IMPORTANCE OF HYDRILLA VERTICILLATA (HYDROCHARITACEAE) AS HABITAT FOR IMMATURE MOSQUITOES AT THE ROSS RIVER RESERVOIR, AUSTRALIA

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ABSTRACT. From November 1990 to November 1992, immature mosquitoes were sampled from the shoreline and from emergent beds of the submerged aquatic plant *Hydrilla verticillata* at the Ross River reservoir, northern Australia. Aerial mapping of *Hydrilla* beds was done in conjunction with sampling to estimate total immature mosquito numbers. Larvae of 7 species were found. *Culex annulirostris, Anopheles annulipes s.l.*, and *Anopheles amictus* comprised 80.4% of the total. Peak larval densities occurred in the late wet season period in both habitat types (March to May) but *Hydrilla* generally supported higher densities, particularly of *An. annulipes s.l.* (43.7% of the total sample), than the shoreline habitats. *Anopheles annulipes* replaced *Cx. annulirostris* as the predominant taxon when 1990–92 data were compared with data for 1985–86. The *Hydrilla* beds supported on the order of $5.6 \times 10^{\circ}$ immatures during the period of peak density. This suggests that where human exposure is of concern, mosquito control in habitats such as *Hydrilla* is warranted.

INTRODUCTION

The Ross River reservoir ($19^{\circ}26'S$, $146^{\circ}45'E$), near Townsville, Australia, increased capacity from $109 \times 10^{\circ}$ liters to $236 \times 10^{\circ}$ liters in 1989, and the surface area tripled at full supply level to 8,090 ha. The dam thereafter was called Stage 2A. Water resource projects have often been associated with increased social, health, and nutritional problems for the surrounding human population (Sornmani and Harinasuta 1988, Birley 1989). To water resource and other non-health-sector personnel, however, relative counts or the mere presence of immature mosquitoes may mean little. It was therefore considered important that the mosquito population using this manmade lake be described in absolute terms.

At the Ross River reservoir, adult mosquito numbers have previously been monitored (Jones et al. 1991, Barker-Hudson et al. 1993, Hearnden and Kay 1995), and arbovirus activity, particularly that of Ross River virus, has been described on the basis of antibody conversion in sentinel chicken flocks (Barker-Hudson et al. 1993) or on alphaviruses detected in mosquitoes (Kay et al. 1996).

During 1983, 1985, and 1986, when the dam was at Stage 1, it was established that larvae of 8 mosquito species used the marginal emergent and floating algal vegetation as habitat (Rae 1990, Kay et al. 1990). Of these, *Culex annulirostris* Skuse comprised up to 98% of all larvae, while *Anopheles annulipes* Walker was second most abundant. During 1985–86, water levels receded during the dry season (usually May–November) and exposed large areas of floating aquatic vegetation, mainly *Hydrilla verticillata*, which provided new habitat for immature mosquitoes. Following the wet season in February 1986, water depths increased, resulting in almost complete disappearance of floating algal beds. Inundated marginal grassland became the primary mosquito breeding habitat (Rae 1990, Kay et al. 1990).

The Stage 2A lake overflowed the new spillway first in April 1989 and subsequently in 1990. Because of the extensive land clearing that had previously occurred, the shoreline was mainly bare and therefore of reduced importance as a source of mosquito breeding. We therefore focused on *Hydrilla* as the primary breeding habitat, but, because of the increased size of the lake to approximately 55 km², it became obvious that remote sensing was required. This paper therefore describes 9 surveys for immature mosquitoes done between 1990–92, of which 3 involved aerial photography and estimation of absolute numbers.

METHODS

Study area: Descriptions of aquatic habitats in the reservoir are given in Hurley et al. (1995). A full description of the surrounding habitats of the study area is given in Kay et al. (1990), Jones et al. (1991), Barker-Hudson et al. (1993), and Hearnden and Kay (1995). Mosquito larvae were sampled from 3 sites in the reservoir (Fig. 1) adjacent to the largest areas of *Hydrilla*. These sites were Big Bay and Ti-tree Bay on the northern side and Ross River on the southwestern side. At each site, both shoreline and *Hydrilla* habitats were sampled.

