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Colonization of Ephemeral Water Bodies in the Wheatbelt of Western Australia by Assemblages of Mosquitoes (Diptera: Culicidae): Role of Environmental Factors, Habitat, and Disturbance

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ABSTRACT Environmental disturbance may have direct and indirect impacts on organisms. We studied the colonization of ephemeral water bodies by mosquitoes (Diptera: Culicidae) in the Wheatbelt region of southwest Western Australia, an area substantially affected by an expanding anthropogenic salinization. Mosquitoes frequently colonized ephemeral water bodies, responded positively to rainfall, and populated smaller water bodies more densely than larger water bodies. We found that the habitat characteristics of ephemeral water bodies changed in association with salinity. Consequently relationships between salinity and abundance of colonizing mosquitoes were direct (salinity–mosquito) and indirect (salinity–water body characteristics–mosquito). Overall, the structure of mosquito assemblages changed with increasing salinity, favoring an increased regional distribution and abundance of *Aedes camptorhynchus* Thomson (Diptera: Culicidae), a vector of Ross river virus (RRV; Togoviridae: Alphavirus). We conclude secondary salinization in the Western Australia Wheatbelt results in enhanced vectorial potential for RRV transmission.

KEY WORDS dryland salinity, community, arbovirus, secondary salinization, temporary water

Disturbance of the environment, which is often anthropogenic and chronic in nature, can create novel environmental conditions, which may have direct and indirect effects on colonizing organisms (Pappas and Pappas 1983, Taniguchi and Tokeshi 2004, Benstead et al. 2005, Doupé et al. 2006, Lacoul and Freedman 2006, Woodcock et al. 2007, Yee and Willig 2007). Populations of mosquitoes (Diptera: Culicidae) are known to respond to a range of environmental conditions, including disturbance, which can have direct or indirect effects on their colonization events (Chase and Knight 2003, Williams 2006, Carver et al. 2009b). Here we study mosquitoes in ephemeral water bodies, a primary source of mosquito production (Williams 2006). We examine the association between a saline disturbance and the aquatic environment and the direct and indirect ecological consequences of this anthropogenic disturbance to mosquitoes.

Salinity is known to affect populations of mosquitoes directly, through physiological tolerance, and indirectly, through biotic characteristics such as habitat, necessary for successful colonization and establishment (Roberts and Irving-Bell 1997, Clark et al. 2004, Silberbush et al. 2005). For example, Klinkenberg et al. (2004) found abundance of the malaria vectors *Anopheles stephensi* Liston increased and *Anopheles culicifacies* s.l. decreased in saline areas of Pakistan, and Roberts and Irving-Bell (1997) found communities of mosquitoes in southern Oman differed among habitat types in association with an environmental salinization.

The inland southwest Western Australia (the Wheatbelt), of which ephemeral water bodies are a characteristic feature, is chronically disturbed by secondary salinity (George et al. 2006). This environmental salinization is caused by historic land clearing and rising ground water tables, which have mobilized large regolith salt loads in the soil column to the surface (McKenzie et al. 2003). Overall, 74% of natural land cover has been cleared in the Wheatbelt (McKenzie et al. 2003). Currently >1,000,000 ha are affected, and secondary salinization is expected to expand to between 3,000,000 and 4,500,000 ha in the next 50 yr (George et al. 2006, Jardine et al. 2007).

Secondary salinity in the Western Australia Wheatbelt is known to create novel environmental conditions in water bodies, particularly to aquatic flora (Halse et al. 2003, Cale et al. 2004, Doupé et al. 2006, Lyons et al. 2007), and changes in populations of mosquitoes have also been documented (Jardine et al. 2008a, Carver et al. 2009b). Jardine et al. (2007) postulated that secondary salinity may influence the ecology of Ross River virus (RRV; Togoviridae: *Alphavirus*) in the Wheatbelt, by promoting dominance of

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mosquito communities by the halotolerant vector *Aedes camptorhynchus* Thomson. This was supported by mosquito surveys and ecological modeling but not human disease incidence (Jardine et al. 2008a, b; Carver et al. 2009a).

Secondary salinization of ephemeral water bodies in the Western Australia Wheatbelt provides an opportunity to investigate the direct and indirect effects of disturbance on mosquitoes. The range of salinity values for which larval mosquito species occur in the Wheatbelt has not been fully described. The relationship of mosquitoes to a range of environmental parameters in the Wheatbelt is also not well understood. In general, the indirect ecological effects of salinity are poorly understood (Rapport et al. 2003, Horrigan et al. 2005). Here we combine environmental data (rainfall, water body dimensions, salinity, and pH) and habitat characteristics (composition of vegetation, algae, detritus, and bare substrate) of ephemeral water bodies to study factors associated with assemblages of mosquitoes in the Wheatbelt of Western Australia. Emphasis is given to the impacts of salinity, because of the severity of this disturbance in the Wheatbelt. We hypothesize that salinity and associated changes in aquatic habitat will favor mosquito communities dominated by *Ae. camptorhynchus*, a vector of RRV.

