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Construction and performance of a novel capture-mark-release moth trap **Cover Page Footnote** We are grateful to Joel Stewart for his help in the early conceptualization of this trap, and to two reviewers for their helpful insights.

Construction and Performance of a Novel Capture-Mark-Release Moth Trap

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Abstract

Mark-recapture studies can provide important information about moth movement as well as habitat preference across a landscape, but to date, such studies tend to be species-specific or require labor-intensive methodologies. To address this challenge, we designed a *capture-mark-release-trap* (CMRT) featuring a cooling unit attached to a black light trap. The CMRT captures and incapacitates moths throughout the night until the morning, when they can be marked on-site and released. Moths captured with the CMRT during summer of 2016 had a recapture rate of 1.6%, similar to those of previous studies. Importantly, because moths are immobilized by the CMRT, they can be handled and marked with ease, reducing the opportunities to damage specimens prior to release. The CMRT can capture a wide array of moth species and may facilitate an increase in the monitoring of moth movement across landscapes.

 \mathbf{Key} $\mathbf{Words} :$ Lepidoptera, mark-release, mark-release-recapture, insect trap, moth, cooling.

Moths are an important taxonomic group across all kinds of landscapes. As the most numerous and diverse group of Lepidoptera, they provide pollination services (MacGregor et al. 2015), break down plant biomass as immatures (Slade et al. 2013), and act as a food source to birds, bats, and other predators (Conrad et al. 2006, Bates et al. 2014). Unfortunately, many moth populations may be in decline (Conrad et al. 2006, Fox et al. 2014), potentially due to land use change (Kozlov 1996) and habitat fragmentation (Fox et al. 2014). Despite their importance, moth movement on a landscape scale has not been well explored, which can help indicate the drivers of moth population decline (Slade et al. 2013).

Exploring moth movement across a landscape can be accomplished using mark-recapture methods, where moths are marked, released and later recaptured. Comparing the biotic and abiotic properties of release and recapture locations, and the terrain in-between, can then provide information about which landscape features impede or facilitate moth movement and dispersal (Dulieu et al. 2007). This is especially important in disturbed habitats that are frequently patchy, surrounded by an

unfriendly matrix (Conrad et al. 2006, Bates et al. 2014).

Mark-recapture methodologies can be broadly divided into two categories: rear-mark-recapture and capture-mark-recapture. Trapping and marking live moths can present challenges and instead, many researchers elect to rear individuals in a lab rather than catching specimens in the field (Shirai and Nakamura 1995, Margaritopoulos et al. 2012). These rear-mark-recapture types of studies tend to be species specific, often only focusing on the movements of one or a few key species of moths (Shirai and Nakamura 1995, Margaritopoulos et al. 2012). Though this methodology is highly effective for species-specific work, it is not practical for examining how landscape factors impact entire moth assemblages.

The alternative method, using a capture-mark-recapture technique, can survey a greater proportion of the local moth assemblage, but presents unique challenges of its own in obtaining live, undamaged specimens. There are a variety of popular moth traps on the market today, but none are designed specifically for capture-mark-recapture work. The black-light trap (BLT) is perhaps the most well-known and widely used trap. It uses low-wavelength UV or LED light to attract insects from the surrounding area (Muirhead-Thomson 1991, White et

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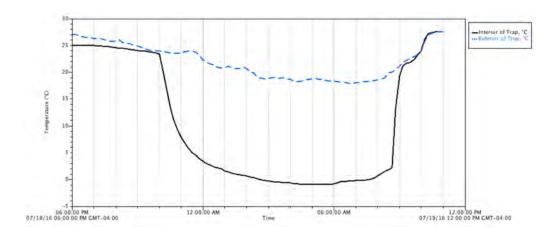


Figure 1. Internal and external temperature of a CMRT, collected with temperature sensors attached to the inside and outside of the trap (respectively).

al. 2016); it is particularly useful where researchers seek to survey an entire moth assemblage. The BLT can be used for *capture-mark-recapture* if the trap's collection container features internal structures (for example, egg-cartons) to reduce moth movement in the collection bucket, which in turn limits moth wing damage. However, this method does not sedate moths and makes moth marking a challenge. The subsequent extra handling of moth specimens can sometimes increase the incidence of moth damage.