Sampling device: We used a white plastic tray $(370 \times 270 \times 50 \text{ mm})$ to sample larvae and calibrated it by placing a known number of larvae in a 1-m² floating enclosure within a large plastic pond. The pond was filled with *Hydrilla* until it was flush with the surface to simulate what occurred in the field. Larvae (a mix of 2nd to 4th instars) were introduced, allowed to settle for 2 min, and then sampled with a single dip. The number sampled by

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Fig. 1. Map of Australia showing the location of the Ross River reservoir near Townsville.

the tray was recorded. This was repeated 10 times each for *Cx. annulirostris* and *An. annulipes*. A third set of samples were done with a 50:50 mix of larvae of both species. There was no significant difference (analyzed by ANOVA) in the mean numbers of larvae sampled for either single-species or the mixed-species samples. The tray sampled 10.70% (mean) of the total larvae within the enclosure so all sample numbers obtained during this study were multiplied by 9.345 to obtain an estimated number of larvae per m².

Larval sampling: Sampling was conducted on 9 occasions (November 1990, and February, April, September, and November in 1991 and 1992) to correspond with the mid-wet (February), late-wet (April), and dry (September and November) seasons.

Shoreline habitats were designated as a 5-m-wide band of water contiguous with the edge of dry land. This habitat designation is distinct from a 3rd breeding habitat composed of muddy pools, hoof prints, ponded water courses, and natural pools inland from the shoreline but within the boundaries of the reservoir land. At each shoreline site, permanent markers were placed at 50-m intervals along a 500-m transect line. At each marker, shoreline dip samples were taken within an area to 5 m out from the water's edge wherever there was floating vegetation or debris. The water depth was always less than 0.5 m. At each sampling point, samples were sieved using a 90-µm mesh to remove excess water, vegetation, and debris. The remaining contents were then preserved in 70% alcohol for identification. All larvae were removed using a low-power light microscope, mounted on slides in Hoyer's solution, and identified to species using a high-power light microscope.

Emergent beds of *Hydrilla* were sampled offshore from the shoreline transect. A preliminary study showed that negligible numbers of larvae were present unless *Hydrilla* was flush with the water surface or emergent. A starting point was set adjacent to the shoreline transect within the boundaries of the *Hydrilla* bed, and dip samples were taken from a small boat at 50-m intervals for a straight-line distance of 500 m, parallel to the shoreline. Depth varied but was always greater than 2 m. The distance between the shoreline and *Hydrilla* transects varied between 50 and 100 m.

Hydrilla mapping: Aerial surveys of the reservoir, to map the area of the surface Hydrilla beds, were done on 3 occasions, corresponding to the April and November 1991 and September 1992 larval sampling dates. Thirty-five-millimeter color transparencies were taken using a polarizing filter from a light airplane at 1,000-m and 5,000-m altitudes, covering the entire surface area of the lake. Flights were done on days where the cloudbase was above 10,000 m and within an hour of midday. All flights were done within 5 days of larval sampling. Surface (emergent) Hydrilla appeared as dark patches in the transparencies and were distinguishable from subsurface beds that appeared lighter in color. This was established by ground-truthing from a small boat after the first aerial survey.

The shape of the reservoir was drawn by obtaining the spillway height at the time of the survey and drawing the edge contour for that height from 1:33,000 topographic maps. The size and shape of the *Hydrilla* beds for each height were drawn onto the maps by comparing the transparencies with landmarks and shoreline characteristics. To estimate the sizes of the *Hydrilla* beds in the reservoir for each site, the maps were scanned and digitized with a computer, and area and *Hydrilla* area, in km², were calculated. The number of immature mosquitoes (larvae and pupae) in the *Hydrilla* at each site was estimated by extrapolating the number of immature per m² to the area of *Hydrilla* present at that site.

Analysis: The distribution of larvae sampled within transects taken from each site, in each month, and in each habitat type were found to be significantly aggregated (Kolmogorov-Smirnov tests; P < 0.01 and variance/mean ratio $\gg 1.0$ for all tests). Mean densities of larvae present in shoreline and Hydrilla habitats were analyzed for site and month of collection by GLM ANOVA (SAS Institute 1988). Multiple-range tests were conducted on all significant main effects to determine similar means. All significance tests were conducted at the 0.05 level of significance.

