Journal of the American Mosquito Control Association, 15(4):479–492, 1999

TOWARD THE INCRIMINATION OF EPIDEMIC VECTORS OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS IN MASSACHUSETTS: ABUNDANCE OF MOSQUITO POPULATIONS AT EPIDEMIC FOCI

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ABSTRACT. Putative epidemic/epizootic eastern equine encephalomyelitis virus (EEE) vector populations were compared at 15 recent (1982–90) human and horse case sites in Bristol and Plymouth counties in southeastern Massachusetts. Carbon dioxide-baited American Biophysics Corporation light traps were used for trapping adult mosquitoes to estimate biting risk in these foci of known transmission. Population data suggest that *Coquillettidia perturbans, Aedes canadensis,* and *Culex salinarius* are more likely vectors of EEE in Massachusetts than *Aedes vexans, Anopheles punctipennis,* and *Anopheles quadrimaculatus.*

Key words Culicidae, abundance, eastern equine encephalomyelitis, epidemic foci

INTRODUCTION

In 1990, Massachusetts experienced its largest eastern equine encephalomyelitis outbreak of this decade. This epidemic resulted in the confirmed infection of 4 humans and 24 horses (Edman et al. 1993). Another epidemic episode involving 10 humans and 21 horses occurred in the early 1980s. These recent outbreaks afforded the opportunity for conducting a retrospective study that could assist in the incrimination of the epidemic vectors of eastern equine encephalomyelitis virus (EEE) in Massachusetts by comparing mosquito populations at various epidemic foci. Historically, eastern equine encephalomyelitis cases in Massachusetts have been concentrated in Plymouth and Bristol counties; most cases in 1990 and in the 1980s also occurred in these 2 counties. This section of Massachusetts has large areas of forested wetlands (Komar and Spielman 1994). This habitat provides developmental, resting, and foraging habitats for many species of mosquitoes, including both the enzootic vector (*Culiseta melanura* (Coquillett)) and the mosquito species that have been considered primary candidates as the epidemic/epizootic or bridge vectors of EEE to humans and horses in Massachusetts. These species include *Coquillettidia perturbans* (Walker), *Aedes canadensis* (Theobald), *Aedes vexans* (Meigen), *Culex salinarius* Coquillett, *Anopheles quadrimaculatus* Say, and *Anopheles punctipennis* (Say) (Vaidyanathan et al. 1997). These species have been judged the most likely bridge vectors in Massachusetts because of certain biological attributes that enable them to transmit EEE from birds to humans and horses.

Vaidyanathan et al. (1997) ranked these 6 potential vectors on the basis of criteria for transmitting arboviruses. First proposed by Chamberlain et al. (1958), these criteria are 1) a vector must be demonstrated to be competent for the arbovirus, 2) a vector must display a feeding pattern that includes both avian and mammalian hosts, 3) the arbovirus must have been isolated from the vector in the field, 4) the flight pattern of the vector must overlap with the host habitat, and finally (emphasized in this study) 5) the vector population must overlap spatially and temporally with foci of disease. We compared putative EEE vector populations at human and horse case sites in Massachusetts to better resolve the 5th criteria.

Aedes canadensis, Ae. vexans, Cq. perturbans, An. quadrimaculatus, Cx. salinarius, and *An. punctipennis* are all attracted to CO_2-baited light traps (Hayes 1962, Newhouse et al. 1966, Carestia and Savage 1967, Schreck et al. 1972, Howard et al. 1988, Kline et al. 1991, Buckley et al. 1994). Human-baited collections directly measure the population density of anthropophagic mosquitoes (Service 1993), but, for long-term trapping, this approach is expensive, time consuming, and difficult to standardize and may expose the collector to health risks (Vaidyanathan and Edman 1997). Carbon dioxide-baited American Biophysics Corporation (ABC) light traps have been compared with human biting collections to evaluate how effectively these traps reflect human biting risk (Vaidyanathan and Edman 1997). Overnight collections predicted approximately 80% of the actual human biting risk by *Aedes, Anopheles,* and *Cq. perturbans* mosquitoes during a 2-h period commencing at sunset. The abundances of *Cx. salinarius* mosquitoes reflected by CO_2-baited ABC traps and 2-h human biting collections were not significantly different (Vaidyanathan and Edman 1997).

MATERIALS AND METHODS

Study sites

The area chosen for this study was the southeastern corner of Massachusetts, specifically Bristol

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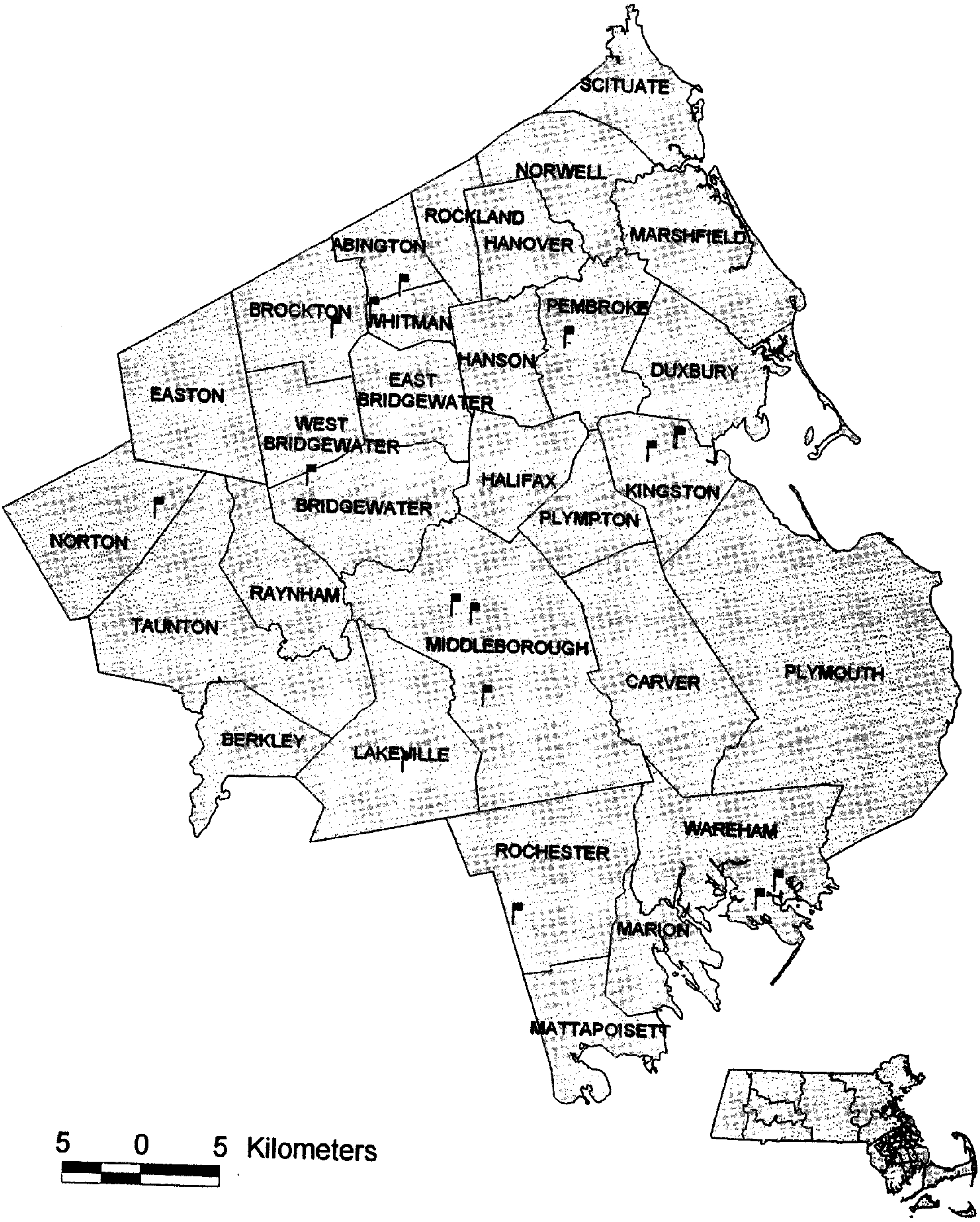

