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DO IT YOURSELF: A MODERNIZED GRAVID TRAP DESIGN FOR MOSQUITO SURVEILLANCE

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ABSTRACT. Gravid traps have become a common and frequently essential surveillance tool for parous *Culex* spp. vectors of West Nile virus and other encephalitis-causing pathogens. The recent closing of BioQuip Products Inc., an entomological supply company, has jeopardized the commercial availability of gravid traps. The Salt Lake City Mosquito Abatement District presents herein a template for making your own gravid trap, but with some modernizations for quieter fans and longer lasting, light weight, lithium battery packs. At the time of writing, the materials cost for the fan ($14 USD), toolbox ($13), cables ($9), ABS pipe ($2.50), aluminum brackets ($10), catch container with lid ($9), trap net ($10), USB battery pack ($35) and the negligible amount of 3D-printed filament ($2), is approximately half the cost (not including labor) of the formerly available commercial model. Additionally, performance validation in the laboratory ($t_{4,9} = 0.1191$, $P < 0.9109$) and within two field sites ($\chi^2 = 0.107$, $P < 0.744$) demonstrated no significant differences in collections of gravid *Culex pipiens*. We do not present an overhaul of the previous gravid trap blueprint, but the quality-of-life updates to the trap design, the feasibility of in-house manufacture, and the mirrored collection efficacy to the commercial model can allow improved maintenance of gravid trap surveillance networks without a commercial supplier.

KEY WORDS *culex*, mosquitoes, surveillance, traps, vector, 3D printing

INTRODUCTION

The Reiter-Cummings style gravid trap (RCGT) is a common tool for surveillance of *Culex* spp. vectors (Allan and Kline 2004). The Reiter-Cummings modification to the original Centers for Disease Control and Prevention (CDC) gravid trap was intended to allow a more compact, easier to transport trap for daily use (Allan and Kline 2004). After improvements to housing and airflow, the RCGT trap has become a tight contender to the originally developed gravid traps (Braks and Cardé 2007) and serves as a staple in many mosquito surveillance operations. The RCGT was available through the now defunct entomological supply company BioQuip Products, Inc. until their closure in 2022, effectively taking prefabricated RCGTs out of circulation. The loss of a major supplier for this trap is reminiscent of the loss of another entomological supply company American Biophysics (Schneider and Hall 2011) and their MM-X counterflow geometry trap (Kline 1999), which was a unique type of research and surveillance tool for gravid (Mboera et al. 2000) and host seeking mosquitoes (Njiru et al. 2006). Subsequent concern about continued service of the RCGT without the originating manufacturer could possibly be resolved with modern tools and e-commerce for in-house fabrication so as not to repeat the loss of additional surveillance tools.

Self-manufacturing is more accessible than ever before (Hoshi et al. 2019, Hiscox and Takken 2021, Wasson-Reinbold and Reiskind 2021) with increasingly simplified trap designs and in-house tools such as laser cutting and 3D printing (Hoshi et al. 2019; Hiscox and Takken 2021). As with virtually any mosquito surveillance tool, resource barriers with shipping, construction/assembly skills, and the lost availability of original parts all limit the long-term operation of any given trap. To help empower programs to continue using the known and useful RCGT, the Salt Lake City Mosquito Abatement District (SLCMAD) has modernized the parts list for the BioQuip RCGT and proposes a design for low-cost upkeep through in-house manufacturing. This enhanced surveillance tool, the Salt Lake gravid trap (SLGT), is easy to manufacture, economic, efficacious, and the design is readily available to all through free-of-charge online 3D modeling programs.

MATERIALS AND METHODS

Design and Construction

The main template for manufacture of the SLGT was the Reiter-Cummings style gravid trap (RCGT) from the now defunct BioQuip (Royce Cummings 2800 series, BioQuip Products, Inc., Rancho Dominguez, CA) equipped with a 6 v, 12 ah sealed lead acid battery (UB6120 AGM type, Universal Power Group, Inc., Coppell, TX). The main physical constraints are matching dimensions for the interior of a toolbox and the mosquito collection container such that everything can be packed within the toolbox. For the SLGT model, we selected a 40.6 cm (16 in) black plastic portable toolbox with metal latches (Husky, The Home Depot, Inc., Atlanta, GA). The interior dimensions of this toolbox are 35 cm × 17.15 cm × 16.5 cm (13.75 in × 6.75 in × 6.5 in) and is paired

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with a square HDPE deli container (2 qt Space-Saver #153202, Carlisle Plastics Company, Inc., New Carlisle, OH) and matching lid (2-4 qt Space-Saver Lid #ST157330, Carlisle Plastics Company, Inc., New Carlisle, OH) that measures 16.5 cm square × 10.16 cm height (6.5 in square × 4 in height).

