRV
	SW97167	"	"	"	"	F	20	NE RRV
	SW97173	"	"	Freshwater larval site, Buffalo Rd, N end of Leschenault Inlet	"	F	20	NE RRV
	SW97174	"	"	"	"	F	20	NE RRV
	SW97176	"	"	"	"	F	20	NE RRV
	SW97178	"	"	"	"	F	20	RRV (PCR only)
	SW97516	10-Dec-13	"	Belvidere, Buffalo Rd, west side of Leschenault Inlet	*Ae. vigilax*	F	20	BFV
	SW97836	04-Feb-14	Dardanup	Pratt Rd swamp, Eaton	*Ae. alboannulatus*	F	20	BFV
Capel								
	SW96520	01-Oct-13	Capel	Intersection of Stirling and Higgins Rds, Ludlow Forest	*Ae. camptorhynchus*	F	20	NE RRV
	SW96521	"	"	"	"	F	20	NE RRV
	SW96524	"	"	"	"	F	20	NE RRV
	SW96525	"	"	"	"	F	20	NE RRV
	SW96526	"	"	"	"	F	20	NE RRV
	SW96536	"	"	"	*Cx. globocoxitus*	F	20	NE RRV
	SW96538	"	"	"	*Culex* species	M	4	NE RRV
	SW96539	"	"	"	*An. annulipes*	F	20	NE RRV
	SW97317	12-Nov-13	"	CALM village, Ludlow Rd North	*Ae. camptorhynchus*	F	20	NE RRV
	SW97414	26-Nov-13	"	Lot 112 Woods Road	"	F	20	NE RRV
	SW97579	10-Dec-13	"	"	"	F	20	BFV
Busselton								
	SW97892	18-Feb-14	Dunsborough	Quindalup, Toby Inlet, West Busselton	*Ae. alboannulatus*	F	20	NE RRV

[1]ID is identity, RRV is Ross River virus, PCR is detection by polymerase chain reaction, BFV is Barmah Forest virus, NE is northern/eastern phenotype of RRV.

Table 7. Details of mosquitoes collected and processed for virus detection, Harvey Estuary sites, southwest of Western Australia, 1 July 2013 to 30 June 2014.[1]

Species	Class	Total	(%)	Processed	Pools	BFV	(MIR)
Ae. (Finlaya) alboannulatus	Bloodfed	5	(<0.1)	0	0		
Ae. (Finlaya) alboannulatus	Female	229	(2.1)	171	27		
Ae. (Finlaya) notoscriptus	Female	276	(2.5)	165	24		
Ae. (Ochlerotatus) camptorhynchus	Bloodfed	132	(1.2)	0	0		
Ae. (Ochlerotatus) camptorhynchus	Female	8396	(77.0)	4375	233	3	(0.7)
Ae. (Ochlerotatus) camptorhynchus	Male	2	(<0.1)	2	1		
Ae. (Ochlerotatus) clelandi	Female	14	(0.1)	7	4		
Ae. (Ochlerotatus) hesperonotius	Female	17	(0.2)	7	3		
Ae. (Ochlerotatus) ratcliffei	Female	25	(0.2)	9	3		
Ae. (Ochlerotatus) vigilax	Female	255	(2.3)	233	24		
An. (Anopheles) atratipes	Female	6	(0.1)	3	3		
An. (Cellia) annulipes s.l.	Female	628	(5.8)	507	40		
An. (Cellia) annulipes s.l.	Male	17	(0.2)	17	1		
Cq. (Coquillettidia) species near *linealis*	Female	14	(0.1)	13	8		
Cs. (Culicella) atra	Female	5	(<0.1)	2	1		
Cx. (Culex) annulirostris	Female	32	(0.3)	18	8		
Cx. (Culex) australicus	Bloodfed	2	(<0.1)	0	0		
Cx. (Culex) australicus	Female	92	(0.8)	52	10		
Cx. (Culex) globocoxitus	Bloodfed	8	(0.1)	0	0		
Cx. (Culex) globocoxitus	Female	620	(5.7)	370	37		
Cx. (Culex) globocoxitus	Male	9	(0.1)	5	3		
Cx. (Culex) quinquefasciatus	Female	2	(<0.1)	2	2		
Cx. species (unidentified) - new or difficult to ID species	Male	53	(0.5)	37	8		
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	41	(0.4)	15	2		
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	27	(0.2)	10	2		
Total		**10907**	**(100.0)**	**6020**	**444**	**3**	**(0.5)**

[1]Mosquitoes collected in May and June 2014 were not processed for virus detection, no mosquitoes were pinned; BFV is Barmah Forest virus, MIR is minimum infection rate per 1000 mosquitoes (Chiang and Reeves 1962)

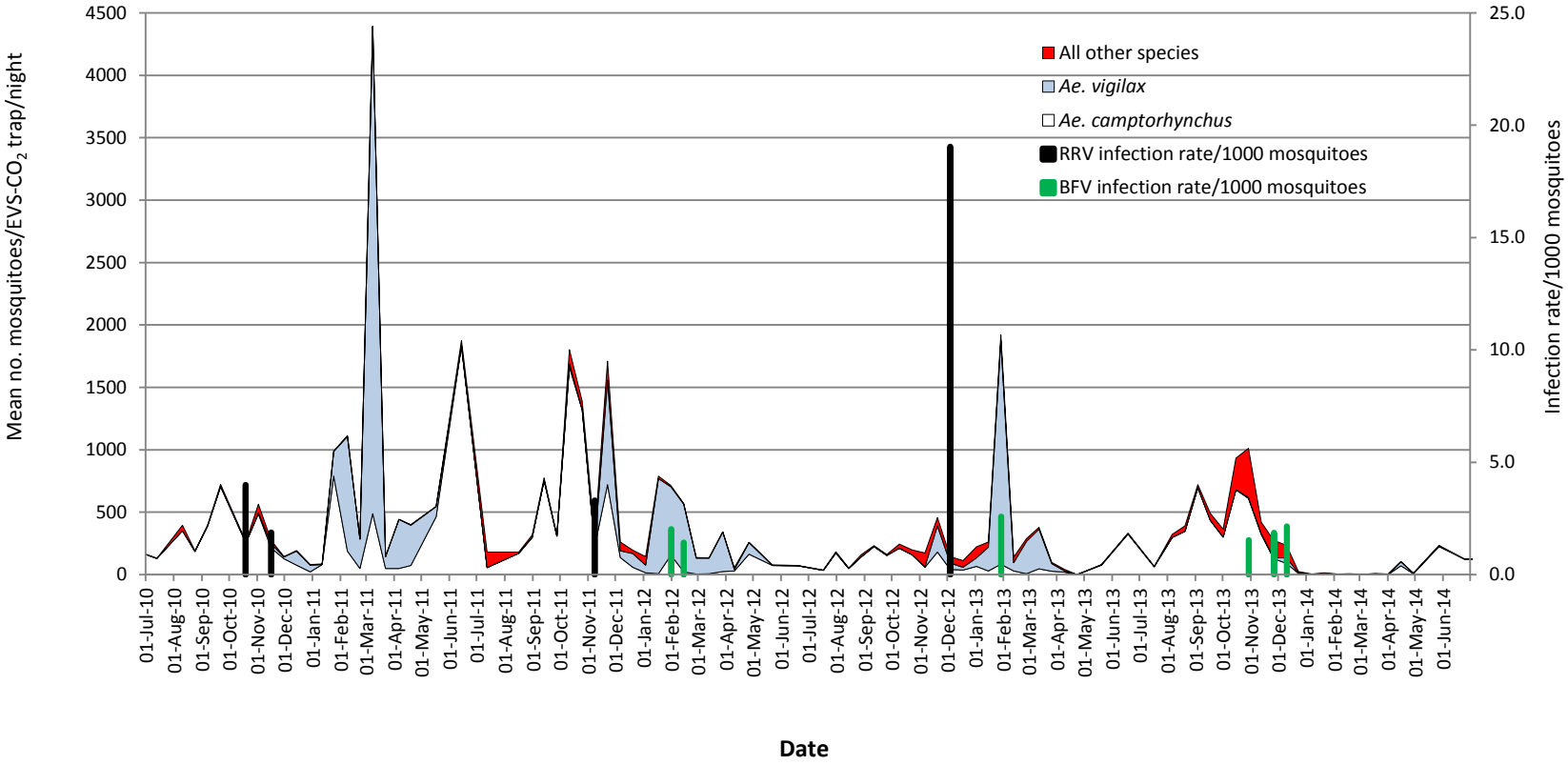

Figure 9. Abundance of adult mosquitoes and their infection rates (all species) with RRV and BFV, Harvey Estuary sites, 1 July 2010 - 30 June 2014.

Leschenault region

A total of 138 traps were set during 23 adult mosquito collection trips in the Leschenault region between July 2013 and June 2014, with successful outcomes for 98.6% of all traps set (Table 8). A total of 40929 mosquitoes belonging to 18 species were collected during the year, 17130 (41.8%) of which were processed for virus isolation (Table 9). The most abundant species collected were *Ae. camptorhynchus* (68.4%) and *Cx. globocoxitus* (10.2%). Vector mosquito abundance was high between September and November 2013, reducing substantially in December and remaining low through to May 2014 (Figure 10) due to reduced impact of tides and rainfall on mosquito breeding sites. There were eight confirmed arbovirus detections from the Leschenault region during the 2013/14 season, including six isolates of RRV (five were of the northern/eastern phenotype and one was detected only by PCR and was not typed further) and two isolates of BFV. All of the RRV detections were from *Ae. camptorhynchus*, whilst *Ae. vigilax* yielded one isolate of BFV and *Ae. alboannulatus* yielded the other BFV isolate. The MIR of mosquitoes for RRV reached 3.0 per 1000 mosquitoes on 12 November 2013, and the peak infection rate for BFV was 8.0 per 1000 mosquitoes on 4 February 2014 (Figure 10). The majority of RRV and BFV detections were from mosquitoes collected along the western and eastern side of the Leschenault Inlet (Belvidere and Freshwater larval site), the exception being one detection of BFV at the Pratt Road swamp collection site in Eaton (Table 6). There were also 37 possible arbovirus detections that were not a recognised alphavirus or flavivirus and further work is required to confirm the presence and identity of an arbovirus in these samples (results not shown). The number of cases of RRV disease (Table 1) in the Leschenault region in 2013/14 (56) was lower than the previous season (103) (ASRL 2012/13 Annual Report). Three cases of BFV were recorded in the Leschenault region between January and June 2014 (Table 2).

Table 8. Summary of the adult mosquito trapping effort (including successful and failed traps) for the mosquito and arbovirus surveillance program in the Leschenault region, July 2013 to June 2014.[1]

Region	Date	Successful	Failed	Total
Leschenault	Wednesday, July 17, 2013	6		6
"	Tuesday, August 06, 2013	6		6
"	Tuesday, August 20, 2013	5	1	6
"	Tuesday, September 03, 2013	6		6
"	Tuesday, September 17, 2013	6		6
"	Tuesday, October 01, 2013	6		6
"	Tuesday, October 15, 2013	6		6
"	Tuesday, October 29, 2013	6		6
"	Tuesday, November 12, 2013	6		6
"	Tuesday, November 26, 2013	6		6
"	Tuesday, December 10, 2013	6		6
"	Monday, December 23, 2013	6		6
"	Tuesday, January 07, 2014	6		6
"	Tuesday, January 21, 2014	6		6
"	Tuesday, February 04, 2014	6		6
"	Tuesday, February 18, 2014	6		6
"	Tuesday, March 04, 2014	6		6
"	Tuesday, March 18, 2014	5	1	6
"	Tuesday, April 01, 2014	6		6
"	Tuesday, April 15, 2014	6		6
"	Tuesday, April 29, 2014	6		6
"	Tuesday, May 27, 2014	6		6
"	Tuesday, June 24, 2014	6		6
Total		**136**	**2**	**138**
	% Successful =	**98.6**		

[1]Only results from successful traps were used to calculate mean mosquito populations/trap/night.

Table 9. Details of mosquitoes collected and processed for virus detection, Leschenault sites, southwest of Western Australia, 1 July 2013 to 30 June 2014.[1]

Species	Class	Total	(%)	Processed	Pools	Pinned	RRV	(MIR)	BFV	(MIR)
Ae. (Finlaya) alboannulatus	Bloodfed	14	(<0.1)	0	0	0				
Ae. (Finlaya) alboannulatus	Female	1347	(3.3)	1045	104	0			1	(1.0)
Ae. (Finlaya) notoscriptus	Bloodfed	1	(<0.1)	0	0	0				
Ae. (Finlaya) notoscriptus	Female	188	(0.5)	111	38	0				
Ae. (Ochlerotatus) camptorhynchus	Bloodfed	262	(0.6)	0	0	0				
Ae. (Ochlerotatus) camptorhynchus	Female	28000	(68.4)	10621	565	0	6	(0.6)		
Ae. (Ochlerotatus) camptorhynchus	Male	5	(<0.1)	2	2	0				
Ae. (Ochlerotatus) camptorhynchus variant	Female	2	(<0.1)	0	0	1				
Ae. (Ochlerotatus) clelandi	Bloodfed	3	(<0.1)	0	0	0				
Ae. (Ochlerotatus) clelandi	Female	918	(2.2)	221	26	0				
Ae. (Ochlerotatus) hesperonotius	Bloodfed	7	(<0.1)	0	0	0				
Ae. (Ochlerotatus) hesperonotius	Female	2623	(6.4)	869	49	0				
Ae. (Ochlerotatus) nigrithorax	Female	11	(<0.1)	6	2	0				
Ae. (Ochlerotatus) nigrithorax	Male	2	(<0.1)	1	1	0				
Ae. (Ochlerotatus) ratcliffei	Female	4	(<0.1)	1	1	0				
Ae. (Ochlerotatus) stricklandi	Female	1	(<0.1)	1	1	0				
Ae. (Ochlerotatus) turneri	Female	16	(<0.1)	4	3	0				
Ae. (Ochlerotatus) vigilax	Bloodfed	3	(<0.1)	0	0	0				
Ae. (Ochlerotatus) vigilax	Female	611	(1.5)	533	53	1			1	(1.9)
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	3	(<0.1)	2	1	0				
Ae. species (unidentified) - new or difficult to ID species	Male	6	(<0.1)	4	3	0				
An. (Anopheles) atratipes	Female	2	(<0.1)	0	0	0				
An. (Cellia) annulipes s.l.	Female	257	(0.6)	114	36	0				
Cq. (Coquillettidia) species near *linealis*	Bloodfed	2	(<0.1)	0	0	0				
Cq. (Coquillettidia) species near *linealis*	Female	633	(1.5)	623	58	0				
Cs. (Culicella) atra	Female	96	(0.2)	53	16	0				
Cx. (Culex) annulirostris	Bloodfed	1	(<0.1)	0	0	0				
Cx. (Culex) annulirostris	Female	58	(0.1)	54	23	0				
Cx. (Culex) australicus	Female	1292	(3.2)	538	61	0				
Cx. (Culex) globocoxitus	Bloodfed	10	(<0.1)	0	0	0				
Cx. (Culex) globocoxitus	Female	4155	(10.2)	2196	158	0				
Cx. (Culex) globocoxitus	Male	8	(<0.1)	2	2	0				
Cx. (Culex) quinquefasciatus	Bloodfed	1	(<0.1)	0	0	0				
Cx. (Culex) quinquefasciatus	Female	226	(0.6)	69	28	0				
Cx. species (unidentified) - new or difficult to ID species	Male	63	(0.2)	22	12	0				
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	63	(0.2)	19	5	0				
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	28	(0.1)	18	5	0				
Unidentifiable *Culex* sp. (too damaged/features missing)	Male	7	(<0.1)	1	1	0				
Total		**40929**	**(100.0)**	**17130**	**1254**	**2**	**6**	**(0.4)**	**2**	**(0.1)**

[1]Mosquitoes collected in May and June 2014 were not processed for virus detection; RRV is Ross River virus, MIR is minimum infection rate per 1000 mosquitoes (Chiang and Reeves 1962), BFV is Barmah Forest virus.

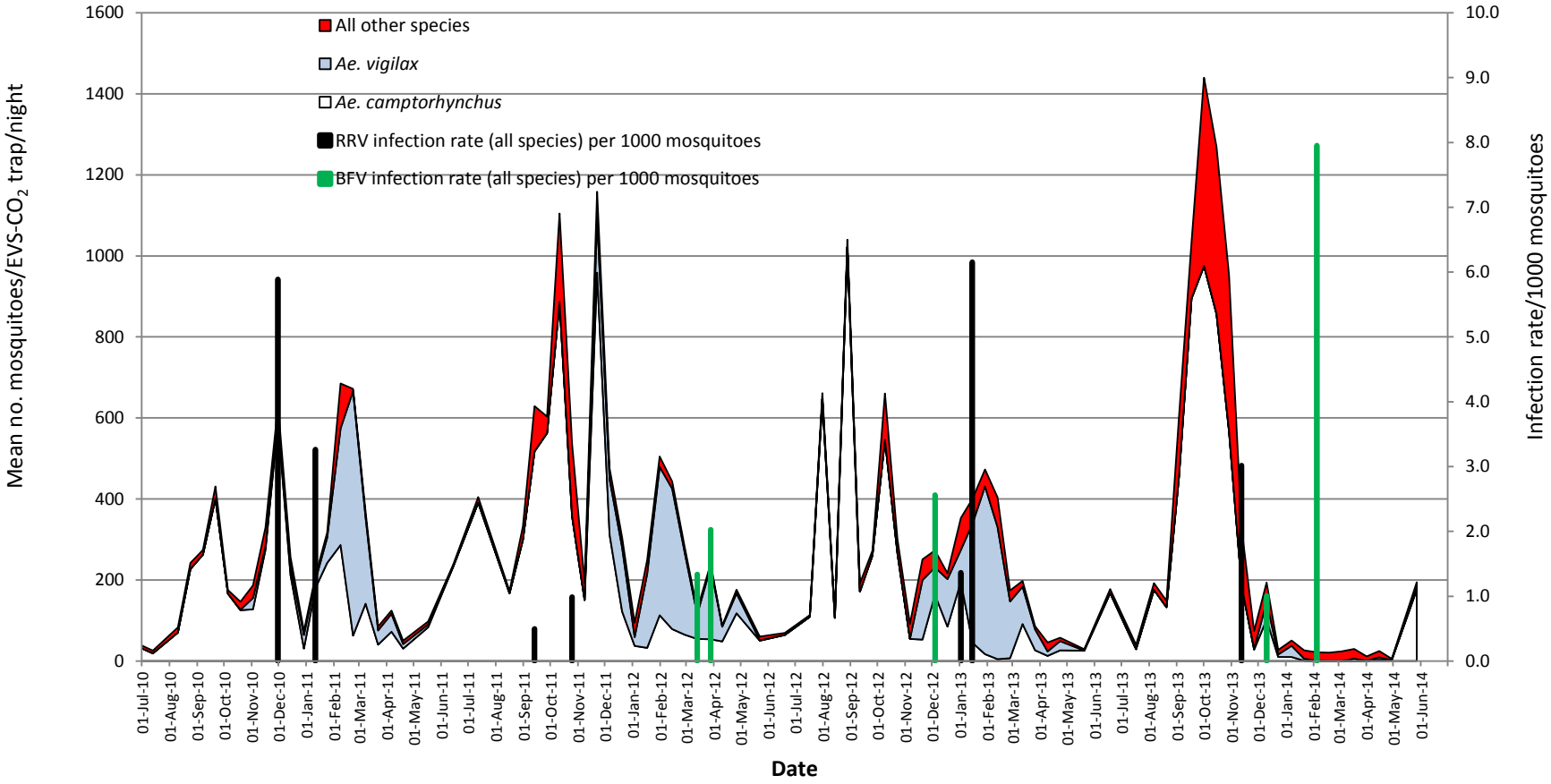

Figure 10. Abundance of adult mosquitoes and their infection rates (all species) with RRV and BFV, Leschenault Inlet sites, 1 July 2010 - 30 June 2014.