Fig. 1. Mosquito trapping sites in southeastern Massachusetts.

and Plymouth counties, where the majority of human and horse cases have occurred historically. In addition, these 2 counties are where the greatest number of virus isolations from mosquito pools have originated since 1982 (Massachusetts State Department of Public Health, State Laboratory files). Only cases that occurred in these 2 counties within the last 15 years were considered in this study. At each site, case families or horse owners were interviewed to confirm case occurrence and to document any travel history.

A map was developed on the basis of the lo-

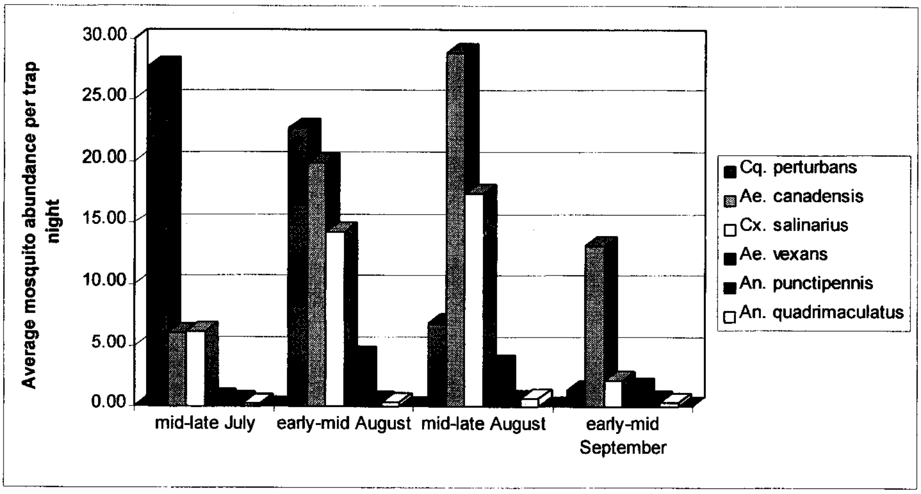

Fig. 2. Biweekly comparison of mosquito abundance per trap night in eastern Massachusetts study area during summer 1996.

cation of horse and human cases as well as virus isolates from mosquito trap sites nearest to these cases (Fig. 1). To create this coverage, we used a United States Geological Survey 1:25,000 datalayer, which was registered to a universal transverse mercator real world coordinate system. This map directed the selection of trap sites for 15 cases with minimum travel history; 11 were from the 1990 outbreak.

Trapping method

ABC traps were used to sample adult mosquito populations at these 15 sites from mid-July to mid-September during the summer of 1996. Traps were equipped with 2 attractants: a photosensitive flickering light that responds to changes in light intensity and starts operating at dusk, and compressed CO_2 that was continuously emitted at 500-ml/min from a storage tank. This flow rate was intended to mimic the average CO_2 discharge from an adult human. Two ABC traps were placed at each of the 15 case sites during 2 consecutive nights each week. Mosquito populations were monitored at each site for the duration of the 8-wk study.

Data analysis

To compare the temporal distributions of each species, the mosquito average abundance was calculated for the entire study area during 4 2-wk intervals designated as mid–late July, early–mid-August, mid–late August, and early–mid-September (Fig. 2). Because each site was selected on the basis of its epidemic history, site was considered to be a fixed factor. A 3-way ANOVA was performed on the log of the average abundance + 1 for the 4 time periods at each site to identify spatial and temporal differences among species (Table 1).

Ranking methods were used to determine which species would be the most likely vector(s) on the basis of abundance. Species were ranked from most to least abundant at each site; a score of 1 was given to the most abundant and a score of up

Table 1. Three-way ANOVA to test the significance of spatial and temporal differences among species.

Source	Type III sum of squares	df	Mean square	F	Significance
Intercept	81.129	1	81.129	1825.839	0.000
SITE	14.321	14	1.023	23.021	0.000
SPECIES	31.757	5	6.351	142.941	0.000
TIME	4.018	3	1.339	30.139	0.000
SITE*SPECIES	22.385	70	0.320	7.197	0.000
SITE*TIME	4.839	41	0.118	2.656	0.000
SPECIES*TIME	8.177	15	0.545	12.269	0.000
Error	9.109	205	0.044		
Total	179.172	354			
Corrected total	95.339	353			

Table 2. Average abundance of putative epidemic mosquito populations at eastern equine encephalomyelitis epidemic foci in southeastern Massachusetts.

Site	*Coquillettidia perturbans*	*Aedes canadensis*	*Culex salinarius*	*Aedes vexans*	*Anopheles punctipennis*	*Anopheles quadrimaculatus*
Norton	22.65	13.13	61.39	0.83	0.04	0.78
NW Middleboro	45.13	5.83	1.21	3.79	0.96	0.38
NE Middleboro	30.58	5.04	0.73	0.46	0.77	0.65
S Middleboro	9.95	5.10	1.05	0.80	1.70	0.05
Lakeville	4.44	2.56	0.00	0.00	0.11	0.67
Rochester	9.87	10.57	1.17	0.09	0.78	0.09
Onset	20.25	1.65	2.40	2.00	0.15	0.10
Wareham	4.83	0.13	4.26	0.61	0.65	0.70
S Kingston	10.00	4.10	1.81	0.19	0.05	0.90
N Kingston	8.25	3.90	1.00	0.90	0.05	0.40
Pembroke	5.88	4.31	0.19	0.31	0.13	0.00
Abington	8.05	11.64	47.09	4.50	0.95	0.82
Whitman	5.04	20.26	4.43	2.52	0.09	0.22
Brockton	3.55	4.20	0.95	5.10	0.45	0.05
Bridgewater	15.18	322.73	43.36	29.73	1.00	0.09
Total average	13.51	27.61	11.20	3.42	0.52	0.39

Table 3. Average mosquito abundance per trap night during 4 biweekly time intervals during summer 1996.

