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DO IT YOURSELF: EVALUATING COMMERCIAL CO_2 REGULATORS FOR SURVEILLANCE NETWORKS USING PRESSURIZED CYLINDERS

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ABSTRACT. Carbon dioxide (CO_2) is a universal attractant for monitoring blood-feeding insects, such as mosquitoes. Although dry ice has been the historical benchmark, compressed gas cylinders can be used in tandem with a gas regulator to control CO_2 flow rate more precisely. The literature is sparse on best practices regarding how to choose or test regulators. We evaluated four commercially available regulator types from beverage and welding suppliers and compared them to a previously tested regulator used at the Salt Lake City Mosquito Abatement District (SLCMAD). Using environmental chambers, we simulated both the temperature drop of spring/fall or summer nights down to 9°C, as well as daytime highs within the seasonal expectations of the central ranges of Utah, up to 42°C. Two regulators failed to maintain calibrations in these screenings. The remainder were vetted by acquiring duplicates and rerunning the simulations with inverted temperature exposures, starting low and heating up, instead of starting warm and cooling down in the first tests. The remaining regulators were tested in the field for validation. After 56 trap cycles with 15 duplicates of three regulator models, general failure rates in real applications all decreased below 5% of total uses. The preexisting regulator used by SLCMAD performed well in simulations, but had double the failure rate of the other screened models. We use this study to highlight the scarcity and importance of conducting evaluations on the existing protocols or equipment for public health vector control programs and provide recommendations for addressing operational usage.

KEY WORDS Attractant, equipment, flow rate, mosquito trap, vector control

INTRODUCTION

The empirical nature of public health vector control elevates the demand for surveillance networks across the globe (Petrić et al. 2014, Aryaprema et al. 2023). Mosquito-focused programs base their intervention decisions on the abundance, diversity, and dispersion characteristics of their geographically relevant species (Petrić et al. 2014, Drakou et al. 2020). These efforts must be monitored through surveillance efforts performed in the field, such as human landing rates, public service requests, manual collections of larvae, and, most rigorously, with mechanical traps for adult mosquitoes (Chen et al. 2011, Sriwichai et al. 2015, Aryaprema et al. 2023). Selectivity to hematophagous insects in trap networks has been subsequently established with gaseous CO_2 (Reeves 1953), improving both the magnitude (Newhouse et al. 1966) and diversity of collections for mosquitoes (Magnarelli 1975, Feldlaufer and Crans 1979), with exceptional specificity to those species with a high vectorial capacity (Reisen et al. 1983, 2000). Historically, CO_2 was added via containers of dry ice (Reeves 1953, Newhouse et al. 1966, Magnarelli 1975, Feldlaufer and Crans 1979, Reisen et al. 1983, 2000). This is effective, widely available in an industrialized country, and was considered cost-effective for upscaling.

The methods used across supporting studies have varied, with dry ice blocks manually and arbitrarily divided (Newhouse et al. 1966), and the pieces wrapped in varying materials such as newspaper (Reeves 1953), plastic (Magnarelli 1975), or foil (Feldlaufer and Crans 1979). Unfortunately, dry ice has many sublimation variables, such as surface area, air temperature, humidity, receptacle shape, and how vapors are expected to escape the receptacle (McPhatter and Gerry 2017, Hafner 2023. It is not a rare occurrence that an operator would find their dry ice completely spent in one night and, on another night, still have some fragments actively sublimating at the time of trap collection (Reeves 1953). As a result, efforts have been made to better regulate CO_2 flow for consistent operation (Reisen et al. 2000). Generally, gas cylinders can function as well or better for collecting mosquitoes via CO_2 baiting (Mboera and Takken 1997, Reisen et al. 2000). A significant aspect of this is the ability to control and modulate CO_2 emission to suit needs, even increasing the CO_2 flow rates well above typical rates for a block of dry ice (Reisen et al. 2000). Gas cylinders also can be stored much easier than dry ice, allowing less frequent deliveries of CO_2 in cylinders versus dry ice and trap preparation can be performed days before deployment.

