

**RELEASE AND CAPTURE TECHNIQUES FOR INSECT POPULATION
MONITORING AND CONTROL**

CROSS-REFERENCE TO RELATED APPLICATIONS

- 5 **[0001]** This application claims priority to provisional application U.S. Ser. No. 63/579,926 entitled “Methods For Conducting Remote Incompatible Insect Technique Programs” filed on August 31, 2023, the entire disclosure of which is incorporated herein by reference for any purpose.

FIELD

- 10 **[0002]** This disclosure is related generally to techniques for combatting insect-borne diseases and more particularly, to systems and methods implementing release and capture techniques for insect population monitoring and control.

BACKGROUND

- 15 **[0003]** Mosquito-borne arboviruses have been increasing in prevalence and geographic distribution over the past several decades. Because there are limited vaccines and drugs available to prevent or treat mosquito-borne arboviruses, efforts to reduce the diseases caused by dengue and other mosquito-borne diseases have historically relied on source reduction and insecticides. Another approach involves vector control, which is the targeted management of mosquito populations to reduce their numbers and interrupt the transmission of arboviruses, including
20 methods such as eliminating breeding sites, applying insecticides, using biological control agents, and introducing genetically modified mosquitoes that are less capable of spreading disease.

- 25 **[0004]** One vector control approach involves the incompatible insect technique (“IIT”) which can employ a naturally occurring bacteria to cause incompatible matings between wild females and trans-infected male mosquitoes. For example, *Aedes aegypti*, the primary vector of dengue, is not naturally infected with *Wolbachia* bacteria but can be trans-infected via microinjection of *Wolbachia* from closely related species. Trans-infected male mosquitoes are incompatible with wild female mosquitoes. For example, eggs laid by wild females after mating with *Wolbachia*-carrying males exhibit cytoplasmic incompatibility and do not hatch, resulting in lower numbers
30 of viable offspring in the next generation. When reared en masse, *Wolbachia*-carrying males can be released to suppress wild populations by reducing the number of viable matings.

SUMMARY

[0005] In one general aspect, a method may include receiving one or more shipping containers for transporting insects, each shipping container containing a plurality of insects. The method may also include for each shipping container of the one or more shipping containers, preparing
5 one or more holding containers by: transferring the plurality of insects to a respective holding container at a first time and providing sustenance for the plurality of insects at the first time. The method may furthermore include releasing the plurality of insects contained in each of the one or more holding containers a predetermined amount of time after the first time.

[0006] In another general aspect, a method may include rearing a first plurality of insects. The
10 method may also include releasing the first plurality of insects at a first time. The method may furthermore include after a first predetermined period of time, capturing a second plurality of insects using one or more traps. The method may in addition include determining a first subset of the second plurality of insects that are also members of the first plurality of insects and determining a property of an insect population based on at least one of the first plurality of
15 insects, the second plurality of insects, the first subset, and the first predetermined period of time.

[0007] In another general aspect, a computer-implemented method may include receiving information about a first plurality of insects, including a first count of the first plurality of released insects. The method may also include after a predetermined period of time, receiving information about a second plurality of captured insects, including a second count of the second
20 plurality of captured insects, where a portion of the second plurality is included in the first plurality. The method may furthermore include determining a third count of the portion and determining a property of an insect population based on at least one of the first count, the second count, the third count, and the predetermined period of time.

BRIEF DESCRIPTION OF THE DRAWINGS

25 [0008] FIG. 1 shows an example flow diagram showing a process for releasing shipped insects for insect population monitoring and control, according to some examples of the present disclosure.

[0009] FIG. 2 shows an example flow diagram showing a process for characterizing insect populations in a region for insect population monitoring and control, according to some examples
30 of the present disclosure.

[0010] FIG. 3 shows an example flow diagram showing a process for releasing and capturing insects for insect population monitoring and control, according to some examples of the present disclosure.

5 [0011] FIG. 4 shows an example flow diagram showing a process for determining dispersal distance following insect release in a region for insect population monitoring and control, according to some examples of the present disclosure.

[0012] FIG. 5A shows an illustration of an example of placement and geographical distribution of insect traps in an example region, according to some examples of the present disclosure.

10 [0013] FIG. 5B shows an example histogram of an example collection of dispersal distance measurements in an example region, according to some examples of the present disclosure.

[0014] FIG. 6 shows an example flow diagram showing a process for determining a recapture rate following insect release in a region for insect population monitoring and control, according to some examples of the present disclosure.

15 [0015] FIG. 7 shows an example flow diagram showing a process for determining a probability of daily survival following insect release in a region for insect population monitoring and control, according to some examples of the present disclosure.

[0016] FIG. 8 shows an example graph of adjusted recapture proportion versus time for use in determining PDS, according to some examples of the present disclosure.

20 [0017] FIG. 9 shows an example flow diagram showing a process for determining a population size estimate following insect release in a region for insect population monitoring and control, according to some examples of the present disclosure.