Seasonal interval	*Coquillettidia perturbans*	*Aedes canadensis*	*Culex salinarius*	*Aedes vexans*	*Anopheles punctipennis*	*Anopheles quadrimaculatus*
Mid–late July	27.73	6.03	6.15	0.63	0.46	0.25
Early–mid August	22.74	19.87	14.28	4.21	0.51	0.33
Mid–late August	6.88	28.86	17.42	3.52	0.57	0.63
Early–mid September	1.32	13.11	2.15	1.55	0.58	0.31

Table 4. Sum of species ranked at each site during the time interval of case occurrence.

Site	*Coquillettia perturbans*	*Aedes canadensis*	*Culex salinarius*	*Aedes vexans*	*Anopheles punctipennis*	*Anopheles quadrimaculatus*
Norton	1	3	2	7	5	4
NW Middleboro	1	2	4	3	6	5
NE Middleboro	1	2	3	7	5	4
S Middleboro	1	2	4	3	5	6
Lakeville	1	7	7	7	7	2
Rochester	2	1	3	5	4	6
Onset	1	2	3	4	7	3
Wareham	1	3	2	4	4	4
S Kingston	1	2	3	4	7	5
N Kingston	1	2	3	4	7	5
Pembroke	1	2	7	3	7	7
Abington	3	2	1	4	5	5
Whitman	2	1	4	3	6	5
Brockton	1	2	3	4	7	7
Bridgewater	4	1	2	3	5	7
Total average	22	34	51	65	87	75

to 6 for the least abundant. If not all 6 species were present at a site, those species would receive scores from 1 to the number of species present. Therefore, if only 3 species were present at a site, the most abundant species would receive a score of 1, the next most abundant a score of 2, and the least abundant a score of 3. If a species was absent, it would receive a score of 7. In case of a tie, the species found in identical quantities would receive the same score in accordance to their abundance relative to the other species at the site. Scores were summed across all sites; species receiving the lowest scores were the most abundant. Six species were compared by this method via two approaches: 1) by ranking at each site with the population data from the time period during which a case had become infected (Table 4) and 2) by ranking at each site with the average of the population data for each site for the entire summer (Table 5).

Once the relative order of abundance was determined for the 6 species, another ranking scheme based on quartiles was used, i.e., 0–3, to express the relation from lowest to highest abundance. The lowest number that could be obtained by the 1st ranking system was 15 (i.e., a species receiving a score of 1 at all 15 sites). The highest number possible was 105 (i.e., a species receiving a score of 7 at all 15 sites). This scheme creates a range of 90, which, divided into quartiles, gives intervals of 15–37.5, 37.5–60, 60–82.5, and 82.5–105. Therefore, a score of 0 would be given to species

Table 5. Sum of species ranked at each site by average summer abundance.

Site	*Coquillettidia perturbans*	*Aedes canadensis*	*Culex salinarius*	*Aedes vexans*	*Anopheles punctipennis*	*Anopheles quadrimaculatus*
Norton	2	3	1	4	6	5
NW Middleboro	1	2	4	3	5	6
NE Middleboro	1	2	4	6	3	5
S Middleboro	1	2	4	5	3	6
Lakeville	1	2	7	7	4	3
Rochester	2	1	3	5	4	5
Onset	1	4	2	3	5	6
Wareham	1	6	2	5	4	3
S Kingston	1	2	3	5	6	4
N Kingston	1	2	3	4	6	5
Pembroke	1	2	4	3	5	7
Abington	3	2	1	4	5	6
Whitman	2	1	3	4	6	5
Brockton	3	2	4	1	5	6
Bridgewater	4	1	2	3	5	6
Total average	25	34	47	62	72	78

Table 6. Average abundance (per trap night) of avian feeding *Culiseta melanura, Culiseta morsitans,* and *Culex restuans/pipiens* populations at eastern equine encephalomyelitis epidemic foci in southeastern Massachusetts.

Site	*Cs. melanura*	*Cs. morsitans*	*Cx. restuans/ pipiens*
Norton	1.74	0.17	0.61
NW Middleboro	7.58	2.42	12.75
NE Middleboro	3.12	0.19	8.65
S Middleboro	2.45	0.20	0.90
Lakeville	1.00	0.00	0.00
Rochester	4.74	2.43	1.43
Onset	0.70	0.05	4.45
Wareham	0.30	0.00	2.22
S Kingston	3.76	0.86	0.71
N Kingston	2.50	0.00	0.40
Pembroke	1.69	0.44	1.69
Abington	2.73	27.09	4.59
Whitman	0.96	0.83	3.09
Brockton	0.40	0.05	1.60
Bridgewater	9.00	1.45	1.45
Total average	2.84	2.41	2.97

showing a sum of ranks within 15–37.5, 1 if within 37.5–60, 2 if within 60–82.5, and 3 if within 82.5–105.

RESULTS

Significant differences were found among sites ($P < 0.000$), among species ($P < 0.000$), and among time intervals ($P < 0.000$) when tested by 3-way ANOVA (Table 1). Tables 2 and 3 and Fig. 2 illustrate these differences. Significant 2-way interactions also were observed. These findings can be interpreted as the differences at each site are not the same for each species ($P < 0.000$); the differences among sites are not the same at each time interval ($P < 0.000$); and the differences among species are not the same at each time interval ($P < 0.000$). Table 3 and Fig. 2 illustrate time by species interactions in which the magnitude of the differences among time points changed with species. Similarly, the differences among species changed over time.

Comparison of putative vectors in the study area

The relative abundances of the 6 potential vectors varied with the trap site (Table 2). In general, the most abundant of the 6 species was *Ae. canadensis,* followed by *Cq. perturbans, Cx. salinarius, Ae. vexans, An. punctipennis,* and *An. quadrimaculatus.* The abundance of *Ae. canadensis* was skewed by the enormous population at the Bridgewater trap site. Without this trap site, the average abundance per trap per night for *Ae. canadensis* was 6.6, making it the 3rd most abundant species in the group.