Gas cylinders require many additional components over dry ice, with a trap set-up requiring the secure mounting of a gas cylinder, appropriate flow controls with choke components, a pressure regulator, and lines extending from and directing the flow of CO_2 (Bibbs et al. 2024). At the scale they are used, the pressure regulators are a significant bottle-neck for the accessibility of gas cylinders for CO_2 because of their cost and maintenance. When maintaining a surveillance network of dozens of traps, set and collected at least weekly, operators

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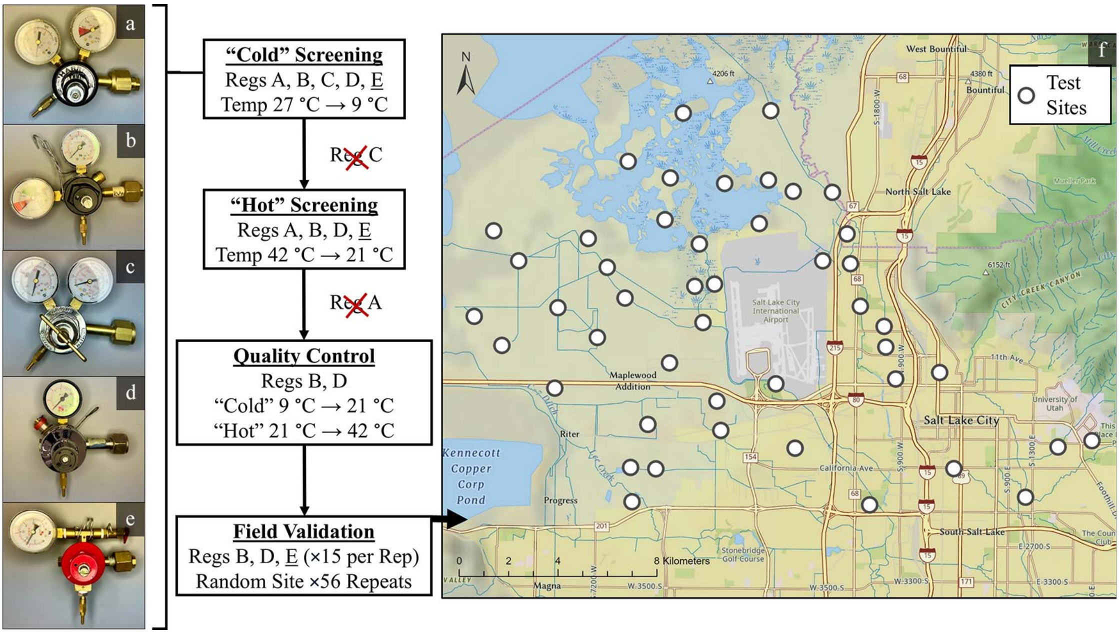

Fig. 1. Single-stage regulators were acquired from beverage and welding supply manufacturers (a-d) and compared to the preexisting, mass-used regulators (e) within the Salt Lake City Mosquito Abatement District for use with pressurized gas cylinders dispensing CO_2. Tolerance tests were conducted using environmental chambers to screen for cold temperature failures, followed by rejection of models that failed. Remaining models were screened again with hot temperature tests, followed by a second round of rejections for failed models. Remaining models were used in quality control tests with duplicates of regulators maintaining calibrations in prior stress tests. Final candidate regulators were deployed alongside the preexisting standard (Regulator E) in sets of 15 for a span of 52 trap nights across the SLCMAD surveillance network (f).

may struggle with systemic failures in their equipment (Ritchie et al. 2008, Chen et al. 2011, Crepeau et al. 2013,). In the case of compressed gas cylinders, the regulators need to be detached and re-attached every time CO_2 is refilled. In addition, calibrations for flow rate are managed with a diaphragm that can be tightened or loosened to change the CO_2 output. This complexity creates points of potential failure because of wear and general stress from environmental conditions including expansion and contraction of the materials during temperature fluctuations. When failure occurs, flow can be blocked or have excessive expulsion of CO_2, which reduces the run time. More importantly, unknown regulator failures could contribute to inaccurate treatment decisions because of higher or lower than normal catches, since surveillance data assumes standard calibrations.

As with many components used in mosquito surveillance programs, regulators are generally made for other industries. In this case, regulators are most available from beverage and welding markets, which are generally indoor applications. Unfortunately, the environmental tolerances of these pre-fabricated models in the field are entirely undescribed in literature. Beyond the effects on the gas itself, questions may arise regarding whether or not the regulator is more reliable in hot or cooler temperatures, or if widespread logistical failures from seasonal changes may compromise the equipment or offset calibrations. To test this, we acquired a subset of off-the-shelf models below $120 USD in online retail (as of 2024) so as to meet a reasonable price point for upscaling. We stress tested the equipment with artificially induced temperature swings and monitored changes to the flow rate calibrations. We then contrasted this with in-use field validation of over 50 trap nights across spring, summer, and fall within the Salt Lake City Mosquito Abatement District (SLCMAD) jurisdiction.