Materials and Methods

Study Area. This study was undertaken in the Great Southern meteorological district of the Western Australia Wheatbelt (Fig. 1). The study area has a Mediterranean climate with hot dry summers (13–29°C mean temperature range and 9% of mean annual rainfall) and mild wet winters (5–15°C mean temperature range and 45% of mean annual rainfall). Annual rainfall declines from ≈600 mm at the western boundary of our study area to 350 mm in the east (Australian Bureau of Meteorology). A large proportion (80–90%) of the region has been cleared for agriculture (Halse et al. 2004), predominantly for cereal crops (wheat and barley) and grazing (by sheep *Ovis aries*), leaving small patches of remnant native vegetation.

Surveys. A random stratified sampling design was used, whereby 1 ha^2 quadrats were stratified across the Great Southern meteorological district of the Western Australia Wheatbelt (Fig. 1) and samples of mosquito larvae (Diptera: Culicidae) were collected from rainfed ephemeral water bodies (size range, 0.2–9,700 m^2) occurring within these quadrats. Quadrats were located in patches of remnant native vegetation corresponding with locations previously used by the Western Australia Department of Environment and Conservation to assess the impacts of secondary salinisation (Keighery et al. 2004). For this study, individual pools within quadrats were used as replicates to assess changes across the Wheatbelt. Surveys were undertaken fortnightly from September to November 2005 (spring) and February to May 2006 (late summer and autumn), giving a total of 11 sampling occasions. These seasons were chosen to maximize the number of mosquitoes, water bodies, the range of water body sizes, and salinities observed in the field (Carver et al. 2009b). Sampling was not conducted in winter because higher rainfall results in fewer saline water bodies. In summer, there are few water bodies because of low rainfall, high temperatures, and evaporation.

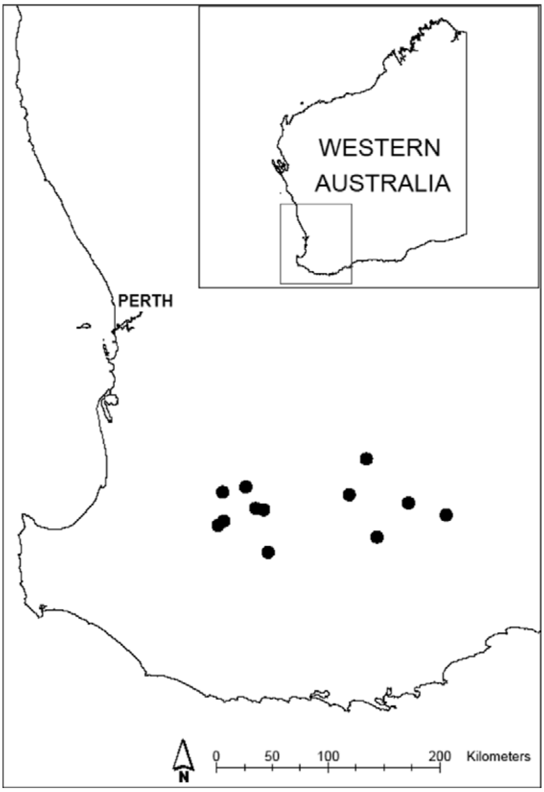

Fig. 1. Locations of sampling quadrats surveyed for mosquito larvae in the Wheatbelt of southwest Western Australia. Site coordinates (latitude, longitude): −33.35628, 116.62067; −33.31329, 116.80671; −33.48809, 116.88669; −33.49884, 116.95164; −33.59043, 116.63058; −33.62621, 116.58273; −33.84380, 116.98703; −33.08636, 117.77947; −33.37755, 117.64134; −33.71999, 117.86499; −33.44393, 118.11712; −33.54058, 118.42258.

At each water body, sampling consisted of sweeping a standard D-frame 500-mm-diameter FBA pond net (250-μm mesh size; Australian Entomological Supplies, Bangalow, New South Wales) through each water body (water body depth, 5–520 mm). Every effort was made to sample all microhabitats evenly within a water body. Up to 10 m^2 surface area was swept per water body, with the total surface area of the water body recorded. In water bodies with a surface area <10 m^2 the whole water body was sampled and the area recorded. All samples were preserved in 70% ethanol and returned to the laboratory, where all mosquitoes were removed and identified to species under a microscope using the taxonomic keys of Liehne (1991). Large samples were homogenized and split into half or quarters for subsampling. Abundance of each mosquito species was standardized to number per square meter of water body surface area (number of mosquitoes/surface area sampled).

The depth, dimensions, and shape of all water bodies were also measured and the surface area calculated. For each water body, salinity and pH was recorded using a YSI-63 m (YSI, Yellow Springs, OH). Habitat was characterized as the percentage of each water body composed of short emergent vegetation (<0.5 m), tall emergent vegetation (>0.5 m), algae (filamentous, mat forming, and floating), detritus (dead and decaying plant material), and bare substratum was assessed (collectively referred to as "habitat"). Additionally, rainfall for water bodies within each stratified quadrat was recorded as the cumulative amount of rain for 14 d preceding sampling. Rainfall data were derived from the weather station of closest proximity to each quadrat (Australian Bureau of Meteorology; weather stations: Collie, Katanning, Lake Grace, Narrogin, and Wagin). Throughout this paper, variables are referred to as environmental (rainfall, water body surface area, depth, pH, and salinity) and habitat (short and tall vegetation, algae, detritus, and bare substrate).