The time of the summer season was then considered in making our species abundance comparison (Fig. 2, Table 3). In the 1st trap period (mid–late July), *Cq. perturbans* was far more abundant (27.7 mosquitoes per trap night) than *Cx. salinarius* (6.2), *Ae. canadensis* (6.0), *Ae. vexans* (0.6), *An. punctipennis* (0.5), and *An. quadrimaculatus* (0.3). In the 2nd time interval (early–mid-August), the following order from highest to lowest abundance was observed: *Cq. perturbans, Ae. canadensis, Cx. salinarius, Ae. vexans, An. punctipennis,* and *An. quadrimaculatus.* In the 3rd time interval (mid–late August), abundance was observed in the following order: *Ae. canadensis, Cx. salinarius, Cq. perturbans, Ae. vexans, An. quadrimaculatus,* and *An. punctipennis.* In the final time interval (early–mid-September), abundance was in the following order from highest to lowest: *Ae. canadensis, Cx. salinarius, Ae. vexans, Cq. perturbans, An. punctipennis,* and *An. quadrimaculatus.*

Other approaches used to compare potential vector populations were 1) ranking species at each site with the abundance data from the time period during which a case had occurred at the site (Table 4) and 2) ranking species at each site with the average of the population data for each site for the entire summer (Table 5). These 2 approaches gave the same result except that the order of the *Anopheles* species was reversed.

Comparison of other mosquito species in the study area

At each of the 15 sites, *Cs. melanura* (the enzootic vector) also was observed (Table 6). At most sites, *Culiseta morsitans* (Theobald) and *Culex restuans/pipiens* (L.) were present as well. Total average abundances per trap night for the summer in

Table 7. Average abundance of *Aedes* species at eastern equine encephalomyelitis epidemic foci in southeastern Massachusetts.

Site	*Ae. excrucians stimuli* and *fitchi*	*Ae. triseriarus*	*Ae. aurifer*	*Ae. cantator*	*Ae. trivittatus*	*Ae. cinereus*	*Ae. taeniorhynchus*	*Ae. sollicitans*
Norton	0.65	0.35	4.83	0.04	0.17	0.43	0.00	0.00
NW Middleboro	0.17	0.50	0.00	0.08	0.04	0.17	0.00	0.00
NE Middleboro	0.54	0.42	0.04	0.00	0.00	0.08	0.00	0.00
S Middleboro	0.15	0.15	0.20	0.00	0.00	0.15	0.00	0.00
Lakeville	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rochester	0.09	0.22	0.09	0.04	0.00	0.04	0.00	0.00
Onset	0.65	0.50	0.35	1.85	0.10	0.05	0.15	0.10
Wareham	0.48	0.00	0.00	1.00	0.00	0.04	3.96	1.65
S Kingston	0.05	0.67	4.71	0.10	0.14	1.86	0.00	0.00
N Kingston	0.30	0.75	0.05	0.25	0.00	0.50	0.00	0.00
Pembroke	0.31	0.13	0.06	0.00	0.00	0.06	0.00	0.00
Abington	0.36	0.55	0.14	0.00	0.64	0.68	0.00	0.00
Whitman	0.22	0.96	0.13	0.09	0.83	8.96	0.00	0.00
Brockton	0.15	5.20	2.50	0.00	7.05	0.95	0.00	0.00
Bridgewater	0.36	3.09	0.18	0.45	0.27	53.82	0.00	0.00
Total average	0.31	0.90	0.89	0.26	0.62	4.52	0.27	0.12

the study area were 2.84 for *Cs. melanura,* 2.41 for *Cs. morsitans,* and 2.97 for *Cx. restuans/pipiens.*

Eight *Aedes* species other than *Ae. canadensis* and *Ae. vexans* were found in the study sites (Table 7). These *Aedes* species included (in order of abundance) *Aedes cinereus* Meigen, *Ae. triseriatus* (Say) *Ae. aurifer* (Coquillett), *Ae. trivittatus* (Coquillett), *Ae. excrucians* (Walker)/ *stimulans/fitchii,* (Felt and Young) *Ae. taeniorhynchus* (Wiedemann), and *Ae. sollicitans* (Walker). All 15 sites had *Ae. excrucians/stimulans/fitchii. Aedes triseriatus* was encountered in all sites except Wareham. *Aedes aurifer* was found in all sites except for Lakeville, Northwest Middleboro, and Wareham. *Aedes cantator* was found in 10 of the 15 sites. *Aedes trivittatus* was found in 8 of the 15 sites. *Aedes cinereus* was found at all sites except for Lakeville. The last 2 species, *Ae. taeniorhynchus* and *Ae. sollicitans,* were found in Onset and Wareham only, near coastal salt marshes.

Population dynamics of the putative vectors at each epidemic foci

Different species were more abundant at different sites (i.e., site-species interactions), and different species predominated during different time intervals. The seasonal population dynamics of these 6 species are shown in Fig. 3. Table 8 contains data on the dates of case occurrence at each site; these are indicated by arrows in Fig. 3.

DISCUSSION

A ranking system was devised by Vaidyanathan et al. (1997) to aid in reducing the list of potential mosquito vectors on the basis of characteristics that an epidemic/epizootic vector must possess in order to transmit EEE. The system ranked each vector character from 3 to 0; a score of 3 was given to the best vector quality of that character, 0 was given to the worst character quality. The list of characters was modified on the basis of this study (Table 9) to include vector competence, host preference, appearance of virus isolates from field-caught mosquitoes, flight range, and temporal and spatial overlap of species at epidemic case sites. Vector competence refers to the ability of a vector to become infected, to support replication, and, finally, to transmit the virus from the salivary glands to a susceptible vertebrate host. In the case of EEE, a good epidemic vector should be catholic in its feeding preference, i.e., it should feed on birds as well as humans and horses. The appearance of EEE isolates in the field, although not an indication of vector competence, does suggest the potential involvement of a mosquito species in transmission. A long flight range from forested resting sites to host-seeking habitats can enhance the chances of a vector serving as a bridge vector between birds and hu-

Table 8. Site, host type, date of onset, and estimated time of infection of eastern equine encephalomyelitis symptoms for cases from 1982 to 1990 based on a 6–10-day incubation period (mean of 8 days) for humans and 4–5 days for horses.

Site	Host	Date of onset	Estimated time of infection
Norton	Human	4 Aug. 1983	Late July
NW Middleboro	Horse	13 Aug. 1990	Early August
NE Middleboro	Horse	13 Aug. 1990	Early August
S Middleboro	Horse	13 Aug. 1990	Early August
Lakeville	Human	12 Aug. 1983	Early August
Rochester	Horse	24 Aug. 1990	Mid–late August
Onset	Horse	29 July 1990	Mid–late July
Wareham	Human	3 Aug. 1990	Late July
S Kingston	Horse	20 Aug. 1982	Early–mid-August
N Kingston	Human	29 Aug. 1990	Mid-August
Pembroke	Horse	20 Aug. 1990	Early–mid-August
Abington	Horse	22 Aug. 1990	Early–mid-August
Whitman	Horse	16 Aug. 1990	Early August
Brockton	Horse	31 July 1990	Mid–late July
Bridgewater	Human	7 Aug. 1990	Early August

mans or horses, especially when these cases occur away from the forest margin.

