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SAMPLING METHODS FOR POTENTIAL EPIDEMIC VECTORS OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS IN MASSACHUSETTS

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ABSTRACT. To determine which of 11 trapping methods best sampled populations of 6 potential epidemic vectors of eastern equine encephalomyelitis (EEE) virus, we compared New Jersey (NJ) light trap, CDC light trap, CDC with octenol, CDC with CO_2, CDC with CO_2 plus octenol, American Biophysics® light trap with flickering light (AB flicker), AB steady light, AB flicker with octenol, AB flicker with CO_2, AB flicker with CO_2 plus octenol, and 3 resting boxes. There was no significant difference between CDC and AB light traps ($P > 0.05$). The addition of octenol increased trap catch with both CDC and AB light traps; however, this increase was not statistically significant ($P > 0.05$). Both brands supplemented with CO_2 collected more *Aedes canadensis, Coquillettidia perturbans, Culex salinarius,* and *Anopheles* spp. than did unbaited light traps ($P < 0.05$). The addition of octenol to CO_2 increased collections of *Anopheles* spp. and decreased collections of *Aedes* spp. and *Cq. perturbans* at some sites, but these changes were not statistically significant ($P > 0.05$). Resting boxes were best for sampling bloodfed or parous *Anopheles* spp. The NJ, AB flicker, and AB steady light traps were not effective for sampling potential vectors of EEE virus.

INTRODUCTION

Culiseta melanura (Coquillet), the enzootic vector of eastern equine encephalomyelitis (EEE) virus along the northeastern coast of the United States, rests and oviposits in cold, acidic, red maple–white cedar swamps and feeds predominantly on passerine birds. Its narrow host range and limited flight range preclude it as an epidemic vector of EEE virus of horses and humans. *Aedes canadensis* (Theobald), *Aedes vexans* (Meigen), *Coquillettidia perturbans* (Walker), *Anopheles quadrimaculatus* Say, *Anopheles punctipennis* (Say), and *Culex salinarius* Coquillet are potential epidemic vectors of EEE virus. The EEE virus has been isolated from all 6 species (Sudia et al. 1968, Grady et al. 1978, Howard et al. 1988, Edman et al. 1993, Ninivaggi and Guirgis 1994). They rest in shaded woods during the daytime but commute to more open or transitional habitats around sunset or early evening. They feed predominantly on mammals, including humans and livestock, but also may feed on enzootic avian reservoirs of arboviruses (LaSalle and Dakin 1982, Weathersbee and Meisch 1990, Edman et al. 1993). All but *Ae. canadensis* host seek in open fields or suburban areas during the evening. *Aedes canadensis* generally feeds on a variety of smaller hosts along the forest ecotone.

New Jersey (NJ) and CDC light traps historically were used to monitor the relative densities and changing population patterns of *Cs. melanura* (Headlee 1932, Sudia and Chamberlain 1962, Matsumoto and Maxfield 1985). The Massachusetts Department of Health uses unbaited CDC light traps within red maple–white cedar swamps and adjacent lowlands to collect *Cs. melanura* for EEE virus isolation (D. Buckley, Massachusetts Department of Health, personal communication). Once an EEE virus isolation is made, CO_2-baited CDC light traps are used to include more species and to increase the numbers of mosquitoes collected.

Traps used to collect *Cs. melanura* usually are placed in swampy, forested habitat, so collections often underestimate mammalophagic species that commute from woods to unforested areas to feed. Therefore, these samples often fail to recover EEE virus from commuter species and provide a limited profile of virus prevalence and transmission among species that may be involved in transmission of virus from avian reservoirs to humans and horses.

We evaluated the ability of CDC light traps, NJ light traps, resting boxes, and a new light trap (AB) manufactured by American Biophysics® of Jamestown, RI, to estimate the diversity and density of potential epidemic vectors of EEE virus.

MATERIALS AND METHODS

Test sites: The Hockomock Swamp, a red maple–white cedar freshwater swamp in the Taunton River basin of southeastern Massachusetts, is enzootic for EEE virus and *Cs. melanura* (A. Decastro, Bristol County Mosquito Control, personal communication). Four sites in this area were selected for trap comparisons in 1994 and 1995 as described by Vaidyanathan and Edman (1997). In 1996, we compared traps along the margin of a forested swamp adjacent to a flood plain in Hadley, MA.

