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MOSQUITO (DIPTERA: CULICIDAE) AND RAINFALL ASSOCIATIONS WITH ARBOVIRUS DISEASE IN EASTERN VICTORIA

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Abstract

Associations between mosquito abundance and Ross River virus (RRV) and Barmah Forest virus (BFV) disease are demonstrated for the Gippsland Lakes region of eastern Victoria, Australia. Significant correlations were obtained between RRV and BFV disease notifications and mosquito counts after lag times ranging from 2–4 months for the dominant mosquito species *Aedes camptorhynchus*, and 0–3 months for the less abundant mosquitoes *Anopheles annulipes*, *Culex australicus* and *Culex globocoxitus*. Correlations between RRV and BFV disease notifications and rainfall were significant after lag times of 3–4 months. Monthly abundance of *Ae. camptorhynchus* was significantly higher during above-average years of RRV notifications, with higher mosquito abundance during November to January. Together, these results clarify some important timelines between rainfall, mosquito abundance and increased arbovirus activity in eastern Victoria.

Keywords: *Aedes camptorhynchus*, Barmah Forest virus, Gippsland Lakes, *Ochlerotatus*, rainfall, Ross River virus.

Introduction

The mosquito fauna of the Gippsland Lake wetlands of eastern Victoria, Australia, is of interest due to its role in the transmission of enzootic Ross River and Barmah Forest viruses to humans (Campbell *et al.* 1989b; Aldred 1993; Russell 1995; Mackenzie *et al.* 1998). The dominant mosquito in the area is the southern saltmarsh mosquito *Aedes camptorhynchus* (Thomson) (see Dhileepan *et al.* 1997), and is a demonstrated vector of Ross River virus (RRV) (Ballard & Marshall 1986; Campbell *et al.* 1989a; Azuolas *et al.* 2003) and suspected vector of Barmah Forest virus (BFV) (Aldred *et al.* 1990; Passmore *et al.* 2002). Less abundant mosquitoes in the region, including *Anopheles annulipes s.l.* Walker, *Culex globocoxitus* Dobrotworsky and *Culex australicus* Dobrotworsky and Drummond, may also play a role in arbovirus ecology, albeit minor (Russell 1995, 2002; Azuolas *et al.* 2003; Barton *et al.* 2004b). For consistency with current practice in other medical entomology journals, in this paper we use the genus *Aedes* Meigen, 1818 in place of *Ochlerotatus* Lynch Arribalzaga, 1891. However, we recognise recent phylogenetic analysis indicates the elevation to generic rank of the subgenus *Ochlerotatus* (see Reinert *et al.* 2004; Reinert *et al*. 2008).

Outbreaks of RRV and BFV disease in Australia have previously been linked to high rainfall and increased mosquito abundance in parts of Queensland (Ryan *et al.* 1999; Kelly-Hope *et al.* 2004a; Gatton *et al.* 2005), the southwest of Western Australia (Woodruff *et al.* 2003; Woodruff *et al.* 2006), and northern Victoria (Wishart 2002; Woodruff *et al.* 2002). Reported outbreaks of RRV disease have occurred in the Gippsland Lakes region in 1989 (Campbell *et al.* 1989b) and 1993 (Norris 1993), and outbreaks of BFV disease in 1993 (Norris & Robinson 1995) and 2002 (Passmore *et al.* 2002). An understanding of the association between the incidence of arbovirus disease, key environmental factors such as rainfall, and increases in vector mosquito populations will contribute to our knowledge of regional arbovirus ecology, and may lead to enhanced predictive abilities for future outbreaks.

The aim of this study was to describe temporal associations between arbovirus notifications in humans, and mosquito abundance and rainfall data from the Gippsland Lakes region of eastern Victoria.

Methods

The Gippsland Lakes are a group of three large lagoons (east to west: Lakes King, Victoria, Wellington) and associated wetlands in coastal eastern Victoria, Australia (147.6°E, 37.9°S). The lakes cover a combined area of approximately 340 km^2 and experience weak tidal influence through an inlet at the eastern extremity (Bird 1978; Walker & Andrewartha 2000).

The Victorian Department of Human Services provided serologically confirmed monthly counts of notifications for RRV (1991–2001) and BFV (1992–2001) disease for the Shires of Wellington and East Gippsland. Population estimates for each Shire were Wellington = 41,183 and East Gippsland = 40,067 (Australian Bureau of Statistics, 2003). Total monthly rainfall and mean monthly minimum temperatures for each Shire was sourced from the Bureau of Meteorology for the localities of Bairnsdale (East Gippsland) and East Sale (Wellington) for the period 1991–2001.