Species with the greatest temporal and spatial overlap are those that are present in highest numbers at an epidemic site (space) during the seasonal interval (time) when transmission takes place. To compare this criterion with the other vector attributes, a score for temporal/spatial overlap of 3 was given to *Cq. perturbans,* 2 to both *Ae. canadensis* and *Cx. salinarius,* 1 to *Ae. vexans,* and 0 for both *Anopheles* species. Note that this ranking is based on populations of mosquitoes caught in CO_2-baited ABC light traps. Depending on the species, collections may overestimate (e.g., *Cx. salinarius*) or underestimate (e.g., *Anopheles* spp.) actual populations in the area (Vaidyanathan and Edman 1997). Regression analysis of human biting collections to CO_2-baited ABC light traps can predict roughly 80% of the actual biting risk by *Cq. perturbans* and *Aedes* spp. (Vaidyanathan and Edman 1997). Trap yields of *Cx. salinarius* were not significantly different from biting collections.

Under this revised ranking scheme, we found that *Cq. perturbans, Ae. canadensis,* and *Cx. salinarius* may be relatively similar in relation to the biological attributes that characterize them as epidemic vectors. Thus, they are more likely to be vectors of EEE in southeastern Massachusetts than are *Ae. vexans, An. punctipennis,* and *An. quadrimaculatus.*

Possibly more than 1 of these species serve as vectors in different years, seasons, and epidemic foci in Massachusetts. Thus, *Cq. perturbans* appears to be the most likely vector for the outbreaks that took place in Northwest, Northeast, and South Middleboro, Lakeville, Onset, Wareham, South and

Table 9. Comparison of the putative eastern equine encephalomyelitis virus (EEE) vectors in Massachusetts according to attributes that characterize them as vectors.

Species	Vector[1]	Host[2]	Isolates[3]	Flight range[4]	Abundance[5]	Total
Coquillettidia perturbans	1	1	3	3	3	11
Aedes canadensis	3	2	2	1	3	11
Culex salinarius	2	3	3	3	2	13
Aedes vexans	0	1	2	3	1	7
Anopheles punctipennis	0	0	1	3	0	4
Anopheles quadrimaculatus	2	1	2	3	1	7

[1] Vector = vector competence: 3, >10% of the infected lab species were found to be competent for the virus; 2, 6–10%; 3, 1–5%; 0, no transmission.

[2] Host = diversity of host range: 3, broad host range; 2, influenced by host availability; 1, narrow host range; 0, extremely narrow host range.

[3] Isolates = EEE isolations from mosquito pools: 3, commonly isolated from the field; 2, occasionally isolated; 1, rarely isolated; 0, never isolated.

[4] Flight range = distance from forest margin when host seeking: 3, >1 km radius; 2, < 1 km radius; 1, remaining close to the forest margin; 0, not leaving the forest.

[5] Abundance = population abundance at epidemic sites during epidemic months compared with other potential vectors present: 3, high abundance; 2, medium abundance; 1, low abundance; 0, least abundance (modified from Vaidyanathan et al. 1997).

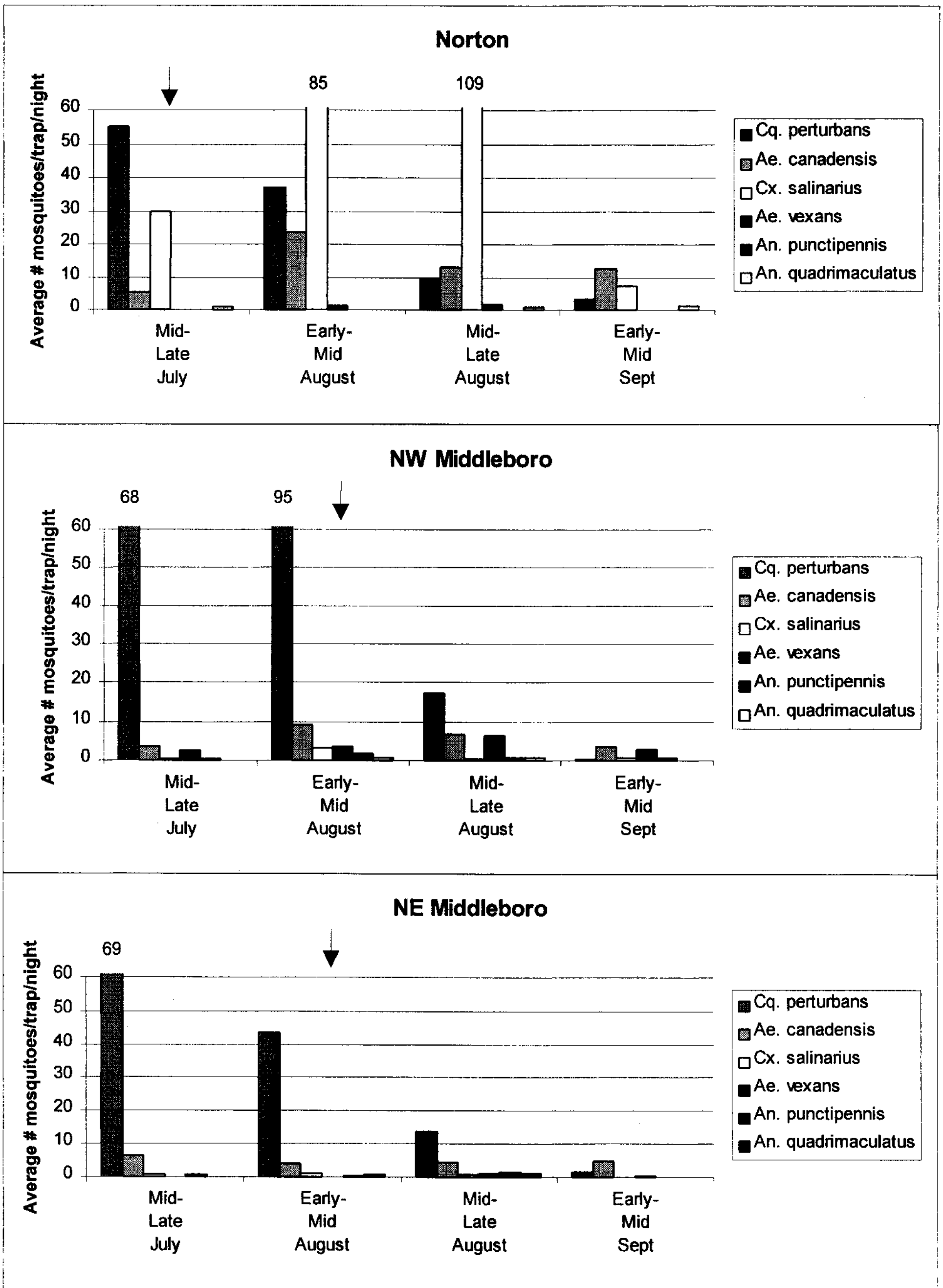

Fig. 3. Average mosquito abundance per trap per night from mid-July to mid-September 1996 at 15 case locations (arrows indicate time of possible transmission).