Capel-Busselton region

Of 138 traps set during 23 trap runs to collect adult mosquitoes at Capel forest sites and Busselton wetlands sites, 94.9% were successful (Table 10). A total of 35627 adult mosquitoes were collected from Capel forest sites between July 2013 and June 2014, 10372 (29.1%) of which were processed for virus isolation (Table 11). The most abundant mosquito species collected was *Ae. camptorhynchus* (86.5%). *Aedes camptorhynchus* populations were greater than the previous season between June and November (ASRL 2012/13 Annual Report), however abundance declined to very low from December through to June 2014 (Figure 11). Ten RRV detections were observed in Capel forest sites, predominantly from *Ae. camptorhynchus*, however single RRV detections were reported in *An. annulipes* s.l., *Cx. globocoxitus* and one pool of four *Cx. globocoxitus* males (Tables 6 and 11). The MIR was greatest (7.7 per 1000 mosquitoes) on 1 October 2013 (Figure 11). The detection of RRV in male *Cx. globocoxitus* is most likely to be due to contamination of the mosquito pool of an infected limb from a female mosquito. There was also one detection of BFV in *Ae. camptorhynchus* on 10 December 2013, and the MIR was 3.2 per 1000 mosquitoes (Table 6, Figure 11). The isolation of RRV from mosquitoes collected at the CALM Village trap site on 1 October 2013 was the first arbovirus isolation for the season, and detections continued through to 10 December 2013.

A total of 16113 adult mosquitoes were collected at Busselton wetlands sites, 7897 (49.0%) of which were processed for virus isolation (Table 12). *Aedes camptorhynchus* (39.5%), *Cx. globocoxitus* (31.4%) and *An. annulipes* s.l. (8.6%) were the most abundant mosquitoes collected. Mosquito abundance was greater than the previous season (ASRL 2012/13 Annual Report), however abundance of the vector *Ae. camptorhynchus* was low during the warmer months (Figure 12). There was a single detection of BFV in *Ae. alboannulatus* collected at Quindalup on 18 February 2014, with a MIR of 6.8 per 1000 mosquitoes (Table 6, Figure 12).

There were also 19 possible detections of arboviruses that were not known alphaviruses or flaviviruses (results not shown). Additional work is required on these possible arbovirus detections to confirm the presence and identity of arboviruses in these samples.

A total of 47 and 39 cases of RRV disease were reported from Capel and Busselton, respectively, in 2013/14 (Table 1), mostly between November 2013 and February 2014. The number of cases in Capel and Busselton was substantially greater than the previous season (ASRL 2012/13 Annual Report). A media release was issued by the WA DOH on 21 October 2013 following eight detections of RRV in mosquitoes collected in the Capel region on 1 October 2013. The media release warned of the increased risk of mosquito-borne disease and advised people in the southwest of the increased risk of RRV disease. A single case of BFV in Capel was reported to the WA DOH and no cases of BFV disease were reported from Busselton between January and June 2014 (Table 2).

Table 10. 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 2013 to June 2014.[1]

Region	Locality	Date	Successful	Failed	Total
Capel-Busselton	Capel (forest)	Wednesday, July 17, 2013	3		3
"	"	Tuesday, August 06, 2013	3		3
"	"	Tuesday, August 20, 2013	3		3
"	"	Tuesday, September 03, 2013	3		3
"	"	Tuesday, September 17, 2013	3		3
"	"	Tuesday, October 01, 2013	3		3
"	"	Tuesday, October 15, 2013	3		3
"	"	Tuesday, October 29, 2013	3		3
"	"	Tuesday, November 12, 2013	3		3
"	"	Tuesday, November 26, 2013	3		3
"	"	Tuesday, December 10, 2013	3		3
"	"	Monday, December 23, 2013	3		3
"	"	Tuesday, January 07, 2014	3		3
"	"	Tuesday, January 21, 2014	3		3
"	"	Tuesday, February 04, 2014	2	1	3
"	"	Tuesday, February 18, 2014	2	1	3
"	"	Tuesday, March 04, 2014	2	1	3
"	"	Tuesday, March 18, 2014	3		3
"	"	Tuesday, April 01, 2014	3		3
"	"	Tuesday, April 15, 2014	3		3
"	"	Tuesday, April 29, 2014	3		3
"	"	Tuesday, May 27, 2014	3		3
"	"	Tuesday, June 24, 2014	3		3
"	Busselton (wetlands)	Wednesday, July 17, 2013	3		3
"	"	Tuesday, August 06, 2013	3		3
"	"	Tuesday, August 20, 2013	3		3
"	"	Tuesday, September 03, 2013	1	2	3
"	"	Tuesday, September 17, 2013	2	1	3
"	"	Tuesday, October 01, 2013	3		3
"	"	Tuesday, October 15, 2013	3		3
"	"	Tuesday, October 29, 2013	3		3
"	"	Tuesday, November 12, 2013	3		3
"	"	Tuesday, November 26, 2013	3		3
"	"	Tuesday, December 10, 2013	2	1	3
"	"	Monday, December 23, 2013	3		3
"	"	Tuesday, January 07, 2014	3		3
"	"	Tuesday, January 21, 2014	3		3
"	"	Tuesday, February 04, 2014	3		3
"	"	Tuesday, February 18, 2014	3		3
"	"	Tuesday, March 04, 2014	3		3
"	"	Tuesday, March 18, 2014	3		3
"	"	Tuesday, April 01, 2014	3		3
"	"	Tuesday, April 15, 2014	3		3
"	"	Tuesday, April 29, 2014	3		3
"	"	Tuesday, May 27, 2014	3		3
"	"	Tuesday, June 24, 2014	3		3
Total			**131**	**7**	**138**
		% Successful =	**94.9**		

[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 11. Details of mosquitoes collected and processed for virus detection, Capel forest sites, southest of Western Australia, 1 July 2013 to 30 June 2014.[1]

Species	Class	Total	(%)	Processed	Pools	RRV	(MIR)	BFV	(MIR)
Ae. (Finlaya) alboannulatus	Bloodfed	8	(<0.1)	0	0				
Ae. (Finlaya) alboannulatus	Female	421	(1.2)	215	45				
Ae. (Finlaya) mallochi	Female	3	(<0.1)	3	2				
Ae. (Finlaya) notoscriptus	Bloodfed	1	(<0.1)	0	0				
Ae. (Finlaya) notoscriptus	Female	48	(0.1)	36	13				
Ae. (Ochlerotatus) camptorhynchus	Bloodfed	367	(1.0)	0	0				
Ae. (Ochlerotatus) camptorhynchus	Female	30822	(86.5)	8603	449	7	(0.8)	1	(0.1)
Ae. (Ochlerotatus) camptorhynchus	Male	5	(<0.1)	2	2				
Ae. (Ochlerotatus) camptorhynchus variant	Female	5	(<0.1)	1	1				
Ae. (Ochlerotatus) clelandi	Female	11	(<0.1)	2	1				
Ae. (Ochlerotatus) hesperonotius	Female	3	(<0.1)	2	2				
Ae. (Ochlerotatus) ratcliffei	Female	738	(2.1)	186	15				
Ae. (Ochlerotatus) turneri	Female	18	(0.1)	3	2				
Ae. species (unidentified) - new or difficult to ID species	Male	6	(<0.1)	2	2				
An. (Anopheles) atratipes	Female	1	(<0.1)	1	1				
An. (Cellia) annulipes s.l.	Bloodfed	5	(<0.1)	0	0				
An. (Cellia) annulipes s.l.	Female	712	(2.0)	289	44	1	(3.5)		
An. (Cellia) annulipes s.l.	Male	1	(<0.1)	1	1				
Cq. (Coquillettidia) species near *linealis*	Female	23	(0.1)	22	10				
Cs. (Culicella) atra	Female	20	(0.1)	7	7				
Cx. (Culex) annulirostris	Female	121	(0.3)	112	24				
Cx. (Culex) australicus	Bloodfed	2	(<0.1)	0	0				
Cx. (Culex) australicus	Female	234	(0.7)	89	15				
Cx. (Culex) globocoxitus	Bloodfed	2	(<0.1)	0	0				
Cx. (Culex) globocoxitus	Female	1914	(5.4)	735	62	1	(1.4)		
Cx. (Culex) globocoxitus	Male	23	(0.1)	11	3				
Cx. (Culex) quinquefasciatus	Female	25	(0.1)	6	5				
Cx. species (unidentified) - new or difficult to ID species	Male	79	(0.2)	39	11	1	(26.5)		
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	5	(<0.1)	2	1				
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	4	(<0.1)	3	1				
Total		**35627**	**(100.0)**	**10372**	**719**	**10**	**(1.0)**	**1**	**(0.1)**

[1]Mosquitoes collected in May and June 2014 were not processed for virus isolation; RRV is Ross River virus, MIR is minimum infection rate per 1000 mosquitoes (Chiang and Reeves 1962), BFV is Barmah Forest virus.

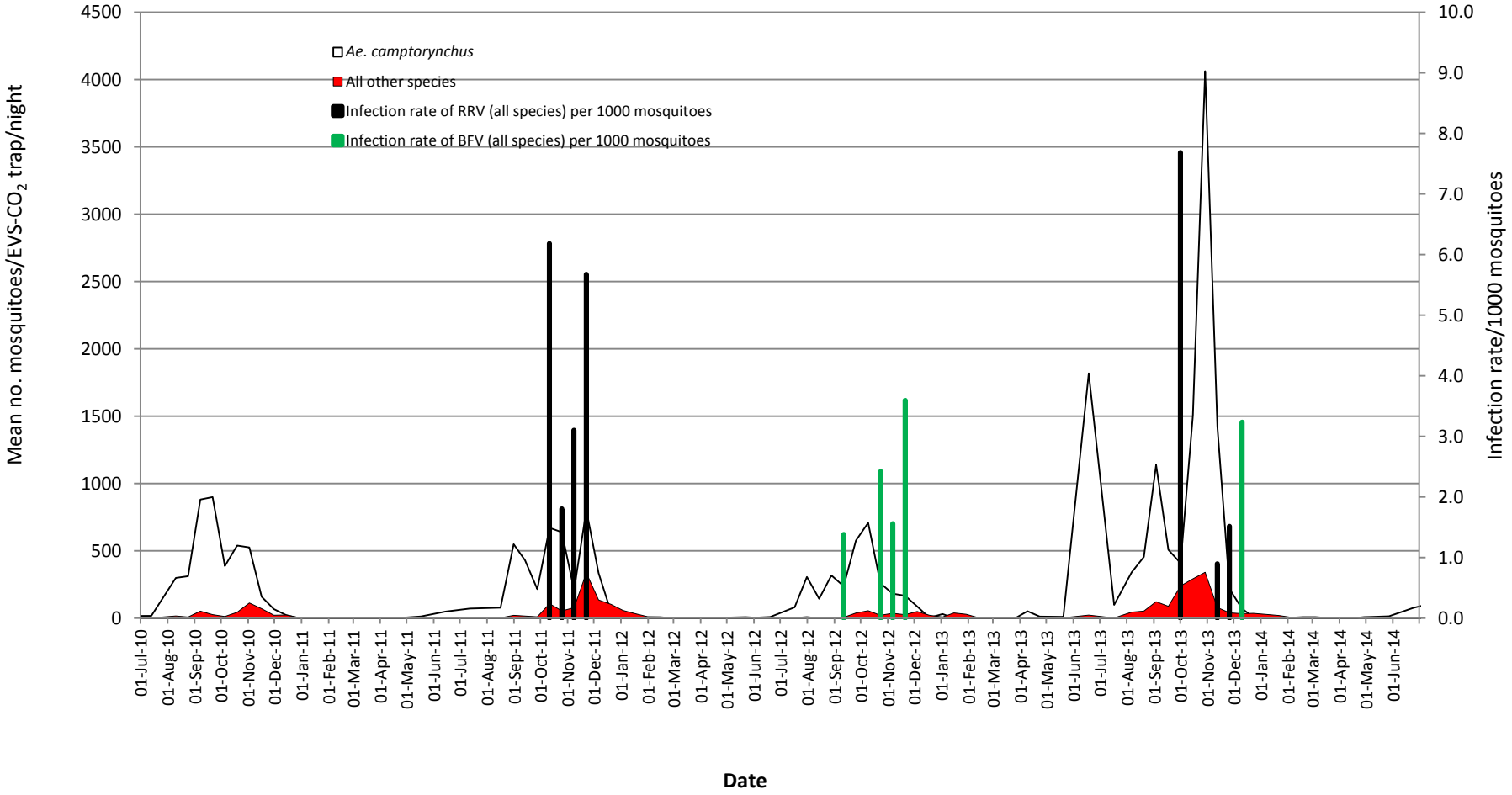

Figure 11. Abundance of adult mosquitoes and their infection rate (all species) with RRV and BFV, Capel forest sites, 01 July 2010 - 30 June 2014.

Table 12. Details of mosquitoes collected and processed for virus detection, Busselton wetland sites, southwest of Western Australia, 1 July 2013 to 30 June 2014.[1]

Species	Class	Total	(%)	Processed	Pools	Pinned	RRV	(MIR)
Ae. (Finlaya) alboannulatus	Bloodfed	45	(0.3)	0	0	0		
Ae. (Finlaya) alboannulatus	Female	1031	(6.4)	1002	81	0	1	(1.0)
Ae. (Finlaya) mallochi	Female	1	(<0.1)	1	1	0		
Ae. (Finlaya) notoscriptus	Bloodfed	2	(<0.1)	0	0	0		
Ae. (Finlaya) notoscriptus	Female	55	(0.3)	44	14	0		
Ae. (Ochlerotatus) camptorhynchus	Bloodfed	120	(0.7)	0	0	0		
Ae. (Ochlerotatus) camptorhynchus	Female	6368	(39.5)	2915	161	0		
Ae. (Ochlerotatus) camptorhynchus variant	Female	1	(<0.1)	1	1	0		
Ae. (Ochlerotatus) clelandi	Bloodfed	7	(<0.1)	0	0	0		
Ae. (Ochlerotatus) clelandi	Female	559	(3.5)	376	26	0		
Ae. (Ochlerotatus) hesperonotius	Female	260	(1.6)	181	19	0		
Ae. (Ochlerotatus) nigrithorax	Female	1	(<0.1)	1	1	0		
Ae. (Ochlerotatus) ratcliffei	Female	12	(0.1)	8	2	0		
Ae. (Ochlerotatus) turneri	Female	7	(<0.1)	3	1	0		
Ae. species (unidentified) - new or difficult to ID species	Male	2	(<0.1)	1	1	0		
An. (Anopheles) atratipes	Female	1	(<0.1)	1	1	0		
An. (Cellia) annulipes s.l.	Female	1382	(8.6)	599	46	5		
An. (Cellia) annulipes s.l.	Male	4	(<0.1)	1	1	0		
Cq. (Coquillettidia) species near *linealis*	Female	1	(<0.1)	1	1	0		
Cs. (Culicella) atra	Female	33	(0.2)	16	7	0		
Cx. (Culex) annulirostris	Female	11	(0.1)	11	8	0		
Cx. (Culex) australicus	Bloodfed	2	(<0.1)	0	0	0		
Cx. (Culex) australicus	Female	512	(3.2)	284	28	0		
Cx. (Culex) australicus variant	Female	1	(<0.1)	0	0	1		
Cx. (Culex) globocoxitus	Bloodfed	6	(<0.1)	0	0	0		
Cx. (Culex) globocoxitus	Female	5057	(31.4)	2146	136	0		
Cx. (Culex) globocoxitus	Male	10	(0.1)	10	3	0		
Cx. (Culex) quinquefasciatus	Female	60	(0.4)	33	12	0		
Cx. (Neoculex) latus	Female	6	(<0.1)	1	1	0		
Cx. species (unidentified) - new or difficult to ID species	Male	495	(3.1)	234	21	0		
Unidentifiable *Aedes* sp. (too damaged/features missing)	Bloodfed	4	(<0.1)	0	0	0		
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	9	(0.1)	6	3	0		
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	2	(<0.1)	0	0	0		
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	46	(0.3)	21	7	0		
Total		**16113**	**(100.0)**	**7897**	**583**	**6**	**1**	**(0.1)**

[1]Mosquitoes collected in May and June 2014 were not processed for virus detection, RRV is Ross River virus, MIR is minimum infection rate per 1000 mosquitoes (Chiang and Reeves 1962).