Analyses. In the first instance, analysis tested whether assemblages of mosquito larvae in water bodies differed between sampling intervals or seasons (spring and autumn). This was examined using a nonparametric permutational-based multivariate analysis of variance (NPMANOVA), which allowed multivariate comparison of uneven category sizes, and was undertaken using the NPMANOVA program (Anderson 2004). Initial analysis showed that mosquito assemblages did not significantly differ between sampling intervals or seasons (NPMANOVA: $F_{10,168} = 1.197$, $P = 0.097$ and $F_{1,178} = 1.676$, $P = 0.137$, respectively); therefore, data were combined for all further analyses.

To examine the relationships between regional rainfall and (1) the number of ephemeral water bodies and (2) percentage of water bodies occupied by mosquitoes, we used regression analyses (linear and logarithmic respectively). NPMANOVAs were used to determine how mosquito assemblages were associated with rainfall, water body surface area, and depth, with these variable categorized (0–10, 10–20, 20–30, 30–40, 40+ mm; 0–1, 1–10, 10–100, 100–1,000, and 1,000–10,000 m^2; and 0–50, 50–100, 100–150, 150–200, and 200+ mm, respectively). Post hoc analyses of pairwise comparisons were used to determine significance between levels. Mosquito assemblage data were square root transformed, to reduce the dominance of abundant species on the analysis and based on Bray-Curtis similarity matrices.

To examine which environmental and habitat variables explained most variation in mosquito density, richness, and the density of each species, a stepwise multiple regression was used. The significance level was set at 0.05. However, to reduce the probability of committing type I statistical errors, α was adjusted for multiple comparisons using the sequential Bonferroni method (Rice 1989), where k is the number of analyses testing the same hypothesis. Before analyses, data were examined for normality using Shapiro-Wilk's test of normality, and non-normal data were normalized by transformation.

To assess how the composition of habitat variables and water pH was influenced by salinity of each water body on each sampling occasion (divided into categories 0–5, 5–10, 10–15, 15–20, and 20+ g/liter^1 total dissolved solids [TDS]), an NPMANOVA was used. Post hoc analyses of pairwise comparisons were used to determine significance between levels. Data were square root transformed and based on Euclidean distance matrices. Data were visually represented by nonmetric multidimensional scaling (NMDS: PRIMER v6, PRIMER-E, Plymouth, United Kingdom) and based on the same transformation and similarity matrix as the NPMANOVA. For visual interpretation, the salinity of each water body was represented by a bubble plots, whereby the size of the bubble on the NMDS reflected the level of salinity. Spearman rank correlation (ρ: the BIOENV procedure in PRIMER v6) was used to assess how much multivariate variation in habitat and pH was explained by salinity. Additionally, relationships between individual environmental and habitat variables were examined using spearman correlation coefficients, with α adjusted for multiple comparisons using the sequential Bonferroni method (Rice 1989).

The final component of the analysis used NPMANOVA to determine how salinity categories influenced mosquito assemblages. Post hoc analyses of pairwise comparisons were used to determine significance between levels. Mosquito assemblage data were square root transformed and based on Bray-Curtis similarity matrices. Data were visually represented by NMDS, based on the same transformation and similarity matrix, and with the salinity of each water body represented by bubbles. The BIOENV procedure was used again, but this time to determine which environmental and habitat variable, or combination of variables, was best associated with the variation in mosquito assemblage data. Similarly, multiple regression was again used to identify relationships between individual mosquito species and salinity. Finally, χ^2 goodness-of-fit analyses examined the frequency of occurrence of each species of mosquito across salinity categories, assuming an equal distribution among salinity categories. A Monte Carlo simulation of the multinomial sampling distribution (200 random samples) was used to estimate the relative probability for mosquitoes, where the expected frequency was <5 in any one salinity category. χ^2 goodness-of-fit analyses were conducted using VassarStats (http://faculty.vassar.edu/lowry/VassarStats.html). All other univariate analyses described were undertaken using SPSS 15.0 (SPSS, Chicago, IL).