Mosquito monitoring data were used from East Gippsland Shire Council (covering Lake King) and Wellington Shire Council (covering Lake Wellington). Briefly, three CO_2-baited light traps were operated weekly near Lake King (May to March 1990–1991, November to March 1991–1994, November 1994) and four traps near Lake Wellington (November-April 1991–2000 and October-April 2000-2001). Details of species composition and patterns of abundance have been described elsewhere (Dhileepan *et al.* 1997; Barton *et al.* 2004a).

We constructed a profile of average monthly abundance of *Ae. camptorhynchus* from Wellington Shire using selected years of the 10 year period in which above-average cases of RRV disease occurred. The years 1993, 1999 and 2001 were classified as above average, and the remaining years below-average relative to the 10-year mean (mean annual RRV notifications = 4.8 ± 4.4 S.D.). An independent t-test was used to test for differences in mosquito abundance between the years of above-average and below-average occurrence of RRV. The purpose of this test was to explore whether years of above average RRV disease is accompanied by a characteristic higher monthly abundance profile. Insufficient mosquito data prevented similar analysis for East Gippsland.

Mosquito trap data were pooled for each Shire with data $\log_{10}(x+1)$ transformed prior to tests for correlation. Spearman's Rank correlation coefficients were calculated between lagged mean monthly mosquito numbers for the four most abundant species (*Ae. camptorhynchus*, *Cx. australicus*, *Cx. globocoxitus*, *An. annulipes*), lagged monthly rainfall and minimum temperatures, and monthly notifications of RRV and BFV disease. Multiple linear regression methods were used to identify which combination of meteorological variables and mosquito species abundance best predicted RRV and BFV notification data. For this analysis both mosquito and notification data were $\log_{10}(x+1)$ transformed to improve homogeneity of variance. Variables in the linear model were selected using the forward-stepwise method in the all-subsets menu of GenStat v10 (VSN International 2008). Correlations and linear regression were not calculated for BFV notifications from Wellington Shire because of very low numbers.

Results

Cases of RRV and BFV disease were lower in Wellington Shire compared to East Gippsland Shire (Table 1). The average 10-year incidence was 11.8/100,000 for RRV and 2.0/100,000 for BFV in Wellington and 44.1/100,000 for RRV and 20.9/100,000 for BFV in East Gippsland. The seasonal pattern of RRV disease notifications was biased towards the warmer summer months, with 73.6% and 69.6% of cases occurring in January to April in Wellington and East Gippsland respectively. A seasonal bias was less obvious for BFV disease, with 37.5% and 51.2% occurred in January to April for Wellington and East Gippsland respectively.

Table 1. Annual total RRV and BFV disease notifications from Wellington Shire and East Gippsland Shire, 1991–2001.

Year	Wellington Shire BFV	East Gippsland Shire BFV	Wellington Shire RRV	East Gippsland Shire RRV
1991	-	-	3	18
1992	0	1	1	24
1993	0	14	6	18
1994	0	5	3	18
1995	0	2	4	1
1996	2	23	4	24
1997	1	11	2	9
1998	1	8	1	7
1999	1	3	15	41
2000	2	6	3	10
2001	1	11	11	24
Total	8	84	53	194

Table 2. Spearman correlation coefficients for RRV and BFV disease notifications and mean monthly numbers of four mosquito species, rainfall and minimum temperature from Wellington and East Gippsland Shires.

	n		0 month	1 month	2 month	3 month	4 month
Wellington							
RRV	60	*Ae. camptorhynchus*	0.377	0.415	0.701**	0.702**	0.828**
RRV	60	*Cx. australicus*	0.311	0.029	0.456*	0.696**	0.545*
RRV	60	*Cx. globocoxitus*	0.443*	0.679**	0.708**	0.593**	0.020
RRV	60	*An. annulipes*	0.763**	0.741**	0.569**	0.457*	0.267
RRV	128	Minimum temperature (°C)	0.370**	0.462**	0.406**	0.276**	0.044
RRV	128	Rainfall (mm)	0.096	0.166	0.076	0.249**	0.353**
East Gippsland							
RRV	27	*Ae. camptorhynchus*	0.264	-0.485	-0.341	0.696*	0.636*
RRV	27	*Cx. australicus*	-0.145	0.196	-0.332	0.242	-0.341
RRV	27	*Cx. globocoxitus*	-0.451	-0.128	-0.409	0.693*	0.028
RRV	27	*An. annulipes*	0.119	-0.494	-0.339	0.156	0.071
RRV	60	Minimum temperature (°C)	0.450**	0.587**	0.555**	0.411*	0.144
RRV	60	Rainfall (mm)	0.118	0.264	0.146	0.241	0.348*
BFV	27	*Ae. camptorhynchus*	0.632	0.422	0.553	0.544	0.509
BFV	27	*Cx. australicus*	-0.018	0.132	-0.123	0.438	-0.244
BFV	27	*Cx. globocoxitus*	0.509	0.553	0.237	0.489	-0.134
BFV	27	*An. annulipes*	0.685	0.044	0.496	0.756	-0.012
BFV	60	Minimum temperature (°C)	0.332*	0.467**	0.432**	0.262	0.056
BFV	60	Rainfall (mm)	-0.058	0.254	0.306	0.218	0.513**