To prepare the trap body, a 7.6 cm (3 in) router blade was used to cut a central hole in the bottom of the toolbox. This hole was then sanded with a deburring tool to smooth the plastic and match the fit for a 7.6 cm (3 in) wide × 15.2 cm (6 in) long ABS pipe section (Cell Core Valencia Pipe, The Home Depot, Inc., Atlanta, GA). The pipe section was routed with a groove 4.5 cm (2.5 in) along the length and encircling the entire pipe (Fig. 1). The groove was fitted with a 7.6 cm (3 in) black o-ring (337 Buna-N 50A Durometer, Sterling Seal & Supply, Inc., Apopka, FL). While facing the toolbox latch, the left lateral end of the toolbox was cut open with a utility knife to match the 11.4 cm (4.5 in) square stencil (Fig. 1) of a USB case fan (120 mm Multifan S3, AC Infinity, Inc., City of Industry, CA). The outer cage of the case fan was unscrewed so that the screw holes could be patterned onto the box (Fig. 1). The cage was mounted on the outside of the toolbox with the fan body inside, with screws connecting through the plastic trap body (Fig. 1).

The opposite lateral end of the toolbox interior was fitted with a 3D printed bracket generated using PLA filament extruded through a 0.6 mm nozzle with set temperature at 210°C (410°F) and 58°C (136°F) bed temperature. The STL (Standard Tessellation Language or Standard Triangle Language) files for this trap are uploaded to ThingiVerse under SLCMAD (https://www.thingiverse.com/slcmad/designs). The bracket was secured with rivets through the exterior of the box. However, the bracket can be substituted with a similar dimensioned piece of metal, wood, thermoformed plastic, or other. The bracket was to seat a USB battery pack (PowerCore 20 100mAh, Anker Innovations Co., Ltd, Hunan, China). Connections were made to the battery using a 90-degree male-to-female USB adapter (USB 3.0, UCEC Tech, Guangdong, China) and then to the fan with the stock USB cable that was provided with the product. Cables were secured to the interior of the toolbox lid by cutting holes in the plastic molding fins and attaching the cables to the holes using a zip tie (Fig. 1).

For the collection container, both the lid and the bottom of the plastic square container were routed with the same size hole as the bottom of the toolbox (Fig. 1). Again, a deburring tool was used to smooth the edges. This time, a 7.6 cm (3 in) circular, knotted ring was 3D-printed as a snap-in lip for the container bottom. Hardware cloth fencing was cut and secured over the hole in the lid using high temperature hot glue around both the hole edges and the outer edges of the metal. The container was completed by using a trap net stocking (P/N 1.41 Cloth for Collection Cup, John W. Hock Co., Gainesville, FL) fed through the inside of the 3D-printed ring and then folded back over the ring in a loop (Fig. 1). Upon snapping the ring into place, the net was secured to the bottom of the container. Aluminum L-brackets ($^{3}/_{4}$ in and $^{1}/_{16}$ in thick Everbilt, The Home Depot, Inc., Atlanta, GA) were cut and riveted into the feet of the two lateral ends of the toolbox (Fig. 1). With a completed SLG unit, usage is near identical to the BioQuip model of RCGT trap: the net is fitted over the gasket end of the ABS pipe, the pipe is slotted into the bottom hole of the toolbox, the collection container is nested, the battery is connected to energize the fan, and infusion water is added to a pan with the trap seated on top. More imagery and extended instructions are included in Supplemental Documents.