RESULTS

Sampling of immatures: A total of 13,620 larvae were examined from 675 samples. Seven species were recorded: Cx. annulirostris (29.9%), Cx. bitaeniorhynchus Giles (0.5%), Cx. starkeae Stone and Knight (0.2%), An. annulipes s.l. (43.7%), An. amictus Edwards (6.8%), An. bancroftii Giles (0.2%), and Aedeomyia catasticta Knab (18.7%).

Species	Effect	df	F	P
Culex annulirostris	Site	2	26.95	0.0001
(n = 16,812)	Month	6	20.02	0.0001
	Habitat	1	7.09	0.008
	Site \times month	12	2.37	0.006
	Site \times habitat	2	8.72	0.0002
	Month \times habitat	6	1.51	0.17
	Site \times month \times habitat	12	0.60	0.84
Anopheles annulipes	Site	2	8.92	0.0002
(n = 24,147)	Month	6	27.97	0.0001
	Habitat	1	41.44	0.0001
	Site \times month	12	3.49	0.0001
	Site \times habitat	2	5.89	0.003
	Month \times habitat	6	3.40	0.003
	Site \times month \times habitat	12	2.72	0.002
Anopheles amictus	Site	2	5.84	0.003
(n = 2,796)	Month	6	9.12	0.0001
	Habitat	1	0.08	0.77
	Site \times month	12	2.46	0.004
	Site \times habitat	2	0.25	0.78
	Month \times habitat	6	2.34	0.03
	Site \times month \times habitat	12	1.37	0.18
All tests	Residual	378		
	Total	419		

 Table 1. ANOVA results for comparison of abundance of each species in different habitat types over the sampling period. Tests were applied to log-transformed data for each species.

Data for all species and individual data for the two most abundant species, An. annulipes and Cx. annulirostris, and for An. amictus are presented below.

Immatures were collected on 8 of 9 occasions. During February 1991, the reservoir was flooding over the spillway and negligible numbers of immatures were sampled only from shoreline habitat.

Mean densities of immature Cx. annulirostris, An. annulipes, and An. amictus were analyzed by ANOVA (Table 1) for each of 3 sites by month, and each site was analyzed for the Hydrilla and shoreline habitat types. Culex annulirostris was equally abundant at Big Bay (mean density ± SE of $47.5 \pm 10.6/m^2$) and Ross River ($44.5 \pm 8.4/m^2$), significantly higher than samples from Ti-tree Bay $(9.3 \pm 1.8/m^2)$. Peak periods of density were strongly seasonal, in the mid- to late-wet season (February and April) for all sites. Overall, immatures were more abundant in samples taken from Hydrilla (36.1 \pm 6.0/m²) than in samples taken from shoreline habitats (32.4 \pm 6.8/m²). The nonsignificant second-order interaction (site \times month \times habitat, Table 1) indicated that, despite differences in densities between sites, seasons, and habitat types, the overall pattern was remarkably consistent.

Mean densities of An. annulipes (Table 1) were generally higher (50.3 \pm 5.6/m²) than those of Cx. annulirostris (34.1 \pm 4.6/m²) but showed similar patterns. Densities were greatest at Ross River (75.1 \pm 12.5/m²); however, no significant differences were detected between mean densities at Big Bay $(43.1 \pm 8.8/\text{m}^2)$ and Ti-tree Bay $(30.3 \pm 5.0/\text{m}^2)$. Seasonality was strong, with populations reaching peak densities in late-wet season (April) throughout the study. Anopheles annulipes showed a similar but stronger preference for Hydrilla habitats (74.5 \pm 10.4/m² mean density compared to $30.1 \pm 5.0/\text{m}^2$ for shoreline habitats) (Table 1). Unlike Cx. annulirostris, seasonal changes in density were not the same for all three sites, with immatures being more abundant for Hydrilla in nearly all months at Big Bay than at the other 2 sites.