Results

Composition of Mosquito Samples. One hundred eighty samples were collected across 11 sampling occasions, comprising eight mosquito species (Table 1). The most commonly encountered (frequency of wa-

Table 1. No. of water bodies that each mosquito species occurred in, mosquito densities (n/m^2) across all samples (n = 180), and relative abundance

	Occurrence	Density		Relative abundance (%)
	[n (%)]	Mean	SE	
Ae. alboannulatus	74 (41.11)	62.21	33.83	43.47
Ae. bancroftianus	1 (0.56)	0.01	0.01	0.01
Ae. camptorhynchus	54 (30.00)	53.85	26.37	37.62
Cx. australicus	50 (27.78)	14.41	5.25	10.07
Cx. globocoxitus	37 (20.56)	9.12	5.16	6.37
An. annulipes	52 (28.89)	3.32	1.57	2.32
Cs. atra	3 (1.67)	0.11	0.08	0.08
Cq. nr linealis	8 (4.44)	0.09	0.05	0.06
Total	155 (86.11)	143.03	44.65	100.00

Mosquitoes were absent from 13.89% (25/180) of water bodies.

ter bodies occupied) and abundant mosquito was *Aedes alboannulatus* Macquart. This was followed by *Ae. camptorhynchus* and *Culex australicus* Dobrotworsky and Drummond. *Culex globocoxitus* Dobrotworsky and *Anopheles annulipes* Giles were also widespread but less abundant. *Aedes bancroftianus* Edwards, *Culiseta atra* Lee, and *Coquillettidia nr linealis* Marks were seldom recorded and consequently excluded from univariate analyses.

Rainfall, Ephemeral Water Bodies, and Colonization by Mosquitoes. As rainfall increased, so did the number of water bodies (linear regression: $r^2 = 0.530$; $F_{1,9} = 10.149$, $P < 0.05$), particularly after rainfall exceeded 10 mm in the preceding 14 d. As rainfall increased, water bodies were composed of significantly greater amounts of emergent vegetation (short and tall), less bare substrate, and had lower pH values (Table 2). Water bodies that had a larger surface area were also significantly deeper, had greater compositions of emergent vegetation (short and tall) and algae, and had lesser amounts of detritus (Table 2). Bare substrate composition was significantly greater in deeper water bodies (Table 2).

As rainfall increased so did the percentage of water bodies to be occupied by mosquitoes (logarithmic regression: $r^2 = 0.614$; $F_{1,9} = 14.345$, $P < 0.01$). Assemblages of mosquitoes were significantly related to rainfall category (NPMANOVA: $F_{4,175} = 2.730$, $P < 0.001$) and surface area of water bodies (NPMANOVA: $F_{4,175} = 2.336$, $P < 0.01$), depicting a gradient of change (rainfall [mm] and surface area [m^2] posteriori tests: 0–10a, 10–20b, 20–30bc, 30–40c, 40+c; and 0–1a, 1–10b, 10–100bc, 100–1,000c, and 1,000–10,000c, respectively). Depth, however, did not influence assemblage structure (NPMANOVA: $F_{4,175} = 1.030$, $P = 0.420$). Mosquito density had a positive relationship with rainfall and a negative relationship with surface area (Table 3; Fig. 2a and b). At the species level, only *Ae. alboannulatus* densities were significantly associated with rainfall and surface area, which were positive and negative associations, respectively (Table 3). Densities of *Ae. camptorhynchus*, however, exhibited a positive trend associated with rainfall (linear regression: $r^2 = 0.029$; $F_{1,178} = 5.234$, $P = 0.023$; critical $\alpha = 0.007$).

Salinity and the Characteristics of Ephemeral Water Bodies. The matrix of habitat and pH variables were significantly related to salinity category (NPMANOVA: $F_{4,175} = 6.220$, $P < 0.001$; Spearman rank correlation $\rho = 0.123$), reflecting a gradient of change (Fig. 3). Saline water bodies were characterized by higher pH values and proportionally less detritus than fresh water bodies (Table 2).

Salinity, Environmental and Habitat Variables, and Mosquitoes. The assemblage structure of mosquitoes changed with increasing salinity, reflecting the gradient (NPMANOVA: $F_{4,175} = 6.651$, $P < 0.001$; Fig. 4a and b). Salinity alone was associated with more variation in mosquito assemblage structure (Spearman rank correlation $\rho = 0.214$) than any other environmental or habitat variable or combination of variables (Fig. 4a). For individual species, salinity was a positive explanatory variable for *Ae. camptorhynchus* density (Table 3) and occurrence up to 20 g/liter TDS (Table 4; Fig. 5). The density of each of the remaining taxa was not related to salinity in the multiple regression (Table 3), but there were upper salinity limits to their distribution (Table 4; Fig. 5). *Ae. bancroftianus*, *Cs. Atra*, and *Cq. nr linealis* were all encountered in fresh water only (Fig. 5). *Cx. australicus*, *Cx. globocoxitus*,

Table 2. Spearman correlation coefficient of environmental and habitat variables (n = 180)

	Rainfall	Surface area	Depth	TDS	Short vegetation	Tall vegetation	Algae	Detritus	Bare substrate
Surface area	0.056								
Depth	−0.099	0.455[a]							
TDS	0.024	0.127	−0.103						
Short vegetation	0.291[a]	0.288[a]	0.07	0.124					
Tall vegetation	0.325[a]	0.241[b]	−0.071	−0.089	−0.092				
Algae	0.056	0.340[a]	−0.115	0.135	0.007	0.249			
Detritus	0.156	−0.365[a]	−0.179	−0.423[a]	−0.227[b]	0.15	−0.267[a]		
Bare substrate	−0.482[a]	−0.073	0.218[b]	0.096	−0.412[a]	−0.510[a]	−0.381[a]	−0.263[a]	
pH	−0.547[a]	0.136	0.182	0.310[a]	−0.410[a]	−0.174	0.091	−0.323[a]	0.526[a]

Correlations were corrected for multiple comparisons with the sequential Bonferroni adjustment. Critical α-values after sequential Bonferroni correction: rainfall, 0.010; surface area, 0.013; depth, 0.007; TDS, 0.007; short vegetation, 0.013; tall vegetation, 0.013; algae, 0.008; detritus, 0.017; bare substrate, 0.025, pH 0.013.