Experimental design: In 1994 and 1995, we used CDC miniature light traps (Sudia and Chamberlain 1962), NJ light traps (Headlee 1932), resting boxes (Nasci and Edman 1981), and AB light traps for 2 summers. Traps, resting boxes, octenol, and CO_2

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Table 1. Number of mosquitoes collected with 8 sampling methods in southeastern Massachusetts in 1994 and 1995.

Mosquito	Flicker+CO_2	Flicker+CO_2+ octenol	CDC	Flicker+ octenol	Flicker	Resting	Steady	NJ
Ae. can[1]	154	147	23	22	2	0	3	5
Ae. vex[2]	6	4	3	0	0	0	0	0
Ae. stim[3]	16	31	11	3	4	0	3	2
Ae. dark[4]	1,214	763	333	337	109	1	2	5
Anophls[5]	98	127	29	17	8	63	1	0
Cs. mela[6]	51	50	170	52	27	16	5	1
Cq. pert[7]	659	210	212	41	42	0	17	7
Culex[8]	85	145	90	24	16	1	15	18
Total	2,283	1,477	871	496	208	81	46	38
Nights in use	31	27	36	34	33	11	13	11
Mean	74	55	24	15	7	3	4	4
SD	104	76	28	21	11	7	8	5

[1] *Aedes canadensis.*
[2] *Aedes vexans.*
[3] *Aedes stimulans, Ae. fitchii, Ae. excrucians.*
[4] *Aedes triseriatus, Ae. abserratus, Ae. cinereus, Ae. aurifer, Ae. trivitattus.*
[5] *Anopheles quadrimaculatus, An. punctipennis, An. walkeri.*
[6] *Culiseta melanura.*
[7] *Coquillettidia perturbans.*
[8] *Culex salinarius, Cx. restuans, Cx. territans.*

were employed as described by Vaidyanathan and Edman (1997). In 1996, we compared AB flicker, AB flicker+octenol, AB flicker+CO_2, AB flicker+CO_2+octenol, CDC light trap, CDC+octenol, CDC+CO_2, and CDC+CO_2+octenol. The octenol emission rate was increased to 2.0 mg/h and traps were operated for 15 days in June 1996.

Data analysis: Nightly means and species means were calculated for each sampling method in 1994 and 1995. Only nightly totals and overall trap means were calculated for each sampling method in 1996 when AB and CDC light traps sampled equivalent numbers. Data were analyzed as described by Vaidyanathan and Edman (1997).

RESULTS

1994 and 1995: A total of 5,500 females were collected with 8 sampling methods over 38 nights (Tables 1 and 2). No significant difference was found between AB flicker and steady traps ($P = 0.18$) or between flicker and NJ traps ($P = 0.10$). The CDC light trap collected an average of 24 females per night, significantly better than either flicker or steady traps ($P < 0.05$). Table 3 is a summary of trap comparisons and corresponding P-values.

The addition of octenol to a flicker trap increased average yield from 6 to 15 females per night; however, the octenol traps were so variable (SD = 21) that this difference was not significant ($P = 0.07$). The addition of CO_2 to a flicker trap significantly increased yield from 6 to 74 females per night ($P < 0.05$). The flicker+CO_2 trap caught significantly more females per night than a flicker+octenol trap ($P < 0.05$). The addition of CO_2 and octenol to a flicker trap significantly increased average trap yield over octenol alone ($P < 0.05$) but not over CO_2 alone ($P = 0.39$). The flicker+CO_2 and flicker+CO_2+octenol traps were significantly better than an unbaited CDC light trap ($P < 0.05$), which was significantly better than a flicker+octenol trap ($P < 0.05$).

Resting boxes were used 11 nights in August. Numbers from resting boxes were not significantly different from those from CDC ($P = 0.54$),

Table 2. Nightly means for 8 sampling methods for mosquitoes in southeastern Massachusetts for 1994 and 1995.