Validation

Suction was verified at the target water level in the basin relative to the respective traps using a handheld anemometer (BT-100 Digital anemometer, BTMETER Zhuhai Electronic Technology Ltd., Zhuhai, CHN) taking a reading ∼2.5-cm (1-in) below the ABS ring underneath an active trap. The average of 3 measuring instances on 5 separate trap units of each model type were taken and averaged. For efficacy validation, laboratory bioassays were conducted with mosquitoes reared and maintained in the SLCMAD insectaries. Species used for validation were 2016 SLCMAD strain *Culex pipiens* L. Mosquito larvae were reared in collection trays and adult flight cages kept at consistent environmental conditions of 28 ± 1°C temperature and 70 ± 5% RH. Colony adults were fed on 10% sucrose solution ad libitum. Adults were allowed 5–10 days for mating before being offered their first blood-meal.

Alfalfa infusion was prepared using 1 g/liter of dried alfalfa cubes (animal feed) in water, then fermented at 27 ± 1°C for 96 hrs. Cohorts of 110 ± 10 female mosquitoes were allowed 72 h post-blood meal to become gravid before being admitted to bioassay tents (BugDorm-2400, MegaView Science Co., Taiwan, China) (Fig. 2). Tents were either loaded with the commercial template Reiter-Cummings style gravid trap or the SLGT trap (Fig. 2). Basins were filled with 2 liters of infusion water and traps were energized for 24 h. After the completion of one trap night, traps were de-energized and the contents of the traps' respective collection containers were removed for mosquito enumeration. Pairwise comparisons were conducted in this manner for five replicates.

Two field sites were assigned from within the SLCMAD trap surveillance network with gravid trap models rotated at each replicate. Six replicates were conducted for each trap at each site. Both traps were baited with 2 liters of alfalfa infusion (recipe as described above) and allowed a 24 h cycle to collect mosquitoes. Collection containers were subsequently returned to the laboratory for sorting and identification under a light dissection scope.

Fig. 1. Parts layout (A) and basic assembly (B) for the Salt Lake gravid trap (SLGT) version of the traditional Reiter-Cummings gravid trap. A computer case fan was used to stencil a pattern on a basic toolbox. Holes were cut such that the fan case pieces could screw directly through the plastic of the toolbox. Additional portholes and brackets were added to allow insert f cables, battery, collection container, and associated funnel pieces for the completed trap. The deployment procedure is identical to prior Reiter-Cummings style traps (C).

Data Analysis

Analysis was conducted using R statistical software (v.4.2.1, The R Foundation for Statistical Computing, Vienna, Austria) via RStudio (v. 3.3.0, RStudio PBC, Boston, MA). Binary choice assays were analyzed using paired 2-sample t-tests between the two gravid trap models. Field data were analyzed using a two-tailed Pearson's χ^2 and Yates' correction in a 2×2 contingency table with trap type and site used as factors and a response variable of female *Culex* spp. collected.