North Kingston, and Brockton; *Ae. canadensis* is the most likely vector in Rochester, Pembroke, Whitman, and Bridgewater; and *Cx. salinarius* is most likely responsible for cases in Norton and Abington. However, a simpler scenario exists. One species may serve as the transmitter at all the sites. *Coquillettidia perturbans, Ae. canadensis,* and *Cx. salinarius* were all present at 14 of the 15 case sites surveyed. Only *Cq. perturbans* was present at the Lakeville site in 1996, but this could have been an atypical year for this site. Lakeville is also the least reliable of the sites because it is where the oldest human case occurred, and we consider humans to be less reliable indicators of transmission location

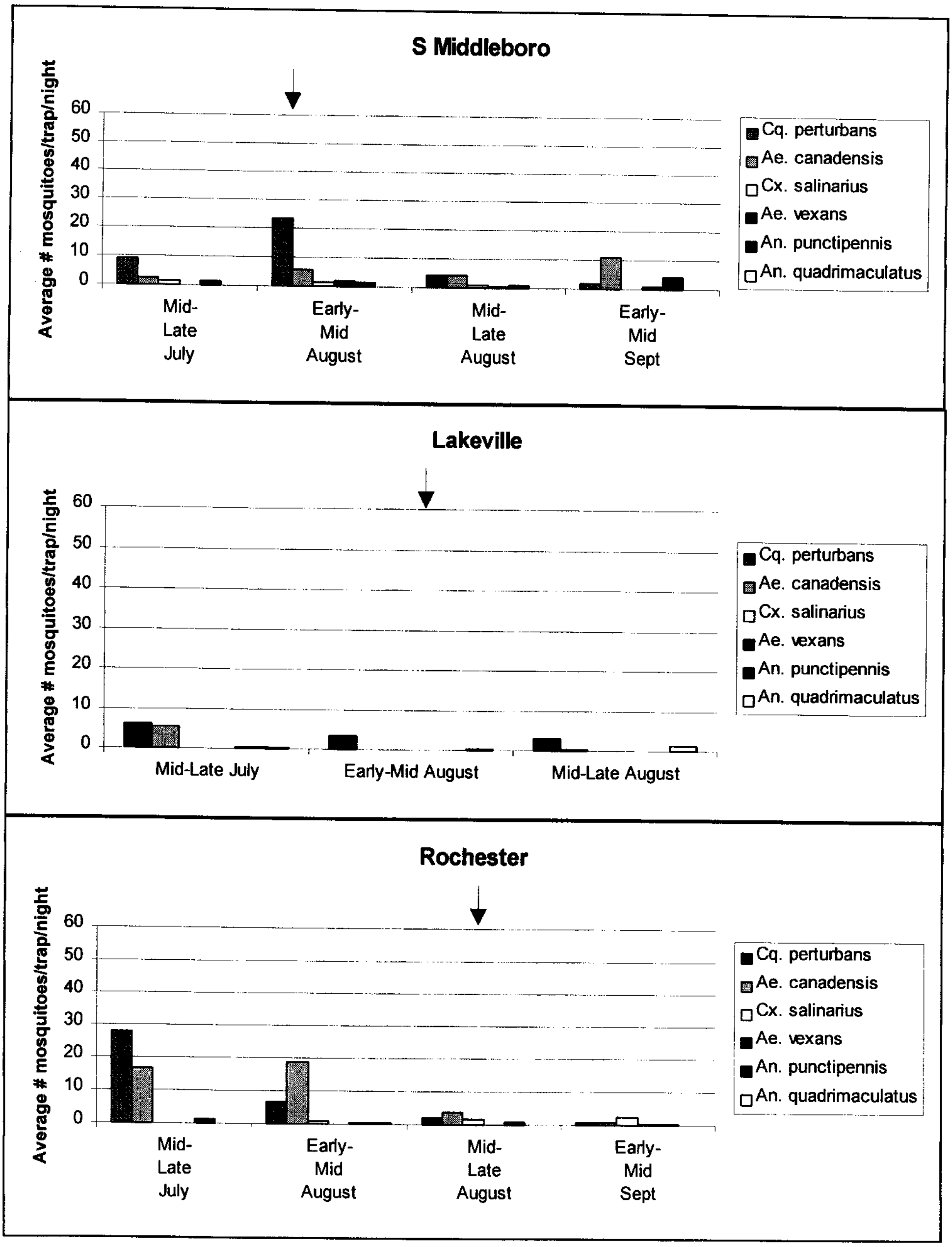

Fig. 3. Continued.

than horses because humans tend to be more mobile.

Although a higher population of a mosquito species (if it is a potential vector) increases the probability that it is responsible for transmission of EEE, this is not necessarily the case. In Wareham and Onset, for example, we found low populations of *Ae. sollicitans*, the putative EEE vector in coastal areas of New Jersey. This species cannot be ruled out as a possible vector at these coastal foci simply because of its low abundance.

Note that year to year variation in population levels is common with at least 2 of the primary candidate vectors: *Cq. perturbans* and *Ae. canadensis*. We cannot know the species diversity and abundance at the sites surveyed in our study at the time when the epidemic occurred. However, mosquito population data were gathered for the Norton