Sample	Flicker+CO_2	Flicker+CO_2+ octenol	CDC	Flicker+ octenol	Flicker	Steady	NJ	Resting
All species	74	55	24	15	7	4	4	3
Aedes[1]	45	35	10	11	4	1	1	0
Coquillettidia perturbans	21	8	6	1	1	1	1	0
Culex salinarius	5	8	5	2	1	1	2	0
Anopheles[2]	3	5	1	1	0	0	0	6

[1] *Aedes canadensis, Ae. vexans, Ae. stimulans s.l., Ae. abserratus, Ae. triseriatus, Ae. trivittatus, Ae. aurifer.*
[2] *Anopheles punctipennis, An. quadrimaculatus, An. walkeri.*

Table 3. Pairwise comparisons using the Wilcoxon signed rank test between sampling methods for all species, 1994 and 1995. If $P > 0.05$, then difference is not significant; the probability that 2 methods are the same is >5%. If $P < 0.05$, then difference is significant. Two-tail P-values are given for normal approximation with continuity correction.

Comparison of methods	P-value
Significant difference present (Trap 1 > trap 2)	
CDC *vs.* flicker	0.00
Flicker+CO_2 *vs.* flicker	0.00
Flicker+CO_2 *vs.* flicker+octenol	0.00
Flicker+CO_2+octenol *vs.* flicker+octenol	0.01
CDC *vs.* AB+steady	0.00
Flicker+CO_2 *vs.* CDC	0.00
Flicker+CO_2+octenol *vs.* CDC	0.00
No significant difference	
Flicker *vs.* steady	0.18
Flicker *vs.* NJ	0.10
Flicker *vs.* flicker+octenol	0.07
Flicker+CO_2 *vs.* Flicker+CO_2+octenol	0.39

flicker+CO_2 ($P = 0.92$), or flicker+CO_2+octenol traps ($P = 0.06$) operated during this same period. Resting boxes were compared only with corresponding light trap nights.

Trap specificity (1994 and 1995): The addition of octenol to an AB flicker light trap did not significantly increase the average yield of female *Aedes* species ($P = 0.12$; Table 2). A combination of CO_2 and octenol significantly increased yield ($P < 0.05$) over octenol alone but not over CO_2 alone ($P = 0.94$).

Aedes vexans numbers were low both years, so we analyzed *Ae. canadensis* independently. There was no significant difference between CDC and flicker ($P = 0.86$), flicker and flicker+octenol ($P = 0.19$), or flicker+CO_2 and flicker+CO_2+octenol ($P = 0.23$). The addition of CO_2 to a flicker light trap sampled significantly more *Ae. canadensis* females than octenol alone ($P < 0.05$). Resting boxes, NJ light trap, and steady light trap were ineffective in sampling *Ae. canadensis.*

Yields of female *Cq. perturbans* from flicker, steady, NJ, and flicker+octenol light traps were not significantly different from one another (all $P > 0.05$; Table 2). The addition of octenol to the AB flicker trap did not significantly affect trap catch ($P = 0.42$), whereas the addition of CO_2 to the AB flicker trap significantly increased trap catch ($P < 0.05$). A combination of CO_2 and octenol significantly increased AB trap catch over octenol alone ($P < 0.05$) but had no significant effect over CO_2 alone ($P = 0.61$).

Flicker+CO_2+octenol traps collected an average of 8 female *Cx. salinarius* per night, the highest for 8 sampling methods ($P < 0.05$; Table 2). The addition of CO_2 ($P < 0.05$) and CO_2+octenol ($P < 0.05$) significantly increased trap catch over unbaited AB traps. Octenol by itself did not significantly increase trap catches by AB flicker traps ($P = 0.41$). Traps baited with only CO_2 collected significantly greater numbers than only octenol ($P < 0.05$). A combination of octenol and CO_2 did not significantly increase yield over CO_2 alone ($P = 0.05$) but did significantly increase yield over octenol alone ($P < 0.05$).

An average of 6 female *Anopheles* spp. were collected nightly from resting boxes, the highest average of the 8 sampling methods (Table 2). A nightly average of 5 females were collected from flicker+CO_2+octenol; however, resting boxes collected more bloodfed or parous females. Resting boxes were significantly better than CDC traps ($P < 0.05$) and flicker+octenol ($P < 0.05$). The addition of octenol, CO_2, and CO_2+octenol to AB flicker traps did not significantly increase collections of *Anopheles* (all $P > 0.05$). Trap catch from flicker+CO_2 was not significantly different from flicker+octenol ($P = 0.12$). A combination of CO_2 and octenol significantly increased trap catch over octenol alone ($P < 0.05$) but had no significant effect over CO_2 alone ($P = 0.09$).