Densities of An. amictus (Table 1) were considerably lower and showed clearly different trends. While the highest densities $(8.1 \pm 1.8/m^2)$ were found in Ross River samples (compared to $4.6 \pm 0.8/m^2$ at Big Bay and $3.9 \pm 1.1/m^2$ at Ti-tree Bay), there was no significant difference in means for either Hydrilla $(5.7 \pm 1.3/m^2)$ or shoreline samples $(5.5 \pm 0.9/m^2)$. Larvae showed no consistent seasonality across sites, being found exclusively in Hydrilla samples in some months (e.g., April 1991 at Ross River and Ti-tree Bay) and in shoreline habitats in others (e.g., September 1991 to February 1992 at Ross River and April 1992 at Big Bay and Ti-tree Bay).

For all species combined, mean densities were highest for Ross River $(165.1 \pm 19.0/m^2)$ when compared with Big Bay $(104.1 \pm 16.2/m^2)$ and lowest for Ti-tree Bay $(58.0 \pm 7.5/m^2)$ (ANOVA, $F_{2.378} = 54.9$, P < 0.0001). Peak densities occurred in the late-wet season (April) for both years $(317.3 \pm 50.8/m^2$ for 1991 and 278.6 $\pm 31.9/m^2$ for 1992), and were lowest for late-dry-season samples (No-





Fig. 2. Location of study sites and relative sizes of the Ross River reservoir and emergent *Hydrilla* beds for each aerial survey; April 1991 (top), November 1991 (middle), and September 1992 (bottom). Each map shows the wall (solid thick line), the margins when at full capacity (dotted thin line), the margin at the time of the survey (solid thin line; Spillway Height, SH, shown in m below each map), and the locations of the *Hydrilla* patches (solid shapes) for each site.

vember) in both years (24.1 \pm 2.7/m² for 1991 and 26.6 \pm 3.2/m² for 1992) (ANOVA, F_{6.378} = 37.8, *P* < 0.0001). Immature densities were significantly higher for samples taken from *Hydrilla* habitats (140.8 \pm 14.4/m²) than for shoreline samples (85.7 \pm 11.3/m²), (ANOVA, F_{1.378} = 59.1, *P* = 0.0035).

Hydrilla mapping: The total area and the area of Hydrilla at each of the study sites (in km²) for April and November 1991 and for September 1992 are shown in Table 2 and Fig. 2. While the total area of the reservoir decreased from approximately 8,800 to 2,200 ha (see Hearnden and Kay 1995), the area of emergent Hydrilla differed according to site and did not reflect seasonal patterns of declining water levels. For example, the largest patch of Hydrilla (approximately 11 km²) appeared at the Ross River site in November 1991 (the end of the dry season) while at Big Bay, the emergent Hydrilla covered just under 0.7 km² for the same period. All other beds ranged from 1.35 to 7.32 km².

Population estimates: During April 1991, November 1991, and September 1992, *Hydrilla* covered 25.7, 37.4, and 37.5%, respectively, of the total area of the reservoir (Table 2). The 3 major sites where transects were done, nominally referred to as Big Bay, Ti-tree Bay and Ross River (Fig. 2), at these times represented 87.8, 94.0, and 92.6%, respectively, of the total emergent *Hydrilla* at the reservoir.

On the basis of mean immature densities by area of *Hydrilla* available as habitat (Table 3), *Cx. annulirostris* populations were greatest during April 1991, from 31 million to 262 million at each of the 3 sites. Similarly for *An. annulipes*, estimates varied from 326 million to 2,799 million. For *An. amictus*, populations were less predictable but, when present, varied from 3.5 million to 153 million at each site.

The estimated numbers of immatures of all 7 species collected from *Hydrilla* at the 3 sites during April 1991, November 1991, and September 1992 were 4,968 million, 258 million, and 475 million, respectively. When this was extrapolated to the total area of *Hydrilla* floating on the reservoir, it produced values of 5,658.5 million, 275.3 million, and 513.6 million, respectively.

Because of the length and varying widths of shoreline suitable as breeding habitat, it was not possible to estimate area and, therefore, absolute abundance. However, on the basis of a known shoreline length of approximately 115 km, with average densities of 85.7 immatures/m² over a 5-m band, the theoretical maximum population would be 49,277,500 immatures. This represents only 2.3% of the average figure for *Hydrilla*.

