

A COMPARISON OF MOSQUITO SPECIES ASSEMBLAGES SAMPLED WITH THREE COMMONLY USED TRAPS AND A NOVEL DESIGN

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ABSTRACT. In nuisance or vector mosquito management, effective sampling is a stepping stone to efficient use of resources, targeted control efforts, and successful reduction of disease transmission. Experimental evidence indicates that there are species biases for certain traps, which in turn implies that the traps used will influence the species make-up of samples collected. A comparative study between 3 CO₂-baited light traps and 2 hay-infusion baited gravid traps—Centers for Disease Control and Prevention (CDC) light trap, American BioPhysics Company (ABC) light trap, Reiter-Cummings (RC) gravid trap, and a convertible gravid/light trap with a novel design—was conducted to test for species specificity of each trap type. It was found that different species of mosquitoes are more likely to be collected in greater numbers with certain traps compared to others, even between the different light traps or gravid traps. In particular, *Coquillettidia perturbans* tended to be collected in greater numbers with CDC light traps, *Culex salinarius* tended to be collected in greater numbers with the experimental convertible light traps, and *Culex pipiens* tended to be collected in greater numbers with the Reiters-Cummings gravid traps over the other traps included in the study ($P \leq 0.05$). The overall species richness of samples was comparable among trap types, with similar performance of the new trap designs as established designs ($P \leq 0.05$).

KEY WORDS *Culex salinarius*, diversity, gravid trap, light trap, sampling, trapping

INTRODUCTION

Mosquito-borne diseases, including West Nile virus (WNV), eastern equine encephalitis virus (EEEV), and La Crosse encephalitis virus, among others, continue to pose a significant risk in temperate areas. Nuisance mosquitoes have economic impacts on tourism, decrease quality of life, and can have other deleterious effects such as stressing dairy cows to the point of reducing milk production (Raña et al. 1971). A major priority in combating mosquito-vectored diseases and nuisance mosquitoes is controlling mosquito abundance through integrated pest management practices that incorporate surveillance, habitat modification, and changes in human behavior, along with pesticide applications that employ the least environmentally damaging yet effective control products available (Gubler et al. 2003).

Effective sampling to identify locations where vectors are present and to monitor population levels along with infection rates allows control measures to be targeted toward medically important mosquitoes and can reduce the environmental and financial costs associated with large-scale, less discriminate pesticide applications while also preventing the failure to initiate control in an area due to a perception that there are few medically important mosquitoes present. The goal when sampling mosquitoes for integrated vector management purposes is to develop an accurate picture of the abundance of species that are most important to public health. In other cases, the

goal of sampling is more ecological in nature, examining the diversity of species or monitoring for new species introduced by commerce or shifting geographical ranges due to climate change. This study focuses on determining the efficacy of widely used mosquito sampling devices along with experimental devices for specific mosquito species and mosquito communities.

Any particular pathogen is normally transmitted by certain mosquito species more than others because different mosquito species have different behavior patterns (e.g., differ in their preferences for hosts) and physiologies that affect their vector competence (Turrell et al. 2001, Reisen et al. 2005, Ledesma and Harrington 2011, Carrington and Simmons 2014). These behavioral differences also imply that certain types of traps may be more effective than others at capturing particular species as shown by Vaidyanathan and Edman (1997), Burkett et al. (2004), and Bhalala and Arias (2009), due to the trap shape, size, colors, airflow, vibration, etc. (Allan and Kline 2004).