1996: A total of 16,318 females were collected with 8 sampling methods operated for 15 nights (Table 4). On the basis of 1994–95 data, NJ and steady light traps and resting boxes were not used in 1996. The CDC light trap collected an average of 13 females per night, not significantly different from AB flicker ($P = 0.53$). The CDC+octenol traps collected an average of 25 females per night, not significantly different from flicker+octenol ($P = 0.85$). The addition of octenol to an AB flicker light trap increased average yield from 9 to 19 (SD = 21) females per night ($P = 0.07$). There was also no significant difference between CDC+CO_2 and flicker+CO_2 ($P = 0.84$). However, CDC+CO_2+octenol was significantly better than AB flicker+CO_2+octenol ($P < 0.05$).

The addition of octenol to a CDC light trap increased average yield from 13 to 25 females per night; however, the octenol traps were so variable (SD = 48) that this difference was not significant ($P = 0.24$). The addition of CO_2 to a CDC trap significantly increased yield from 13 to 319 females per night ($P < 0.05$). The CDC+CO_2 trap caught significantly more females per night than CDC+octenol ($P < 0.05$). The addition of CO_2 and octenol to a CDC trap significantly increased average trap yield over octenol alone ($P < 0.05$) but not CO_2 alone ($P = 0.23$).

Trap specificity (1996): There was no significant difference between unbaited AB and CDC light traps for any species (Table 5). *Aedes canadensis, Aedes stimulans* (Walker) s.l.g., and dark-legged *Aedes* were equally represented by flicker+CO_2, flicker+CO_2+octenol, CDC+CO_2, and CDC+CO_2+octenol. More *Aedes* spp. were sampled with CO_2 plus octenol *vs.* CO_2 alone, but

Table 4. Number of mosquitoes collected with 8 sampling methods in western Massachusetts in 1996.

Mosquito	Flicker	Flicker+ octenol	Flicker+ CO_2	Flicker+ CO_2+ octenol	CDC	CDC+ octenol	CDC+CO_2	CDC+CO_2+ octenol
Ae. can[1]	8	13	426	529	9	9	290	335
Ae. vex[2]	0	0	4	4	0	2	1	10
Ae. stim[3]	5	7	130	136	0	2	149	163
Ae. dark[4]	24	47	1,077	984	19	33	1,225	1,017
Anophls[5]	3	20	113	257	6	10	164	211
Cs. mela[6]	1	0	0	7	0	0	5	0
Cq. pert[7]	93	190	1,253	2,166	145	302	2,855	1,523
Culex[8]	5	10	84	85	9	14	102	27
Total	139	287	3,087	4,168	188	372	4,791	3,286
Nights in use	15	15	15	13	15	15	15	9
Mean	9	19	206	321	13	25	319	365
SD	9	21	226	204	18	48	288	202

[1] *Aedes canadensis.*
[2] *Aedes vexans.*
[3] *Aedes stimulans, Ae. fitchii, Ae. excrucians.*
[4] *Aedes triseriatus, Ae. abserratus, Ae. cinereus, Ae. aurifer, Ae. trivitattus.*
[5] *Anophleles quadrimaculatus, An. punctipennis, An. walkeri.*
[6] *Culiseta melanura.*
[7] *Coquillettidia perturbans.*
[8] *Culex salinarius, Cx. restuans, Cx. territans.*

this difference was not significant ($P > 0.05$). The addition of octenol to AB and CDC light traps did not significantly increase numbers of *Aedes* spp. sampled. *Aedes vexans* populations were too poorly represented to predict anything.

Addition of octenol to AB and CDC light traps increased collections of *Cq. perturbans*; however, this increase was not significant (Table 5). Addition of CO_2 to AB and CDC light traps significantly increased collections of *Cq. perturbans* ($P < 0.05$). The combination of CO_2 and octenol did not significantly increase collections over CO_2 alone in either AB or CDC light traps.

Culex salinarius was sampled best by light traps supplemented with CO_2. There was no significant increase by adding octenol to CO_2, and in some cases, this actually decreased the number of host-seeking females sampled. All *Anopheles* spp. were better represented in light traps supplemented with both CO_2 and octenol *vs.* either CO_2 alone or octenol alone ($P < 0.05$).