MATERIALS AND METHODS

Tolerance tests—general procedure

Five single-stage gas regulators were acquired from online-accessible vendors, labeled A (Model 201 Sku 3002260, Harris Products Group, Monroe, OH), B (3741-br, Taprite Micro Matic, Inc., San Antonio, TX), C (Series 30, Miller Electric Mfg, LLC, Appleton, WI), D (Model 841, Micro Matic USA, Inc., Brooksville, FL), and E (Model 810, Micro Matic USA, Inc., Brooksville, FL) (Fig. 1a-1e). Regulator E was a discontinued model that was no longer available for purchase, but has served as the historical regulator model already deployed for operational surveillance by SLCMAD). All models were fitted with the adaptors and 0.0075" choke as previously described (Bibbs et al. 2024). Each of the five regulator types were calibrated to 300 ml/min at

Fig. 2. (a) A field-deployed Salt Lake City (SLC) mosquito trap with contained transport and housing for gas cylinders. This unit is functionally equivalent to miniature CDC-style traps. (b) Regulator attachment to gas cylinders are fed through transport housing. (c) Cargo of battery, trap, trap net, cylinder, and attached regulator are packaged into the housing for transport.

the beginning of each trial, with stressors labeled as either "cold" or "hot" based on the extremity of the temperature the regulators were housed in. All calibrations and performance checks were made using 1 kg-capacity CO_2 cylinders and a flowmeter (Gas Flowmeter w/Copper Connector, JIAWANSHUN td., China). The theoretical release for calibrated regulators should reach 847 g per day [24 h $\times$ 60 min $\times$ 0.3 liter/min $\times$ 1.96 g/L of CO_2 at standard temperature and pressure (STP)].

Separately, a cohort of regulators was stressed and recalibrated only when the regulator completely failed to show the progressive degradation in performance. The logic was that, for operational usage, regulators would only be recalibrated at regular intervals that may not be daily. But if a total failure is detected, such as by a prematurely empty CO_2 cylinder or the confirmation of zero gas flow, then units would be recalibrated ad libitum to keep regular surveillance in operation. In all trial types, cylinder valves were fully opened, and the unit was allowed to flow for 24 continuous h within the prescribed assay conditions. Flow rates were measured and recorded after occupying an initial temperature (phase 1) for 16 h and again after occupying the final temperature for the remainder of the observation window (phase 2). Cylinders were replaced after each phase 2 measurement regardless of trial type. Tests were conducted with the stipulation that a useful regulator must be resilient in both the "cold" and the "hot" trials to be carried forward to field validation. In all cases, whether screening or doing quality control assessments, measurements were repeatedly taken at the end of each phase over 9 replicates.

Tolerance tests—model screening, changing temperature high to low

The "cold" trial reflects temperature extremes similar to those in the field during the spring and fall