Some traps are designed to collect particular species such as the Fay-Prince trap, which is designed with contrasting colors and guide rails to specifically target *Aedes aegypti* (L.) and *Culex quinquefasciatus* Say (Fay and Prince 1970), or the BG Sentinel, that uses counterflow and a proprietary mix of lactic acid and other odors to collect *Ae. aegypti* (Maciel-de-Freitas et al. 2006). *Culex melanura* (Coquillett), an EEEV vector, though it blood feeds on birds and lays eggs in standing water, is notoriously difficult to capture using common light or gravid traps and is best sampled using resting boxes (Howard et al. 2011). When *Ae. albopictus* (Skuse) was invading the US mainland, one of the major issues for many mosquito control districts was identifying infestations because detection through trapping was thought to be unreliable until the introduction of the BG Sentinel, which although

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designed for *Ae. aegypti* was more effective for *Ae. albopictus* than other widely used models (Farajollahi et al. 2009).

Even variations of trap types designed to sample the same specific host- or oviposition-site seeking vectors may have sampling biases at a community level. Carbon dioxide (CO₂)-baited light traps and gravid traps are 2 of the most common types of devices used for vector mosquito surveillance. Comparative studies between multiple models of gravid traps or different models of CO₂ traps for particular species efficacies are useful for improving vector sampling efficacy and formed the impetus for the current study.

The experimental design was intended to compare the species caught with 3 commonly used commercial mosquito traps and an experimental design with multiple configurations. The objective was to compare the sampling efficiency of these different traps for a variety of mosquito species of interest, as well as for mosquito community-level characteristics.

MATERIALS AND METHODS

This study also tested the efficacy of an experimental trap design with multiple configurations referred to as a Convertible Gravid/Light Trap (CGLT) developed by one of the authors (LH). The CGLT is a compact convertible sampling device that can be used as either a gravid or a CO₂ light trap (Figs. 1–3). It can also be configured for use as a resting box trap, but that was not part of this study. Among the suite of commercial traps that were compared were the American Biophysics Company light trap marketed by Clarke Mosquito (“ABC light trap”; St. Charles, IL); the CDC light trap marketed by The John W Hock company (Gainesville, FL); and the Reiter-Cummings gravid trap marketed by Bio-Quip (“RC gravid trap”; Compton, CA; Fig. 4), in addition to the CGLT light trap and the CGLT gravid trap. Using the protocols employed by Pennsylvania’s West Nile Virus Program, when operating the 3 commonly used traps, the light bulbs were removed from the ABC and the CDC trap to reduce by-catch, and the light sensor for the fan motor was covered on the Reiter-Cummings trap to prevent sample loss during daylight hours.

The CGLT is a compact, convertible trap that can be used as either a CO₂ light trap or a gravid trap. The trap body is constructed using 3-inch (7.62-cm) schedule 40 PVC pipe and couplings (Charlotte Pipe and Foundry Company, USA) with a fan powered by a 6-volt DC motor (John W. Hock) at the exhaust end and a removable, clear collection chamber, also made of 3-inch (7.62-cm) schedule 40 PVC pipe (United States Plastic Corp.) between the intake and the fan (Fig. 1). The lack of fan pass-through in this technology is intended to result in less damaged insects collected and higher quality samples for identification. Black burlap cloth encircles the intake opening to create visual contrast. In light-trap mode, the trap

base is hung vertically beneath a 2-liter insulated beverage container having four 1-cm holes drilled in the sides that is filled with dry ice. In gravid-trap mode, the trap base is placed horizontally and inserted through an opening into the side of an 8-liter black plastic bucket filled to bottom lip of the opening with hay infusion.

Sites for trials were chosen based on Pennsylvania’s West Nile Program’s regular surveillance data in Lebanon County, PA. A variety of sites were selected to ensure diversity in habitat and potential variety of mosquito species inhabiting these locations. Sites included wastewater treatment plants, wetlands, flooding terrains, and industrial sites rich with discarded containers. In total, 19 different sites were used, with, on average, 2 replicates collected at each site over a 2-year period. Each replicate (randomized complete block) comprised 1 night’s collections from the 5 trap treatments set simultaneously at the same site. Trap malfunction (e.g., battery failure, tampering by passers-by, and a fallen tree) was recorded and these data excluded from the analysis, and, excluding these rare events, 39 blocks of usable data were collected.