Table 6 is a summary of trap comparisons and corresponding p-values.

DISCUSSION

The AB light traps with a steady light source were comparable with those with a flickering light source and were not used the 2nd summer (Table 3) because flicker uses less battery power. Flicker and CDC light traps also consistently collected higher total numbers than NJ light traps. Size, convenience, and greater trap yield clearly favor AB flicker or CDC light traps over NJ traps for extended surveillance.

Resting boxes were poor indicators of *Aedes* spp. and *Cq. perturbans* abundance but were the best indicators of *Anopheles* spp. in southeastern Massachusetts; resting boxes were not used in western Massachusetts. Resting boxes tended to collect engorged or parous females and provided more blood-fed *Anopheles* from which to recover virus.

Table 5. Nightly means for 8 sampling methods for mosquitoes in western Massachusetts for 1996.

Sample	Flicker	Flicker+ octenol	Flicker+ CO_2	Flicker+ CO_2+ octenol	CDC	CDC+ octenol	CDC+CO_2	CDC+ CO_2+ octenol
All species	9	19	206	321	13	25	319	365
Aedes canadensis	1	1	28	41	1	1	19	37
Other *Aedes*[1]	2	4	81	86	1	2	92	132
Coquillettidia perturbans	6	13	84	167	10	20	190	169
Culex spp.[2]	0	1	6	7	1	1	7	3
Anopheles[3]	0	1	8	20	0	1	11	23

[1] *Aedes vexans, Ae. stimulans s.l., Ae. abserratus, Ae. triseriatus, Ae. trivitattus, Ae. aurifer.*
[2] *Culex salinarius, Cx. restuans, Cx. territans.*
[3] *Anopheles punctipennis, An. quadrimaculatus, An. walkeri.*

Table 6. Pairwise comparisons using the Wilcoxon signed rank test between sampling methods for all species, 1996. If $P > 0.05$, then difference is not significant; the probability that 2 methods are the same is >5%. If $P < 0.05$, then difference is significant. Two-tail *P*-values are given for normal approximation with continuity correction.

Comparison of methods	*P*-value
Significant difference present (Trap 1 > trap 2)	
CDC + CO_2+octenol *vs.* flicker+CO_2+octenol	0.03
CDC+CO_2 *vs.* CDC	0.00
CDC+CO_2+octenol *vs.* CDC	0.01
No significant difference	
CDC *vs.* flicker	0.53
CDC+octenol *vs.* flicker+octenol	0.85
CDC+CO_2 *vs.* flicker+CO_2	0.84
CDC *vs.* CDC+octenol	0.24

The AB light traps and CDC light traps sampled roughly the same number of female mosquitoes whether they were unbaited or supplemented with CO_2, octenol, or both. Although 1994–95 data show that the unbaited CDC light trap was significantly better than an unbaited flicker light trap, results from 1996, when densities were higher, found no difference. Also, in 1994 and 1995, we sampled in *Cs. melanura* habitat, where unbaited CDC light traps took the highest numbers of *Cs. melanura.* In 1996, we sampled near a forested marsh in western Massachusetts in an area ideal for *Aedes, Anopheles,* and *Cq. perturbans* but not for *Cs. melanura.* Because the CDC light trap no longer sampled *Cs. melanura,* no significant difference was found between the total number of mosquitoes collected in it and in the AB flicker light trap.

The AB flicker+octenol and CDC+octenol traps were not significantly different from one another ($P = 0.85$). Both traps had a high standard deviation and neither increase was significant. Still, the trend suggests that octenol could play a role in mosquito sampling, especially for mosquitoes that feed on large mammals.

Previous authors have reported an increase in collections of *Cq. perturbans, Cx. salinarius,* and *Aedes* spp. collected with an emission of 3.0 mg/h of octenol to CDC light traps (Kline et al. 1990a, 1991b). We used octenol emission of 0.5 mg/h added to AB flicker light traps in 1994 and 1995, which may have been inadequate to increase trap catch significantly. We increased emission rate to 2.0 mg/h in 1996 and did sample more mosquitoes, emphasizing the role of this volatile material in the host-seeking behavior of some species, but the increase still was not significant.