[a] $P < 0.001$.

[b] $P < 0.01$.

TDS, total dissolved solids.

Table 3. Stepwise multiple regression between environmental and habitat variables and mosquito density (n/m^2), richness, and the density of each mosquito species (n = 180)

	Predictors	R^2	Regression coefficient	SE	$t_{1,178}$	P
Density	(Constant)	0.280	0.394	0.283	1.390	0.166
	Surface area		−0.378	0.055	−6.915	<0.001
	Rainfall		0.744	0.207	3.594	<0.001
	Tall vegetation		0.012	0.004	3.050	0.003
Richness	(Constant)	0.091	1.856	0.105	17.663	<0.001
	Bare substrate		−0.011	0.003	−4.209	<0.001
Ae. alboannulatus	(Constant)	0.261	2.948	1.178	2.502	0.013
	pH		−3.294	1.123	−2.934	0.004
	Surface area		−0.217	0.047	−4.627	<0.001
	Rainfall		0.571	0.204	2.803	0.006
Ae. camptorhynchus	(Constant)	0.270	1.076	0.347	3.102	0.002
	TDS[a]		0.832	0.108	7.730	<0.001
	Algae		−0.007	0.002	−3.031	0.003
	pH		−0.155	0.052	−2.979	0.003
Cx. australicus	(Constant)	0.140	0.915	0.127	7.178	<0.001
	Short vegetation		−0.389	0.079	−4.912	<0.001
	Bare substrate		−0.007	0.002	−4.285	<0.001
Cx. globocoxitus	(Constant)	0.120	0.691	0.106	6.496	<0.001
	Short vegetation		−0.310	0.066	−4.686	<0.001
	Bare substrate		−0.005	0.001	−3.546	<0.001
An. annulipes	(Constant)	0.090	0.080	0.034	2.318	0.022
	Algae		0.005	0.001	4.186	<0.001

Predictors are corrected for multiple comparisons with the sequential Bonferroni adjustment. Critical α-values after sequential Bonferroni correction: density, 0.007; richness, 0.006; *Ae. alboannulatus*, 0.007; *Ae. camptorhynchus*, 0.007; *Cx. australicus*, 0.006; *Cx. globocoxitus*, 0.006; *An. annulipes*, 0.006. *Ae. bancroftianus*, *Cs. Atra*, and *Cq. nr linealis* were excluded from analysis because of infrequent occurrence (Table 1).

[a] TDS, total dissolved solids.

and *An. annulipes* were encountered more commonly at 5–10 g/liter TDS than what would be expected assuming an equal distribution among salinity categories (Table 4). The upper limit for occurrence of *Cx. australicus*, *Cx globocoxitus*, and *An. annulipes* was 12.5, 9.3, and 17.5 g/liter TDS, respectively (Table 4; Fig. 5). *Ae. alboannulatus* had a wider salinity range than either *Culex* species or *An. annulipies* but was not recorded beyond 23.7 g/liter TDS (Table 4; Fig. 5). *Ae. camptorhynchus* was present in fewer than expected water bodies at 0–5 g/liter TDS, but the occurrence of this species exceeded expectations at all salinities >5 g/liter TDS (Table 4) and was recorded in water bodies as saline as 51.6 g/liter TDS (Fig. 5).

Variables other than salinity were associated with species abundances (Table 3). However, in some cases, salinity correlated with these variables (Table 2). Densities of *Ae. alboannulatus* declined as pH increased (Table 3), and pH was positively correlated with salinity (Table 2). *Cx. globocoxitus* densities were lower in water bodies with a greater composition of short emergent vegetation, and there was a positive, but nonsignificant, trend between salinity and short emergent vegetation (linear regression: r^2 = 0.023, $F_{1,178}$ = 4.125, P = 0.044; critical α = 0.006; Table 2 and 3).