For each block, traps were set evenly spaced approximately 10 m apart, in a line, within a homogeneous area, and specific traps were randomly assigned to a position within the block each night using dice. The line of traps was set parallel to nearby adult mosquito cover (e.g., vegetation, tire pile, abandoned building). Tripods were used for hanging light traps at a uniform height (1 m), and the gravid traps were placed on the ground (Fig. 5). At the time the traps were deployed, the dry ice chambers of the light traps were filled to capacity (2.25 kg). After agitation of the container, the pan of the RC gravid trap was filled with hay infusion to a depth of 2.5 cm, and the CGLT gravid trap (CGLT-G) bucket was filled to the brim of its side opening with the same hay infusion solution from the same container as was used for the RC gravid trap. The time period of mosquito-trapping for each block was approximately 24 h, being set during the day before dusk and collected during the next day after dawn and before dusk that day. After collection, the sample chambers were placed on dry ice to kill and preserve the samples until they could be identified to species, using taxonomic keys (Darsie and Hutchinson 2009).

The hay infusion was made by placing approximately 5 pounds (2.25 kg) alfalfa hay in a mesh bag and allowing it to ferment for a week in a 65-gallon (246-liter) trash can filled with water and 0.5 liter milk or 30 ml milk albumin. Dry ice was purchased in pelleted form from AirGas (Radnor, PA).

To investigate community-level performance of the traps, we explored the datasets using a principal components analysis (PCA) ordination method and compared the overall species richness of the collections depending on trap type (Potts and Elith 2006). In order to perform a PCA with species abundance data we performed a transformation of the data based on the