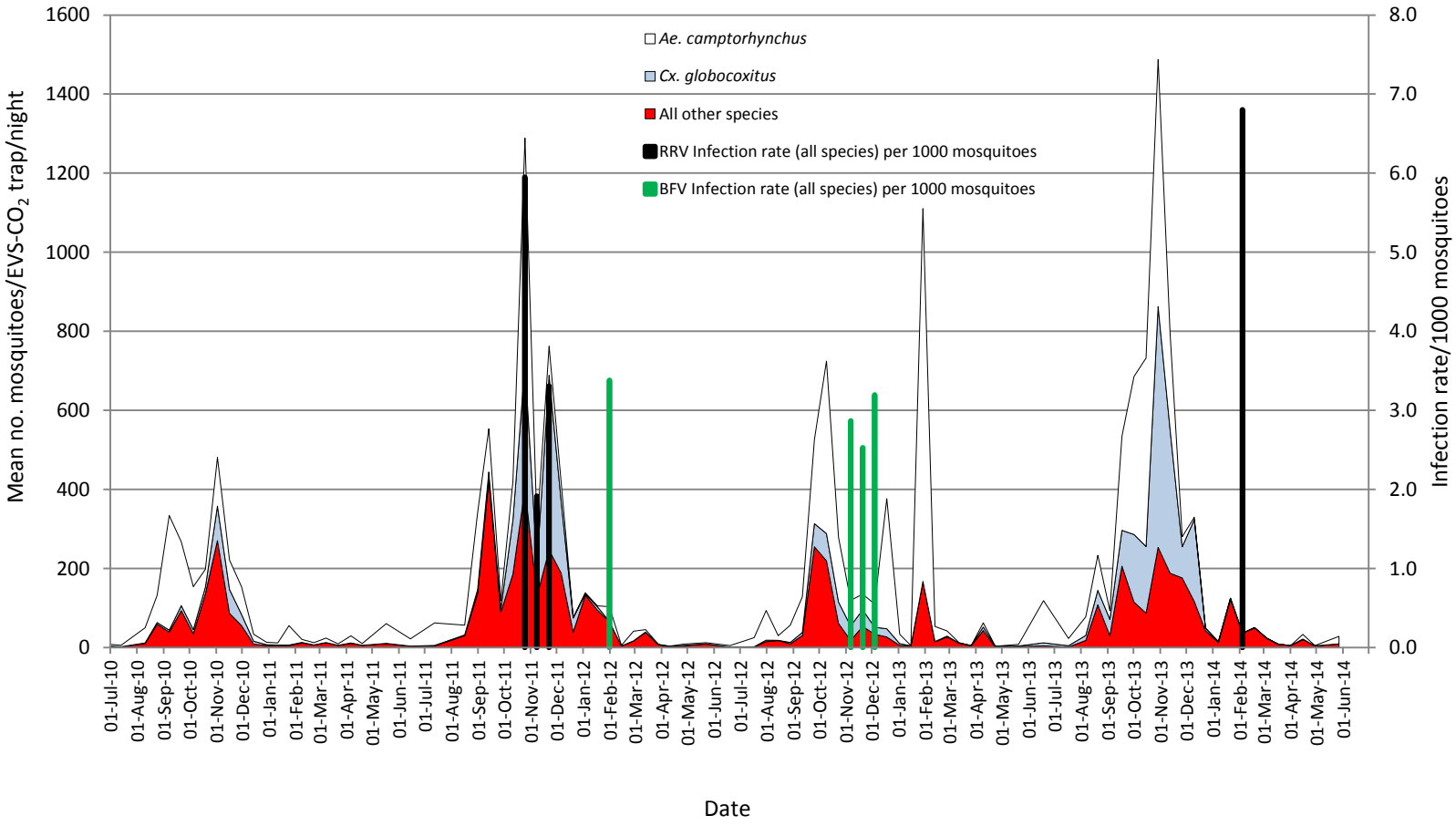

Figure 12. Abundance of adult mosquitoes and their infection rate (all species) with RRV and BFV, Busselton wetland sites, 01 July 2010 - 30 June 2014.

Kimberley

Results of mosquito trapping and virus isolations in 2013 for the Kimberley region are presented in this report, as well as details of mosquito trapping carried out in 2014. Mosquito identifications from the 2014 collections are yet to be processed for virus isolation. This will be completed in 2015 and the results will be presented in the 2014/15 Annual Report.

2013 field and laboratory results

The annual collecting trip to investigate flavivirus activity throughout the Kimberley region was carried out between 18 March and 21 March 2013 (Table 13). A total of 48 traps were set with a 97.9% success rate. All of the traps were set in the west Kimberley region. Mosquito species collected, their percentage composition of the overall total, processing details and viruses yielded at all localities within each town or community in 2013 are summarised in Tables 14A-G. Virus isolates are listed in Table 15, species and locations yielding arbovirus isolates are summarised in Tables 16 and 17, respectively, and the infection rates of mosquitoes for arboviruses are shown in Table 18.

Table 13. Details of Kimberley 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**
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 Peninsula	"	Willie Creek	20-Mar-13	4	0	4
"	Coconut Wells	"	Locality and environs	"	2	0	2
"	Broome	"	Town 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			

Of the estimated 104135 mosquitoes collected in 2013, 15847 (15.2%) were processed for virus isolation in 872 pools, yielding 26 arbovirus isolates: 16 RRV (northern/eastern phenotype), 8 BFV and 2 KOKV (Tables 15-18). Most virus isolates were from *Ae. vigilax* (50.0%) and *Cx. annulirostris* (26.9%), with low numbers of isolates from another four species (Tables 15 and 16). The RRV isolates were from a wide range of species, although most were from *Ae. vigilax* and *Cx. annulirostris*. The BFV isolates were from *Ae. vigilax* (5), *Ae. (Macleaya)* sp. (1), *Ae. phaecasiatus* (1) and *Cx. annulirostris* (1). This is the first arbovirus isolate from *Ae. phaecasiatus* in WA. The KOKV isolates were from *Ae. vigilax*. There were no isolations of MVEV or KUNV from mosquitoes collected in the Kimberley region in 2013. Most isolates were from Broome, Roebuck Plain and Coconut Wells (12) and Derby (including the Gibb River Road transect) (11) (Table 17).

The infection rate of RRV in mosquitoes was generally greatest in *Aedes* mosquitoes (Table 18). For BFV, the infection rate was greatest in *Ae. phaecasiatus* (500.0 per 1000 mosquitoes) and *Ae. (Macleaya)* sp. (15.4 per 1000 mosquitoes), however only low numbers of these species were collected. The infection rate of *Ae. vigilax* for KOKV was 3.8 per 1000 mosquitoes at Broome and 1.8 per 1000 mosquitoes at Derby. The predominance of alphaviruses rather than flaviviruses, and probably reflects a shorter time-frame between heavy rainfall and adult mosquito collections, favouring increased alphavirus activity.

Generally, 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. An active monsoon and TC Rusty 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. Above average rainfall continued into May and June 2013 in northern parts of WA.

A total of 4508 serum samples from 28 chicken flocks across WA were tested for antibodies to flaviviruses during 2012/13 (ASRL 2012/13 Annual Report, Appendix 1). 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 seasonal flavivirus activity, and was also the lowest level of flavivirus activity observed since 1995/96 when just two seroconversions to KUNV were detected in March-April 1996. The absence of MVEV or KUNV in mosquitoes collected in the west Kimberley region in March 2013 reflects the results of the sentinel chicken flavivirus surveillance program (ASRL 2012/13 Annual Report), and may indicate that the adult mosquito collections were conducted before the onset of low-level MVEV and KUNV activity in the region.

West Kimberley

Mosquito abundance at Broome (Town and environs) was lower than the previous season (152 per trap versus 417 per trap) (Table 14A). The dominant species were *Ae. notoscriptus* (19.4%), *Cx. annulirostris* (17.4%), *Ae. vigilax* (12.5%) and *Cx. quinquefasciatus* (11.1%). Arbovirus detections comprised six isolates of RRV and one flavivirus. The RRV isolates were from *Ae. vigilax* (2), with single RRV isolates from *Ae. normanensis*, *Cx. annulirostris*, *Cx. sitiens* and a pool of damaged *Culex* mosquitoes. The KOKV isolate was from *Ae. vigilax*. Mosquito abundance was substantially greater at the Roebuck Plain trap sites (Table 14B), where the mean abundance was 7658 per trap, similar to the previous season (8208 per trap). *Culex annulirostris* dominated the collections (89.8%), and there were three isolations of RRV from this species as well as a single isolate of RRV from a pool of damaged *Culex* mosquitoes. Vector abundance was lower at Willie Creek (1106 per trap), and major species comprised *Ae. vigilax* (62.9%) and *Cx. annulirostris* (15.5%) (Table 14C). The alphavirus BFV was detected in *Ae. vigilax*, *Cx. annulirostris* and tree-hole breeding *Ae. (Macleaya)* species. At Coconut Wells mosquito abundance was just 326 per trap, substantially lower than the previous season (1168 per trap) (Table 14D). The most common species comprised *Cx. annulirostris* (61.2%) and *Ae. vigilax* (28.0%). BFV was detected in *Ae. phaecasiatus*, the first time an arbovirus isolate has been detected in this species in WA.

At Willare, mosquito abundance was high (1748 per trap) but less than the previous season (4978 per trap) (Table 14E). The most abundance species comprised *Ae. normanensis* (59.8%), *Culex*

annulirostris (13.1%) and *Ae. vigilax* (6.8%). A notable result was the collection of an unusual *Aedes* mosquito at Willare, and Prof. Richard Russell and Mr Peter Whelan have confirmed that it is not recorded in the Culicidae of the Australasian Region (Lee et al. 1984). No viruses were detected in mosquitoes collected at Willare in 2013.

Mosquito abundance was low at Derby compared with the previous season. *Aedes vigilax* was the dominant species at trapsites closer to town (Table 14F), whilst *Cx. annulirostris* was slightly more abundant than *Ae. vigilax* in trap sites along the Gibb River Road (Table 14G). Barmah Forest virus was isolated from three pools of *Ae. vigilax* collected at town sites, and RRV (4), BFV (1) and KOKV (1) was isolated from the same species along the Gibb River Road transect. In addition, there were two isolations of RRV from *Cx. annulirostris* along the Gibb River Road transect.

2014 field results

Field work was carried out in the northeast from 15-17 April 2014 (Table 19). A total of 53 adult mosquito traps were set in the west Kimberley region, with a success rate of 98.1%. Results of mosquito and arbovirus detections will be presented in the 2014/15 ASRL Annual Report.

Table 14A. Results of mosquito trapping and virus isolations from Broome (Town and environs), west Kimberley.
Trap dates: 18 and 20 March 2013 (14 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	Virus isolates[1]
Ae. (Finlaya) notoscriptus	Bloodfed	1	(<0.1)	0	0	0	
Ae. (Finlaya) notoscriptus	Female	413	(19.4)	413	24	0	
Ae. (Finlaya) pecuniosus	Female	1	(<0.1)	1	1	0	
Ae. (Macleaya) E.N. Marks' species No. 147	Female	1	(<0.1)	1	1	0	
Ae. (Macleaya) species	Bloodfed	1	(<0.1)	0	0	0	
Ae. (Macleaya) species	Female	168	(7.9)	168	13	0	
Ae. (Macleaya) species	Male	186	(8.7)	186	11	0	
Ae. (Macleaya) tremulus	Female	7	(0.3)	7	2	0	
Ae. (Ochlerotatus) normanensis	Female	17	(0.8)	17	3	0	1 RRV
Ae. (Ochlerotatus) phaecasiatus	Female	1	(<0.1)	1	1	0	
Ae. (Ochlerotatus) vigilax	Bloodfed	4	(0.2)	0	0	0	
Ae. (Ochlerotatus) vigilax	Female	266	(12.5)	266	18	0	1 KOKV, 2 RRV
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	4	(0.2)	3	1	1	
Ae. species (unidentified) - new or difficult to ID species	Male	1	(<0.1)	1	1	0	
Cx. (Culex) annulirostris	Bloodfed	8	(0.4)	0	0	0	
Cx. (Culex) annulirostris	Female	371	(17.4)	371	20	0	1 RRV
Cx. (Culex) annulirostris variant	Female	1	(<0.1)	0	0	1	
Cx. (Culex) quinquefasciatus	Bloodfed	3	(0.1)	0	0	0	
Cx. (Culex) quinquefasciatus	Female	236	(11.1)	236	15	0	
Cx. (Culex) sitiens	Female	119	(5.6)	119	12	0	1 RRV
Cx. species (unidentified) - new or difficult to ID species	Male	2	(0.1)	2	1	0	
Tripteroides (Polylepidomyia) punctolateralis	Female	3	(0.1)	3	3	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Bloodfed	1	(<0.1)	0	0	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	53	(2.5)	53	7	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	5	(0.2)	0	0	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	258	(12.1)	258	15	0	1 RRV
Total		**2131**	**(100.0)**	**2106**	**149**	**2**	**6 RRV, 1 KOKV**

[1]RRV is Ross River virus, Flavi is flavivirus.

Table 14B. Results of mosquito trapping and virus isolations from Roebuck Plain (Plain and environs), west Kimberley.[1]
Trap dates: 19 March 2013 (11 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools	Virus isolates[2]
Ae. (Macleaya) species	Female	7	(<0.1)	1	1	
Ae. (Mucidus) alternans	Female	8	(<0.1)	1	1	
Ae. (Neomellanoconion) lineatopennis	Female	24	(<0.1)	3	1	
Ae. (Ochlerotatus) phaecasiatus	Female	13	(<0.1)	2	1	
Ae. (Ochlerotatus) vigilax	Female	335	(0.4)	35	7	
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	28	(<0.1)	1	1	
An. (Cellia) amictus	Female	95	(0.1)	5	2	
An. (Cellia) annulipes s.l.	Female	3	(<0.1)	2	1	
An. (Cellia) hilli	Female	67	(0.1)	9	3	
Cx. (Culex) annulirostris	Bloodfed	2013	(2.4)	0	0	
Cx. (Culex) annulirostris	Female	75626	(89.8)	5115	207	3 RRV
Cx. (Culex) quinquefasciatus	Female	11	(<0.1)	1	1	
Cx. (Culex) sitiens	Female	488	(0.6)	116	9	
Cx. (Culiciomyia) pullus	Female	7	(<0.1)	1	1	
Cx. species (unidentified) - new or difficult to ID species	Male	101	(0.1)	6	3	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	213	(0.3)	19	4	
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	247	(0.3)	0	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	4951	(5.9)	274	15	1 RRV
Total		**84237**	**(100.0)**	**5591**	**258**	**4 RRV**

[1]No mosquitoes were pinned. [2]RRV is Ross River virus.

Table 14C. Results of mosquito trapping and virus isolations from Dampier Peninsula (Willie Creek), west Kimberley.
Trap date: 20 March 2013 (4 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	Virus isolates[1]
Ae. (Macleaya) E.N. Marks' species No. 147	Female	3	(0.1)	2	1	0	
Ae. (Macleaya) species	Female	118	(2.7)	65	5	1	1 BFV
Ae. (Macleaya) species	Male	117	(2.6)	77	4	0	
Ae. (Macleaya) tremulus	Female	60	(1.4)	44	2	0	
Ae. (Mucidus) alternans	Female	3	(0.1)	1	1	0	
Ae. (Ochlerotatus) phaecasiatus	Female	1	(<0.1)	1	1	0	
Ae. (Ochlerotatus) vigilax	Bloodfed	7	(0.2)	0	0	0	
Ae. (Ochlerotatus) vigilax	Female	2780	(62.9)	1226	50	0	1 BFV
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	1	(<0.1)	1	1	0	
Ae. (Subgenus *Nov*.) *daliensis*	Female	4	(0.1)	2	2	0	
Cx. (Culex) annulirostris	Female	686	(15.5)	426	19	0	1 BFV
Cx. (Culex) quinquefasciatus	Female	3	(0.1)	1	1	0	
Cx. (Culex) sitiens	Bloodfed	1	(<0.1)	0	0	0	
Cx. (Culex) sitiens	Female	168	(3.8)	84	6	0	
Unidentifiable (too damaged/features missing)	Female	76	(1.7)	76	3	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	282	(6.4)	103	5	0	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	2	(<0.1)	2	1	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	1	(<0.1)	0	0	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	109	(2.5)	69	5	0	
Total		**4422**	**(100.0)**	**2180**	**107**	**1**	**3 BFV**

[1]BFV is Barmah Forest virus.

Table 14D. Results of mosquito trapping and virus isolations from Broome (Coconut Wells), west Kimberley.[1]
Trap date: 20 March 2013 (4 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools	Virus isolates[2]
Ae. (Finlaya) notoscriptus	Female	1	(0.1)	1	1	
Ae. (Macleaya) species	Female	9	(0.7)	7	2	
Ae. (Neomellanoconion) lineatopennis	Female	1	(0.1)	1	1	
Ae. (Ochlerotatus) phaecasiatus	Female	2	(0.2)	2	1	1 BFV
Ae. (Ochlerotatus) vigilax	Bloodfed	2	(0.2)	0	0	
Ae. (Ochlerotatus) vigilax	Female	366	(28.0)	333	14	
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	5	(0.4)	5	2	
Cx. (Culex) annulirostris	Bloodfed	30	(2.3)	0	0	
Cx. (Culex) annulirostris	Female	799	(61.2)	671	28	
Cx. (Culex) sitiens	Bloodfed	1	(0.1)	0	0	
Cx. (Culex) sitiens	Female	25	(1.9)	20	2	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	21	(1.6)	21	1	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	43	(3.3)	41	3	
Total		**1305**	**(100.0)**	**1102**	**55**	**1 BFV**

[1]No mosquitoes were pinned. [2]BFV is Barmah Forest virus.

Table 14E. Results of mosquito trapping and virus isolations from Willare (Town and environs), west Kimberley.[1]
Trap date: 21 March 2013 (4 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned
Ae. (Macleaya) species	Female	42	(0.6)	21	4	0
Ae. (Macleaya) species	Male	1	(<0.1)	1	1	0
Ae. (Mucidus) alternans	Female	9	(0.1)	4	3	0
Ae. (Neomellanoconion) lineatopennis	Bloodfed	4	(0.1)	0	0	0
Ae. (Neomellanoconion) lineatopennis	Female	90	(1.3)	20	1	0
Ae. (Ochlerotatus) normanensis	Bloodfed	493	(7.0)	0	0	0
Ae. (Ochlerotatus) normanensis	Female	4183	(59.8)	1028	42	0
Ae. (Ochlerotatus) vigilax	Female	473	(6.8)	121	7	0
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	3	(<0.1)	1	1	0
Ae. species (unidentified) - new or difficult to ID species[2]	Female	5	(0.1)	0	0	1
Ae. species (unidentified) - new or difficult to ID species	Male	3	(<0.1)	1	1	0
An. (Cellia) amictus	Female	5	(0.1)	1	1	0
An. (Cellia) annulipes s.l.	Bloodfed	3	(<0.1)	0	0	0
An. (Cellia) annulipes s.l.	Female	99	(1.4)	32	4	0
An. (Cellia) hilli	Female	5	(0.1)	1	1	0
Cx. (Culex) annulirostris	Bloodfed	44	(0.6)	0	0	0
Cx. (Culex) annulirostris	Female	917	(13.1)	294	14	0
Cx. (Culex) palpalis	Female	1	(<0.1)	1	1	0
Cx. (Culex) sitiens	Female	3	(<0.1)	1	1	0
Unidentifiable *Aedes* sp. (too damaged/features missing)	Bloodfed	9	(0.1)	0	0	0
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	538	(7.7)	124	7	0
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	1	(<0.1)	1	1	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	62	(0.9)	20	3	0
Total		**6993**	**(100.0)**	**1672**	**93**	**1**

[1]No viruses were isolated. [2]This mosquito was examined by Prof. Richard Russell and Mr Peter Whelan who confirmed this species is not recorded in Lee et al. (1984).

Table 14F. Results of mosquito trapping and virus isolations from Derby (Town and environs), west Kimberley.[1]
Trap date: 21 March 2013 (9 traps, 1 failure).