The AB flicker+CO_2 and CDC+CO_2 trap yields were not significantly different from one another ($P = 0.84$). The addition of CO_2 to an AB flicker trap increased average yield from 7 to 74 (SD = 104) females per night ($P < 0.05$) for 1994–95; the average increased from 9 to 258 (SD = 226) in 1996. The addition of CO_2 to a CDC light trap increased average yield from 12 to 319 (SD = 288) females per night ($P < 0.05$). Previous studies have found that the addition of CO_2 to light traps increases trap catches of *Aedes* spp., *Cq. perturbans, Cx. salinarius,* and *An. quadrimaculatus* and provides a more accurate estimate of nuisance mosquito problems (Newhouse et al. 1966, Carestia and Savage 1967, Magnarelli 1974, Buckley et al. 1994). We found that the addition of CO_2 significantly increased trap catch over AB flicker and CDC light traps alone for *Aedes* spp., *Cq. perturbans, Cx. salinarius,* and *Anopheles* spp. (all $P > 0.05$). Light traps supplemented with CO_2 collected approximately 10–29 times the numbers of mosquitoes collected by unbaited traps. Because AB and CDC light traps collected similar numbers of target species, both traps supplemented with CO_2 are equally useful in arbovirus surveillance.

The AB and CDC light traps supplemented with CO_2 were significantly better than traps supplemented with octenol ($P < 0.05$). Takken and Kline (1989) found that octenol emitted at 1.57–2.26 mg/h attracted mosquitoes in numbers similar to CO_2 released at 200 ml/min in the Everglades. We found that octenol increases trap catch relative to unbaited traps, but emissions between 0.5 and 2.0 mg/h attract significantly fewer host-seeking females than CO_2 released at 400–500 ml/min.

The CDC+CO_2+octenol traps collected significantly greater numbers of host-seeking females than flicker+CO_2+octenol ($P < 0.05$). The CDC+CO_2+octenol traps collected some species not found in other traps, such as *Aedes implicatus* Vockeroth, *Aedes intrudens* Dyar, and *Wyeomyia smithii* (Coquillet). Overall, there was no significant difference between CDC+CO_2+octenol and CDC+CO_2 ($P = 0.23$) or between flicker+CO_2+octenol and flicker+CO_2 ($P = 0.39$).

Light traps supplemented with CO_2 alone may collect lower numbers overall than traps with CO_2 plus octenol, but CO_2 alone was more effective for sampling *Ae. canadensis* and *Cq. perturbans* (Table 2). The addition of octenol to CO_2 may increase catch size for some species and decrease it for others (Kline et al. 1990a, 1990b). Studies have shown that light traps supplemented with a combination of CO_2 plus octenol have collected greater numbers of *Aedes* spp., *Cq. perturbans,* and *Cx. salinarius* (Kline et al. 1990a, 1991b). We sampled lower numbers of *Ae. canadensis* and *Cq. perturbans* with this combination in southeastern Massachusetts. In western Massachusetts, we sampled more *Ae. canadensis* and *Cq. perturbans* per night with the same combination. This chemical combination also has yielded ambiguous results with *An. quadrimaculatus* (Kline et al. 1990a, 1990b, 1991a). We noticed collections of *Cx. salinarius* and *Anopheles* spp. increased significantly with this combination, but

sample sizes varied dramatically from site to site, emphasizing the importance of regional differences in sampling and the danger of extrapolating light trap data from one site (and population) to another. Because *Cx. salinarius* and the 2 *Anopheles* species (*punctipennis* and *quadrimaculatus*) have similar flight ranges and nocturnal/seasonal periodicities, a light trap supplemented with CO_2 and octenol could be more effective for sampling these 3 species in particular.

The CDC and AB light traps appear to sample about the same numbers of host-seeking females whether unbaited or supplemented with octenol or CO_2. Only CDC light traps supplemented with both chemical attractants were significantly better than AB light traps with this combination. The CDC traps cost somewhat less, but AB traps have the following potential advantages: 1) collect almost no nontarget insects (e.g., moths, beetles, crane flies, wasps); 2) are easier to assemble and disassemble; 3) are lighter, more compact, and easier to suspend; 4) are easier to connect to CO_2 gas cylinders, or add dry ice or octenol packets to; and 5) use less battery power (flicker light option).

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