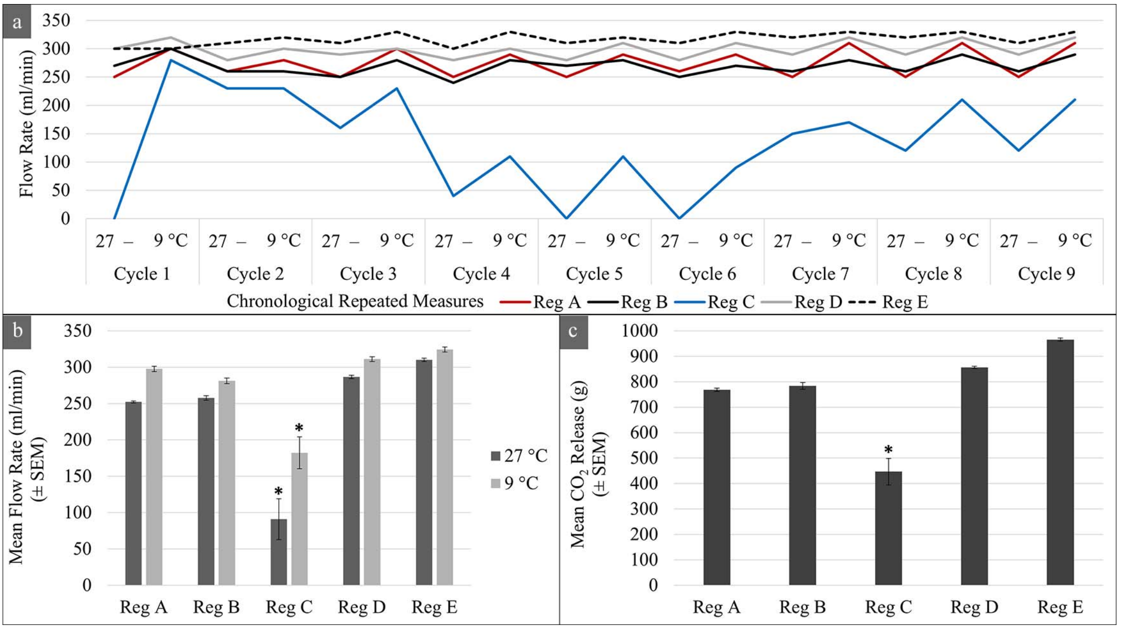

Fig. 3. Varying models of regulator were screened by acclimating to 27°C for 16 h (phase 1), then brought down to 9°C for 8 h to simulate a nightly temperature drop during spring/fall. Flow rate measures were taken on regulators that were continuously oscillated for 9 cycles (a) ($F_{4,44} = 67.72$, $P < 0.001$). Mean flow rates were assessed on units that were recalibrated each trial (b) ($F_{4,44} = 45.79$, $P < 0.0001$). Mean CO_2 release was also taken after a 24-h total cycle (c) ($F_{4,44} = 62.45$, $P < 0.0001$). Model C failed to retain calibrations during study and had significantly varying outputs.

season; cold nights may be as low as 0°C with warm day time temperatures reaching close to 21°C (NWS 2024). The cylinder-regulator setup (Fig. 2a-2c) was placed in an environmental chamber (Thermo Scientific 3920 Large Capacity Environmental Chamber) maintained at 27°C overnight during phase 1. After recording the flow rates following the 16-h acclimation, each setup was chilled to 9°C for the second phase of the "cold" trials. The flow rate was measured for each regulator at the end of each stage and the total mass of CO_2 expelled in the 24-h period was calculated with a before and after weight measurement of each cylinder after phase 2 measurements. Regulator C was not carried forward to the "hot" tests because of inability to maintain calibrations when chilled (Fig. 3a).

The "hot" trial reflects temperature extremes similar to those in the field during the summer season with daytime temperatures as high as 41°C and cooler nighttime temperatures reaching below 21°C (NWS 2024). Phase 1 for this cylinder-regulator setup was a 16-h acclimation at 42°C, after which each setup was brought down to 21°C during phase 2 of the "hot" trials. The flow rate was measured for each regulator at the end of each phase and the total mass of CO_2 expelled in the 24-h period was again calculated with a before and after weight measurement of each cylinder after phase 2. Regulator A was not carried forward to the quality control tests because of consistently irregular flow rates only when hot (Fig. 4a).

Tolerance tests—quality control, changing temperature low to high

To streamline effort in the field, additional quality control replicates were conducted on regulators B and D to verify their consistency for use in field validations. Quality control from the manufacturer of the regulators may play a role in the observed outcomes with tested regulators. To control for this, a second round of "cold" and "hot" trials was conducted with five duplicated regulators of models B and D. Regulator E was omitted from this test because over 30 units have already been in use with SLCMAD for more than five years. For regulators B and D, the 10 duplicates were labeled as D1, D2, D3, D4, D5, B1, B2, B3, B4, B5 for convenience, where B1/D1 were the same regulators used in the screening trials. The same methods were used to calibrate each regulator as with the first set of trials, whereby initial calibrations were made and then repeated measurements on flow rate were taken for a 9-wk duration.