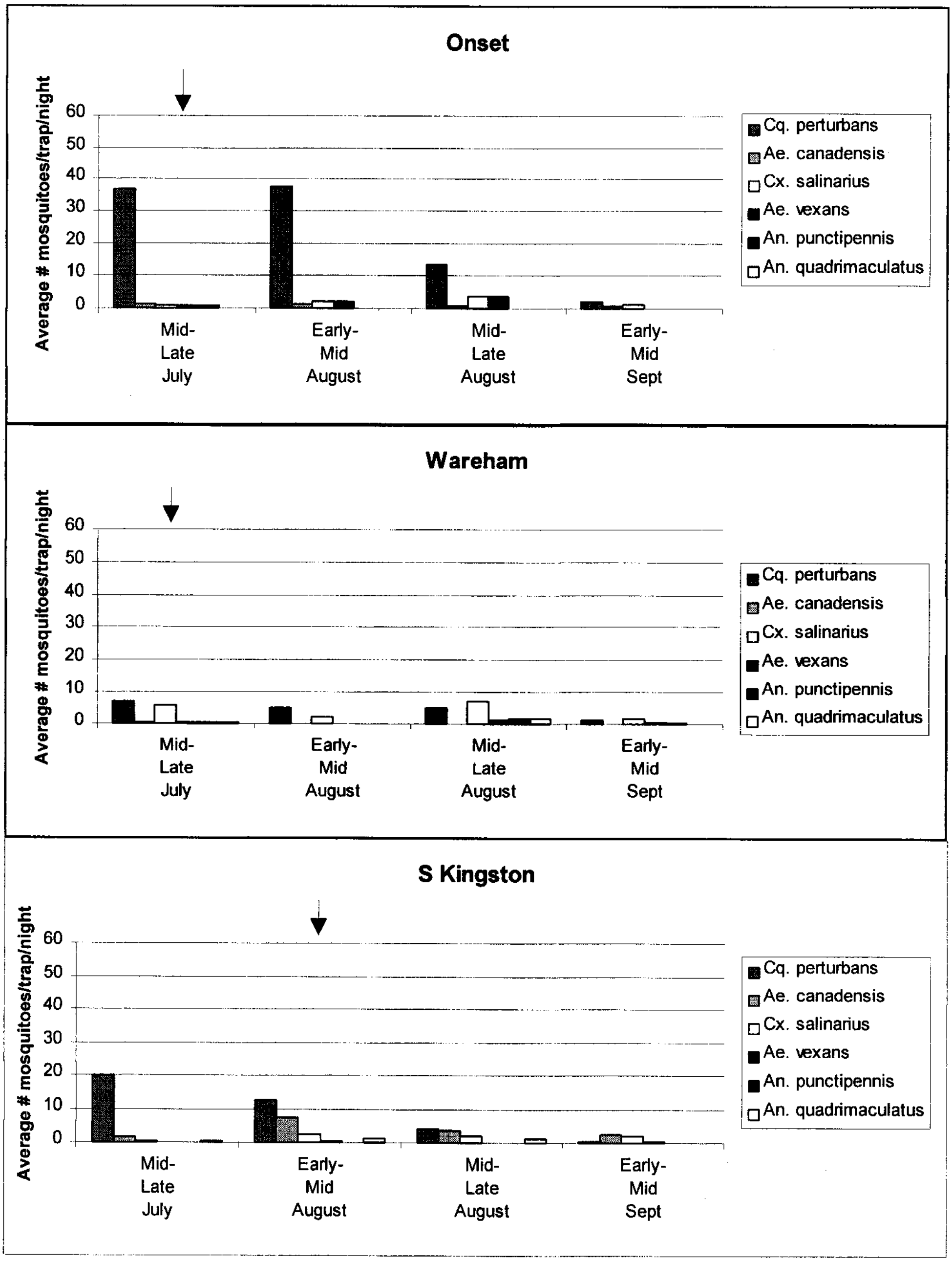

Fig. 3. Continued.

epidemic site the year after the 1983 epidemic (Alan DeCastro, personal communication) and for Kingston, Middleboro, and Brockton at sites near these epidemics during the summer when these epidemics occurred (Ken Ek, personal communication). In late July 1984, the most abundant species at the Norton epidemic site was *Cq. perturbans,* followed by *Cx. salinarius,* and *Ae. vexans. Anopheles quadrimaculatus,* and *Ae. canadensis* were present but in low numbers. These abundance rankings coincide with our finding that *Cq. perturbans* and *Cx. salinarius* were the most abundant species at this site in late July 1996. Surveillance data are also available from 1990 for the cities of Kingston, Middleboro, and Brockton. Although not taken exactly at the case sites, these data are from the nearest sites to these 3 1990 cases. Additionally, these data were from the same

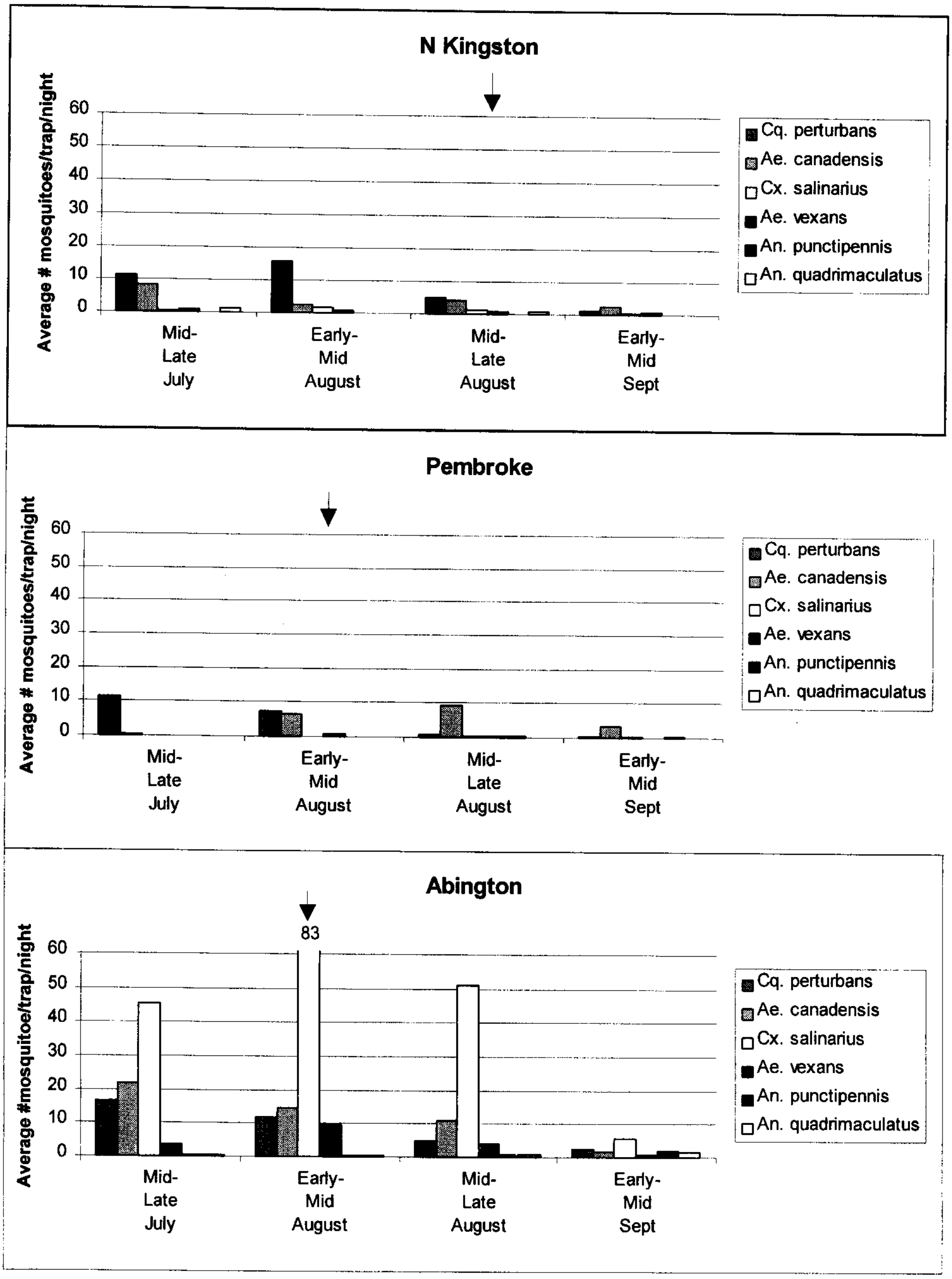

Fig. 3. Continued.