[0018] FIG. 10 illustrates a simplified block diagram depicting an example device for implementing one or more example methods of the description, according to some examples of the present disclosure.

25 **DETAILED DESCRIPTION**

[0019] Examples are described herein in the context of systems and methods for implementing release and capture techniques for insect population monitoring and control. Those of ordinary skill in the art will realize that the following description is illustrative only and is not intended to be in any way limiting. For example, some features described with respect to rearing and
30 releasing mosquitoes are applicable to any other insect. Reference will now be made in detail to

implementations of examples as illustrated in the accompanying drawings. The same reference indicators will be used throughout the drawings and the following description to refer to the same or like items.

5 [0020] In the interest of clarity, not all of the routine features of the examples described herein are shown and described. It will, of course, be appreciated that in the development of any such actual implementation, numerous implementation-specific decisions must be made in order to achieve the developer's specific goals, such as compliance with application- and business-related constraints, and that these specific goals will vary from one implementation to another and from one developer to another.

10 [0021] The IIT technique introduced above refers generally to method of controlling pest populations by releasing insects that have been biologically modified to be incompatible with the local population, typically through sterilization or the introduction of genetically modified, incompatible insect strains. When these modified insects mate with the wild population, they may result in no viable offspring, leading to a gradual decrease in the population of the target
15 species. The methods disclosed herein can be used to implement an IIT program as well as various other pest control programs based on the introduction of genetically modified insects. For example, the methods are similarly compatible with sterile insect technique ("SIT") programs. In an IIT program, the insects used are conditionally sterile (e.g., males with a particular genetic characteristic may be unable to mate with wild females but can still mate with females with the
20 same characteristic). In contrast, in an SIT program, the male insects are sterile and unable to successfully mate with any females.

[0022] In one example IIT program, for regions plagued by mosquito-borne illness, male mosquitoes containing the endosymbiont *Wolbachia* can be used as a tool to suppress wild mosquito populations using the IIT technique. IIT programs reduce wild mosquito populations
25 via incompatible matings between released males and wild females to reduce the number of viable offspring produced in the next generation. Successful programs rely on the regular release of incompatible males to outcompete wild males for female mates.

[0023] Existing approaches to IIT have relied on local production of *Wolbachia* males to support regular releases of incompatible males. However, local production of *Wolbachia* males
30 presents several logistical challenges. Setting up and maintaining specialized rearing facilities can be costly and resource-intensive, particularly in regions with scarce resources or lower logistical support. Such regions may likewise lack skilled staffing to manage and operate rearing

facilities for limited-production operations. Additionally, establishing local production facilities in each region of concern may not be practical or scalable.

[0024] These challenges can be addressed using the release and capture techniques for insect population monitoring and control disclosed herein. In one aspect, the techniques involve packing and shipping mosquitoes internationally over long distances for a remote IIT program. Upon arrival at the remote destination, mark-release-recapture (“MRR”) techniques can be for estimating parameters such as the dispersal distance, probability of daily survival (“PDS”), and efficiency of traps used in the release areas. Additionally, MRR approaches can be used to evaluate differences in survival and dispersal between released mosquitoes using different release methods to help refine the release technique. MRR approaches can also be used to estimate the population size of the local mosquito population to inform the number of *Wolbachia* males needed to outnumber wild male competitors in sufficient numbers (e.g., a ratio of ~10:1 may be used in some regions). The effectiveness of these MRR techniques can be improved when used in concert with the release and capture techniques for insect population monitoring and control disclosed herein.

[0025] One illustrative example involves the survival and dispersal of *Wolbachia Aedes aegypti* males at a release site separated by over thousands of miles from a centralized production facility. In this example, the males are shipped using specialized shipping containers configured to house a large number of compressed insects in a sedentary state. The shipping containers for transporting insects are received at the region targeted for release. Each shipping container contains a large number of insects (e.g., thousands of mosquitoes). In preparation for release, one or more intermediate holding containers are then prepared by transferring the insects from each shipping container to a respective holding container. The holding containers may be, for example, larger containers, configured to allow the insects to recover after shipping. Sustenance, such as sugar and water, is added to the holding containers. Then, after a period of time has elapsed to allow the sedentary insects to recover, the insects are released from the holding containers. The use of the holding containers as intermediate step before release can improve the performance of an IIT program.

[0026] These illustrative examples are given to introduce the reader to the general subject matter discussed herein and the disclosure is not limited to these examples. The following sections describe various additional non-limiting examples illustrating techniques for release and capture for insect population monitoring and control. While some examples will be described in given in the context of an IIT program involving adult *Wolbachia* males, the techniques

disclosed herein may be used for control and monitoring of any insect population, in particular flying insects, such as other species of mosquitoes, fruit flies, tsetse flies, and so on.