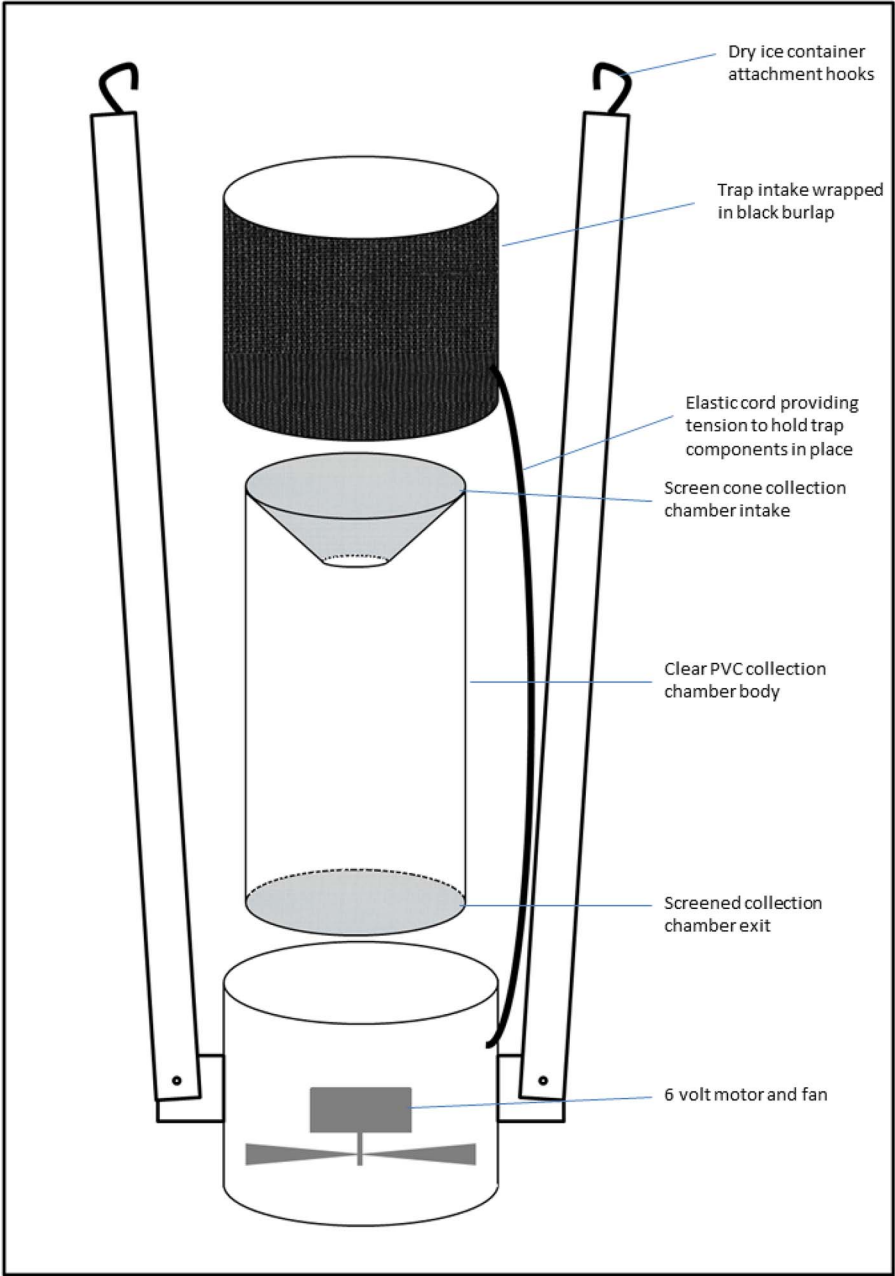


Fig. 1. A schematic of the novel convertible trap (CGLT).

Hellinger distance, following Legendre and Gallagher (2001) and Borcard et al. (2018). Additionally, data for species captured in 10 or more replicates were also analyzed for each species using a Hurdle model, a 2-part test for zero-inflated data. The Bonferroni correction was used to account for multiple comparisons. The analyses were conducted with R (R Core Team, 2018), using the packages *FactoMineR* (Lê et al. 2008) and *vegan* (Oksanen et al. 2022) for the multivariate

analyses and *pscl* (Jackman et al. 2015) for the Hurdle model.

RESULTS

Species-by-species trap performance

Thirty-nine randomized complete-block replicates were obtained over 2 years. Fourteen different species



Fig. 2. CGLT CO₂-baited light trap configuration.

were captured over the course of the study: *Ae. vexans* (Meigen), *Anopheles punctipennis* (Say), *An. quadrimaculatus* Say, *Coquillettidia perturbans* (Walker), *Cx. pipiens* L., *Cx. restuans* Theobald, *Cx. salinarius* Coquillett, *Ae. canadensis* (Theobald), *Ae. japonicus* (Theobald), *Ae. triseriatus* (Say), *Ae. trivittatus* (Coquillett), *Psorophora columbiae* (Dyar and Knab), *Ps. ferox* (von Humboldt), and *Ps. horrida* (Dyar and Knab).

Histograms of species captured in 10 or more replicates are displayed in Fig. 3, along with statistical test results, and demonstrate differential capture rates for the traps tested. Several other species were captured in numbers of blocks that were too low to allow a determination of any significant differences among the trap types' capture abilities: *Ae. canadensis* (2 blocks), *Ae. japonicus*, (7 blocks), *Ae. triseriatus* (5 blocks), *Ps. columbiae* (6 blocks), *Ps. ferox* (6 blocks), and *Ps. horrida* (3 blocks).

Across-species trap-type performances

We performed a principal component analysis of the data in order to visualize how the sampled mosquito communities related to the specific trap types (Fig. 6). The first 2 axes of the PCA explained 17.8% and 11.2% of the variation in the data, respectively, and the species contributing most strongly to the variation explained by these 2 dimensions were *An. quadrimaculatus*, *Ps. columbiae*, *Ae. vexans*, and *Ae. trivittatus*. The 2 gravid type designs tended to cluster together and were associated with species assemblages consisting of *Cx. pipiens*, *Cx. restuans*, and *Ae. triseriatus*. The light trap designs showed more variability in the assemblages they were associated with, perhaps indicating a greater sensitivity

to the species present in different environments than the gravid traps.