Species	Class	No. collected	(%)	No. processed	No. pools	Virus isolates[2]
Ae. (Finlaya) notoscriptus	Female	21	(0.7)	21	3	
Ae. (Macleaya) E.N. Marks' species No. 147	Female	8	(0.2)	8	1	
Ae. (Macleaya) species	Bloodfed	1	(<0.1)	0	0	
Ae. (Macleaya) species	Female	53	(1.6)	46	6	
Ae. (Macleaya) species	Male	26	(0.8)	24	6	
Ae. (Mucidus) alternans	Female	33	(1.0)	22	5	
Ae. (Neomellanoconion) lineatopennis	Female	36	(1.1)	34	3	
Ae. (Ochlerotatus) normanensis	Bloodfed	7	(0.2)	0	0	
Ae. (Ochlerotatus) normanensis	Female	114	(3.5)	72	8	
Ae. (Ochlerotatus) vigilax	Bloodfed	29	(0.9)	0	0	
Ae. (Ochlerotatus) vigilax	Female	2234	(69.2)	1125	49	3 BFV
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	29	(0.9)	14	4	
Cx. (Culex) annulirostris	Bloodfed	14	(0.4)	1	0	
Cx. (Culex) annulirostris	Female	543	(16.8)	511	27	
Cx. (Culex) quinquefasciatus	Female	7	(0.2)	7	4	
Cx. (Culex) sitiens	Female	2	(0.1)	2	2	
Cx. (Culiciomyia) pullus	Female	2	(0.1)	2	1	
Mimomyia (Eto) elegans	Female	3	(0.1)	3	1	
Mimomyia (Eto) elegans	Male	3	(0.1)	3	1	
Tripteroides (Polylepidomyia) punctolateralis	Female	8	(0.2)	8	2	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Bloodfed	3	(0.1)	0	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	37	(1.1)	18	5	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	15	(0.5)	14	3	
Total		**3228**	**(100.0)**	**1935**	**131**	**3 BFV**

[1]No mosquitoes were pinned. [2]BFV is Barmah Forest virus.

Table 14G. Results of mosquito trapping and virus isolations from Derby (Gibb River Road transect), west Kimberley.
Trap date: 21 March 2013 (9 traps, 1 failure).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	Virus isolates[1]
Ae. (Finlaya) notoscriptus	Female	3	(0.2)	3	2	0	
Ae. (Macleaya) species	Female	2	(0.1)	2	2	0	
Ae. (Mucidus) alternans	Female	11	(0.6)	9	4	0	
Ae. (Neomellanoconion) lineatopennis	Female	3	(0.2)	2	2	0	
Ae. (Ochlerotatus) normanensis	Bloodfed	15	(0.8)	0	0	0	
Ae. (Ochlerotatus) normanensis	Female	237	(13.0)	153	8	0	
Ae. (Ochlerotatus) vigilax	Bloodfed	7	(0.4)	0	0	0	
Ae. (Ochlerotatus) vigilax	Female	648	(35.6)	512	23	0	4 RRV, 1 BFV, 1 KOKV
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	5	(0.3)	4	4	0	
Ae. (Ochlerotatus) vigilax - variant with mottled tergites	Female	2	(0.1)	1	0	1	
Ae. species (unidentified) - new or difficult to ID species	Male	1	(0.1)	1	1	0	
An. (Cellia) annulipes s.l.	Female	2	(0.1)	2	1	0	
Cx. (Culex) annulirostris	Bloodfed	36	(2.0)	0	0	0	
Cx. (Culex) annulirostris	Female	766	(42.1)	519	22	0	2 RRV
Cx. (Culex) palpalis	Female	2	(0.1)	1	1	0	
Cx. (Culex) quinquefasciatus	Female	1	(0.1)	1	1	0	
Cx. species (unidentified) - new or difficult to ID species	Male	2	(0.1)	1	1	0	
Tripteroides (Polylepidomyia) punctolateralis	Female	1	(0.1)	1	1	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Bloodfed	2	(0.1)	0	0	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	22	(1.2)	14	2	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	51	(2.8)	35	4	0	
Total		**1819**	**(100.0)**	**1261**	**79**	**1**	**6 RRV, 1 BFV, 1 KOKV**

[1]RRV is Ross River virus, BFV is Barmah Forest virus, KOKV is Kokobera virus.

Table 15. Details of virus isolations from mosquitoes collected in the Kimberley region during the 2013 wet season.

Locality	Code	Species	Collection site	Date	Virus[1]
Broome (Town and environs)	K79976	*Aedes vigilax*	Lullfitz Drive	18-Mar-13	KOKV
"	K80012	"	Antheous Way	"	NE RRV
"	K80029	*Culex* species damaged	New Roebuck Estate	"	NE RRV
Roebuck Plain (Plain and environs)	K80262	*Cx. annulirostris*	Roebuck Plain Station Homestead	19-Mar-13	NE RRV
"	K80265	"	"	"	NE RRV
"	K80266	"	"	"	NE RRV
"	K80299	*Culex* species damaged	Roebuck Plain Station cattle grid	"	NE RRV
Dampier Peninsula (Willie Creek)	K80389	*Ae. (Macleaya)* sp.	Willie Creek house - Jacaranda tree	20-Mar-13	BFV
"	K80395	*Ae. vigilax*	"	"	BFV
"	K80408	*Cx. annulirostris*	"	"	BFV
Broome (Coconut Wells)	K80476	*Ae. phaecasiatus*	Steins Property	"	BFV
Broome (Town and environs)	K80531	*Ae. normanensis*	New development approx. 500m N. of racetrack	"	NE RRV
"	K80532	*Ae. vigilax*	"	"	NE RRV
"	K80534	*Cx. annulirostris*	"	"	NE RRV
"	K80535	*Cx. sitiens*	"	"	NE RRV
Derby (Town and environs)	K80633	*Ae. vigilax*	Derby jetty	21-Mar-13	BFV
"	K80707	"	Derby sewage overflow	"	BFV
Derby (Gibb River Road transect)	K80746	"	Mowanjum Community Sewage lagoon	"	BFV
"	K80763	"	Yabbagoody Claypan	"	NE RRV
"	K80764	"	"	"	NE RRV
"	K80765	"	"	"	NE RRV
"	K80769	"	"	"	NE RRV
"	K80772	*Cx. annulirostris*	"	"	NE RRV
"	K80776	"	"	"	NE RRV
"	K80786	*Ae. vigilax*	Leprosarium Road	"	KOKV
Derby (Town and environs)	K80828	"	Derby Light Aerodrome	"	BFV

[1]NE RRV is northern/eastern phenotype of RRV, BFV is Barmah Forest virus, KOKV is Kokobera virus.

Table 16. Mosquito species yielding arboviruses in the Kimberley region in March 2013.

	Virus[1]		
Species	**RRV**	**BFV**	**KOKV**
Aedes (Macleaya) species damaged		1	
Ae. normanensis	1		
Ae. phaecasiatus		1	
Ae. vigilax	6	5	2
Culex annulirostris	6	1	
Cx. sitiens	1		
Cx. species damaged	2		
Total	**16**	**8**	**1**

[1]RRV is Ross River virus, BFV is Barmah Forest virus, KOKV is Kokobera virus.

Table 17. Virus isolations from localities in the Kimberley region in March 2013.

	Virus[1]		
Locality	**RRV**	**BFV**	**KOKV**
Broome (Town and environs)	6		1
Roebuck Plain (Plain and environs)	4		
Dampier Peninsula (Willie Creek)		3	
Broome (Coconut Wells)		1	
Derby (Town and environs)		3	
Derby (Gibb River Road transect)	6	1	1
Total	**16**	**8**	**2**

[1]RRV is Ross River virus, BFV is Barmah Forest virus, KOKV is Kokobera virus, Flavi is a flavivirus.

Table 18. Infection rates of mosquitoes with arboviruses at major study sites in the Kimberley region in March 2013.

		Infection rate[1]		
Location	**Species**	**RRV**	**BFV**	**KOKV**
Broome (Town and environs)	*Aedes normanensis*	58.8		
"	*Ae. vigilax*	7.6		3.8
"	*Culex annulirostris*	2.7		
"	*Cx. sitiens*	8.4		
"	*Cx.* species (damaged)	3.9		
Roebuck Plain	*Cx. annulirostris*	0.6		
"	*Cx.* species (damaged)	3.6		
Dampier Peninsula	*Ae. (Macleaya) sp.*		15.4	
"	*Ae. vigilax*		0.8	
"	*Cx. annulirostris*		2.3	
Broome (Coconut Wells)	*Ae. phaecasiatus*		500.0	
Derby (Town and environs)	*Ae. vigilax*		2.7	
Derby (Gibb River Rd transect)	*Ae. vigilax*	7.8	1.9	1.9
"	*Cx. annulirostris*	3.8		
Total[2]		**1.0**	**0.5**	**0.1**

[1]Calculated per 1000 mosquitoes; RRV is Ross River virus, BFV is Barmah Forest virus, KOKV is Kokobera virus.
[2]Calculated for all mosquitoes collected at all locations.

Table 19. Details of Kimberley meteorological regions, localities and suburbs where mosquito sampling was conducted in 2014.

				Trap outcomes		
Meteorological region	**Town or region**	**Locality**	**Date**	**Successful**	**Failed**	**Total**
Northeast	Wyndham	Parry's Creek region	15-Apr-14	8	1	9
"	"	Wyndham (Six Mile)	"	4	0	4
"	"	Wyndham (Three Mile)	"	3	0	3
"	"	Wyndham (Port)	"	1	0	1
"	Kununurra	Irrigation area	16-Apr-14	13	0	13
"	"	Town and environs	"	5	0	5
"	"	"	17-Apr-14	11	0	11
"	"	Packsaddle Plain	"	7	0	7
Total				**52**	**1**	**53**
			% Successful =	98.1%		

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

Results of virus isolation from mosquito collections conducted at Nullagine and Marble Bar in the eastern Pilbara and the De Grey River catchment area and Port Hedland in the north east Pilbara region following heavy rainfall and flooding associated with the passage of TC Rusty in February 2013 are shown below (Tables 20 and 21; Figure 13). A total of 92 traps were set with a success rate of 97.8% (Table 20).

Table 20. Details of meteorological regions, localities and suburbs where opportunistic mosquito sampling was conducted in 2013.

				Trap outcomes		
Meteorological region	**Town or region**	**Suburb**	**Date**	**Successful**	**Failed**	**Total**
East Pilbara	Nullagine	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	De Grey River catchment	Bush	"	7	0	7
"	Port Hedland	East of town	"	1	1	2
"	"	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
"	De Grey River catchment	Bush		1	0	1
"	Port Hedland	East of town	"	1	0	1
"	"	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	Town and environs	25-Mar-13	14	0	14
"	Nullagine	"	26-Mar-13	14	0	14
Total				**90**	**2**	**92**
		% Successful =	97.8			

Mosquito abundance at Nullagine was similar in March and April (Tables 21A and B), although there was a shift of abundance from *Aedes* species to an increased abundance of *Cx. annulirostris*. Similar abundance and species were observed at Marble Bar (Tables 21C and D). There were two arbovirus detections, one from Nullagine in early March and one from Marble Bar between 13-15 March 2013. Both arboviruses were not a recognised Australilan alphavirus or flavivirus and require additional work for identification.

Mosquito populations were substantially greater at Port Hedland and environs (Tables 21E – 21V). *Culex annulirostris* was the dominant species at all locations except for Redbank on 16 March 2013, where *Ae. vigilax* was the most abundant species (70%), however the collection size was small (Table 21O). No arboviruses were detected in mosquitoes collected at Port Hedland and environs in March 2013.

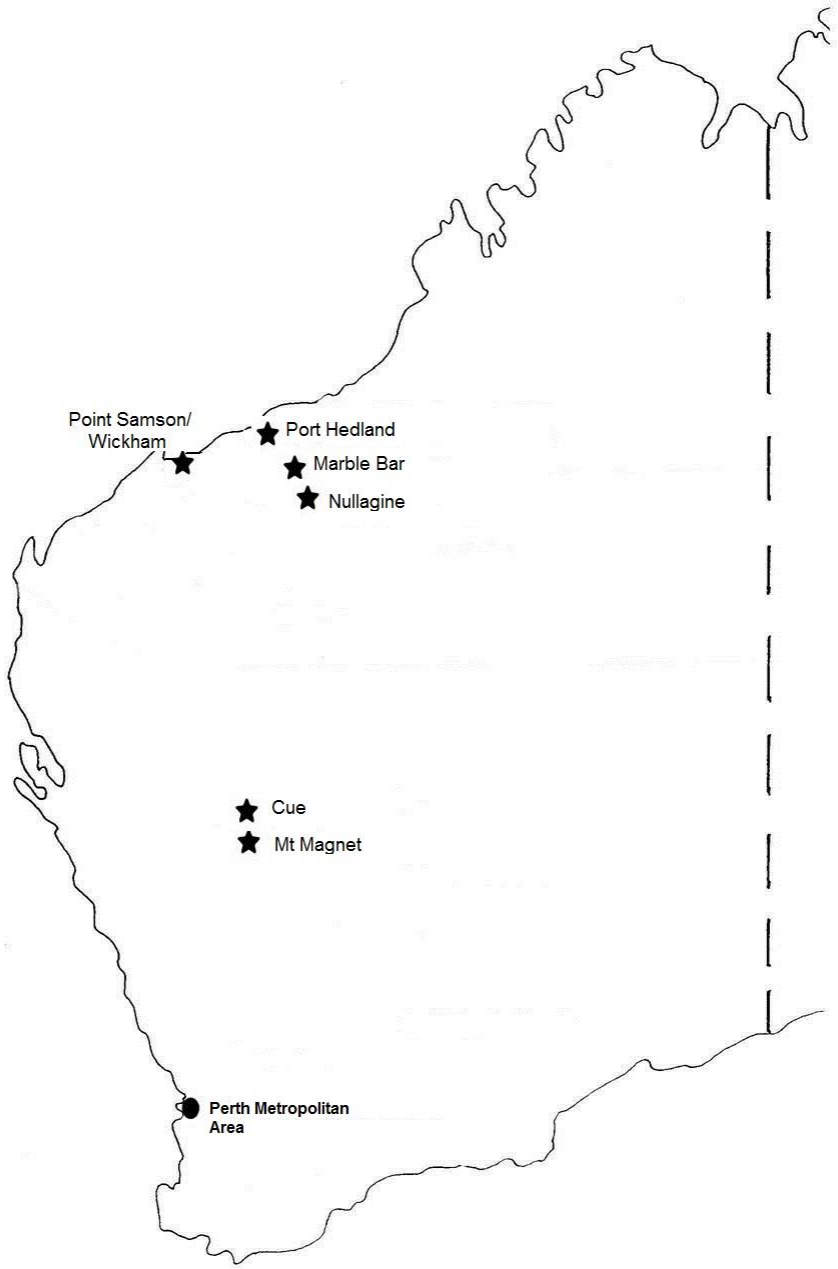

Figure 13. The locations (represented by a star) where opportunistic studies of mosquito and arbovirus activity were conducted in 2012/13 (Port Hedland, Marble Bar and Nullagine) and 2013/14 (Point Samson/Wickham, Mt Magnet and Cue).

Table 21A. Results of mosquito trapping and virus isolations from Nullagine (Town and environs), east Pilbara, March 2013.
Trap dates: 13 March 2013 (14 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	Virus isolates[1]
Ae. (Finlaya) mallochi	Female	1	(0.2)	1	1	0	
Ae. (Macleaya) species	Female	4	(0.7)	4	3	0	
Ae. (Macleaya) species	Male	34	(5.9)	34	5	0	
Ae. (Macleaya) tremulus	Female	27	(4.7)	27	6	0	
Ae. (Mucidus) alternans	Female	7	(1.2)	7	5	0	
Ae. (Ochlerotatus) E.N. Marks' species No. 159	Female	1	(0.2)	1	1	0	
Ae. (Ochlerotatus) E.N. Marks' species No. 85	Female	4	(0.7)	3	3	1	
Ae. (Ochlerotatus) eidsvoldensis	Bloodfed	1	(0.2)	0	0	0	
Ae. (Ochlerotatus) eidsvoldensis	Female	8	(1.4)	8	7	0	
Ae. (Ochlerotatus) normanensis	Bloodfed	4	(0.7)	0	0	0	
Ae. (Ochlerotatus) normanensis	Female	82	(14.1)	82	14	0	1Non A/F
Ae. (Ochlerotatus) pseudonormanensis	Bloodfed	2	(0.3)	0	0	0	
Ae. (Ochlerotatus) pseudonormanensis	Female	37	(6.4)	37	9	0	
Ae. (Pseudoskusea) bancroftianus	Female	3	(0.5)	3	1	0	
Ae. species (unidentified) - new or difficult to ID species	Male	16	(2.8)	16	4	0	
An. (Cellia) annulipes s.l.	Female	25	(4.3)	25	8	0	
An. (Cellia) annulipes s.l.	Male	1	(0.2)	1	1	0	
An. species (unidentified) - new or difficult to ID species	Male	3	(0.5)	3	2	0	
Cx. (Culex) annulirostris	Bloodfed	2	(0.3)	0	0	0	
Cx. (Culex) annulirostris	Female	89	(15.3)	89	13	0	
Cx. (Culex) E.N. Marks' species No. 92	Female	9	(1.6)	8	5	1	
Cx. (Culex) quinquefasciatus	Female	56	(9.7)	56	6	0	
Cx. (Culex) starckeae	Female	1	(0.2)	1	1	0	
Cx. (Lophoceraomyia) species	Female	15	(2.6)	15	1	0	
Cx. (Lophoceraomyia) species	Male	5	(0.9)	5	1	0	
Cx. species (unidentified) - new or difficult to ID species	Male	3	(0.5)	3	3	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Bloodfed	11	(1.9)	0	0	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	80	(13.8)	80	10	0	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	1	(0.2)	1	1	0	
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	48	(8.3)	48	8	0	
Total		**580**	**(100.0)**	**558**	**119**	**2**	**1Non A/F**

[1]P10042; not a known alphavirus or flavivirus and further work is required to identify this isolate.

Table 21B. Results of mosquito trapping and virus isolations from Nullagine (Town and environs), east Pilbara, March 2013.[1]
Trap dates: 26 March 2013 (14 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned
Ae. (Macleaya) E.N. Marks' species No. 121	Female	1	(0.2)	1	1	0
Ae. (Macleaya) E.N. Marks' species No. 125	Female	15	(3.7)	15	4	0
Ae. (Macleaya) species	Female	52	(12.7)	52	11	0
Ae. (Macleaya) species	Male	15	(3.7)	15	5	0
Ae. (Mucidus) alternans	Female	2	(0.5)	2	2	0
Ae. (Ochlerotatus) eidsvoldensis	Female	1	(0.2)	1	1	0
Ae. (Ochlerotatus) normanensis	Female	4	(1.0)	4	4	0
Ae. (Ochlerotatus) pseudonormanensis	Female	3	(0.7)	3	3	0
Ae. (Pseudoskusea) bancroftianus	Female	4	(1.0)	4	3	0
An. (Cellia) amictus	Female	11	(2.7)	11	5	0
An. (Cellia) annulipes s.l.	Bloodfed	3	(0.7)	0	0	0
An. (Cellia) annulipes s.l.	Female	39	(9.5)	37	11	2
Cx. (Culex) annulirostris	Bloodfed	3	(0.7)	0	0	0
Cx. (Culex) annulirostris	Female	198	(48.4)	198	16	0
Cx. (Culex) bitaeniorhynchus	Female	1	(0.2)	1	1	0
Cx. (Culex) crinicauda	Female	1	(0.2)	1	1	0
Cx. (Culex) E.N. Marks' species No. 92	Female	1	(0.2)	0	0	1
Cx. (Culex) quinquefasciatus	Female	51	(12.5)	51	9	0
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	1	(0.2)	1	1	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	3	(0.7)	3	1	0
Total		**409**	**(100.0)**	**400**	**79**	**3**

[1]No viruses were isolated.