* p < 0.05, ** p < 0.01 (two-tailed)

Several significant correlations were obtained between mosquito abundance, rainfall, minimum temperatures and RRV notifications in Wellington Shire (Table 2). For *Ae. camptorhynchus* a lag of 2, 3 or 4 months was significant. For *Cx. australicus* a lag of 2, 3 or 4 months was significant. For *Cx. globocoxitus* a lag period of 0, 1, 2 and 3 months was significant. *Anopheles annulipes* was significantly correlated after 0, 1, 2 and 3 months. Minimum temperature was significantly correlated with RRV notifications after lags of 0, 1, 2 and 3 months. Rainfall was significantly correlated after 3 and 4 months.

For RRV in East Gippsland (Table 2), *Ae. camptorhynchus* was significantly correlated after 3 and 4 months. For *Cx. globocoxitus* a lag of 3 months was significant. Not significant correlations were obtained for either *Cx. australicus* or *An. annulipes*. As for Wellington Shire, minimum temperature was correlated after 0, 1, 2 and 3 months. Rainfall was significantly correlated after 4 months.

For BFV in East Gippsland (Table 2), no mosquito species were significantly correlated after any time lags. Minimum temperature was correlated after 0, 1 and 2 months, and rainfall after 4 months.

Multiple linear regression results for Wellington Shire (Table 3) indicate a combination of mosquito species, rainfall and minimum temperature contributes to explaining patterns of RRV notifications. *Culex australicus* and *An. annulipes* were both included in the linear models after lags of 0 and 1 month, *Cx. globocoxitus* after 3 and 4 months and *Ae. camptorhynchus* after 2, 3 and 4 months. Rainfall had a positive regression coefficient after 3 and 4 months.

For East Gippsland Shire (Table 3), as for Wellington Shire, a combination of mosquito species, rainfall and minimum temperature contributed to explain patterns of arbovirus notifications. For RRV, *Aedes camptorhynchus* was included in the linear models, and had a positive coefficient after time lags of 0 and 4 months. *Anopheles annulipes* was included in the linear models after 0 and 1 month. Minimum temperature and rainfall and were only included in the linear models after 0 and 1 month respectively. For BFV, all mosquito species were included in the linear model after 0 months. *Culex australicus* was the only mosquito species included in the linear model after 3 months. *Aedes camptorhynchus* was also included in the linear models after 2 and 4 months. Minimum temperature was included in models, but had a negative regression coefficient, after 3 and 4 months. Rainfall did not feature in any models for BFV.

Numbers of *Ae. camptorhynchus* were significantly higher during years of above-average RRV notifications compared to below-average years of notifications (t = -3.07, d.f. = 58, P<0.01). The highest mosquito numbers occurred during November and December in above-average disease years, with average trap captures exceeding 1,000 mosquitoes per pooled trap count (Fig 1). In below-average years there was little differentiation in mosquito numbers between months.

Discussion

We found significant correlations between RRV and BFV disease, mosquito abundance, rainfall and minimum temperature for the Gippsland Lakes region after specific monthly time lags. The existing knowledge is that *Ae. camptorhynchus* readily feeds on humans (Dobrotworsky 1965), is the dominant species in the region (Dhileepan *et al*. 1997) and is a competent vector of at least RRV (Ballard & Marshall 1986). This supports our findings of a significant relationship between RRV disease notifications and abundance of this mosquito species. The lag period of 2–4 months for *Ae. camptorhynchus* can be accounted for by the time necessary for the sequence of events between the development and emergence of mosquitoes from larval habitat, infection of the mosquito following a successful blood meal from a viraemic vertebrate host, and transmission to a human host at its second blood meal. Subsequently, the incubation period in humans for RRV is usually 7–9 days (Mackenzie *et al*. 1994).