Other popular methods in capture-mark-recapture studies include pheromone traps and flight intercept traps. Pheromone and bait traps use a chemical attractant rather than a light to attract moths. The chemical attractant tends to be taxonomically specific making the trap efficient for studies surveying one or a few species at a time (Furlong et al. 1995, Margaritopoulos et al. 2012), but not ideal for capture-mark-recapture studies that aim to survey and/or track a representative proportion of the greater moth assemblages. Finally, flight intercept traps typically involve setting up a large sheet or net and collecting anything that flies onto it. These traps often lack an attractant and have been shown to collect fewer moths and with lower species richness than BLTs (Butler et al. 1999).

There are many ways to collect moths, yet a gap exists for a trapping method designed specifically for *capture-mark-recapture* purposes at the moth community level. In this present study, we detail the construction of a novel *capture-mark-recapture* trap (the CMRT) to collect a diverse moth species assemblage in urban woodlot.

The CMRT combines the efficacy of standard BLTs for attracting moths and an on-site cooling mechanism that allows the moths to be sedated with cold air over night until they are marked and released in the morning.

Methods

Trap Construction. We constructed a CMRT using the basic structure of a BLT with the addition of a cooling unit being used in the place of a standard collection bucket. The CMRT therefore consists of a light and vane structure, a cooling component, and a power source. The light and vane components are from BioQuip (parts no. 2851U and 2851A; Rancho Dominguez, CA). We modified the BLT structure by adding an adjusted portable 12V cooler (Koolatron 18 quart Compact Cooler from Amazon.com), with an opening cut in the top for the funnel to fit into, and a live collection bag (For construction details see box 1). The bulb used in a 12V BLT-type trap generally has an attraction radius of up to 30m a forest habitat (Truxa and Konrad 2012, Merckx et al. 2014).

The trap is powered using heavy-duty deep cycle 12V batteries. The batteries are kept in plastic toolboxes to protect them from rain and make transport to and from field sites easier. Both the cooler and the UV black light bulb are connected to batteries via timers so that they can be set to run at specific times during the night and early morning hours.

Study site. The CMRT, was tested in the Hudson Woodlot (42° 41 58 N, 84° 28 32 N) in East Lansing, Michigan, USA. The 7.7

Table 1. Capture and recapture data. Recapture percentage is calculated as the sum total number of moths that were recaptured (*italics underlined*) divided by the total number of moths caught and dusted (**bold**).

			Total # of	# of	Total Catch	
			Moths	Dusted Moths	Abundance	
		# of Traps	Caught and	that were	for	Recaptured
Date	Event	Deployed	Dusted	Recaptured	Trap Type	Species
07/19/16	Capture	4	276		276	
07/21/16	Recapture	9		1	697	Orthodes majuscula
07/22/16	Recapture	9		0	427	
07/26/16	Capture	4	238		238	
07/27/16	Recapture	9		2	624	Noctuid sp. (x2)
07/28/16	Recapture	9		4	408	Spilosoma virginica, Noctuid sp. (x3)
07/29/16	Recapture	9		1	270	Striacosta albicosta
08/02/16	Capture	4	149		149	
08/03/16	Recapture	9		2	557	Striacosta albicosta (x2)
08/04/16	Recapture	9		1	443	$Eurois\ occulta$
08/05/16	Recapture	9		0	401	
08/09/16	Capture	4	279		279	
08/10/16	Recapture	9		3	643	Xestia dolosa Orthodes majuscula, Oreta rosea
08/11/16	Recapture	9		1	177	$Noctuid\ sp.\ (x2)$

ha, mixed-age woodlot is surrounded by agricultural fields south of the Michigan State University campus. The woodlot is primarily comprised of sugar maple (*Acer saccharum* Marshall), beech (*Fagus grandifolia Ehrh.*), red oak (*Quercus rubra* L.), and basswood (*Tilia americana* L.).