The gravid traps caught, per average trap night, the greatest variety of mosquito species (Fig. 8). However, this seems to be because they were extremely effective at catching *Cx. pipiens* and *Cx. restuans*, whereas light traps in environments dominated by those species often caught very few, if any, mosquitoes. These *Culex* spp. were ubiquitous, being found together in almost every block (Fig. 7). The other species were not so common, with only *Ae. vexans* being found in more than half of the blocks. For species other than *Cx. pipiens* and *Cx. restuans*, the light traps were shown to be statistically equivalent with each other and more effective than the gravid traps for detecting the large variety of non-*Culex* species present at some sites (Fig. 8).

DISCUSSION

Differential capture rates were found for the traps used in this study for different species, indicating that the trap design selected for sampling can greatly influence the sample results. The type of trap used can not only greatly influence which species and the diversity of species that are able to be detected, but can also influence the relative numbers of certain species that will be collected. Thus, when conducting surveillance, this preference by certain species for certain traps should be considered.

The novel CGLT traps that were tested were shown to often be as effective in sampling certain important target species of mosquito as the standard, currently used commercial mosquito traps. As with the other devices, for some species the novel traps were superior to the commercial traps, and for others they



Fig. 3. CGLT hay infusion-baited gravid trap configuration.

appeared to be less effective. Because of the sample quality, compactness, and versatility of the CGLT traps, they may prove to be a valuable addition and more easily portable and deployable alternative to the standard sampling arsenal used by professionals in the field.

For some species, the traditionally used commercial traps performed better. *Anopheles quadrimaculatus* and *Cq. perturbans*, for instance, were most effectively caught with the CDC light trap. *Culex pipiens* and *Cx. restuans*, vectors of WNV, were captured at the highest

rates with RC traps, with CGLT capturing the second most, and both gravid traps capturing them at much greater levels than the light traps. *Aedes trivitatus* was captured most effectively with CDC light traps, and all 3 light traps were more effective than the 2 gravid traps. For many species, the new CGLT designs performed as well as the standard commercial traps. *Aedes vexans*, vector of dog heartworm and bridge vector for WNV and EEEV, were captured at higher rates with the CGLT-L, followed with the ABC light trap, with these light traps more effective than the CDC light trap or the



Fig. 4. Commercial traps tested. From left to right: ABC light trap, CDC light trap, RC gravid trap.



Fig. 5. Experimental setup.

gravid traps. *Culex salinarius* was also captured in the CGLT-L traps at greater rates than in the ABC, CGLT-G, and RC traps.

Certain species such as *Ae. vexans*, *An. quadrimaculatus*, *Cq. perturbans*, *Cx. salinarius*, and *Ae. trivittatus* were captured at higher rates in the light traps than in gravid traps, while the reverse was true for *Cx. pipiens* and *Cx. restuans*, which matches previous findings (Williams and Gingrich 2007).

We found species specificity among the light traps, and patterns also emerged with regard to species vulnerability to capture among the gravid traps. This may have to do with slight differences in their design or colors (Bidleingmayer 1994) or air flow. For instance, the location between the CO₂ release point and the intake opening for the light traps is different for the CGLT-L, CDC, and ABC traps. The CDC trap has a CO₂ release point to the side and below the trap intake opening, whereas the release points for the ABC and CGLT-L traps are above the intake point. The CGLT-L has a greater distance between the release point and the trap intake than the ABC. The ABC and the CDC both have black rain shields that also served to funnel mosquitoes approaching from below toward the intake, but also directed away those approaching from above. The CGLT-L had no rain shield.