There were also cases where variables were significantly associated with the density of mosquito species

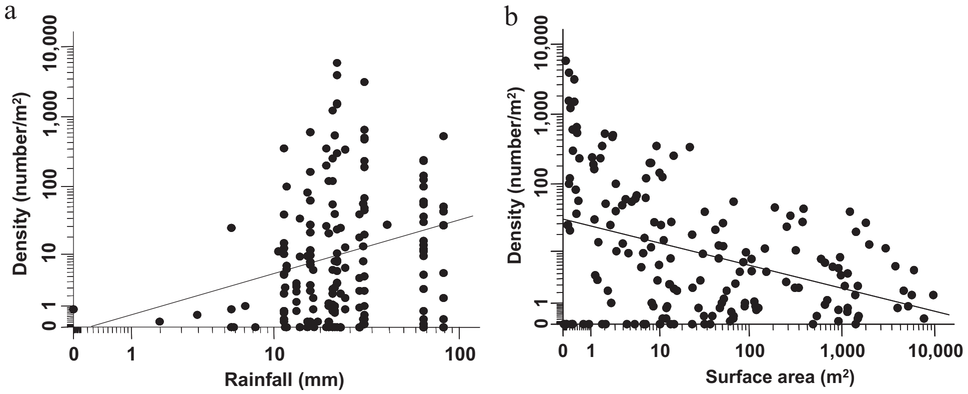

Fig. 2. Relationship between the density of mosquitoes (n = 180) and (a) rainfall and (b) the surface area of ephemeral water bodies (linear regressions of the log-transformed data plus predictor and constant coefficients (±SE): r^2 = 0.064, $F_{1,178}$ = 12.148, P < 0.001, 0.779 (0.224) and −0.069 (0.312); and r^2 = 0.154, $F_{1,178}$ = 32.346, P < 0.001, −0.331 (0.058), and 1.479 (0.106).

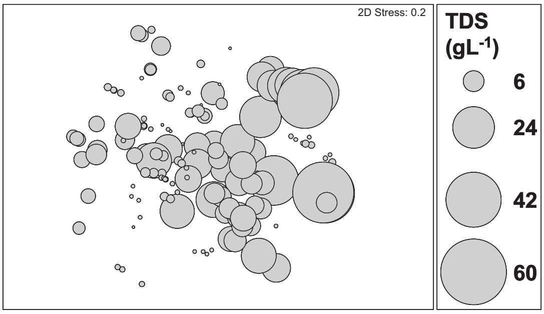

Fig. 3. NMDS ordination of habitat composition (short and tall emergent vegetation, algae, detritus, and bare substrate) and pH variables for each water body ($n = 180$). The size of each bubble represents the salinity (total dissolved solids) of each water body.

but not correlated in the expected direction with salinity, suggesting that, in these cases, variables were independently associated with the density of mosquito species. *Cx. globocoxitus* were less abundant when bare substrate levels were high (Table 3). The richness of mosquitoes and densities of *Cx. australicus* and *Cx. globocoxitus* were reduced in water bodies with a greater composition of bare substrate (Table 3). Densities of *Ae. camptorhynchus* declined as pH increased (Table 3). Finally, the overall density of culicid fauna increased in water bodies with a greater composition of tall emergent vegetation (Table 3).

Discussion

In this study, we principally found that (1) ephemeral water bodies are widely colonized by mosquitoes, (2) changes in the habitat characteristics of water bodies reflected a gradient of salinity disturbance, and (3) the colonization dynamics of mosquitoes is directly and indirectly (for *An, alboannulatus* and *Cx. globobocoxitus* only) associated with the salinity of water bodies. In support of our hypothesis, we found these changes favored abundance and occurrence of *Ae. camptorhynchus.* With the exceptions of *Ae. bancroftianus, Cs. Atra,* and *Cq. nr linealis,* which were seldom recorded, culicid fauna occurred widely across our study region (occurrence at 7–9 of the 12 quadrats for each species). Thus, for these common species, their relationship to salinity could be assessed. Overall, our study suggests that, as secondary salinity expands in the Wheatbelt, the distribution and abundance of the RRV vector, *Ae. camptorhynchus,* is likely to increase.

Salinity exerts a direct physiological pressure on mosquitoes (Patrick and Bradley 2000, Roberts 1996, Clark et al. 2004, Barton and Aberton 2005), favoring species, such as *Ae. alboannulatus* and *Ae. camptorhynchus,* with a wide salinity tolerance and selecting against more halosensitive species. Our analysis did not find a significant linear relationship between the density of some abundant species (such as *Cx. australicus, Cx. globocoxitus,* and *An. annulipes*) and salinity. Nevertheless upper salinity limits of distribution were observed. Our results and evidence from the literature may indicate mosquitoes selectively oviposit based on the salinity of water bodies, which is supported by laboratory and field studies (Trimble and Wellington 1979, Bailey et al. 1981, Pappas and Pappas 1983, Roberts 1996, Silberbush et al. 2005, Carver et al. 2008). Our data may also suggest indirect relationships occurred between the abundance of *Ae. alboannulatus* and *Cx. globocoxitus* and water body salinity.

A significant focus of this study was the relationship between *Ae. camptorhynchus,* salinity, and other environmental conditions. We anticipated an association between *Ae. camptorhynchus* abundance and rainfall, because *Ae. camptorhynchus* lays desiccation-resistant eggs that hatch when inundated with water (Dhileepan et al. 1997, Barton et al. 2004) but only observed a positive trend. Other studies have found the abundance of *Ae. camptorhynchus* significantly linked to rainfall (Woodruff et al. 2003, Barton et al. 2004). It is possible that a significant relationship may have been observed if rainfall data had been collected at the site rather than the nearest weather station.