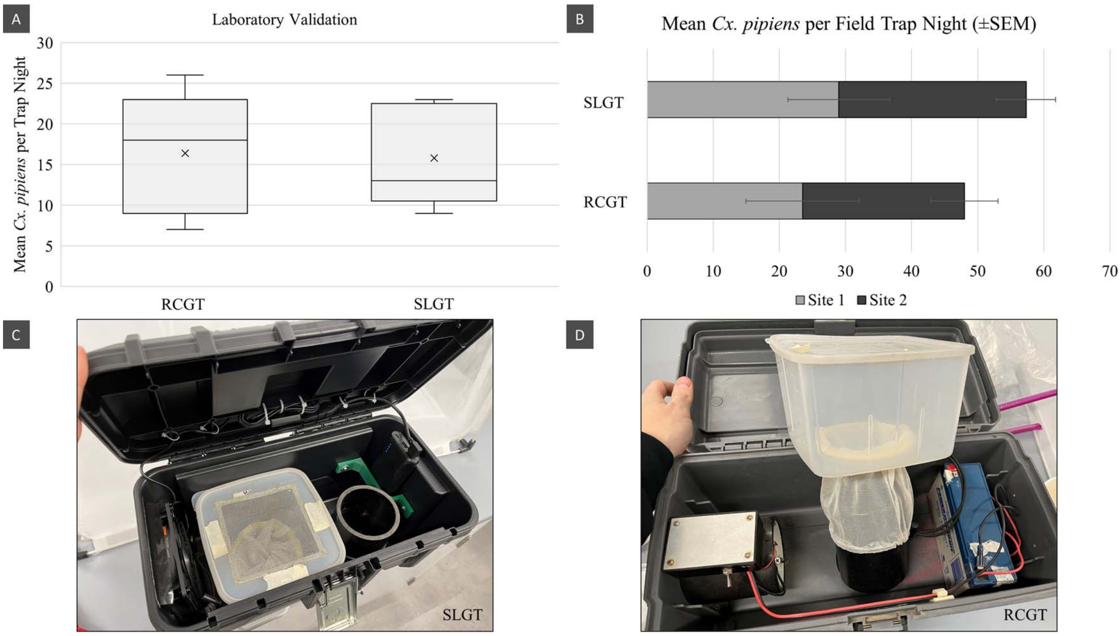

Fig. 2. A) Laboratory validation of the BioQuip model Reiter-Cummings gravid trap and Salt Lake gravid trap. Mean, nightly gravid *Culex pipiens* recovery in the assays is visualized with a box and whisker blot, where the "x" denotes the mean and the median (center line) and quantiles (outer box borders) are flanked by the variance (I-bars). B) Mean, nightly field collection data at two sites for wild *Cx. pipiens* with a stacked bar graph showing the similarity in performance between trap models. Standard error of the mean is represented with I-bars on the respective bars. C) Salt Lake gravid trap (SLGT). D) BioQuip model Reiter-Cummings gravid trap (RCGT).

RESULTS

Air flow measurements tracked suction for both traps at ~12 km/h at the water surface. The SLGT was lighter weight because of the lithium USB battery pack. The case fan was also noticeably quieter than the original 6-v motor. At the time of this writing, the materials cost for the fan ($14 USD), toolbox ($13), cables ($9), ABS pipe ($2.50), aluminum brackets ($10), catch container with lid ($9), trap net ($10), the USB battery pack ($35) and the negligible amount of 3D printed filament ($2) is approximately half the cost (not including labor) of the formerly available commercial model. Collections of gravid *Cx. pipiens* in bioassay tents was not significantly different between the two trap models (Fig. 2; $t_{4,9} = 0.1191$, $P < 0.9109$). Field validation echoed the insignificant difference in collections between traps (Fig. 2; $\chi^2 = 0.107$, $P < 0.744$).

DISCUSSION

Throughout both laboratory and field validations, the SLGT design and the commercial BioQuip design for the RC trap performed equivalently. Ultimately, the goal of SLCMAD with this SLGT design is to address trap replacement, repair, and durability. Failure to collect surveillance data can lead to sub-optimal decision trees and severance from historical data (Ritchie et al. 2007, Hiscox and Takken 2021). Retention of known trap models and minimizing failure rates in trap operation, therefore, dictate the reliability of a surveillance program (Chen et al. 2011, Crepeau et al. 2013). It is our hope that by offering a reproduceable RCGT design, we can help other programs establish or maintain their surveillance networks without risk of being disconnected from historical operational and scientific knowledge.

Fewer innovations and modifications to surveillance technology are available for *Culex* spp. in the current research climate. Management for invasive *Aedes* spp. has led to the development of the Gravid *Aedes* Trap (Ritchie et al. 2014) and the autocidal gravid ovitraps (Mackay et al. 2013) for lethal removal of ovipositing mosquitoes. The multifunctional mosquito trap is a hybrid between gravid and conventional sentinel traps for invasive *Aedes* spp. without requiring multiple trap styles (Reinbold-Wasson and Reiskind 2021). Much effort was already dedicated to evaluating the CDC-style gravid traps and the later RCGT modifications (Allan and Kline 2004, Braks and Cardé 2007) but there has otherwise been a distinct lack of contemporary revisions for *Culex* spp. gravid trapping. Our SLGT trap is not intended to be a reinvention of the gravid trap. However, there is room for continued improvements or technological updates to various traps, regardless of vector target. In-house manufacture with 3D printing is still a novel concept in mosquito operations and could yield more modern trap designs for gravid mosquito collections. In this case, updates to the RC trap with the SLGT design successfully replicated the BioQuip commercial model and are interchangeable when it reaches time to retire the original traps. We encourage all surveillance practitioners to seek new ways to update, improve, reduce cost, and simplify trap designs for streamlined operations.

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