For the gravid traps, the position of the intake opening in relation to the water level of the grass infusion was different. The CGLT-G's intake is located laterally, allowing uninhibited access to the entire surface of the attractant and capturing mosquitoes from the side with a vertical intake opening. The RC trap's intake is located ventrally, and mosquitoes had to pass under the trap to explore the entire surface of the

attractant and capturing mosquitoes from above with a horizontal intake opening.

The colors of the intakes were similar in color (black), but the CGLT-G had black burlap encircling white plastic while the RC's intake was solid black plastic. The body of the CGLT trap was clear, white, and metallic, whereas the body of the RC was gray. Mosquitoes likely are sensitive to these sorts of nuances, and these differential sensitivities may possibly translate into different behavioral reactions to the traps, depending on the species. Further research will be needed to clarify and define any behavioral reasons for these differences.

The uniform height at which the traps were deployed may also have preferentially trapped particular species over others. For the light traps, some species may have preferred to feed or rest at higher or lower heights than this particular trap level (Swanson and Adler 2010, Votýpka et al. 2010), and a change in trap height could possibly have increased the performance of the light traps for such species. For the gravid traps, the vertical distribution of individuals of a species in the environment due to their reproductive behaviors would seem to be able to influence their capture levels in the current study. For example, *Ae. hendersonii* is often found in the same locale as *Ae. triseriatus*, but *Ae. hendersonii* lay eggs in tree holes at higher elevations with *Ae. triseriatus* laying eggs nearer the ground (Scholl and DeFoliart 1977). No specimens of *Ae. hendersonii* were captured in this study, using the on-ground gravid traps, but these traps did catch small numbers of *Ae. triseriatus* even though it was possible that *Ae. hendersonii* females were in the area.

One element we did not consider was whether differences in trap design (within light trap and gravid trap

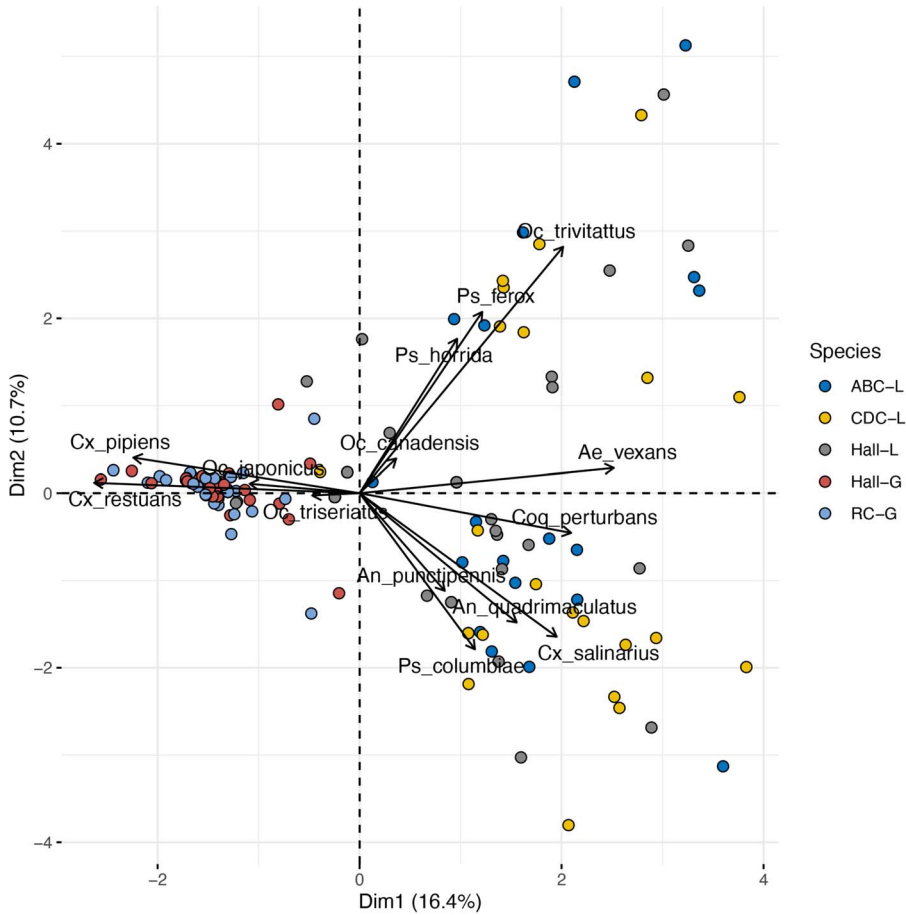


Fig. 6. A principal components analysis plot showing the first 2 axes. Trap types are indicated by color.

