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SCIENTIFIC NOTE

THE EFFICIENCY OF BIOGENTS SENTINEL 2.0 TRAPPING AND HUMAN-LANDING CATCHING METHODS TO CALCULATE HUMAN BITING RATES

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ABSTRACT. To calculate human biting rates for various mosquito species, we performed simultaneous collections for 15 wk at 6 ecologically variable sites in Hungary. Of the dominant species, the relative abundance of *Aedes vexans*, *Ae. sticticus*, and *Coquillettidia richiardii* showed a significant positive correlation between CO_2 + Biogents lure and human landing catch (HLC). The relative abundance of *Culex pipiens* was significantly lower in the HLC samples than in the CO_2 + BG lure samples. Of the invasive species, *Aedes korecius* was found more frequently in HLC, while *Ae. japonicus* was more common in CO_2 + BG lure samples. Estimated human biting rates, determined with the 2 collection methods, showed no significant differences at high mosquito density (100–120 bites/h/person), but there was considerable variation at low mosquito biting rates. Therefore, correcting the CO_2 + BG lure trapping data to include only species biting humans provides estimates approaching the values of the HLC. Our study confirmed that while HLC is the gold standard method for determining the human biting rate, provided appropriate data adjustments are made, trapping methods performing automated data collection can provide similar data while reducing the exposure of the data collector.

KEY WORDS Carbon dioxide, control, Europe, monitoring, mosquitoes

The search for an automated method that provides data similar to human biting rate for mosquitoes would be most useful in estimating mosquito numbers in a wide range of studies: for the surveillance of invasive species (Dennett et al. 2005, Becker et al. 2013, Sáringer-Kenyeres et al. 2020), for the study of pathogen vectors (Jöst et al. 2011, L'Ambert et al. 2012), and to evaluate mosquito control measurements (Kröckel et al. 2006, Drago et al. 2012).

Unfortunately, mosquito trapping methods differ significantly in their attractiveness, with large variations in the species collected and their abundance (Jupp et al. 1980), and carbon dioxide (CO_2) trapping being the most effective method for sampling a broad range of mosquito species (Newhouse et al. 1966). The essence of the CO_2 trapping method is that the trap simulates the CO_2 release of large mammals (and humans), which is a critical factor for the host-seeking response of female mosquitoes (Kellogg 1970). Previous studies have found that CO_2 release rates can substantially affect the number of mosquitoes caught. For example, dry ice is significantly more efficient (collecting ~3.5 times more mosquitoes) than solutions using yeast or other means that generate lower rates of CO_2 (Oli et al. 2005).

The composition and abundance of species differ significantly between CO_2 trapping and human-landing catch (HLC) (Rubio-Palis and Curtis 1992, Rubio-Palis et al. 2012, Gao et al. 2015). Although CO_2 trapping is efficient and excludes the problem of variation in efficiency between individual HLC collectors and is a human exposure-free technique (Tangena et al. 2015), HLC is considered the gold standard method for measuring mosquito density of vector importance (Service 1993, Wotodjo et al. 2015). Because the measurements required to determine the justification for mosquito control should be sensitive only to species attacking humans, other methods can yield misleading results potentially resulting in unneeded exposure of the ecosystem and the human environment to unnecessary pesticide applications.

While several studies have been carried out in the USA on purpose-adapted comparisons of different collecting methods, few studies have been conducted in Europe. Therefore, the main questions of our study on comparing various collections methods are the following: (1) Can a difference of species composition and abundance be detected locally among the samples collected with CO_2 + BG lure trap and HLC? And (2) What is the relationship between the human biting rates calculated based on the CO_2 + BG lure samples and HLC?

We performed simultaneous collections with CO_2 + BG lure and HLC for 15 wk (week 20 to week 34) during 2021. Data collection took place at 6 sites

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(settlements/codes: Badacsonytördemic [BT], Dorog [DO], Keszthely [KE], Mecsér [ME], Pécs [PE], and Tiszafüred [TF]). During site selection, we strived to ensure that they were scattered throughout Hungary, providing a variety of ecological and meteorological conditions.

Biogents Sentinel 2.0 traps were used for CO_2 + BG lure trapping. The trap fan was operated from an electricity network, and the main lure was CO_2 applied uniformly at a pressure of 3 bar from a 5 kg bottle via CO_2 nozzle, but a single BG lure (human scent odor; mixes of ammonia, lactic acid, and caproic acid) unit per trap was also used to increase the collection of *Aedes* species. Based on our experience from previous studies, the volume of outgassing of CO_2 emissions is essential. Wu et al. (2020) found 0.3 liter/min CO_2 flow to be the most efficient in terms of the collection efficiency of *Ae. albopictus* (Skuse), using BG-Sentinel traps. It corresponds to the amount of CO_2 applied at 3 bar in the present study.