DISCUSSION

Of 22 taxa of adult mosquitoes collected by CO₂supplemented encephalitis virus surveillance (EVS) light traps from 1991 to 1993 (Hearnden and Kay

Date	Spillway height (m)	Area (km ²)	Area of Hydrilla (km ²)			
			Big Bay	Ross River	Ti-tree Bay	Total reservoir
April 1991	38.0	54.33	2.37	7.32	2.59	13.98
November 1991	36.9	44.45	0.68	11.02	3.92	16.61
September 1992	35.3	35.53	5.48	1.35	5.50	13.31

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 Table 3. Density (no./m²) and total estimated number of immatures per Hydrilla site for Culex annulirostris, Anopheles annulipes, and An. amictus (and all species combined).

Site	Date	Density (no./m ²)	Estimated number of immatures
Culex annulirostris			
Big Bay	April 1991	110.93	262,600,000
	November 1991	7.01	4,800,000
	September 1992	7.01	38,400,000
Ross River	April 1991	24.48	179,200,000
	November 1991	7.01	77,200,000
	September 1992	17.48	23,600,000
Ti-tree Bay	April 1991	12.24	31,700,000
	November 1991	0.00	0
	September 1992	2.62	14,400,000
Anopheles annulipes			
Big Bay	April 1991	236.62	560,000,000
	November 1991	6.07	4,100,000
	September 1992	20.93	114,700,000
Ross River	April 1991	382.49	2,799,000,000
	November 1991	7.01	77,200,000
	September 1992	4.39	5,900,000
Ti-tree Bay	April 1991	125.78	326,000,000
	November 1991	4.39	17,200,000
	September 1992	17.86	81,800,000
Anopheles amictus	•		
Big Bay	April 1991	0.00	0
	November 1991	5.23	3,600,000
	September 1992	1.78	9,700,000
Ross River	April 1991	20.93	153,000,000
	November 1991	0.00	0
	September 1992	3.46	4,700,000
Ti-tree Bay	April 1991	8.69	22,500,000
	November 1991	1.78	7,000,000
	September 1992	2.62	14,400,000
All species			
Big Bay	April 1991	361.46	855,600,000
	November 1991	26.17	17,800,000
	September 1992	34.95	191,500,000
Ross River	April 1991	499.49	3,655,300,000
	November 1991	21.87	241,000,000
	September 1992	39.34	53,000,000
Ti-tree Bay	April 1991	176.43	457,300,000
	November 1991	16.63	65,200,000
	September 1992	41.96	230,900,000

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1995), 7 were found using floating beds of *Hydrilla* verticillata as breeding habitat in this study.

In contrast to previous sampling of Hydrilla during 1985-86, when Cx. annulirostris comprised 90-98% of immatures, aquatic stages of An. annulipes s.l. were predominant, comprising 43.7%. This taxon is recognized as being a species group (Booth et al. 1987), and larvae are found typically among filamentous green algae in streams and rockpools and in a wide variety of freshwater habitats with or devoid of emergent vegetation (Lee et al. 1987). From February to April 1943, An. annulipes var. mastersi was implicated as the vector in a malaria outbreak at Sellheim and Charters Towers, north Queensland (Black 1972), and Ross River, Barmah Forest, and Murray Valley encephalitis viruses have been isolated from this taxon (Russell 1995).

Both An. annulipes s.l. and Cx. annulirostris reached peak densities of immatures and adults (Hearnden and Kay 1995) during the late wet season. For example, in April 1991, mean densities of these 2 taxa reached 382 and 111 immatures/m² in emergent Hydrilla at Ross River and Big Bay, respectively. Anopheles amictus generally occurred at densities $<5/m^2$ at Ross River during April 1991. For all species, the peak immature densities for April 1991 and 1992 of $317/m^2$ and $279/m^2$ is comparable to the $272/m^2$ recorded for January–March 1985 (Kay et al. 1990).