designs) lead to differences in their ability to sample infected mosquitoes. As these diseases are not static within an individual vector (Isaia et al. 2020) and can affect mosquito behavior/senses (Cator et al. 2013, Vogels et al. 2017, Stanczyk et al. 2019), it is possible that mosquitoes at different points of the transmission cycle will have behavioral/sensory differences that will affect their responses to particular traps.

This study provides comparative data that show that these novel CGLT-L and CGLT-G trap types can be considered as supplements to the standard mosquito sampling arsenal when appropriate. One possible field application of CGLT models could be for sampling the variety of *Culex* species in an area, particularly *Cx. salinarius*. Additionally, when transportation of equipment is an issue, or when there is a concern that samples might become degraded because of contact with the fans of some standard commercial traps, a different trap type such as the CGLT series may be appropriate for these situations.

Important implications can be drawn from this study relevant to the goals of mosquito control districts, mosquito-vectored disease programs, and ecological studies/

monitoring. Trap selection will influence sampling results, and utilizing a toolbox of sampling devices is pragmatic. Accurate sampling allows for more efficient use of resources, targeted control efforts, prevention of failure to initiate control when inaccurate sampling might falsely indicate that there are too few target mosquitoes present to be concerned about, and more success in nuisance or vector mosquito management. Detection of new or understudied species may require a sampling device other than what is currently being used or several different devices to more fully sample all the present species. A diverse set of sampling devices is key for sampling such a diverse group of insects as mosquitoes.