Aedes camptorhynchus was previously found to be abundant in salinity affected regions of the Wheatbelt

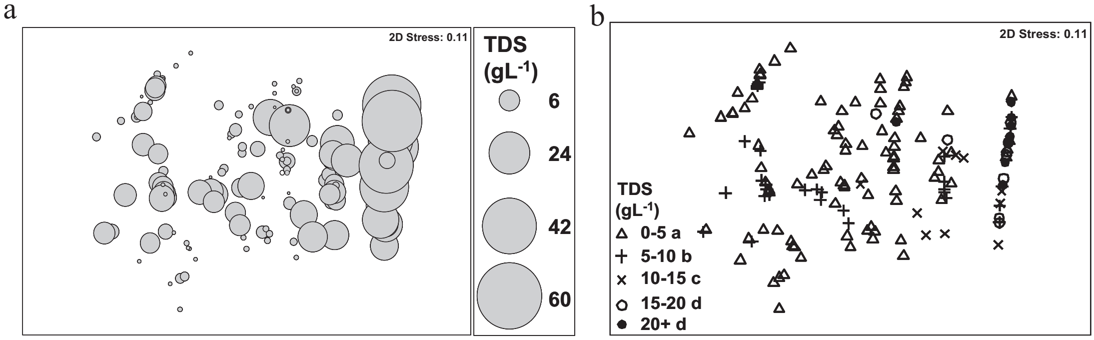

Fig. 4. NMDS ordinations of salinity and mosquito assemblages ($n = 180$): (a) ordination of mosquito assemblages in water bodies, with the salinity of the water body represented by size of each bubble; and (b) the relatedness of mosquito assemblages between salinity (total dissolved solids) categories (NPMANOVA: $F_{4,175} = 6.651$, $P < 0.001$), with post hoc comparisons of categories represented on the legend by a, b, c, and d.

Table 4. χ^2 goodness of fit analyses of differences in frequency of occurrence of commonly encountered mosquito species among water bodies (Table 1) between salinity categories (TDS), assuming an equal distribution among salinity categories

	Observed/expected frequency [TDS (g/liter)]					Goodness of fit test		
	0–5	5–10	10–15	15–20	20+	χ^2	df	P
Culicidae	98/101.6	29/25	10/9.5	9/7.8	9/11.2	1.416	4	0.843
Ae. alboannulatus	56/48.5	9/11.9	6/4.5	2/3.7	**1/5.3**	6.636	4	0.125
Ae. camptorhynchus	**16/35.4**	12/8.7	**9/3.3**	**9/2.7**	**8/3.9**	40.739	4	<0.001
Cx. australicus	33/32.8	**14/8.1**	3/3.1	**0/2.5**	**0/3.6**	10.402	4	0.030
Cx. globocoxitus	23/24.3	**4/6**	**0/2.3**	**0/1.9**	**0/2.7**	17.636	4	0.010
An. annulipes	38/34.1	**13/8.4**	**0/3.2**	1/2.6	**0/3.8**	10.950	4	0.015

Numbers in bold are salinity categories where deviations between observed and expected values are largest. Monte Carlo simulations (200 random samples from the multinomial distribution) were used to estimate significance where the expected frequency was <5. *Ae. bancroftianus*, *Cs. Atra*, and *Cq. nr linealis* were excluded from analysis because of infrequent occurrence (Table 1; Fig. 5).

by Jardine et al. (2008a), who made broad comparisons of areas relatively unaffected with areas affected by secondary salinity. This study also found the occurrence and abundance of this species to be directly and positively associated with the salinity of water bodies, suggesting salinity is the mechanism for expansion of distribution and abundance of *Ae. camptorhynchus.* Furthermore, *Ae. camptorhynchus* is the major vector of RRV in southern Australia (Harley et al. 2001, Russell 2002). This species is commonly encountered around coastal areas where salt marshes are abundant (Dhileepan et al. 1997, Barton et al. 2004). However, our surveys showed that *Ae. camptorhynchus* also occurs infrequently in fresh water habitats, and laboratory studies have also shown that larvae survive well in fresh water (Barton and Aberton 2005). Factors that determine why *Ae. camptorhynchus* is more abundant in saline water have not been studied. It is possible *Ae. camptorhynchus* may be competitively inferior in fresh water or may be released from pressures of predation as water body salinity increases.

Our study found densities of *Ae. camptorhynchus* related to two environmental and one habitat variable. As such, efforts to study or control larvae of this species may benefit from considering variables identified here. For example, the negative relationship between pH and abundance of *Ae. camptorhynchus* is in contrast to pH and abundance relationships of some other *Aedes* species (Williams et al. 1993, Paradise and Dunson 1997, Honório et al. 2006). Therefore, targeted management of *Ae. camptorhynchus* may benefit from examining the pH, in addition to salinity, of water bodies before expending resources on vector control.