Carbon dioxide trapping was performed once a week at each site for 1 h, always within the main activity period of the mosquitoes (1830 to 2230 h). The collections were made on days when weather conditions were optimal for mosquitoes (rainless, windless).

Simultaneously with the CO_2 + BG lure trapping, HLC were performed at a distance of 50–60 m from the traps so that the collections did not interfere with each other. Catching with aspirators was performed in 10-min intervals. The aspirator was 15 cm long, 3 cm in diameter, thick-walled glass tubes with a concave cone at the end. HLC samplings were carried out by the authors, acting as attractors.

The material collected was stored dry, with separate samples for site, date, and collection method.

The mosquito specimens were identified using the keys of Becker et al. (2003) with Tanaka et al. (1979) and Versteirt et al. (2012) for the identification of *Ae. japonicus* (Theobald) and *Ae. koreicus* (Edwards) adults. The nomenclature followed Sáringer-Kenyeres et al. (2018).

Per sample, we determined (a) the species number, (b) the Shannon diversity, (c) the number of specimens per hour, (d) the number of bites per hour, and (e) the relative abundance of the collected species in the samples. The number of bites per hour was determined by multiplying the number of individuals collected in 10 min by 6 at HLC and based on the overall abundance of species attacking humans at CO_2 + BG lure trapping. To determine the species' significance in human mosquito harm, we used the categories of Tóth et al. (2022), using a pooled database collected with various methods over several decades (categorized by having importance in human mosquito harm = species with share of >2% in material collected with HLC).

The data sets collected at the 6 sites were integrated into 1 database. To assess the association between the data collected with CO_2 + BG lure and with HLC, we used *t*-tests and built linear models.

Statistical procedures were performed with the PAST 2.16 (Hammer et al. 2001) and CANOCO 4.5 (Braak and Smilauer 2002) software packages.

We collected 2,201 individuals of 16 mosquito species during the study. Out of 90 collection times (6 × 15), CO_2 + BG lure trapping was effective (at least 1 mosquito specimen caught) 67 times and HLC 60 times. Sixteen species were caught with CO_2 + BG lure trapping and 12 with HLC.

The number of species collected per sample was significantly higher ($t = 3.966$; $P < 0.001$) for samples collected with CO_2 + BG lure (mean ± SE of species number: 2.61 ± 0.19) than for HLC (1.73 ± 0.11). The number of individuals per hour was substantially higher ($t = -3.173$; $P = 0.002$) for HLC (53.39 ± 8.20) than CO_2 + BG lure (25.39 ± 3.99). Shannon diversity did not differ between samples collected with CO_2 + BG lure and HLC (0.855 ± 0.142 and 0.69 ± 0.116, $t = 0.897$; $P = 0.390$) or between localities: CO_2 + BG lure/HLC (BT/1.269/0.849; DO/0.815/0.271; KE/0.342/0.702; ME/0.995/0.930; PE/0.578/0.426; TF/1.134/0.966).

Of the dominant species, the relative abundance of *Ae. vexans* (Meigen), *Ae. sticticus* (Meigen), and *Coquillettidia richiardii* (Ficalbi) in samples collected with CO_2 + BG lure and HLC showed a significant positive correlation with linear models (Fig. 1). On the other hand, the relative abundance of *Culex pipiens* L. in samples collected with CO_2 + BG lure was independent of the relative abundance of the species detected with HLC, as catch with the latter was rare (Fig. 1). Several other species were caught including *Ae. koreicus* and *Ae. japonicus* but formed only 2.65 % (on 1 site) and 0.46 % (on 3 sites) of the total catch, in numbers too low for detailed analysis.

The main indicator used to determine the human biting rate (bite/hour/person) measured with the different methods used here (CO_2 + BG lure trapping, HLC, CO_2 + BG lure trapping results restricted to species attacking humans) were not substantially different at high mosquito densities of 100–120 bites/hour/person (Fig. 2). However, there were substantial differences between the three methods at medium and low mosquito nuisance levels (Fig. 2). At the medium and low densities, the corrected values sometimes were close to the human collection values but at other times were not.

Our results confirmed that while CO_2 + BG lure trapping collects biting mosquitoes efficiently, it also catches species that do not typically feed on humans. For *Cx. pipens*, far more were caught with the CO_2 + BG lure than HLC, which is consistent with Gao et al. (2015), who had significantly higher individual numbers of *Cx. pipiens* in samples collected with CO_2 + BG lure trapping than in HLC samples.