Table 21C. Results of mosquito trapping and virus isolations from Marble Bar (Town and environs), east Pilbara, March 2013.
Trap dates: 14 and 15 March 2013 (15 traps, 1 failure).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned	Virus isolates[1]
Ae. (Macleaya) E.N. Marks' species No. 121	Female	1	(0.2)	1	1	0	
Ae. (Macleaya) E.N. Marks' species No. 125	Female	21	(4.7)	20	5	1	
Ae. (Macleaya) species	Female	19	(4.3)	19	6	0	
Ae. (Macleaya) species	Male	31	(7.0)	31	7	0	
Ae. (Mucidus) alternans	Bloodfed	1	(0.2)	0	0	0	
Ae. (Mucidus) alternans	Female	11	(2.5)	11	6	0	
Ae. (Ochlerotatus) E.N. Marks' species No. 71	Female	1	(0.2)	1	1	0	
Ae. (Ochlerotatus) E.N. Marks' species No. 85	Female	5	(1.1)	5	2	0	
Ae. (Ochlerotatus) eidsvoldensis	Female	4	(0.9)	4	3	0	
Ae. (Ochlerotatus) normanensis	Bloodfed	7	(1.6)	0	0	0	
Ae. (Ochlerotatus) normanensis	Female	37	(8.3)	37	8	0	
Ae. (Ochlerotatus) pseudonormanensis	Bloodfed	1	(0.2)	0	0	0	
Ae. (Ochlerotatus) pseudonormanensis	Female	9	(2.0)	9	6	0	
Ae. species (unidentified) - new or difficult to ID species	Male	2	(0.5)	2	1	0	
An. (Cellia) amictus	Female	1	(0.2)	1	1	0	
An. (Cellia) annulipes s.l.	Bloodfed	4	(0.9)	0	0	0	
An. (Cellia) annulipes s.l.	Female	32	(7.2)	32	7	0	
An. species (unidentified) - new or difficult to ID species	Male	5	(1.1)	5	3	0	
Cx. (Culex) annulirostris	Bloodfed	7	(1.6)	0	0	0	
Cx. (Culex) annulirostris	Female	108	(24.3)	108	13	0	
Cx. (Culex) bitaeniorhynchus	Female	1	(0.2)	1	1	0	
Cx. (Culex) E.N. Marks' species No. 92	Female	1	(0.2)	1	1	0	
Cx. (Culex) quinquefasciatus	Female	27	(6.1)	27	4	0	
Cx. species (unidentified) - new or difficult to ID species	Male	1	(0.2)	1	1	0	
Unidentifiable (too damaged/features missing)	Male	2	(0.5)	2	2	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Bloodfed	12	(2.7)	0	0	0	
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	63	(14.2)	63	8	0	
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	13	(2.9)	13	3	0	1NonA/F
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	17	(3.8)	17	6	0	
Total		**444**	**(100.0)**	**411**	**96**	**1**	**1NonA/F**

[1]P10161; not a known alphavirus or flavivirus and further work is required to identify this isolate.

Table 21D. Results of mosquito trapping and virus isolations from Marble Bar (Town and environs), east Pilbara, March 2013.[1]

Trap dates: 25 March 2013 (14 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Macleaya) E.N. Marks' species No. 125	Female	4	(0.6)	4	1
Ae. (Macleaya) species	Female	34	(5.3)	34	8
Ae. (Macleaya) species	Male	24	(3.7)	24	7
Ae. (Mucidus) alternans	Female	2	(0.3)	2	2
Ae. (Ochlerotatus) eidsvoldensis	Female	2	(0.3)	2	2
Ae. (Ochlerotatus) normanensis	Female	3	(0.5)	3	2
Ae. (Ochlerotatus) pseudonormanensis	Female	7	(1.1)	7	3
Ae. (Ochlerotatus) vigilax	Female	2	(0.3)	2	2
An. (Cellia) amictus	Female	1	(0.2)	1	1
An. (Cellia) annulipes s.l.	Bloodfed	2	(0.3)	0	0
An. (Cellia) annulipes s.l.	Female	12	(1.9)	12	5
An. species (unidentified) - new or difficult to ID species	Male	1	(0.2)	1	1
Cx. (Culex) annulirostris	Bloodfed	23	(3.6)	0	0
Cx. (Culex) annulirostris	Female	517	(79.9)	517	26
Cx. (Culex) quinquefasciatus	Bloodfed	1	(0.2)	0	0
Cx. (Culex) quinquefasciatus	Female	12	(1.9)	12	2
Total		**647**	**(100.0)**	**621**	**62**

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

Table 21E. Results of mosquito trapping and virus isolations from the De Grey River catchment area, northeast Pilbara, March 2013.[1]

Trap dates: 15 March 2013 (7 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned
Ae. (Macleaya) E.N. Marks' species No. 125	Female	1	(<0.1)	0	0	1
Ae. (Macleaya) species	Female	22	(0.6)	15	6	0
Ae. (Macleaya) species	Male	9	(0.2)	6	4	0
Ae. (Mucidus) alternans	Female	40	(1.0)	27	6	0
Ae. (Ochlerotatus) eidsvoldensis	Female	8	(0.2)	6	3	1
Ae. (Ochlerotatus) normanensis	Bloodfed	100	(2.5)	0	0	0
Ae. (Ochlerotatus) normanensis	Female	1137	(28.7)	838	38	0
Ae. (Ochlerotatus) pseudonormanensis	Bloodfed	5	(0.1)	0	0	0
Ae. (Ochlerotatus) pseudonormanensis	Female	94	(2.4)	93	6	0
Ae. (Ochlerotatus) vigilax	Female	69	(1.7)	31	5	0
Ae. species (unidentified) - new or difficult to ID species	Male	2	(0.1)	1	1	0
An. (Cellia) amictus	Female	1	(<0.1)	1	1	0
An. (Cellia) annulipes s.l.	Female	13	(0.3)	13	3	0
Cx. (Culex) annulirostris	Bloodfed	15	(0.4)	0	0	0
Cx. (Culex) annulirostris	Female	2163	(54.6)	824	36	0
Cx. (Culex) sitiens	Female	7	(0.2)	6	1	1
Unidentifiable *Aedes* sp. (too damaged/features missing)	Bloodfed	1	(<0.1)	0	0	0
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	80	(2.0)	69	7	0
Unidentifiable *Anopheles* sp. (too damaged/features missing)	Female	3	(0.1)	3	2	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	188	(4.7)	71	6	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Male	1	(<0.1)	1	1	0
Total		**3959**	**(100.0)**	**2005**	**126**	**3**

[1]No viruses were isolated.

Table 21F. Results of mosquito trapping and virus isolations from the De Grey River catchment area, northeast Pilbara, March 2013.[1]

Trap dates: 24 March 2013 (1 trap, no failure).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Ochlerotatus) normanensis	Female	7	(0.4)	2	1
Ae. (Ochlerotatus) vigilax	Female	49	(2.5)	13	1
An. (Cellia) annulipes species B	Female	4	(0.2)	1	1
Cx. (Culex) annulirostris	Female	1879	(96.6)	500	20
Cx. (Culex) sitiens	Female	7	(0.4)	2	1
Total		**1946**	**(100.0)**	**518**	**24**

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

Table 21G. Results of mosquito trapping and virus isolations from Port Hedland (East of town), northeast Pilbara, March 2013.[1]

Trap dates: 15 and 16 March 2013 (3 traps, 1 failure).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned
Ae. (Macleaya) E.N. Marks' species No. 121	Female	4	(0.1)	3	1	0
Ae. (Macleaya) E.N. Marks' species No. 76	Female	1	(<0.1)	1	1	0
Ae. (Macleaya) species	Female	3	(0.1)	2	1	0
AE. (Macleaya) tremulus	Female	2	(<0.1)	2	1	0
Ae. (Mucidus) alternans	Female	20	(0.4)	15	2	2
Ae. (Ochlerotatus) normanensis	Bloodfed	14	(0.3)	0	0	0
Ae. (Ochlerotatus) normanensis	Female	770	(14.4)	600	22	2
Ae. (Ochlerotatus) vigilax	Female	95	(1.8)	65	5	1
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	5	(0.1)	5	2	0
Cx. (Culex) annulirostris	Bloodfed	9	(0.2)	0	0	0
Cx. (Culex) annulirostris	Female	4256	(79.8)	855	35	2
Cx. (Culex) sitiens	Female	4	(0.1)	4	1	0
Cx. (Culex) starckeae	Female	1	(<0.1)	1	1	0
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	13	(0.2)	11	2	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	138	(2.6)	28	4	0
Total		**5335**	**(100.0)**	**1592**	**83**	**7**

[1]No viruses were isolated.

Table 21H. Results of mosquito trapping and virus isolations from Port Hedland (East of town), northeast Pilbara, March 2013.[1]

Trap dates: 24 March 2013 (1 trap, no failure).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Macleaya) species	Female	5	(<0.1)	1	1
Ae. (Ochlerotatus) normanensis	Female	88	(0.4)	27	3
Ae. (Ochlerotatus) vigilax	Female	712	(3.3)	102	6
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	120	(0.6)	10	3
Cx. (Culex) annulirostris	Bloodfed	80	(0.4)	0	0
Cx. (Culex) annulirostris	Female	19750	(92.9)	1844	75
Cx. (Culex) sitiens	Female	3	(<0.1)	1	1
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	16	(0.1)	5	2
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	494	(2.3)	60	4
Total		**21268**	**(100.0)**	**2050**	**95**

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

Table 21I. Results of mosquito trapping and virus isolations from Port Hedland (Port), northeast Pilbara, March 2013.[1]

Trap dates: 16 March 2013 (1 trap, no failure).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Macleaya) species	Male	2	(2.4)	2	1
Ae. (Macleaya) tremulus	Female	4	(4.7)	4	1
Ae. (Ochlerotatus) vigilax	Female	2	(2.4)	2	1
Cx. (Culex) annulirostris	Female	58	(68.2)	58	3
Cx. (Culex) quinquefasciatus	Female	13	(15.3)	13	1
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	6	(7.1)	6	1
Total		**85**	**(100.0)**	**85**	**8**

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

Table 21J. Results of mosquito trapping and virus isolations from Port Hedland (Port), northeast Pilbara, March 2013.[1]

Trap dates: 24 March 2013 (1 trap, no failure).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Macleaya) species	Female	2	(5.3)	2	1
Ae. (Macleaya) species	Male	4	(10.5)	4	1
Ae. (Ochlerotatus) vigilax	Female	1	(2.6)	1	1
Cx. (Culex) annulirostris	Female	16	(42.1)	16	1
Cx. (Culex) quinquefasciatus	Female	2	(5.3)	2	1
Cx. (Culex) sitiens	Female	13	(34.2)	13	1
Total		**38**	**(100.0)**	**38**	**6**

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

Table 21K. Results of mosquito trapping and virus isolations from Port Hedland (Town of Port Hedland), northeast Pilbara, March 2013.[1]

Trap dates: 16 March 2013 (1 trap, no failure).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Macleaya) species	Male	4	(0.6)	3	1
Ae. (Ochlerotatus) vigilax	Female	5	(0.7)	4	1
Cx. (Culex) annulirostris	Bloodfed	7	(1.0)	0	0
Cx. (Culex) annulirostris	Female	623	(89.4)	450	18
Cx. (Culex) quinquefasciatus	Female	1	(0.1)	1	1
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	57	(8.2)	41	2
Total		**697**	**(100.0)**	**499**	**23**

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

Table 21L. Results of mosquito trapping and virus isolations from Port Hedland (Town of Port Hedland), northeast Pilbara, March 2013.[1]

Trap dates: 24 March 2013 (1 trap, no failure).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Macleaya) species	Female	1	(0.5)	1	1
Ae. (Ochlerotatus) vigilax	Female	3	(1.6)	3	1
Cx. (Culex) annulirostris	Bloodfed	1	(0.5)	0	0
Cx. (Culex) annulirostris	Female	173	(92.0)	173	7
Cx. (Culex) quinquefasciatus	Female	2	(1.1)	2	1
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	8	(4.3)	8	1
Total		**188**	**(100.0)**	**187**	**11**

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

Table 21M. Results of mosquito trapping and virus isolations from Port Hedland (Pretty Pool), northeast Pilbara, March 2013.[1]

Trap dates: 16 March 2013 (2 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Macleaya) species	Female	18	(22.5)	18	1
Ae. (Macleaya) species	Male	17	(21.3)	17	1
Ae. (Ochlerotatus) vigilax	Female	13	(16.3)	13	2
Cx. (Culex) annulirostris	Bloodfed	1	(1.3)	0	0
Cx. (Culex) annulirostris	Female	27	(33.8)	27	2
Cx. (Culex) crinicauda	Female	1	(1.3)	1	1
Tripteroides (Polylepidomyia) punctolateralis	Female	1	(1.3)	1	1
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	2	(2.5)	2	1
Total		**80**	**(100.0)**	**79**	**9**

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

Table 21N. Results of mosquito trapping and virus isolations from Port Hedland (Pretty Pool), northeast Pilbara, March 2013.[1]

Trap dates: 24 March 2013 (2 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Macleaya) species	Bloodfed	1	(0.5)	0	0
Ae. (Macleaya) species	Female	18	(8.5)	18	1
Ae. (Macleaya) species	Male	18	(8.5)	18	1
Ae. (Ochlerotatus) vigilax	Female	20	(9.4)	5	2
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	1	(0.5)	1	1
Cx. (Culex) annulirostris	Bloodfed	1	(0.5)	0	0
Cx. (Culex) annulirostris	Female	149	(70.0)	149	7
Cx. (Culex) quinquefasciatus	Female	1	(0.5)	1	1
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	1	(0.5)	1	1
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	3	(1.4)	3	2
Total		**213**	**(100.0)**	**196**	**16**

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

Table 21O. Results of mosquito trapping and virus isolations from Port Hedland (Redbank), northeast Pilbara, March 2013.[1]

Trap dates: 16 March 2013 (1 trap, no failure).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Mucidus) alternans	Female	1	(5.0)	1	1
Ae. (Ochlerotatus) vigilax	Female	14	(70.0)	14	1
Cx. (Culex) annulirostris	Female	5	(25.0)	5	1
Total		**20**	**(100.0)**	**20**	**3**

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

Table 21P. Results of mosquito trapping and virus isolations from Port Hedland (Redbank), northeast Pilbara, March 2013.[1]

Trap dates: 24 March 2013 (1 trap, no failure).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Ochlerotatus) vigilax	Female	22	(28.2)	22	1
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	1	(1.3)	1	1
Cx. (Culex) annulirostris	Female	48	(61.5)	48	2
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	2	(2.6)	2	1
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	5	(6.4)	5	1
Total		**78**	**(100.0)**	**78**	**6**

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

Table 21Q. Results of mosquito trapping and virus isolations from Port Hedland (South Hedland), northeast Pilbara, March 2013.[1]

Trap dates: 16 March 2013 (2 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned
Ae. (Macleaya) tremulus	Female	3	(0.5)	3	1	0
Ae. (Ochlerotatus) normanensis	Bloodfed	13	(2.1)	0	0	0
Ae. (Ochlerotatus) normanensis	Female	194	(31.8)	194	9	0
Ae. (Ochlerotatus) vigilax	Female	6	(1.0)	6	2	0
Ae. (Subgen. *Nov.*) E.N. Marks' species No. 160	Female	1	(0.2)	0	0	1
Cx. (Culex) annulirostris	Bloodfed	4	(0.7)	0	0	0
Cx. (Culex) annulirostris	Female	356	(58.3)	356	15	0
Cx. (Culex) quinquefasciatus	Bloodfed	1	(0.2)	0	0	0
Cx. (Culex) quinquefasciatus	Female	5	(0.8)	5	1	0
Cx. (Culex) sitiens	Female	1	(0.2)	1	1	0
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	9	(1.5)	9	1	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	18	(2.9)	18	2	0
Total		**611**	**(100.0)**	**592**	**32**	**1**

[1]No viruses were isolated.

Table 21R. Results of mosquito trapping and virus isolations from Port Hedland (South Hedland), northeast Pilbara, March 2013.[1]

Trap dates: 24 March 2013 (2 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Macleaya) species	Female	5	(0.2)	1	1
Ae. (Ochlerotatus) normanensis	Female	9	(0.3)	2	1
Ae. (Ochlerotatus) vigilax	Female	55	(1.7)	13	2
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	1	(<0.1)	1	1
Cx. (Culex) annulirostris	Bloodfed	39	(1.2)	0	0
Cx. (Culex) annulirostris	Female	2999	(94.6)	982	40
Cx. (Culex) quinquefasciatus	Female	3	(0.1)	2	1
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	5	(0.2)	0	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	55	(1.7)	28	2
Total		**3171**	**(100.0)**	**1029**	**48**

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

Table 21S. Results of mosquito trapping and virus isolations from Port Hedland (Spinifex Hill), northeast Pilbara, March 2013.[1]

Trap dates: 16 March 2013 (1 trap, no failure).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Ochlerotatus) vigilax	Bloodfed	1	(0.2)	0	0
Ae. (Ochlerotatus) vigilax	Female	6	(1.2)	6	1
Ae. (Subgenus *Nov.*) *daliensis*	Female	1	(0.2)	1	1
Cx. (Culex) annulirostris	Bloodfed	3	(0.6)	0	0
Cx. (Culex) annulirostris	Female	427	(87.7)	427	18
Cx. (Culex) quinquefasciatus	Female	1	(0.2)	1	1
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	48	(9.9)	48	2
Total		**487**	**(100.0)**	**483**	**23**

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

Table 21T. Results of mosquito trapping and virus isolations from Port Hedland (Spinifex Hill), northeast Pilbara, March 2013.[1]
Trap dates: 24 March 2013 (1 trap, no failure).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Macleaya) species	Female	1	(0.2)	1	1
Ae. (Macleaya) tremulus	Female	1	(0.2)	1	1
Ae. (Ochlerotatus) vigilax	Female	4	(0.7)	4	1
Cx. (Culex) annulirostris	Bloodfed	6	(1.1)	0	0
Cx. (Culex) annulirostris	Female	496	(88.9)	450	18
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	50	(9.0)	45	2
Total		**558**	**(100.0)**	**501**	**23**

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

Table 21U. Results of mosquito trapping and virus isolations from Port Hedland (Wedgefield), northeast Pilbara, March 2013.[1]
Trap dates: 16 March 2013 (2 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned
Ae. (Macleaya) tremulus	Female	4	(0.2)	1	1	0
Ae. (Ochlerotatus) normanensis	Female	5	(0.2)	5	1	0
Ae. (Ochlerotatus) vigilax	Female	47	(2.0)	38	3	0
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	1	(<0.1)	1	1	0
Cx. (Culex) annulirostris	Bloodfed	15	(0.6)	0	0	0
Cx. (Culex) annulirostris	Female	2122	(90.2)	695	29	0
Cx. (Culex) quinquefasciatus	Female	26	(1.1)	8	2	0
Cx. (Culex) sitiens	Female	2	(0.1)	2	1	0
Cx. species (unidentified) - new or difficult to ID species	Male	4	(0.2)	1	1	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	126	(5.4)	37	3	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Hermaphrodite	1	(<0.1)	0	0	1
Total		**2353**	**(100.0)**	**788**	**42**	**1**

[1]No viruses were isolated.