Another focus of this study was the relationship between a range of environmental parameters and mosquitoes. We found a large amount of variation in mosquito density and occurrence was not accounted for in our analyses, exemplifying the variable nature of ephemeral water bodies and complexity of environmental variables that influence occurrence and abundance of mosquito species. Williams (2006) suggested ephemeral water bodies are frequently unsaturated, because not all organisms have encountered or entered. Accordingly, the variation in our mosquito data may reflect stochastic encounters of mosquitoes with water bodies (Srivastava 2005, Williams 2006). Other factors, such as biotic interactions may also account for unexplained variation in this study, and is subject to further study (Carver et al. 2009a).

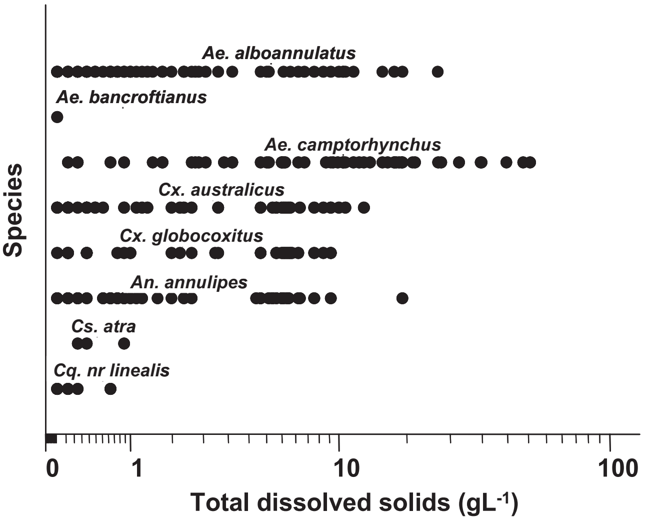

Fig. 5. Relationship between salinity and the occurrence of each mosquito species (n = 180).

Rainfall and water body surface area were, however, environmental parameters with significant relationships to the density of mosquitoes. As rainfall increased, so did the number of ephemeral water bodies, and more water bodies are located in depressions occupied by vegetation, implying rainfall relates positively to an increased variety of ephemeral water body habitat. Mosquito larval density was negatively related to water body surface area. Other studies have also found low abundance of mosquitoes in larger, more permanent aquatic habitats in the Wheatbelt (Halse et al. 2004; Pinder et al. 2004, 2005). Lindsay et al. (2007) suggested that low encounters with mosquitoes, in these studies, may be caused by sampling technique. A similar sampling procedure was used in this study with good success, and we argue that water body area is a more parsimonious explanation of the density of mosquito larvae.

The effect of salinity and other environmental factors on the habitat characteristics of ephemeral water bodies in this study was also assessed. We evaluated gross changes in water body structure. Aspects of habitat, such as emergent vegetation, were not assessed to species level, which could be a caveat in this study. In addition, ephemeral water bodies in this study occurred in a diverse range of areas and heterogeneity among areas, instability of water body persistence, and variability in water body size may account for unexplained variation in their composition. Nevertheless, we found secondary salinization was significantly associated with changes in the composition of the habitat of water bodies. Our study supports others, which have found relationships between terrestrial, riparian, and aquatic vegetation and salinity (Cramer and Hobbs 2002; Davis et al. 2003; Lymbery et al. 2003; Pinder et al. 2004, 2005; Strehlow et al. 2005; Doupé et al. 2006; Seddon et al. 2007), such as the replacement of grasses and rushes, in fresh water areas, with salt tolerant succulents such as *Sarcocornia* sp. in saline areas (Lymbery et al. 2003).

Conclusions. As water bodies become saline, there are direct physiological effects on biota (Patrick and Bradley 2000, James et al. 2003, Hassell et al. 2006). Habitat in secondary salinized ecosystems is simplified, and both salinity and habitat are likely to have a cumulative effect on the occurrence of other organisms (Davis et al. 2003, Doupé et al. 2006, Lyons et al. 2007). Our study has found both direct (salinity–mosquito) and indirect (salinity–environment/habitat–mosquito) associations between the disturbance of secondary salinization and the abundance of mosquitoes. Indirect associations between salinity and mosquitoes were limited to *Ae. alboannulatus* and *Cx. globocoxitus.*

Mosquitoes in the Wheatbelt and presumably their functional properties in food web dynamics (filter feeders and prey) persist in saline disturbed areas (at least up to 20 g/liter TDS). Salinity and its effects on habitat (Lymbery et al. 2003, Doupé et al. 2006, Lyons et al. 2007), however, influence the composition of mosquito communities leading to a dominance of *Ae. camptorhynchus,* a known vector of RRV, in saline areas. Stressed ecosystems can have implications for human welfare (Rapport et al. 1998, Ostfeld and LoGiudice 2003). A more abundant regional population of *Ae. camptorhynchus* in the Wheatbelt has potential to influence RRV activity in saline areas (Carver et al. 2009a).

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