Based on our results, the human-biting rate (number of bites/person/hour) can be quantified in the case of samples collected with a CO_2 + BG lure trap only after the number of individuals of each

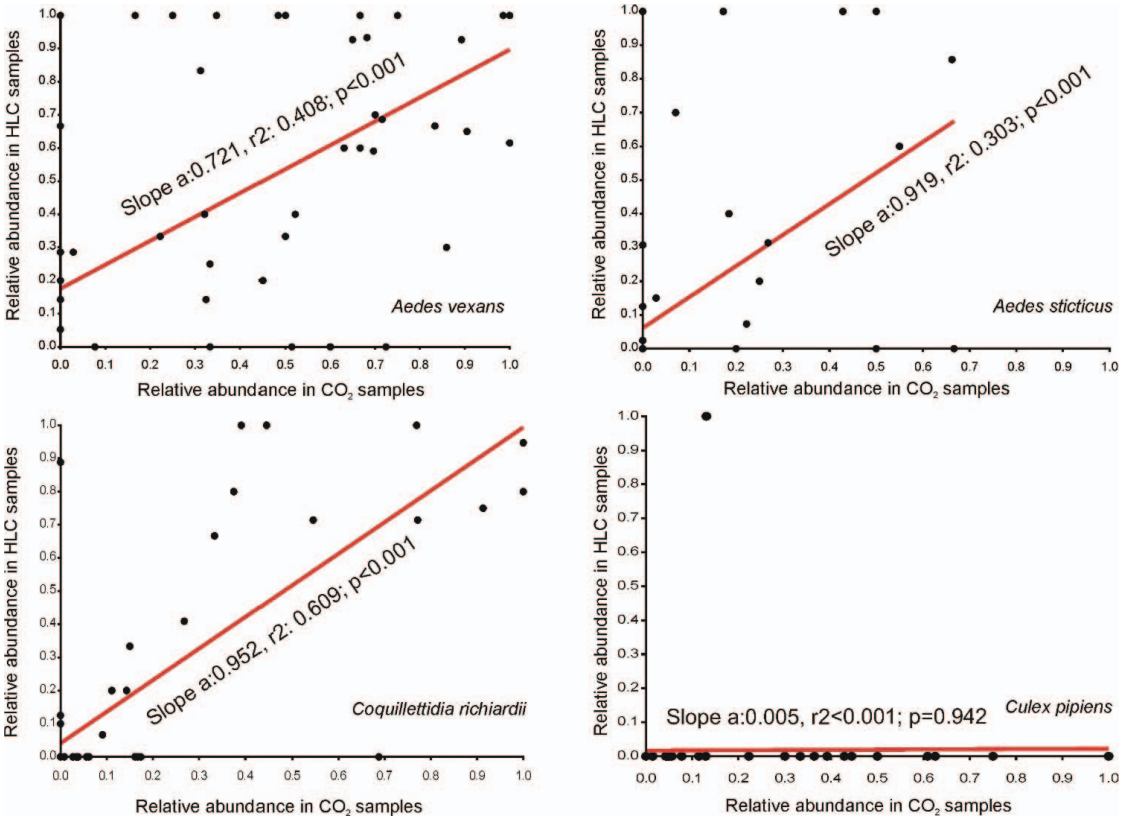

Fig. 1. Relative abundance of *Aedes vexans*, *Ae. sticticus*, and *Coquillettidia richiardii* in samples collected with Biogents Sentinel 2.0 (CO_2) and human landing collection (HLC) showed significant ($P < 0.001$) correlation with linear models. The relative abundance of *Culex pipiens* in samples collected by Biogents Sentinel 2.0 was totally independent of the abundance of this species detected by HLC.

species in the samples have been determined. By eliminating the counts for species that do not bite people or do so at a low level, the data of the CO_2 + BG lure trap can be used as an estimate of the human biting rate.

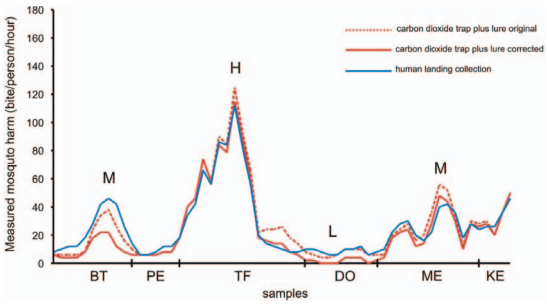

Fig. 2. Differences in the biting rate per hour estimated with the Biogents Sentinel 2.0 (CO_2 + BG lure), the CO_2 + BG results corrected to include only human-biting species, and human landing collection at high (H), medium (M), and low (L) mosquito densities over the sampling period.

In tropical areas, where HLC poses a significant health risk to the collector, automated collection tools are recommended, provided the data are adjusted for nonbiting and low-biting species (Govella et al. 2016). However, some checking using HLC is also essential in these cases to obtain an accurate insight into the human biting rate (Duo-quan et al. 2012). As with HLC, using a human-baited double-net trap seems to be a usual method that maintains human attractiveness, while being safe (Tangena et al. 2015, Gao et al. 2018).

In summary, our studies confirmed that provided the data are corrected for the catch of non- or low-human-biting species of mosquito, trapping methods performing automated data collection provide a safe accurate alternative to human landing collection methods.

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