However, during this round of quality control trials the 10 regulators were stressed by reversing the temperature sequence for "cold" and "hot". For "cold" trials this meant acclimating phase 1 at 9°C and then heating the assemblies to 21°C during phase 2. For "hot" trials this meant acclimating phase 1 at 21°C and then heating the assemblies to 41°C. All other testing details were conducted identically to the screening portion, with the addition of the aforementioned regulator duplicates. As before, flow rate was measured after each phase and before/after CO_2

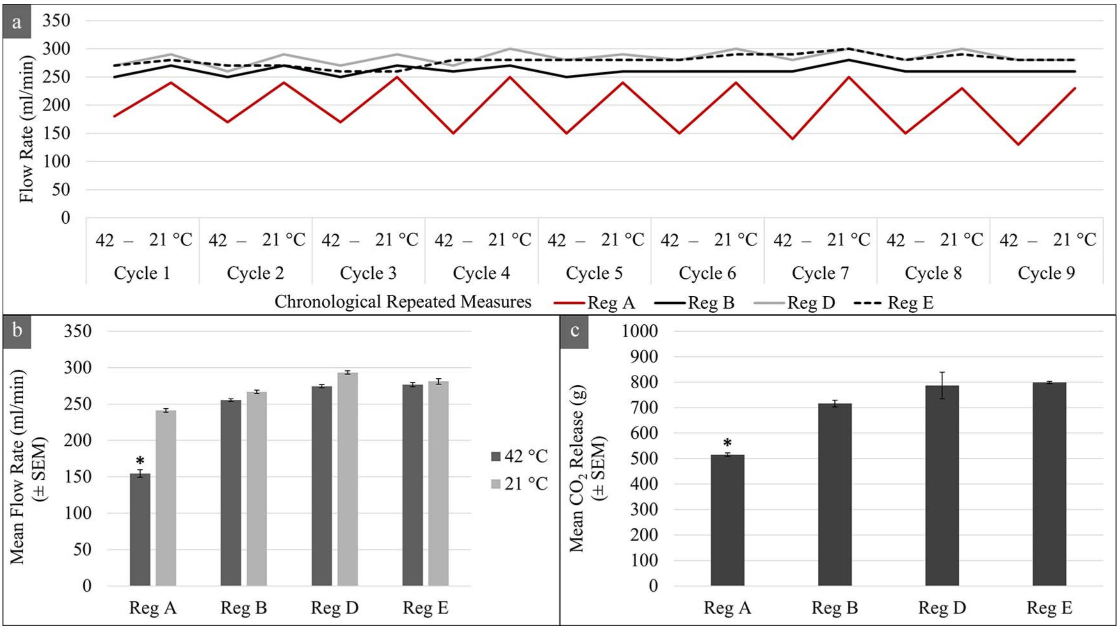

Fig. 4. Varying models of regulator were screened by acclimating to 42°C for 16 h (phase 1), then brought down to 21°C for 8 h to simulate a nightly temperature drop during summer. Flow rate measures were taken on regulators that were continuously oscillated for 9 cycles (a) ($F_{3,51} = 60.18$, $P < 0.001$). Mean flow rates were assessed on units that were recalibrated each trial (b) ($F_{3,35} = 296.59$, $P < 0.0001$). Mean CO_2 release was also taken after a 24-h total cycle (c) ($F_{4,44} = 61.06$, $P < 0.0001$). Model A fluctuated significantly in flow rates only while hot.

masses were taken after phase 2 for both sets of environmental conditions.

Field validations

Consistency of measures during quality control testing supported carrying forward both regulators B and D. Regulator E was included as a comparison group since SLCMAD already used these as their main regulator for more than 5 years. A total of fifteen duplicate regulators of B (#1-15), D (#16-30), and E (#31-45) were simultaneously deployed within the preexisting surveillance network of the SLCMAD, spanning a geography that includes urban/residential tracts, rural wetland, and industrial transition zones (Fig. 1f). From April 1 to October 31, 2024, a total of 56 trap-nights were conducted across each of 45 surveillance locations, yielding a total effort of 2,520 deployments of regulators into the field. Regulators were assigned with a random sequence generator (no duplicated numbers) across this network during each trap night for the duration of the study (Fig. 2a). Regulators (Fig. 2b) were paired with the SLC Trap and transport container developed in Bibbs et al. (2024) (Fig. 2c). High and low temperatures were recorded in the area for each trap night. Trap failures because of regulator malfunctions, such as by CO_2 not flowing or CO_2 excessively dispensed, were recorded for every deployment. Failure events were corrected by recalibrating the regulators at the SLCMAD facility and then redeploying on the next trap night.