The significant correlations between the less abundant *An. annulipes*, *Cx. australicus* and *Cx. globocoxitus* with RRV notifications indicate these mosquito species may play a role in the epizootic cycle. The mixed time

Table 3. Summary of multiple linear regression results for monthly mosquito abundance, rainfall and minimum temperature as predictors of RRV and BFV notifications. (annu = *An. annulipes*, aust = *Cx. australicus*, camp = *Ae. camptorhynchus*, glob = *Cx. globocoxitus*, min = minimum temperature (°C), rain = rainfall (mm)).

	Equation	R^2	F	d.f.	*p*
Wellington					
0 months	RRV=0.244(annu)+0.241(aust)-0.003(rain)+0.182	0.342	11.20	3	<0.001
1 month	RRV=0.218(aust)+0.176(annu)+0.034(min)-0.246	0.338	11.06	3	<0.001
2 months	RRV=0.217(camp)+0.156(aust)-0.114	0.245	10.55	2	<0.001
3 months	RRV=0.132(camp)+0.130(glob)+0.002(rain)+0.092(aust)-0.253	0.337	8.51	4	<0.001
4 months	RRV=0.136(camp)+0.004(rain)-0.097(annu)+0.128(glob)-0.026(min)+0.108(aust)+0	0.419	8.08	6	<0.001
East Gippsland					
0 months	RRV=1.003(annu)+0.081(min)+0.145(camp)-0.536(glob)+0.405(aust)-0.979	0.264	2.29	5	0.106
1 month	RRV=-0.325(camp)+0.00395(rain)+0.449(annu)+0.758	0.261	3.24	3	0.050
2 months	no significant terms, no variables selected				
3 months	RRV=0.170(glob)+0.372	0.065	2.39	1	0.138
4 months	RRV=0.221(camp)-0.012	0.122	3.93	1	0.061
0 months	BFV=0.662(annu)+0.438(camp)-0.538(aust)+0.223(glob)-0.672	0.453	3.69	4	0.048
1 month	BFV=0.344(glob)+0.002(rain)-0.271(aust)-0.063	0.298	2.98	3	0.078
2 months	BFV=0.132(camp)-0.081	0.047	1.69	1	0.216
3 months	BFV=0.394(aust)-0.035(min)+0.176	0.298	3.97	2	0.048
4 months	BFV=0.162(camp)-0.426(annu)-0.058(min)+0.256(aust)+0.331	0.396	3.29	4	0.057

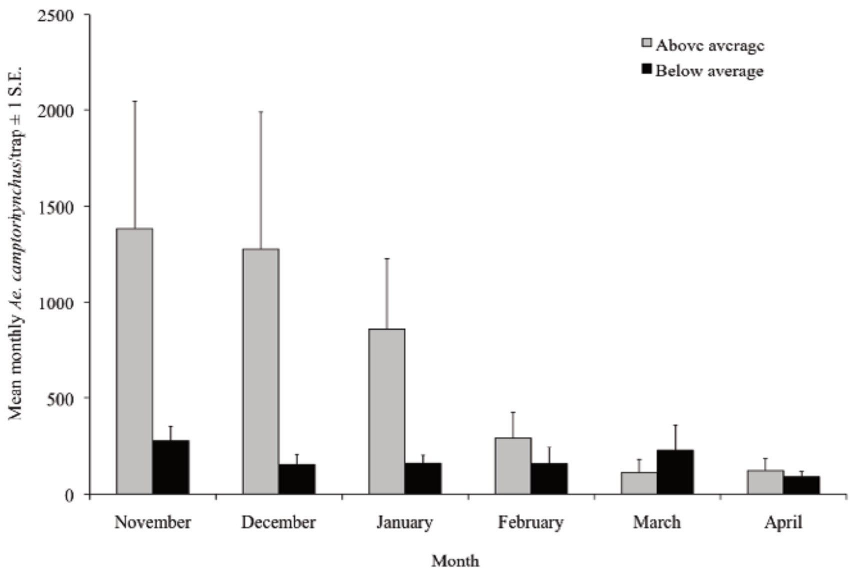

Figure 1. Mean monthly *Ae. camptorhynchus*/trap during above-average (1993, 1999, 2001) and below-average (1991–1992, 1994–1998, 2000) years of RRV disease for Wellington Shire.

lags in Wellington Shire of 0–3 months for *An. annulipes* and *Cx. globocoxitus*, and 2–4 months for *Cx. australicus* are difficult to clarify, but may be attributable to later seasonal peak in numbers during December-January compared to *Ae. camptorhynchus* (Barton *et al.* 2004b). We suggest these less abundant mosquito species might act as bridging vectors and could play a role in amplifying or prolonging virus activity in local bird and animal populations on which these species are known to feed (Dobrotworsky 1965), and on which *Ae. camptorhynchus* also feeds (Dobrotworsky 1960, 1965). The relationship between the time involved for the amplification of RRV and BFV in susceptible vertebrate hosts and the feeding preferences of the four mosquito species included in this study may be an important underlying factor contributing to the range of time lags obtained in this study.