Table 21V. Results of mosquito trapping and virus isolations from Port Hedland (Wedgefield), northeast Pilbara, March 2013.[1]
Trap dates: 24 March 2013 (2 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Ochlerotatus) vigilax	Female	530	(9.9)	142	6
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	44	(0.8)	15	1
Cx. (Culex) annulirostris	Bloodfed	25	(0.5)	0	0
Cx. (Culex) annulirostris	Female	4315	(80.6)	793	32
Cx. (Culex) quinquefasciatus	Female	22	(0.4)	3	1
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	12	(0.2)	4	1
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	22	(0.4)	0	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	383	(7.2)	69	4
Total		**5353**	**(100.0)**	**1026**	**45**

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

Opportunistic adult mosquito trapping carried out in WA, 2013/2014

The ASRL assisted personnel from MBDC DOH (Ms Susan Harrington and Dr Peter Neville) to collect mosquitoes using EVS/CO_2, BG-sentinel traps and ovitraps following diagnosis of a case of dengue at Point Samson, in the Pilbara region of WA (Figure 13, Table 22). Mosquitoes collected in EVS/CO_2 and BG-sentinel traps were processed for virus isolation. In addition, the ASRL processed mosquitoes collected by personnel from MBDC DOH (Dr Andrew Jardine and Mr Ryan Janes) at Mt Magnet (Figure 13) in 18-19 March 2014 following heavy rainfall in the area and complaints about mosquitoes in the area (Table 25A and B).

Table 22. Details of adult mosquito trapping (EVS/CO_2 and BG-sentinel) in the Pilbara region in October 2013 and January 2014.

Meteorological region	Locality	Suburb	Date	Successful	Failed	Total
Pilbara (De Grey)	Point Samson	Town and environs	18-Oct-13	14		14
"	"	"	19-Oct-13	8		8
"	Wickham	"	"	9		9
"	Point Samson	"	21-Oct-13	4		4
"	"	"	10-Jan-14	18		18
"	Cossack	"	11-Jan-14	3	1	4
"	Point Samson	"	"	12		12
"	Wickham	"	"	1		1
"	Point Samson	"	12-Jan-14	9		9
"	"	"	13-Jan-14	5		5
"	Wickham	"	"	10		10
"	Point Samson	"	14-Jan-14	2		2
"	Wickham	"	"	9		9
"	Cheeditha	"	15-Jan-14	3		3
"	Point Samson	"	"	1		1
"	Roebourne	"	"	4		4
"	Wickham	"	"	1		1
"	Point Samson	"	16-Jan-14	2		2
"	Wickham	"	"	2		2
Total				**117**	**1**	**118**
			% Successful =	99.2		

Mosquito abundance at Point Samson was extremely low in October 2013 (Table 23A), with a mean of nine mosquitoes collected per trap. *Culex sitiens* was the most abundant species, comprising 21.4% of the total collected. Various *Ae. (Macleaya)* species and *Ae. vigilax* was also more common (19.2% and 17.9%, respectively), followed by *Cx. quinquefasciatus* (12.4%). Mosquito abundance was slightly greater at Wickham, however still very low (18.9 per trap) (Table 23B). Various *Ae. (Macleaya)* species were most abundant (64.7%), followed by *Cx. quinquefasciatus* (11.2%). No exotic container-breeding mosquito species of concern (*Ae. aegypti* or *Ae. albopictus*) were identified, although other container-breeding species were collected. No arboviruses were detected in homogenates of mosquitoes collected during these surveys.

Table 23A. Results of mosquito trapping and virus isolations from Point Samson (Town and environs), Pilbara in 2013.[1]
Trap dates: (18, 19 and 21 October 2013; 26 traps, no failures; includes 7 BG traps).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Macleaya) E.N. Marks' species No. 125	Female	16	(6.8)	16	7
Ae. (Macleaya) species	Female	10	(4.3)	10	8
Ae. (Macleaya) species	Male	19	(8.1)	19	8
Ae. (Ochlerotatus) vigilax	Female	42	(17.9)	42	11
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	1	(0.4)	1	1
An. (Cellia) annulipes s.l.	Female	2	(0.9)	2	2
Cx. (Culex) annulirostris	Female	2	(0.9)	2	2
Cx. (Culex) quinquefasciatus	Bloodfed	1	(0.4)	1	1
Cx. (Culex) quinquefasciatus	Female	29	(12.4)	29	12
Cx. (Culex) sitiens	Female	50	(21.4)	50	12
Cx. species (unidentified) - new or difficult to ID species	Male	43	(18.4)	43	10
Unidentifiable (too damaged/features missing)	Female	1	(0.4)	1	1
Unidentifiable (too damaged/features missing)	Male	1	(0.4)	1	1
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	1	(0.4)	1	1
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	4	(1.7)	4	3
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	12	(5.1)	12	6
Total		**234**	**(100.0)**	**234**	**86**

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

Table 23B. Results of mosquito trapping and virus isolations from Wickham (Town and environs), Pilbara in 2013.[1]
Trap date: (19 October 2013; 9 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned
Ae. (Macleaya) E.N. Marks' species No. 125	Female	44	(25.9)	41	4	3
Ae. (Macleaya) species	Female	26	(15.3)	26	2	0
Ae. (Macleaya) species	Male	40	(23.5)	40	5	0
Ae. (Ochlerotatus) vigilax	Female	14	(8.2)	14	4	0
An. (Cellia) annulipes s.l.	Female	4	(2.4)	4	3	0
Cx. (Culex) annulirostris	Female	1	(0.6)	1	1	0
Cx. (Culex) australicus	Female	2	(1.2)	2	1	0
Cx. (Culex) quinquefasciatus	Female	19	(11.2)	17	7	2
Cx. (Culex) sitiens	Female	15	(8.8)	15	6	0
Cx. species (unidentified) - new or difficult to ID species	Male	5	(2.9)	5	1	0
Total		**170**	**(100.0)**	**165**	**34**	**5**

[1]No viruses were isolated.

Adult mosquito abundance was slightly higher in January 2014. At Point Samson the abundance was about four times greater (mean of 42/trap) than in October 2013 (Table 24A), and *Ae. vigilax* dominated the collection (67.8%). Similar mosquito abundance was observed at Cossack, Wickham, Roebourne and Cheeditha (Table 24B-E). At Cossack, the most abundant species were *Ae. vigilax* (70.1%) and *Cx. sitiens* (Table 24B). Temporary ground-pool breeding *Ae. vigilax, Ae. normanensis* and *Ae. (Ochlerotatus)* E.N.M. species no. 85 were the most abundant species at Wickham, comprising 30.2%, 17.8% and 11.9% of the collections, respectively (Table 24C). In contrast, *Ae. (Macleaya)* sp., *Cx. annulirostris* and *Ae. normanensis* were the most abundant mosquito species at Roebourne (Table 24D). A very low abundance of mosquitoes was observed at Cheeditha (nine per trap) (Table 24E).

Table 24A. Results of mosquito trapping and virus isolations from Point Samson (Town and environs), Pilbara in 2014.[1]

Trap date: (10, 11, 12, 13, 14, 15 and 16 January 2014; 49 traps, no failures; includes 22 BG traps).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned
Ae. (Macleaya) E.N. Marks' species No. 121	Female	1	(<0.1)	0	0	1
Ae. (Macleaya) E.N. Marks' species No. 125	Bloodfed	1	(<0.1)	1	1	0
Ae. (Macleaya) E.N. Marks' species No. 125	Female	171	(8.3)	164	25	7
Ae. (Macleaya) species	Bloodfed	1	(<0.1)	0	0	0
Ae. (Macleaya) species	Female	136	(6.6)	136	23	0
Ae. (Macleaya) species	Male	170	(8.3)	170	31	0
Ae. (Ochlerotatus) E.N. Marks' species No. 159	Female	1	(<0.1)	1	1	0
Ae. (Ochlerotatus) vigilax	Bloodfed	7	(0.3)	0	0	0
Ae. (Ochlerotatus) vigilax	Female	1396	(67.8)	1395	80	1
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	86	(4.2)	85	16	1
Ae. (Ochlerotatus) vigilax variant	Female	5	(0.2)	0	0	5
Ae. species (unidentified) - new or difficult to ID species	Male	2	(0.1)	2	1	0
Cx. (Culex) quinquefasciatus	Female	10	(0.5)	10	8	0
Cx. (Culex) sitiens	Female	36	(1.7)	36	4	0
Cx. species (unidentified) - new or difficult to ID species	Male	9	(0.4)	9	8	0
Unidentifiable (too damaged/features missing)	Female	1	(<0.1)	1	1	0
Unidentifiable (too damaged/features missing)	Male	3	(0.1)	3	3	0
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	18	(0.9)	18	8	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Bloodfed	1	(<0.1)	1	1	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	3	(0.1)	3	3	0
Total		**2058**	**(100.0)**	**2035**	**214**	**15**

[1]No viruses were isolated.

Table 24B. Results of mosquito trapping and virus isolations from Cossack (Town and environs), Pilbara in 2014.[1]

Trap date: (11 January 2014; 4 traps, 1 failure).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned
Ae. (Macleaya) species	Male	1	(0.4)	1	1	0
Ae. (Ochlerotatus) E.N. Marks' species No. 85	Female	1	(0.4)	1	1	0
Ae. (Ochlerotatus) normanensis	Female	1	(0.4)	1	1	0
Ae. (Ochlerotatus) vigilax	Bloodfed	2	(0.9)	0	0	0
Ae. (Ochlerotatus) vigilax	Female	162	(70.1)	164	9	0
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	9	(3.9)	9	1	0
Ae. (Ochlerotatus) vigilax variant	Female	4	(1.7)	0	0	4
Cx. (Culex) annulirostris	Female	1	(0.4)	1	1	0
Cx. (Culex) sitiens	Female	50	(21.6)	50	4	0
Total		**231**	**(100.0)**	**227**	**18**	**4**

[1]No viruses were isolated.

Table 24C. Results of mosquito trapping and virus isolations from Wickham (Town and environs), Pilbara in 2014.[1]

Trap date: (11, 13, 14, 15 and 16 January 2014; 11 traps, no failures; includes 5 BG traps).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned
Ae. (Macleaya) E.N. Marks' species No. 125	Female	31	(7.4)	31	10	0
Ae. (Macleaya) species	Female	14	(3.3)	14	5	0
Ae. (Macleaya) species	Male	21	(5.0)	21	8	0
Ae. (Mucidus) alternans	Female	2	(0.5)	2	2	0
Ae. (Ochlerotatus) E.N. Marks' species No. 159	Female	22	(5.2)	20	2	2
Ae. (Ochlerotatus) E.N. Marks' species No. 159	Female	9	(2.1)	9	2	0
Ae. (Ochlerotatus) E.N. Marks' species No. 85	Bloodfed	1	(0.2)	0	0	0
Ae. (Ochlerotatus) E.N. Marks' species No. 85	Female	50	(11.9)	50	5	1
Ae. (Ochlerotatus) normanensis	Bloodfed	2	(0.5)	0	0	0
Ae. (Ochlerotatus) normanensis	Female	75	(17.8)	75	7	0
Ae. (Ochlerotatus) vigilax	Female	127	(30.2)	125	12	0
Ae. (Ochlerotatus) vigilax - two spots on tergites variant	Female	7	(1.7)	7	4	0
Cx. (Culex) annulirostris	Female	24	(5.7)	233	8	1
Cx. (Culex) quinquefasciatus	Female	8	(1.9)	8	6	0
Cx. (Culex) sitiens	Female	18	(4.3)	18	7	0
Cx. species (unidentified) - new or difficult to ID species	Male	5	(1.2)	5	3	0
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	5	(1.2)	5	3	0
Total		**421**	**(100.0)**	**623**	**84**	**4**

[1]No viruses were isolated.

Table 24D. Results of mosquito trapping and virus isolations from Roebourne (Town and environs), Pilbara in 2014.[1]

Trap date: (15 January 2014; 4 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools	No. pinned
Ae. (Macleaya) E.N. Marks' species No. 125	Female	27	(18.2)	27	3	0
Ae. (Macleaya) species	Female	6	(4.1)	6	1	0
Ae. (Macleaya) species	Male	27	(18.2)	27	3	0
Ae. (Ochlerotatus) E.N. Marks' species No. 159	Female	15	(10.1)	15	1	0
Ae. (Ochlerotatus) E.N. Marks' species No. 85	Female	4	(2.7)	4	3	0
Ae. (Ochlerotatus) normanensis	Female	20	(13.5)	20	2	0
Ae. (Ochlerotatus) vigilax	Female	5	(3.4)	5	1	0
An. (Cellia) annulipes s.l.	Female	2	(1.4)	2	1	0
Cx. (Culex) annulirostris	Female	39	(26.4)	39	3	0
Cx. (Culex) palpalis	Female	1	(0.7)	0	0	1
Cx. (Culex) sitiens	Female	1	(0.7)	1	1	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	1	(0.7)	1	1	0
Total		**148**	**(100.0)**	**147**	**20**	**1**

[1]No viruses were isolated.

Table 24E. Results of mosquito trapping and virus isolations from Cheeditha (Town and environs), Pilbara in 2014.[1]

Trap date: (15 January 2014; 3 traps, no failures).

Species	Class	No. collected	(%)	No. processed	No. pools
Ae. (Ochlerotatus) E.N. Marks' species No. 159	Female	2	(7.4)	2	1
Ae. (Ochlerotatus) E.N. Marks' species No. 85	Female	6	(22.2)	6	2
Ae. (Ochlerotatus) normanensis	Female	10	(37.0)	10	2
Cx. (Culex) annulirostris	Female	9	(33.3)	9	3
Total		**27**	**(100.0)**	**27**	**8**

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

Mosquito abundance was very low at both Mt Magnet and Cue (Tables 25A-B). On average, just eight mosquitoes were collected per trap at Mt Magnet, and the most abundant species was *Cx. quinquefasciatus*. At Cue the average number of mosquitoes per trap was 14. A large variety of mosquito species was collected, however the most abundant species comprised *Ae. sagax* (41.8%), *Ae. (Macleaya)* E.N.M. species no. 125 (12.5%), *Cx. quinquefasciatus* (7.8%) and *An. annulipes* s.l. (5.9%). No viruses were isolated from mosquitoes collected at either location.

Table 25A. Results of mosquito trapping and virus isolations from the Shire of Mt Magnet (Town and environs), Murchison.[1]
Trap date: 18 March 2014 (4 traps, no failures).

Species	Class	Total collected	(%)	No. processed	No. pools	No. pinned
Ae. (Macleaya) E.N. Marks' species No. 125	Female	3	(9.4)	3	3	0
Ae. (Macleaya) species	Male	3	(9.4)	3	2	0
Ae. (Pseudoskusea) bancroftianus	Female	5	(15.6)	5	3	0
An. (Cellia) annulipes s.l.	Female	1	(3.1)	1	1	0
An. (Cellia) annulipes species B	Female	1	(3.1)	1	1	0
Cx. (Culex) quinquefasciatus	Female	19	(59.4)	18	3	1
Total		**32**	**(100.0)**	**31**	**13**	**1**

[1]Mosquito trapping conducted by MBDC DOH personnel; no viruses were isolated.

Table 25B. Results of mosquito trapping and virus isolations from the Shire of Cue (Town and environs), Murchison.[1]
Trap dates: 18 and 19 March 2014 (18 traps, 1 failure).

Species	Class	Total collected	(%)	No. processed	No. pools	No. pinned
Ae. (Finlaya) notoscriptus	Female	2	(0.8)	2	2	0
Ae. (Macleaya) E.N. Marks' species No. 125	Female	32	(12.5)	32	3	0
Ae. (Macleaya) species	Bloodfed	1	(0.4)	0	0	0
Ae. (Macleaya) species	Female	8	(3.1)	8	4	0
Ae. (Macleaya) species	Male	28	(10.9)	28	5	0
Ae. (Mucidus) alternans	Female	1	(0.4)	1	1	0
Ae. (Ochlerotatus) E.N. Marks' species No. 159	Female	5	(2.0)	5	2	0
Ae. (Ochlerotatus) E.N. Marks' species No. 71	Female	2	(0.8)	2	2	0
Ae. (Ochlerotatus) E.N. Marks' species No. 85	Female	3	(1.2)	2	2	1
Ae. (Ochlerotatus) eidsvoldensis	Female	1	(0.4)	1	1	0
Ae. (Ochlerotatus) sagax	Bloodfed	6	(2.3)	0	0	0
Ae. (Ochlerotatus) sagax	Female	107	(41.8)	104	13	3
Ae. (Pseudoskusea) bancroftianus	Female	4	(1.6)	4	3	0
An. (Cellia) annulipes s.l.	Female	15	(5.9)	15	7	0
An. (Cellia) annulipes s.l.	Male	1	(0.4)	1	1	0
An. (Cellia) annulipes species A	Female	1	(0.4)	1	1	0
An. (Cellia) annulipes species D	Female	2	(0.8)	2	1	0
Cx. (Culex) annulirostris	Bloodfed	1	(0.4)	0	0	0
Cx. (Culex) annulirostris	Female	3	(1.2)	3	2	0
Cx. (Culex) quinquefasciatus	Bloodfed	2	(0.8)	0	0	0
Cx. (Culex) quinquefasciatus	Female	20	(7.8)	20	5	0
Cx. species (unidentified) - new or difficult to ID species	Male	3	(1.2)	3	1	0
Unidentifiable *Aedes* sp. (too damaged/features missing)	Female	6	(2.3)	6	3	0
Unidentifiable *Culex* sp. (too damaged/features missing)	Female	2	(0.8)	2	2	0
Total		**256**	**(100.0)**	**242**	**61**	**4**

[1]Mosquito trapping was conducted by MBDC DOH personnel; no viruses were isolated; approximately 40 Ceratopogonidae (*Culicoides*) were also processed for virus isolation.