Data analysis

Statistical testing on continuously tested flow rates for phase 1 and phase 2 measurements were calculated using a repeated measures ANOVA test with a paired t-test and Bonferroni correction. Zero flow rate values were transformed to 0.0001 ml/min for calculations. Independently measured means of CO_2 release and phase 1/phase 2 flow rates were analyzed using ANOVA/Tukey HSD. Mean flow rates and CO_2 release from the quality control tests of regulator B and D were analyzed with paired t-tests within measurement groups. Flow rates from tolerance tests and failures in the field were summarized with negative binomial regressions with the temperature data collected for the cycle of use. All statistical analyses were conducted using R v. 4.2.0 (R Core Team 2022).

RESULTS

In "cold" trial tolerance tests, regulator C failed to maintain calibrations throughout continuously cycling temperatures (Fig. 3a). Degradation of flow calibrations were significant across regulators ($F_{4,44} = 67.72$, $P < 0.001$), with ranking from high to low outputs reflecting E > D > A = B > C ($\alpha = 0.005$). Flow rates for both the 27°C phase 1 ($F_{4,44} = 45.79$, $P < 0.0001$) and the 9°C phase 2 ($F_{4,44} = 30.11$, $P < 0.0001$) were significantly reduced from the other models (Fig. 3b). The mean CO_2 released was significantly less for model

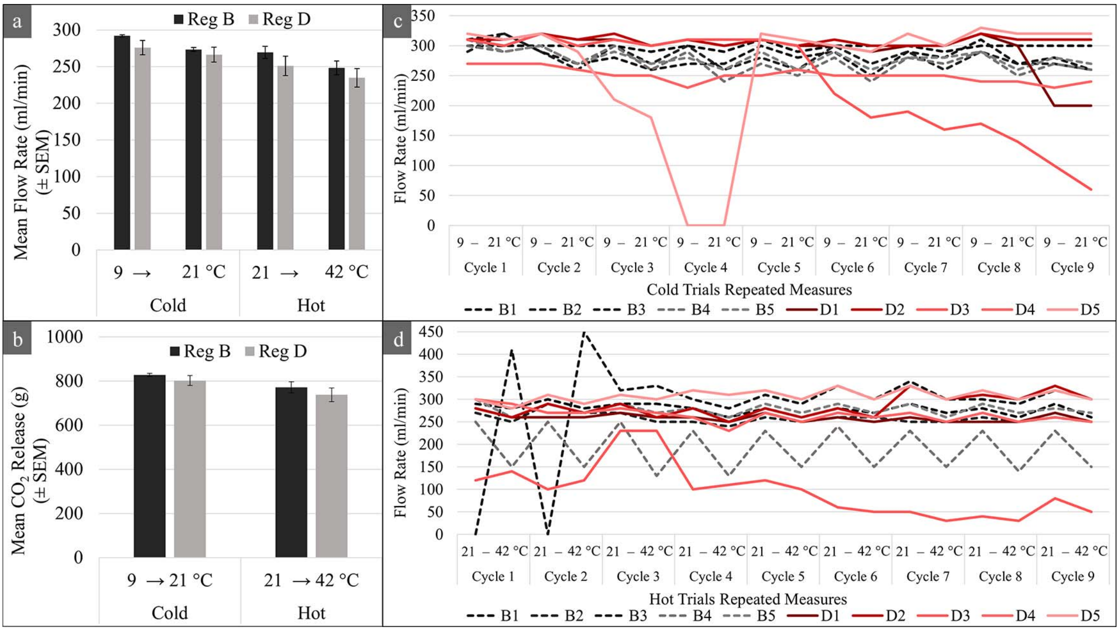

Fig. 5. Regulators of models B and D, chosen from the prior screenings, were quality checked by acquiring 5 identical units of each model (B or D, #1-5). Tests were conducted by acclimating to "cold" conditions of 9°C for 16 hours (P1), then brought up to 21°C for 8 h to simulate a temperature increase following a spring/fall night. A second series was conducted by acclimating to "hot" conditions of 21°C for 16 h (P1), then brought up to 42°C for 8 h to simulate a temperature increase following a summer night. Mean flow rates were assessed on units that were recalibrated each trial (a). Mean CO_2 release was also taken after a 24-h total cycle (b). Flow rate measures were taken on regulators that were continuously oscillated for 9 cycles in either the "cold" (c) or hot (d) conditions. Mean performance was not significantly different between regulators. Individual regulator performance indicates observable "cold" condition failures for model D and "hot" condition failures for both B and D.