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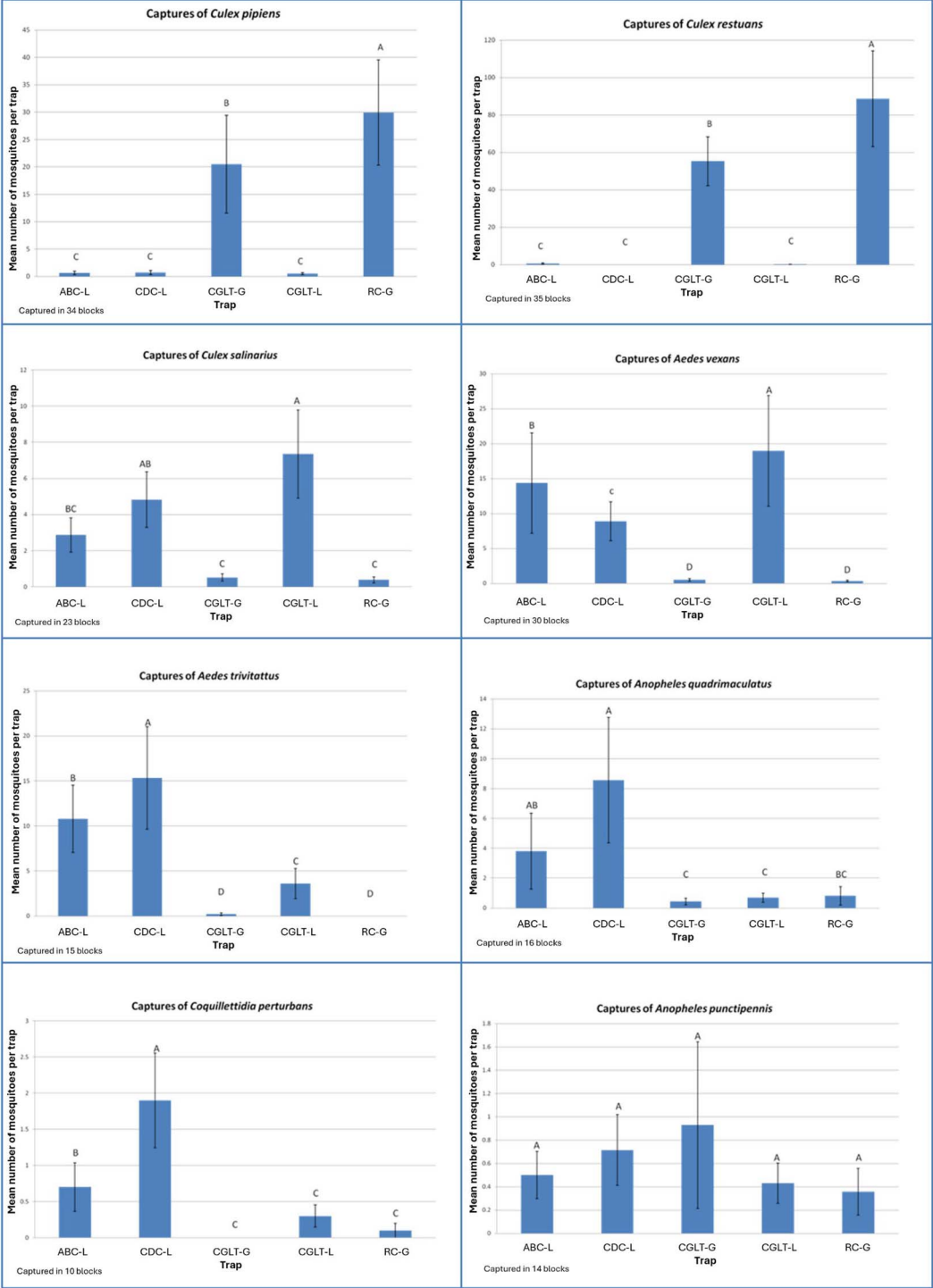


Fig. 7. Bar plots of average catches for each trap type for various mosquito species as tested by the Hurdle model with the Bonferroni correction. Having no letters in common indicates statistical significance at $P \leq 0.05$.

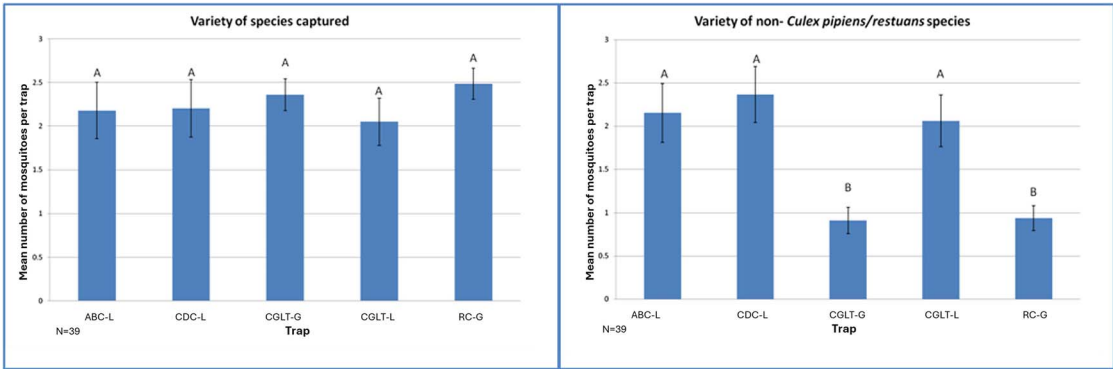


Fig. 8. Average species diversity associated with each trap type as tested by the Hurdle model with the Bonferroni correction. Having no letters in common indicates statistical significance at $P \leq 0.05$.

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