Trap Testing. Trapping was conducted over 4 weeks in summer 2016 from July 18 through August 11. Four CMRTs were set at the center of the woodlot, about 10 meters from one another (in a square formation) and set to run from 10:00pm at night until to 9:00am the next morning. The traps were not intended to be independent samples, rather they were clustered to increase their attracting ability. Onset HOBO pendant temperature loggers (UA-002-08; Bourne, MA) were attached to the interior and exterior of the traps to monitor temperature changes through the course of the night. The following morning, the cold-sedated moths were removed from the mesh collection bag and dusted with UV dust using a small paintbrush before being released near the capture site (Solar Color Dust brand UV sensitive dust; Winter Haven, FL). Moth captures were monitored at release sites until they dispersed.

Recaptures were collected with nine conventional BLTs, deployed for 2–3 nights throughout the woodlot, set at 50m, 100m, and 150m away from the original capture site, in each cardinal direction. Different colors of dust were used to demarcate different initial trapping events in case moths were captured later than the trapping week in which they were marked. The BLTs were equipped with pest strips containing 2,2-dichlorovinyl dimethylphosphate to kill recaptured moths; dead specimens were preferred upon recapture so that positive species identifications could be made.

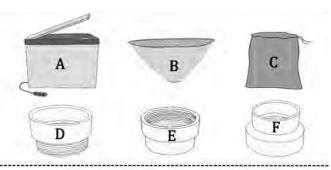
Results

The CMRT initially captured 942 moths pooled over four capturing nights. Moths were recaptured at a rate of 1.6% (Table 1). The recaptures were in the families Noctuidate, Erebidae, and Geometridae.

The CMRT appeared to have cooled and incapacitated the moths that it captured; no moths were moving inside the collection bucket upon retrieval. After marking, it

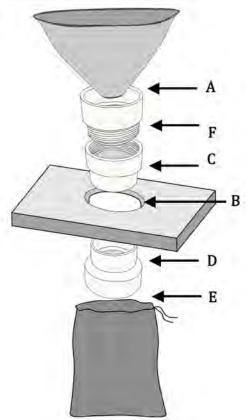
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Box 1: CMRT Construction Parts needed for the CMRT (A) 12V portable cooler with a top-opening lid, (B) funnel from a standard black light trap, (C) mesh insect collection bag, (D) 4" PVC/DWV male adapter, (E) 4" PVC/DWV cleanout (female) adapter, (F) 4" x 3" PVC/DWV reducer.

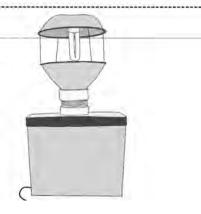


Assembly instructions for the CMRT

- (A) Use window and door grade insulating foam sealant to attach the metal funnel to the 4" PVC/DWV male adapter. For a secure fit, apply foam to both the inside and outside of the adapter where it joins the funnel.
- (B) Cut a 4" hole in the lid of the 12V portable cooler. This can be done by removing the lid from the cooler and using an orbital jig saw after a pilot hole has been drilled. Reattach the lid to the cooler after the hole has been cut.
- (C) Use window and door grade insulating foam sealant to secure the 4" PVC/DWV cleanout (female) adapter to the cooler lid, through the newly cut hole.
- (D) Use PVC cement to attach the 4" x 3" PVC/DWV reducer to the bottom of the PVC/CWV cleanout adapter. The 3" side of the reducer fits into cleanout adapter and the 4" side of the reducer opens into the cooler below.
- (E) Attach an insect collecting bag to the 4" opening of the PVC/DWV reducer. The reducer provides a natural lip that the bag can be cinched around. Alternatively, an artificial lip can be made on the bottom edge of the reducer using a zip-tie or heavy-duty caulk.
- (F) Screw the 4" x 3" PVC/DWV reducer (with funnel attached) into the 4" PVC/DWV cleanout (female) adapter when the trap is ready to be deployed.