Rainfall and minimum temperature was significantly associated with BFV and RRV in East Gippsland Shire, and with RRV notifications in Wellington Shire. The 4 month lag in RRV notification maxima following rainfall, for both Wellington and East Gippsland Shires could be attributed to the sequence of events described above for correlations with mosquito abundance, but with rainfall as the instigating factor for egg hatching, followed (for *Ae. camptorhynchus*) by approximately 26 days for larval development at temperatures of 20°C in brackish water (Barton & Aberton 2005). The extended effect of minimum temperature from 0 to 3 months may be the result of seasonal influence, with overnight temperatures increasing through summer.

The results of the linear regression for both shires indicate that combinations of mosquito species, rainfall and minimum temperature are able to explain patterns of arbovirus disease occurrence. The results were clearer for Wellington, compared to East Gippsland, with *Ae. camptorhynchus* and rainfall becoming more important after 2–4 months. The inclusion of the less abundant mosquitoes in some regression models, particularly after 0–2 months, further highlights their likely role in the epizootic cycle.

No previous studies have documented the time lags between rainfall and RRV or BFV notifications in the Gippsland region. This finding is therefore particularly important in establishing rainfall as a central climatic factor dominating the ecology of RRV and BFV in the Gippsland Lakes, as has been shown for rainfall and RRV in the Murray Valley of northern Victoria (Woodruff *et al.* 2002).

Any large rainfall event may have an impact on the incidence of RRV or BFV virus disease by promoting vector breeding and creating suitable habitat for mosquito oviposition and development. The period between rain events and peaks in *Ae. camptorhynchus* populations has been recorded as 0–2 months in Wellington Shire and 1 month in East Gippsland (Barton *et al.* 2004b). These time periods fit within the time lags established in this paper for arboviral disease outbreaks (3–4 months for RRV), and a more complete picture of mosquito and disease patterns following rainfall has been attained.

The rainfall thresholds that trigger mosquito breeding events are closely related to water levels in the lake system. Rises in water levels of less than 0.5 metres can vastly increase areas of inundated saltmarsh (Barton *et al*. 2005). Following these increases, sites may be inundated for extended time periods caused by subsequent inflows from the surrounding catchment. The relationship between lake water levels, rainfall and other climatic and hydrologic factors on mosquito habitat is complex and could be further explored to better partition causative effects on mosquito numbers.

The profile of monthly numbers of *Ae. camptorhynchus* during above-average years of RRV disease gives an indication of the pattern of abundance during a year of increased RRV activity. Averaged catches from the four traps sites across Wellington Shire exceeded 1000 mosquitoes per trap during November and December. Further mosquito monitoring data are required in East Gippsland before a similar profile can be used to define mosquito abundance during years of above-average RRV or BFV disease.

The lower incidence of BFV relative to RRV is reflected elsewhere in Australia and is, at least partially, due to the different ecologies of their respective vertebrate hosts and vectors (Russell 1995, 2002). Seasonal activity for RRV was greatest from January to April, which occurs between 1–3 months earlier than that for more northern areas in Queensland where the principal vector is *Aedes vigilax* (Skuse) (see Russell 2002;

Gatton *et al.* 2004; Kelly-Hope *et al.* 2004b), but similar to the southwest of Western Australia where *Ae. camptorhynchus* is the major vector in coastal areas (Russell 1995, 2002).

Further collection of mosquito surveillance, rainfall and RRV and BFV notification data from the Gippsland Lakes region will allow for more detailed and sophisticated analysis, as has been achieved for other parts of Australia (Gatton *et al.* 2005; Woodruff *et al.* 2006). This will become important as predicted changes in climate patterns begin to influence aspects of the ecology of arboviruses and their mosquito vectors (Hu *et al.* 2006; Tong *et al.* 2007). The associations between mosquitoes, rainfall and arboviral disease occurrence in the Gippsland lakes region contributes to the growing body of knowledge on Ross River and Barmah Forest virus ecology across Australia.

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