The 34–37% coverage of the Stage 1 in 1985– 86 (Kay et al. 1990) is also similar to the 26–37% estimates in 1991–92. The use of remote sensing is standard practice for broadscale topographical survey and has been used for aquatic weeds (Martyn et al. 1987, Service 1993). We believe that the digitizing of such data into computerized format will expedite future mosquito surveys and aid water board management of potential pest and medical problems caused by mosquitoes.

The February rains of both 1986 and 1991 resulted in submergence of *Hydrilla*, thus terminating it as a productive mosquito-breeding habitat. As the dry season progressed, our data indicate that the area of emergent *Hydrilla* did not simply reflect declining water levels in the reservoir. It is likely that such changes were influenced by turbidity and depth profiles for each site (J. Balciunas, personal communication) as well as by herbivory by the numerous waterfowl living around the lake.

Whereas the larval densities in emergent Hydrilla had similarities between Stages 1 and 2A, major differences were related to the relative contributions of emergent Hydrilla and shoreline habitats. In 1985–86, transects through expansive flooded grassland and marsh indicated immature densities of 592–2,704/m² (Kay et al. 1990). Our data for 1991–92 indicate that, on average, densities in Hydrilla (141/m²) were 1.64 times higher than those for shoreline (85.7/m²). With progressive establishment of emergent vegetation along the shoreline and in the shallow marshy areas, it can be expected that this will favor increased production of species such as *Cx. annulirostris.* There is evidence based on adult collections that this is ongoing (Hearnden and Kay 1995). *Culex annulirostris* is a major vector of arboviruses such as Ross River virus, Barmah Forest virus, Murray Valley encephalitis, and Kunjin virus in Australia (Russell 1995). During February 1991 and March 1992, alphaviruses, mainly Ross River virus, were isolated from adult *Cx. annulirostris, Ae. normanensis,* and *An. amictus* (Kay et al. 1996) from various sites on the reservoir, including Big Bay and Ross River.

Our population estimates of immature mosquitoes in emergent Hydrilla indicate that peak numbers of 5,658.5 million were reached during April 1991. Although we have no data on survivorship, Rae (1990) estimated that 0.4-18.5% of Cx. annulirostris reached adulthood in flooded grassland at the Ross River reservoir. Even on this basis, we believe that larval control is warranted, especially during the late-wet season (March-May). Shoreline habitat also may warrant control when it has properly established, but, at present, it could only be contributing a maximum of 2.3% of the numbers produced by Hydrilla. Two questions are relevant: 1) what is the method of choice for control of mosquito breeding in Hydrilla and 2) what will be the expected impact of such control?

Since the 1980s, Hydrilla has been a problem in various reservoirs and waterways administered by the Tennessee Valley Authority (TVA). Their goals have been to effect control of aquatic macrophytes where they conflict with multipurpose use, e.g., recreation, and to minimize the application of herbicides, since extensive plant colonies persist from year to year despite such treatments (E. L. Snoddy and J. C. Cooney, unpublished data). In the USA, grass carp, Ctenopharyngodon idella Val., have been used with some success as herbivores (Sutton and Vandiver 1986, Leslie et al. 1987). It is doubted, however, that the stocking of sterile (triploid) fish at recommended rates (Cassani and Canton 1985) would be considered because of potential risk to Australian waterways. Hydrilla also has been the target for biological control using a range of stem-boring and leaf-mining insects (Balciunas et al. 1993), but, although a specialist U.S. Department of Agriculture unit is situated near the Ross River dam, development of appropriate programs would be costly and time-consuming. Mechanical harvesting has been tried downstream in the Ross River, but it is noted that in the reservoir itself, this may enhance the spread of Hydrilla via propagules from uncollected fragments.

The desirable option, therefore, would seem to be along TVA policy guidelines for aquatic macrophytes that do not conflict with public use of the reservoir. Biorational insecticides such as *Bacillus thuringiensis israelensis* and s-methoprene should be applied during periods of greater risk, i.e., late-

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wet season at least. The TVA carries out larval control of An. quadrimaculatus (Say) and Cx. erraticus (Dyar and Knab) in areas where human exposure to mosquitoes is a concern (E. L. Snoddy and J. C. Cooney, unpublished data).

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