C than the other types (Fig. 3c; $F_{4,44} = 62.45$, $P < 0.0001$). As a result, model C was considered a total loss and omitted from further testing.

In "hot" trial tolerance tests, regulator A consistently released less CO_2 only while heated but resumed acceptable calibration points once coming down to room temperature (Fig. 4a). Degradation of flow rates were significant across regulators ($F_{3,51} = 60.18$, $P < 0.001$), with ranking from high to low outputs reflecting E = D ≥ B > A ($\alpha = 0.0083$). Correspondingly, the flow rates for regulator A were significantly lower than the models B, D, and E during the 42°C phase 1 ($F_{3,35} = 296.59$, $P < 0.0001$), but not during the 21°C phase 2 (Fig. 4b). Similarly, the mean CO_2 released was also significantly less for model A as compared to the other models (Fig. 4c; $F_{4,44} = 61.06$, $P < 0.0001$). Model A was not selected for further testing as a result.

Performance was not significantly different with mean flow rates (Fig. 5a) or mean CO_2 release (Fig. 5b) between regulators B and D when conducting quality control replicates with five duplicates of each model. When parsing out the individual performance of each duplicate, regulator D had three of five regulators lose calibrations when oscillating between a 9°C phase 1 and 21°C phase 2 (Fig. 5c). When conducting a 21°C phase 1 and 42°C phase 2, regulator B had two of five units lose calibration, whereas regulator D had one of five units unable to maintain calibration (Fig. 5d). Across all testing, both during screening and quality control assessments, there was a general trend of lower flow rates whereas in hot conditions and higher flow rates after cooling (relative to the prior acclimated temperature).

Field validations were devised from total failures out of 15 duplicates for each of regulator B, D, and E per night of use. The mean failure rate across the entire season was less than 5% for all models (Fig. 6a), but model E was observed to fail twice as frequently as the others ($F_{2,55} = 296.15$, $P < 0.001$). Across the season, 10, 13, and 24 failures were observed among regulators B, D, and E, respectively. There was no particular trend across the failures from the field, whether correlating with the difference between daily high and low temperatures (Fig. 6b), nightly lows (Fig. 6c), or daily highs (Fig. 6d). This is likely confounded by the low overall number of failures relative to the total number of trap nights.

DISCUSSION

Overall, commercially pre-fabricated regulators can work within an acceptable tolerance of extreme temperatures and maintain a reasonably low rate of overall failure in the field despite not being manufactured for use in mosquito abatement operations. It was notable to