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 are 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 14 shows the locations of sentinel flocks in the 2013/14 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

From July to September 2013 rainfall was below to very much below average throughout the Kimberley, Pilbara and Gascoyne regions. From October 2013 through to February 2014 the

Kimberley and Pilbara regions experienced above to very much above average rain. The Gascoyne region experienced average rainfall during the same period, except for November and January when there was above average rain. Isolated showers and thunderstorms were confined to the Kimberley region until mid-October when they extended to the Pilbara, Northern Interior and parts of northern, central, and eastern WA. Thunderstorms brought unseasonal rains to the Pilbara later in October. The Kimberley saw regular thunderstorm activity during November and the first tropical cyclone (TC) for the season, TC Alessia caused heavy rain near the north Kimberley coast on the 23 and 24 November. Above average rainfall across northern WA in December was associated with a weak low in the Timor Sea followed by the passage of TC Christine later in the month. Rain was particularly heavy from 23 December 2013 as the low in the Timor Sea moved toward the north Kimberley region. Severe TC Christine made landfall on the Pilbara coast between Whim Creek and Roebourne on 30 December and caused very heavy rain. Some sites in the Pilbara received their highest daily rainfall on record. A number of sites in the Kimberley, east Pilbara and east Gascoyne recorded their wettest January on record, mainly due to the passage of a deep tropical low and associated cloud band during the middle of the month. An active monsoon in the first half of February brought some very heavy falls to the east Kimberley. Monsoonal activity across northern parts of WA in March was weaker than usual with no significant impact from tropical low pressure systems or tropical cyclones, although TC Gillian did reform northwest of WA to bring some rain to northern WA toward the end of the month. Rain was above average in April and May 2014 over large parts of northern WA. Significant rain occurred on the 26 April in the west Pilbara as a result of slow moving thunderstorms. Above average rain in May was caused by a northwesterly flow behind a high pressure system in the middle of the month bringing rain to parts of northern and inland WA. Rainfall in June was below to very much below average throughout northern WA.

Flavivirus activity

The level of flavivirus activity in sentinel chickens in northern WA in 2013/14 was low (Table 27). Seroconversions were detected in 15 of the 4,798 samples tested (0.31%), which was higher than the 2012/13 season (ASRL 2012/13 Annual Report), but still low. Low level activity associated with the end of the 2012/13 season was detected in the west Kimberley and continued to October 2013. One KUNV infection was detected at Roebuck Plain, one MVEV seroconversion was detected at Broome, and two flavivirus infections of unidentified specificity (not MVEV or KUNV) were detected at Roebuck Plains. The WA DOH issued a media alert in early May reminding travellers and residents to take precautions against mosquito bites following late season flooding in the Pilbara and Gascoyne regions in May to June 2014. Flavivirus activity commenced late in the 2013/14 season, after generally above average rainfall between November 2013 and February 2014 followed by two months of very much below average or average rainfall in March and April in the Kimberley region. The first seroconversion for the season occurred in mid-May, when a KUNV seroconversion was detected in the Derby flock, in the west Kimberley. In the same month, antibodies to KUNV were detected at Ophthalmia Dam in the Pilbara region, followed by seroconversions to KUNV (3), MVEV (2) and an unknown flavivirus infection at Roebuck Plains, in the west Kimberley region. These results triggered the release of a second media alert by the WA DOH in mid-June, advising travellers and residents in the Kimberley and Pilbara regions of the increased risk of mosquito-borne disease and the need to take precautions against mosquito bites. Activity of KUNV and an unidentified flavivirus infection were detected at Ophthalmia Dam in July 2014. Overall 11 flavivirus seroconversions were detected in sentinel chickens in the 2013/14 season, and the majority of those (63.6%) were due to KUNV infection. Activity of MVEV was only detected at Roebuck Plain in the west Kimberley region of WA during the 2013/14 season.

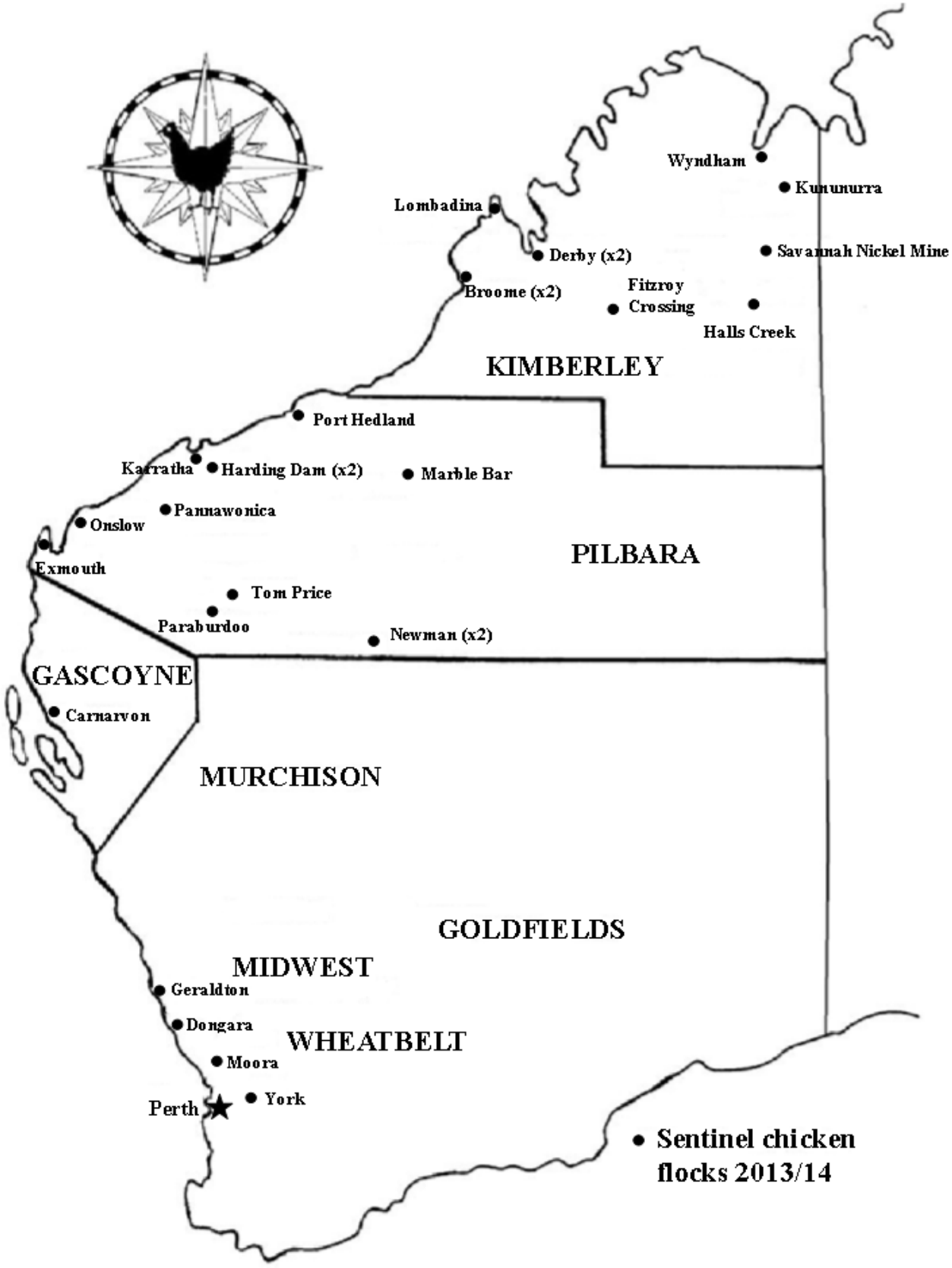

Figure 14. Locations of towns, communities, dams and mining centres at which flocks of chickens were located for surveillance of flavivirus activity in WA in 2013/14 (numbers in brackets indicate the number of flocks at that locality).

Table 27. Summary of WA Flavivirus Surveillance program, 2013/14*
Sentinel chicken flocks are tested for infection with Murray Valley encephalitis and Kunjin viruses.

July - December 2013																								
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	12	0	12	0	11	0	12	0	12	0	71	0
Kununurra	12	0	12	0	12	0	24	0	12	0	12	0	84	0
Savannah Nickel mine	13	0	13	0	13	0			5	0	14	0	58	0
Halls Creek	33	0	21	0	20	0	22	0	11	0	20	0	127	0
Fitzroy Crossing	10	0	20	0	10	0	10	0			12	0	62	0
Derby site 1	22	0	22	0	22	0	22	0	11	0			99	0
Derby site 2	22	0	22	0	22	0	19	0	10	0			95	0
Lombadina	NO FLOCK												0	0
Broome	9	0			8	1	7	0					24	1
					1M									
Roebuck Plains	19	2	14	0	12	0	12	1	23	0	12	0	92	3
	1F 1K						1F							
PILBARA														
Port Hedland	8	0	8	0	7	0					12	0	35	0
Karratha	18	0	18	0	18	0	27	0	12	0	12	0	105	0
Harding Dam 1	22	0	32	0	20	0	20	0	11	0	11	0	116	0
Harding Dam 2	22	0	33	0	20	0	19	0	11	0	11	0	116	0
Marble Bar	13	0	13	0			25	0					51	0
Pannawonica	19	0	19	0	18	0	25	0	11	0	12	0	104	0
Tom Price	14	0	14	0	13	0	12	0	12	0	12	0	77	0
Paraburdoo	18	0	18	0	18	0	16	0	11	0	12	0	93	0
Onslow	16	0	15	0	8	0	23	0	12	0	12	0	86	0
Ophthalmia Dam	16	0	8	0	16	0	16	0	12	0	12	0	80	0
Newman Shire	20	0	10	0	20	0	20	0	12	0	12	0	94	0
Exmouth	24	0	24	0	36	0	24	0	24	0	12	0	144	0
GASCOYNE														
Carnarvon	27	0	17	0	19	0	19	0	11	0	11	0	104	0
MID-WEST/WHEATBELT														
Moora	21	0			11	0	9	0	19	0			60	0
Geraldton	12	0	12	0	24	0			24	0	12	0	84	0
Dongara	33	0			20	0	22	0	24	0	12	0	111	0
York	22	0			11	0	11	0	12	0			56	0

Table 27 (continued). Summary of WA Flavivirus Surveillance program, 2013/14*
Sentinel chicken flocks are tested for infection with Murray Valley encephalitis and Kunjin viruses.

January - June 2014														
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	24	0	12	0	12	0					11	0	59	0
Kununurra	16	0	16	0	8	0	8	0	8	0	8	0	64	0
Savannah Nickel mine	14	0	12	0	4	0	7	0	12	0	12	0	61	0
Halls Creek	19	0	20	0	20	0	29	0	20	0	20	0	128	0
Fitzroy Crossing	24	0					11	0	22	0	22	0	79	0
Derby site 1	24	0	24	0	24	0	12	0	36	0	24	0	144	0
Derby site 2	24	0	24	0	24	0	12	0	35	1 (1K)	22	0	141	1
Lombadina	9	0			8	0	6	0	12	0	10	0	45	0
Broome			12	0			11	0			11	0	34	0
Roebuck Plains	18	0	17	0	9	0	8	0	9	6 (3K 2M 1F)	3	0	64	6
PILBARA														
Port Hedland			12	0	11	0	9	0					32	0
Karratha	24	0	12	0	24	0	36	0	22	0	22	0	140	0
Harding Dam 1	19	0	14	0	17	0	18	0	27	0	18	0	113	0
Harding Dam 2	21	0	20	0	19	0	18	0	27	0	18	0	123	0
Marble Bar			22	0	11	0			11	0			44	0
Pannawonica	23	0	20	0	18	0	27	0	18	0	18	0	124	0
Tom Price	24	0	22	0	22	0	31	0	9	0	18	0	126	0
Paraburdoo	24	0	24	0	24	0	36	0	12	0	24	0	144	0
Onslow	24	0	11	0	12	0	3	0	3	0	15	0	68	0
Ophthalmia Dam	24	0	24	0	24	0	24	0	24	1 (1K)	22	3 (2K 1F)	142	4
Newman Shire	24	0	24	0	24	0	24	0	24	0	24	0	144	0
Exmouth	12	0	22	0	30	0	18	0	18	0	18	0	118	0
GASCOYNE														
Carnarvon	22	0	31	0	10	0	27	0	18	0	10	0	118	0
MID-WEST/WHEATBELT														
Moora	12	0	12	0	12	0	36	0	12	0	12	0	96	0
Geraldton	23	0	11	0	22	0	11	0	33	0	22	0	122	0
Dongara	12	0	12	0	15	0			12	0	12	0	63	0
York			12	0							12	0	24	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 serology

Occasionally the ASRL is asked to assist with arbovirus serological testing by other organisations such as the WA Department of Agriculture and Food (DAF) using epitope blocking ELISAs or neutralisation assays. In 2013/14 the ASRL tested four donkey sera and two horse sera from stations in northern WA for flavivirus antibodies in the epitope blocking ELISA using 3H6, 10C6 and 31112G. Both horses were flavivirus antibody positive but did not test positive in the MVEV-specific (10C6) and KUNV-specific (31112G) assays. Three of the donkeys were MVEV antibody-positive and the fourth donkey was broadly reactive in both the MVEV and KUNV assays.

Section 8: Other research projects

Detection and preliminary characterisation of a novel yellow fever group flavivirus isolated from mosquitoes from northern WA

Recently a novel flavivirus in the yellow fever group was detected in mosquitoes collected in northern WA. The flavivirus, named Fitzroy River virus (FRV), was isolated from mosquitoes collected at Fitzroy Crossing in 2011 (three isolates; ASRL 2011/12 Annual Report) and Kununurra, Wyndham and Billiluna in 2012 (13 isolates; ASRL 2012/13 Annual Report). The majority of isolates were from *Ae. normanensis*. The virus was typed as a flavivirus after isolation in C6/36 cells and PSEK cells and analysis using a panel of monoclonal antibodies (Broom et al. 1998). Viral RNA was extracted and amplified in the NS5-3'UTR of the genome by RT-PCR (Poidinger et al. 1996) at PathWest Laboratory Medicine WA. Preliminary phylogenetic analysis by Dr Glenys Chidlow (PathWest) revealed the viruses were identical, novel and closely related to Sepik and Wesselsbron viruses. These viruses are members of the yellow fever group and are known to infect humans and livestock (Weyer et al. 2013, Woodroofe and Marshall 1971, Olson et al. 1983), although infection with Sepik virus is rare**.** Whole genome sequencing of the prototype virus by Mr Simon Williams at The University of Columbia in New York (USA) revealed FRV is most closely related to Sepik virus at the nucleotide and amino acid level over the entire polyprotein coding region. Given the widespread distribution of FRV in northern WA, it may have a wider distribution across northern Australia. The likely vertebrate hosts and vectors of FRV are unknown, although the large number of FRV isolates from *Ae. normanensis* may implicate this species in transmission. Host-feeding studies show that *Ae. normanensis* takes blood meals from cattle, pigs, horses, dogs, marsupials and humans (Johansen et al. 2009, Lee et al. 1984). Additional work is required to investigate the potential role of this new flavivirus in human and/or animal disease in Australia. Studies including serological surveys, replication kinetics and assessment of virulence in a mouse model are being conducted in collaboration between the ASRL, Dr Lorna Melville (Berrimah Veterinary Laboratories, Northern Territory) and Prof. Roy Hall and Dr Natalie Prow (University of Queensland, Queensland) with financial assistance from the FIMMWA, WA DOH.

Comparison between virus isolation and PCR for detection of Ross River and Barmah Forest viruses in mosquito pools from the southwest of WA

The ASRL detects Ross River and Barmah Forest viruses in mosquitoes by virus isolation, taking between eight and 13 days for detection (Section 5). Molecular tools such as polymerase chain reaction (PCR) have the capacity to greatly increase the speed that these and other arboviruses can be detected. A trial was conducted in 2013/14 comparing virus isolation with a multiplex real-time PCR. All homogenates of mosquitoes collected from the routine monitoring sites in the southwest of WA between September 2013 and April 2014 were tested in parallel by routine virus isolation in the ASRL and multiplex real-time PCR at PathWest Laboratory Medicine WA (Table 28). A total

Table 28. Results of parallel testing of mosquito homogenates from the southwest of WA between August 2013 and April 2014 by virus isolation and PCR.[1]

		Initial virus isolation		Reisolation			Multiplex real-time PCR		
Sample number	Collection run	C636	Vero/PSEK	C636	Vero/PSEK	Result	First Ct-value	Second Ct-value	Result
DC57568	01.10.13	GM	GM	Syncytia (3/4 wells)	Did not isolate (tried 3 times)	Neg	38	31	RRV
SW96520	"	GM	GM	Sncytia (2/4 wells)	CPE	RRV	32	30	RRV
SW96521	"	Syncytia	CPE	Syncytia	CPE	RRV	18	13	RRV
SW96524	"	Syncytia	CPE	Syncytia (3/4 wells)	CPE (3/4 wells)	RRV	30	28	RRV
SW96525	"	Syncytia	CPE	Syncytia (1/4 wells)	CPE (1/4 wells)	RRV	34	28	RRV
SW96526	"	GM	GM	Syncytia (2/4 wells)	CPE	RRV	31	31	RRV
SW96536	"	Syncytia	CPE	Syncytia (1/4 wells)	CPE (1/4 wells)	RRV	33	28	RRV
SW96538	"	Syncytia	CPE	Syncytia	CPE	RRV	24	21	RRV
SW96539	"	Syncytia	CPE	Syncytia	CPE	RRV	33	30	RRV
DC57908	29.10.13	GM	CPE?	GM	CPE	BFV	37	31	BFV
DC57910	"	Cells clumped	GM	Cells clumped	CPE (1/4) CPE (2/4 wells)	pending	34	29	BFV
DC57911	"	Syncytia	CPE	Syncytia (1/4 wells)	CPE	BFV	22	22	BFV
DC58064	"	Syncytia (1/2 wells)	CPE (1/2 wells)	Syncytia	CPE	BFV	BFV failed	28	BFV
SW97167	12.11.13	Syncytia	CPE	Syncytia	CPE	RRV	29	17	RRV
SW97173	"	Cells clumped	CPE	Cells clumped	CPE	RRV	26	23	RRV
SW97174	"	Cells clumped	CPE	Cells clumped	CPE	RRV	19	15	RRV
SW97176	"	Cells clumped	CPE	Cells clumped	CPE	RRV	25	22	RRV
SW97178	"	Cells clumped	GM	GM	Did not isolate (tried 3 times)	Neg	27	19	RRV
SW97317	"	Syncytia	CPE	Syncytia	CPE	RRV	27	19	RRV
SW97164	"	Syncytia (1/2 wells)	CPE (1/2 wells)	Syncytia	CPE	RRV	Neg	23	RRV
DC58220	26.11.13	Syncytia	CPE	Syncytia	CPE	BFV	24	23	BFV
SW97414	"	Syncytia	CPE (1/2 wells)	Syncytia	CPE	RRV	27	22	RRV
DC58315	10.12.13	Syncytia	CPE	Syncytia	CPE	BFV	20	19	BFV
SW97516	"	GM	CPE	Syncytia	CPE	BFV	26	26	BFV
SW97579	"	Syncytia (1/2 wells)	CPE	Syncytia	CPE	BFV	Neg	22	BFV
SW97836	04.02.14	Syncytia	CPE	Syncytia	CPE	BFV	26	25	BFV
SW97892	18.02.14	Syncytia	CPE	Syncytia	CPE	RRV	17	15	RRV

[1]GM is good monolayer, CPE is cytopathic effect, Neg is negative, RRV is Ross River virus, BFV is Barmah Forest virus.

of 3,595 homogenates were tested and 27 samples initially tested positive by PCR or virus isolation. Of these, 25 were PCR positive and 22 were virus isolation positive. One sample was only RRV positive by virus isolation (subsequently it also tested positive by PCR), one sample was only BFV

positive by virus isolation (it also subsequently tested positive by PCR) and five samples were only PCR positive, including the first detection of RRV in the Peel region for the season. It is likely the two samples initially virus isolation positive and PCR negative contained low titres of RRV and BFV as only one of two wells of C6/36 cells inoculated with these samples developed evidence of arbovirus infection. After repeat testing of all samples with discrepancies between the two methods, overall two more samples tested positive to RRV by PCR whilst the number of samples with evidence of BFV infection was equal (results not shown). These results provide substantial data supporting adoption of PCR for detection of RRV and BFV in mosquito homogenates. Further assessment is being conducted between August and December 2014, with the support of the FIMMWA DOH funding. More rapid detection of virus-infected mosquitoes will improve the capacity of the WA DOH and Local Government Authorities to manage and minimise the impact of arboviral disease in WA. This project was a collaboration between the ASRL, Clinical Prof. David Smith and Dr Glenys Chidlow at PathWest Laboratory Medicine WA.