A fully assembled capture-mark-recapture trap (CMRT).



typically it took 10–15 minutes for moths to warm up and fly away after the marking treatment was applied.

The interior of CMRTs was approximately 19°C cooler than the surrounding environment (Figure 1), effectively making the trapped individuals too cold to move. The CMRT took about 2 hours to sufficiently cool after it was turned on. Once it reached its low temperature, near 0°C, it continued running until it was turned off the following morning by the project team, prior to 9:00am.

Discussion

The CMRT may be an effective tool for moth *capture-mark-recapture* initiatives. Trapped moths are subdued by the cold temperatures inside the trap, long enough to be marked before release, and show no signs of damage upon release. The low attraction radius is ideal for studies that are monitoring a local habitat, as it is less likely the traps will catch moths from adjacent habitats.

The 1.6% recapture rate we observed (n=15 of 942 marked individuals) is consistent with other mark recapture research. For example, a study using 12-volt actinic light traps had a recapture rate of 3.88% (Dulieu et al. 2007). Another study using pheromone-baited traps had recapture percentages ranging from 1.3% and 2.5% for male codling moths (Judd et al. 2010). While these recapture rates may seem low, moths are highly agile making high recapture rates rare. It is unlikely that the moths were adversely affected by dusting with UV dust as previous studies have marked moths using similar dust with no reported impact on moth mortality (Cameron et al. 2002, Botero-Garcés and Isaacs 2004, Judd et al. 2010).

It should be noted, that the purpose of the CMRT is not necessarily to identify and record the types of moths present in a given habitat. Moths can be notoriously difficult to identify, and for most Lepidopterists, it would not be possible to identify all of the individuals collected by a CMRT, in short order, in the field. Typically, to identify and record the types of moths present in a habitat, a pesticide strip is added to a BLT and dead specimens are brought back to a lab for identification. Instead, the CMRT is designed for capture-mark-recapture studies. By definition, any individual that is captured, marked but not recaptured, cannot be included as part of a capture-mark-recapture dataset. Therefore, identifying all of the moths present at the initial capture event in the CMRT is not necessary for its intended application. That said, a high-resolution camera could be used to take photos of CMRT

captures, prior to re-release should this kind of data be desired.

We caution that the effects of long term effect of cooling of moths has not been explored and could vary from species-to-species, family-to-family, and from one geographic extent to another. For example, species that are bivoltine, or with a flight season that includes cold summer or fall nights, could be more cold tolerant than species with short mid-summer flight seasons. Furthermore, assemblages in northern temperate regions may be more cold tolerant than assemblages in southern or tropical regions. Prior to using this kind of trap for a mark-recapture study, we recommend that the post-cooling survivorship of moths from a given research landscape be explored. This would involve capturing moths with the cooling trap, releasing them in a controlled environment (e.g., a rearing cage) and observing post-cooling mortality rates. The cooling mechanism on the trap can then be easily modulated, using a timer, to turn the trap on-and-off at appropriate intervals to maintain the desired temperature inside the trap.

This CMRT combined with a simple marking method that is easy to carry out in the field simplifies *capture-mark-recapture* studies and may avoid some of the challenges involved with other trapping methods. There is no need to move captured moths from the site in order to incapacitate and mark them, which decreases the risk of moth damage or mixing of samples while they are handled. The non-specific nature of the CMRT-BLT combination allows a diverse assemblage of moths to be monitored. Given the increased interest in monitoring movement of moth assemblages, the CMRT has a wide array of applications.

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We are grateful to Joel Stewart for his help in the early conceptualization of this trap, and to two reviewers for their helpful insights.

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