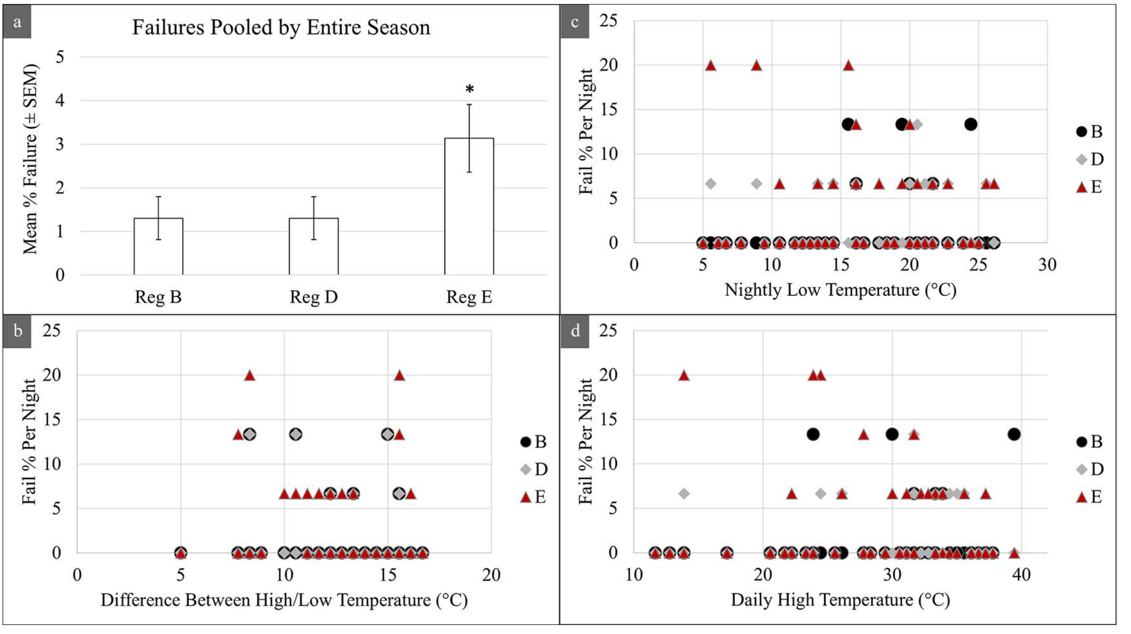

Fig. 6. During field validation, 15 units of regulators B, D, and E (the benchmark already in use) were deployed for 2,520 trap events across an established mosquito surveillance network within the Salt Lake City Mosquito Abatement District. The cumulative mean failure rate of each regulator was taken for the entire season (a). The nightly failure rate percentages were plotted for the daily difference in high-to-low (b), daily low (c), and daily high (d) temperatures across the monitoring period. No specific correlations to temperature were observed in the field. Regulator E had significantly higher overall rates of failure ($F_{2,55} = 296.15$, $P < 0.001$) than the other models, but all models were below an average fail rate of 5% of uses during the season.

SLCMAD staff that their default regulator E that is already used in our surveillance network had the worst reliability in the field. It is possible that this result is partially because of the preexisting age and wear on these regulators from routine field usage. However, this regulator is discontinued from sale from Micromatic, and so may be a moot point in the future operations of their program. However, this result highlights the importance of periodically evaluating a program's tools, even down to the individual manufacturers or suppliers. A variable CO_2 release rate, whether by dry ice or an improperly tuned regulator set-up, may create an inaccurate perception of mosquito activity and pathogen transmission risk (McPhatter and Gerry 2017).

The mosquito surveillance literature provides little advice to find or test regulators for deployment with adult mosquito traps, despite the casual mentions of their use as a default part of surveillance equipment (Reisen et al. 2000, Silver 2007, McPhatter and Gerry 2017). Regardless, there are some quality metrics that may be helpful for identifying the working models for a program's surveillance needs. Simulating temperature flux relative to seasonal use patterns stresses regulators to the point of failure. Furthermore, continuous measurements over repeated cycles of temperature can eliminate models that are not suitable for high-stress use. In addition, testing duplicates of the same model of interest can help reduce errors in judgements. For example, in our data we eliminated regulator A after the first sequence of "hot" tests. Yet one of the duplicates for regulator B mirrored the flow rate errors. This could mean that our exclusion of model A was not necessary, if we had tested more manufacturing duplicates of that particular regulator.

Our field validations were performed in the hot, arid, and high elevation area of Salt Lake City. However, humidity and high-low pressure fluctuation, such as in the southeastern United States, could easily result in different sources of error in potentially suitable regulators. Fortunately, these conditions can be recreated in an environmental chamber. We recommend that using evaluation methods as demonstrated in this study can be a valid screening tool. We also encourage mosquito surveillance/control programs to be cognizant of their particular environmental stressors when evaluating equipment. One piece of guidance that may be useful is our observation across several iterations of these trials that regulators tended to error toward lower flow rates while under hot conditions and higher flow rates after cooling (relative to the prior acclimated temperature). This could allow some anticipation of the type of failure one could expect given local seasonal conditions. For example, a chilled regulator may yield an empty CO_2 cylinder more often whereas a sun-heated regulator may be more likely to restrict flow in spite of the calibrations. Such failures with lures are also observed in dry-ice baited traps as well (McPhatter and Gerry

2017), but the complexity of regulators, and users greater demand for consistency may increase noted failure rates (Ritchie et al. 2008, Chen et al. 2011, Crepeau et al. 2013).

Gas cylinders are already a widely accepted tool in mosquito surveillance (Silver 2007). However, it is often overlooked that evaluation of surveillance equipment is necessary to better understand the reliability of those tools. Ultimately, the data presented suggests that off-the-shelf regulators from beverage and welding suppliers, even when limited in scope to lower cost models, can be reliable for field deployment in surveillance programs. Consistency and reliability of surveillance data is paramount for public health protection and data interpretation.

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