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

Sera from sentinel chickens in WA are routinely screened using the monoclonal antibody (Mab) 3H6 in an epitope blocking ELISA (Hall et al. 1995) to detect evidence of flavivirus infection. If positive in the 3H6 assay, additional testing using mabs 10C6 and 31112G in Murray Valley encephalitis virus (MVEV)-specific and Kunjin virus (KUNV)-specific assays, respectively. However further testing of some flavivirus-negative sera (determined in the 3H6 assay) in the 10C6 and 31112G assays indicates that some flavivirus infections may be missed in the 3H6 assay. Previous studies with 6B6C-1 (Blitvich et al. 2003a, b) and 4G2 (Henchal et al. 1982) showed these Mabs may be useful for improved detection of flavivirus infections in sentinel chickens. Epitope blocking ELISAs were used to test a panel of sentinel chicken sera previously determined to be positive or negative using 3H6, 10C6 and 31112G mabs. Sera from MVEV and KUNV laboratory-infected chickens were also tested. The percentage inhibition of each monoclonal antibody was calculated for each test serum. The sensitivity and specificity of each assay was determined, and the data was used to determine appropriate positive/negative cut-offs using modified receiver-operated characteristic (ROC) analyses (SPSS Statistics version 22). Preliminary results show that 6B6C-1 is superior to 3H6 and 4G2 for detection of flavivirus infections. Mab 6B6C-1 detected 98% of MVEV and KUNV infections, whilst 3H6 and 4G2 detected 96% and 92%, respectively. Adoption of 6B6C-1 for screening chicken sera for flavivirus infection may increase the sensitivity and specificity of the sentinel chicken flavivirus surveillance program. It is hoped that improved specificity and timing of warnings of increased activity of these viruses may reduce the impact of these viruses on humans in WA.

The ASRL also investigated the potential for a new KUNV-specific monoclonal antibody P10F8 (University of Queensland) for detection of KUNV seroconversions in sentinel chickens. Sera from sentinel chickens and laboratory-infected chickens were tested in an epitope blocking ELISA using P10F8 and results were compared with the KUNV-specific assay using monoclonal antibody 31112G. In our hands, P10F8 detected fewer KUNV infections in sentinel chickens than 31112G, and results of ELISAs on sera from laboratory-infected chickens revealed that P10F8 may also detect some MVE infections. A UWA MSc Infectious Diseases student (Ms Dulcie Lautu) assisted with the 6B6C-1, 4G2 and P10F8 ELISAs during her research project (Section 9).

Analysis of 22 years of sentinel chicken seroconversions, human case data and rainfall to investigate the effectiveness of the flavivirus surveillance program in WA

Sentinel chicken seroconversion data, human case data and Bureau of Meteorology rainfall records were analysed over a 22 year period to investigate the effectiveness of the sentinel chicken

surveillance program for MVEV and the association between rain and MVEV activity. Negative binomial regression was used to analyse MVEV seroconversions in sentinel chickens, human case and rainfall data from northern WA from 1990 to 2011 (SPSS Statistics version 21). The models developed using this data were used to test sentinel chicken and rainfall data in 2012 and 2013. All but two human cases of MVE were preceded by sentinel chicken seroconversions. Rainfall in the previous three months was significantly associated with sentinel chicken seroconversions and human cases. In addition, seroconversions predicted human cases in the models. The model predicted seroconversions in the Kimberley region in 2012, however seroconversions in the Pilbara region in early 2012 were not predicted, possibly due to localised activity of MVEV in the vicinity of dams that may not be representative of the level of MVEV activity elsewhere in the Pilbara region. This study showed that sentinel chicken seroconversions and rainfall provide early warning of MVEV activity in northern WA, and supports the current MVE contingency plan that requires a media statement be issued across a region after detection of a single seroconversion in a region. This study involved collaboration with Prof. Linda Selvey, Prof. John Mackenzie and Ms Catarina Antao (Curtin University), Dr Michael Lindsay (MBDC, WA DOH), Clinical Professor David Smith (PathWest Laboratory Medicine WA), Dr Annette Broom (previously of the ASRL, UWA) and Dr Cheryl Johansen, and has been accepted for publication in BMC Infectious Diseases.

Emergence of a new lineage of dengue virus type 2 identified in travellers returning to Western Australia from Bali, 2010-2012

Dengue is currently the most rapidly spreading mosquito-borne viral disease of humans, and is endemic in most tropical and sub-tropical countries. An estimated 390 million infections occur annually and nearly 75% of the current global dengue disease burden is borne by people who live in Southeast Asia and the Western Pacific region. DENV serotype and genotype data is lacking in many parts of the region, limiting our attempts to understand the observed patterns of hyperendemicity and disease severity. Many countries in the region are popular tourist destinations, and dengue has been identified as a cause of travel-related illness in people returning from endemic countries. We sequenced the E gene of DENV isolated from travellers returning to Western Australia from 5 countries throughout Asia between 2010-2012. The majority of DENV originated in Indonesia, predominantly Bali, a popular travel destination for Australians. We identified hyperendemic transmission of all four DENV serotypes in Bali in 2010; circulating DENV included dominant local strains which had circulated for several years in Indonesia and Singapore as well as strains more recently introduced into Bali from other countries in the region. Finally, we show the emergence of a new lineage of DENV2 (Cosmopolitan genotype) in 2011-2012, which should be monitored. Travellers may act as sentinels and provide important information on DENV genotypes and linages circulating in countries where locally generated detailed genetic data may not be available. This study was undertaken by Mr Timo Ernst, Ms Suzi McCarthy, Dr Glenys Chidlow, Mr Dagwin Luang-Suarkia, Professor Edward Holmes, Clinical Professor David Wmith and Dr Allison Imrie and has been accepted for publication in PLOS Neglected Tropical Diseases.

Section 9: 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 investigated 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. Aziz also showed that multiple sublineages of G1 are circulating, including one that has only been detected in northern WA. The circulation of multiple genotypes and sub-lineages of MVEV in northern WA provide evidence of complex transmission cycles of viruses with potential involvement of different vectors or hosts. 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, neuroinvasiveness, survival time and 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 submitted his PhD thesis for examination in September 2013, and has now been passed. 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 Dr. 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 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 1960s 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 Dr. Allison Imrie, Prof. Geoffrey Shellam, Dr Paul Effler (WA DOH), Dr David Smith (PathWest Laboratory Medicine WA), Dr John McBride (James Cook University) and Dr. Cheryl Johansen. The laboratory component of this work has been completed.

Alternative monoclonal antibodies for the detection of antibodies specific for Murray Valley encephalitis and Kunjin viruses.

In 2013 Dulcie Lautu undertook a Masters of Infectious Diseases research project in the ASRL. Dulcie's project assessed the sensitivity of flavivirus group-reactive monoclonal antibodies 6B6C-1 and 4G2 and the KUNV-specific monoclonal antibody P10F8 for detection of antibodies to MVEV and KUNV in sentinel chicken sera. When sufficient sera was available, parallel serological testing was conducted using serum neutralisation assays, however there was a poor correlation between ELISA and neutralisation results. Overall Dulcie concluded that 6B6C-1 could be considered for routine detection of flavivirus infections in sentinel chickens. Further details of this project are provided in Section 7. Dulcie's research project was supervised by Assoc. Prof. Cheryl Johansen and Dr. Jay Nicholson.

Application of the anti-double stranded RNA monoclonal antibody 3G1 for detection of arboviruses in mosquitoes from Western Australia.

Najeeb Chachar is a Masters of Infectious Diseases student at UWA. The research project carried out by Najeeb involved investigating the application of an anti-double stranded RNA monoclonal antibody 3G1 (developed at The University of Queensland) for detection of arbovirus infections in C6/36 cell monolayers inoculated with mosquito homogenates (Obrien, 2013). Initially a panel of homogenates previously shown to be infected or uninfected were inoculated and assayed with 3G1 in a fixed cell ELISA. Najeeb then tested all homogenates of mosquitoes collected by the ASRL in the southwest of WA between 1 January 2014 and 4 March 2014, and results were compared with detection using the routine virus isolation procedure. This analysis is continuing in collaboration with Prof. Roy Hall and students and staff in his laboratory at The University of Queensland in order to fully evaluate 3G1. This monoclonal antibody was also used successfully by Assoc. Prof. Cheryl Johansen to detect BFV in serum taken from an Eastern Grey Kangaroo during a mass fatality event in NSW (unpublished results). This project was supervised by Dr. Cheryl Johansen and Dr Jay Nicholson.

The effect of boric acid and commercial honey on viral RNA detection on FTA® cards

Moaaz Malik is a Masters of Infectious Diseases student who conducted a three month research project in the ASRL in semester 1, 2014. Moaaz investigated the possibility of using boric acid and commercial honey as substitutes to other insecticides and medical grade honey on FTA® cards, which have been used to detect arbovirus activity (van den Hurk et al. 2014). Although insecticides containing fiprinol have been used successfully to increase the sensitivity of FTA® cards placed in mosquito traps, most states in Australia require a pesticide license to use fiprinol, so Moaaz tested boric acid as an alternative insecticide without licensing requirements. FTA® cards were inoculated with RRV or KUNV and tested for up to 14 days for detectable levels of viral RNA using real-time RT-PCR. Over the two week period, there was no evidence that the addition of boric acid inhibited the ability to detect either virus. Commercial honey was also compared with medical grade Medihoney® and preliminary data suggests commercial honey had no deleterious impact on viral RNA detection and would be a less expensive option in WA. Although Moaaz' experiment testing mosquito feeding and mortality rates when exposed to honey cards containing boric acid was inconclusive, his results indicated there are possible alternatives to fiprinol and medical grade honeys currently used in honey-baited FTA® card systems. This project was supervised by Dr. Cheryl Johansen, Dr. Jay Nicholson and Miss Shani Wong.

Molecular epidemiology of Murray Valley encephalitis virus in 2011 and Barmah Forest virus in 2012/13, Western Australia

Joanna Chua is a Masters of Infectious Diseases student at UWA. Joanna's research project involved phylogenetic analysis of the E gene of isolates of MVEV from mosquitoes collected by the ASRL in northern WA during a large outbreak of MVE (Knope et al. 2013). Joanna also obtained partial nucleotide sequences of BFV isolates from the southwest of WA during a reported large outbreak of BFV disease in 2012/13. Molecular phylogeny of the MVEV isolates from northern WA showed the isolates clustered with lineage 1. Interestingly, the phylogenetic analyses of BFV isolates revealed an insertion in the 3' non-coding region compared to the prototype strain of BFV. This project was supervised by Dr. Allison Imrie and Clinical Prof. David Smith (PathWest Laboratory Medicine WA).

Does vector mosquito and arbovirus surveillance predict incidence of RRV disease in the southwest of WA?

Anita Williams is undertaking studies towards a Master of Applied Epidemiology (MAE) at the Australian National University (ANU) in Canberra. One of Anita's research projects is to analyse vector mosquito abundance and mosquito infection rates with RRV at the 21 ASRL long-term mosquito and arbovirus monitoring sites between Mandurah and Dunsborough in the southwest of WA since 2002, particularly in relation to incidence of RRV disease in those regions. This project aims to assess the capacity of the ASRL to provide advance warning of increased activity of RRV. It will also establish which monitoring sites are most predictive of RRV disease risk, and therefore which sites are most important for future surveillance and RRV disease risk assessment. Anita's MAE supervisors are Prof. Tom Riley (UWA), Dr Paul Armstrong (WA DOH) and Dr Martyn Kirk (ANU), and this project is supervised by Dr. Cheryl Johansen and Dr. Michael Lindsay (MBDC, WA DOH).

Section 10: Publications of the group in 2013/14

1. Namekar, M., Ellis, E.M., O'Connell, M., Elm, J., Park, S.Y., **Imrie, A.** and Nerurkar, V.R. (2013). Evaluation of a new commercially available immunoglobulin M capture enzyme-linked immunosorbent assay for diagnosis of dengue virus infection. Journal of Clinical Microbiology 51: 3106-3106.
2. **Nicholson J.,** Ritchie S.A., and van den Hurk A.F. (2014). *Aedes albopictus* (Diptera: Culicidae) as a potential vector of endemic and exotic viruses in Australia. *Journal of Medical Entomology* 51:661-669.
3. Pacioni, C., **Johansen, C.A.,** Mahony, T.J., Parkinson, J., O'Dea, M., Robertson, I., Wayne, A.F. and Ellis, T. (2014). A virological investigation into declining woylie populations. *Australian Journal of Zoology* 61:446-453.
4. Young, P.R., Ng, L.F.P., Hall, R.A., Smith, D.W. and **Johansen, C.A.** (2014). Arbovirus Infections. In: G.C. Cook and A. Zumla (eds), *Manson's Tropical Infectious Diseases,* 23rd Edition. Elsevier Science Ltd., Edinburgh, United Kingdom. http://dx.doi.org.10.1.016/B978-0-7020-5101-2.00015-7.

Section 11: Formal presentations by the group in 2013/14

1. Dr Cheryl Johansen presented lectures entitled "Introduction to vector-borne diseases", "Introduction to arboviruses" and "Some medically important arboviruses" to Masters of Infectious Diseases students at The University of Western Australia, Nedlands, July and August 2013.

2. Dr Cheryl Johansen presented a short seminar entitled "Surveillance of medically important arboviruses in WA" at the WA Department of Health Combined CLAG forum at Shenton Park, July 2013.
3. 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 2013.
4. Dr Cheryl Johansen presented a lecture entitled "Murray Valley encephalitis and Kunjin virus activity in Western Australia at the Western Australian Department of Health 2013 Mosquito Management Course held in Mandurah, October 2013.
5. Dr Jay Nicholson presented a seminar entitled "Overview of ASRL program, including new surveillance initiatives" to the School of Pathology and Laboratory Medicine, The University of Western Australia, March, 2014.

Section 12: 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 Tarun Weeramanthri (Executive Director of Public Health and Clinical Services Division) 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, Dr Andrew Jardine, Mrs Amber Douglas, Mr Ryan Janes and Dr Abbey Potter of the MBDC, DOH, for their continuing logistical and technical assistance with our program and for many invaluable discussions about the program during 2013/14.
- The valuable contributions of the people involved in the sentinel chicken program for detection of flaviviruses in northern WA in 2013/14 are listed in Table 29.
- 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 and organisations who must be acknowledged for their assistance include:
  - 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 Parks and Wildlife 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, cell cultures and virus strains, many useful discussions about aspects of the program and collaborative research on innovative methods for remote area mosquito trapping;

Table 29. Personnel involved in management and bleeding of sentinel chickens in WA.

Personnel	Institution/Location (town or community)
Mr Tahi Morton, Mr John Coles	Shire of Wyndham- East Kimberley, Kununurra
Mr Cameron Heavens, Mr Haydn van Locken, Mr Lyndell Dudley, Ms. Jo Gibson	Savannah Nickel Mine, East Kimberley
Mr Musa Mono, Mr Steven Bai, Ms Hannah Davis	Shire of Halls Creek, Halls Creek
Ms Melanie Houghton, Mr Ken McLeod, Mr Tim Stuckey	Shire of Derby / West Kimberley, Derby
Mr Chris Kloss, Ms Lisa Stevens	Private residents, Derby
Roebuck Plains Station personnel, Ms Sarah Mason, Ms Dimity Hargrave	Roebuck Plains Station
Ms Sarah Morris, Mr Chicky Clements	Nirrumbuk Aboriginal Corporation, Broome
"Cedric"	Lombadina flock carer
Dr Heather Lyttle, Ms Phillipa Rose, Ms Jodie Bennett	Pilbara Public Health Unit, Port Hedland
Mr Craig Watts, Mr Eugene Wiedemann, Mr Leon Myburg, Ms Michelle Jordan, Ms Corey King	Shire of Roebourne, Karratha
Mr and Mrs Kevin and Debbie Cutmore, Mr Rob Trieb	Water Corporation, Harding Dam
Ms Jay Gordon, Mr Liam Ryder	Shire of Carnarvon
Ms Helen Mitchell, Ms Debbie Cook	Private residents, Marble Bar
Mr Bill Hardy, Mr Mick Dunne, Mr Aden Broocker, Mr Tim Brokenshire, Ms Samantha Tointon	Shire of Ashburton (Tom Price, Paraburdoo, Pannawonica and Onslow)
Dr Yvonne Ogiers	Shire of Newman, Newman
Mr Ken Cameron, Ms Jo Wall	Shire of Exmouth, Exmouth
Mr Don McLeod	Private resident, Carnarvon
Ms Maurie Struwig, Mr Viraj Ballanthuda Achchige, Mr Mark Chadwick	Shire of Greenough-Geraldton
Mr Felix Neuweiler	Shire of Irwin, Dongara
Mr Sean Harris	Private resident, Moora
Mr James Fisher	Private resident, York

- 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 and Ms Nina Kurucz (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;

- Dr Cassie Jansen (North Metro Public Health Unit, Queensland Health) for advice on surveillance using honey-baited FTA cards in mosquito traps;
- Professor Scott Ritchie (James Cook University) for collaborative research on innovative mosquito traps for arbovirus surveillance;
- Assoc. Professor Linda Selvey (Curtin University) for collaborative research on longitudinal analyses of flavivirus seroconversions in sentinel chickens, human cases of MVE and rainfall;
- Dr Lorna Melville (Berrimah Veterinary Laboratory, Darwin) for advice and collaborative serological studies on Fitzroy River virus;
- Dr Mark O'Dea (Western Australian Department of Agriculture) for helpful advice and assistance with various aspects of the surveillance and research program;
- 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 2013/14.

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Appendix 1: Sentinel Chicken Surveillance Results 2012/13

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

Appendix 1 (continued). Summary of WA Flavivirus Surveillance program, 2012/13*
Sentinel chicken flocks 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.