year as these 3 cases. These data indicate that the most abundant species was consistently *Cq. perturbans* during the time of transmission, which coincides with our findings. *Aedes vexans* also was present in low numbers in Kingston and Middleboro during these epidemics; *Cx. salinarius* was present but in low numbers in Brockton. Most of the epidemics described here occurred in 1990, and the landscape of many of these sites had not changed to any great extent in the 6 years before we conducted our survey. Retrospective data can therefore be informative. Detailed study of the landscape in these epidemic sites can provide additional useful information to assess the vector potential of these species, especially for populations of *Cq. perturbans, Ae. canadensis,* and *Cx. salinarius* (see Moncayo et al. in press). Other recent experiments evaluating the survival of virus-infected *Cq. perturbans* suggest that *Ae. canadensis* and *Cx. salinarius* may be more suited for EEE

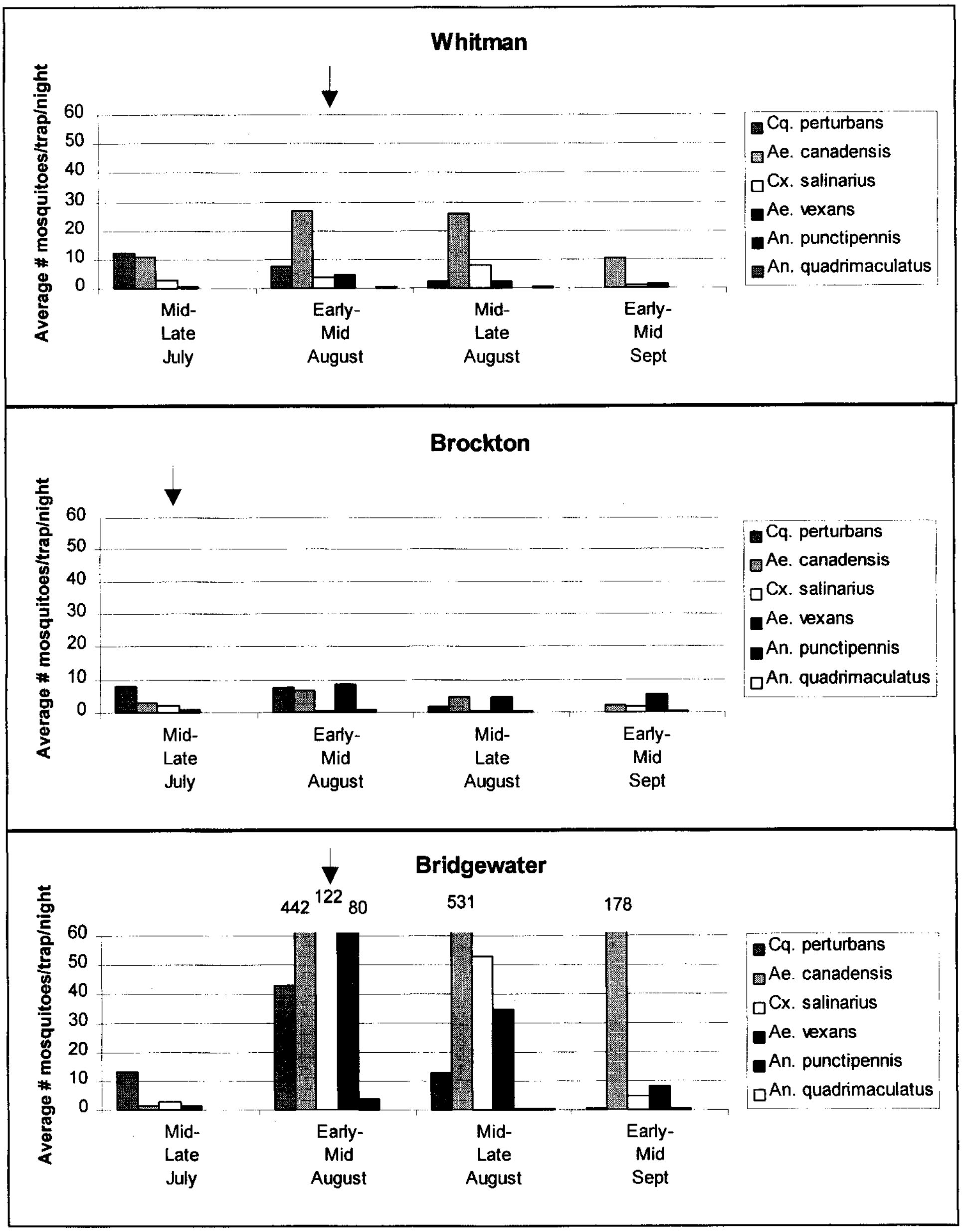

Fig. 3. Continued.

transmission than is *Cq. perturbans* despite the high population densities of *Cq. perturbans* at all epidemic sites from July to mid-August (Moncayo, unpublished data).

Two recent atypical human cases bring into question many previous assumptions about the transmission of EEE in Massachusetts. One case involved a young boy without travel history living in Springfield, far west of the endemic foci and any previous human case. Moreover, this late season (October) case in 1995 occurred during a year when no virus isolation was made from the enzootic vector in the enzootic focus in the eastern part of the state. The 2nd atypical case occurred in 1998 within the enzootic area but was 6 wk earlier than any previous human case and occurred at a time (onset of symptoms in June) when all mosquito populations were unusually low except for spring *Aedes*.

ACKNOWLEDGMENTS

We thank Donald Buckley (Massachusetts Department of Health State Lab), Ken Ek (Plymouth

County Mosquito Control), and Alan DeCastro (Bristol County Mosquito Control) for the mosquito surveillance information they provided. Barbara Warner and Ralph Timperi (Massachusetts Department of Health) provided the human case data; horse case data were provided by the Massachusetts State Veterinarian Office. This study was supported by a grant from the Massachusetts Department of Public Health and Hatch support from the Massachusetts Agricultural Experiment Station.

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