[0027] Turning first to FIG. 1, FIG. 1 shows an example flow diagram showing a process 100 for releasing shipped insects for insect population monitoring and control, according to some examples of the present disclosure. This process, and any other processes described herein, is illustrated as a logical flow diagram, each operation of which represents a sequence of operations. The order in which the operations are described is not intended to be construed as a limitation, and any number of the described operations can be combined in any order and/or in parallel to implement the process. Process 100 can be used for packing, shipping, and delivering healthy, competitive insects for an IIT program.

[0028] At block 110, the operations include receiving one or more shipping containers for transporting insects, in which each shipping container containing a number of insects. For example, consider one example of a IIT program for reducing the mosquito population in a region using certain transinfected adult male mosquitoes. In this example, a strain of *Aedes aegypti* males containing wAlbB *Wolbachia* (hereinafter, “*Wolbachia*” mosquitoes) can be made by backcrossing males from a wild-type colony to a *Wolbachia*-infected *Aedes aegypti* colony for several (e.g., five) generations. The strain designation “wAlbB” is shorthand for the bacteria type, the bacteria's host species, and the specific strain of the bacteria. The larvae may be reared using, for example, automated rearing and sex sorting methods described in the art.

[0029] In some examples, batches of mosquito larvae (e.g., thousands at a time) can be reared using rearing bags filled with water inside a storage and retrieval robot and fed a suitable mosquito larvae diet (e.g., 1.75 mg diet per larvae). Then, after one week, the pupae can be sieved to remove most females and transferred to an adult sex sorting machine to separate males from residual females. The sorted *Wolbachia* male mosquitoes can then be decanted into small containers (e.g., 11.5 x 11.5 x 18.5 cm) for up to a few days and fed a suitable diet prior to shipping (e.g., 10% sucrose solution). In some examples, the small containers are chilled (e.g., to about 3 degrees Celsius) to sedate the *Wolbachia* mosquitoes. In some examples, the *Wolbachia* mosquitoes can be transferred into small, foam-lined shipping containers that resemble plastic pucks (e.g., 70 x 70 x 23 mm). The *Wolbachia* mosquitoes may be kept chilled (e.g., at about 10 degrees Celsius) and compressed prior to transit. A number of pucks of live, chilled and compressed *Wolbachia* mosquitoes may be placed into packing sleeves inflated with air to maintain pressure during transit.

[0030] The packing sleeves may then be shipped to the target release region using any suitable shipping method such as mail, express mail, freight, rail, train, road, overland truck, ship, air, sea, and so on. For example, the packing sleeves can be combined in insulated boxes (e.g., 40.6 x 40.6 x 50.8 cm) containing up to several tens of thousands mosquitoes each.

5 [0031] At block 120, for each shipping container of the one or more shipping containers, one or more holding containers is prepared by performing at least the following two operations. At block 130, number of insects in each shipping container is transferred to a respective holding container at a first time. For example, within 24 hours of receipt following transit, the packed mosquitoes can be unpacked into holding containers such as small hand-release containers (e.g.,
10 11.5 x 11.5 x 18.5 cm). The holding containers may be sized to permit the insects to move about the holding container as part of the recovery process. In this example, the first time would be 24 hours. At block 140, sustenance for the number of insects in each holding container is provided at the first time. For example, after being unpacked into holding containers as described in block 130, the mosquitoes can be held overnight with access to 10% sucrose solution until release the
15 following day.

[0032] Examples of holding containers include substantially cylindrical or rectangular boxes. For example, the holding containers may be a substantially cylindrical box or prism. One end of the holding container can be capped, and the other end configured to mate with a shipping container of the one or more shipping containers to enable the insects to easily transfer from the
20 shipping container to the holding container. The holding containers may be configured for hand-release of the insects, vehicle release, or a passive release method.

[0033] At block 150, the number of insects contained in each of the one or more holding containers is released after a predetermined amount of time after the first time. For example, where the mosquito release was targeted for the following day, the predetermined amount of time
25 could be about 12 to 24 hours after the first time. Thus, instead of releasing insects directly from the containers are shipped in after receipt at the release site destination, they are first transferred to intermediate holding containers, where they have access to water, sugar, or other sustenance, and can recover from shipment conditions.

[0034] Using the holding containers as an intermediate container for the predetermined period
30 of time can have several benefits. Packed and chilled mosquitoes may take some amount of time to recover after packing and shipping which could reduce their ability to disperse, especially in the first day after release. Giving insects the opportunity to recover from shipping conditions

(e.g., compression, colder temperatures, etc.) may increase their fitness upon release. Giving insects access to sustenance such as water or sugar prior to release may also increase their fitness and provide them with increased reserves in the field. Having a method for holding insects after receipt, without negative impacts on fitness and longevity, can provide more operational flexibility, and enables insects to be released when logistics/biological timing is optimal. Moreover, releasing insects from their shipping containers into hand containers or other intermediate containers can allow more flexibility when selected a method of release. For example, the available release methods may includes ones that do not require shipping containers only.

- 10 [0035] Upon release, MRR techniques may be employed to characterize insect populations in a region. In one example use of MRR, *Wolbachia Aedes aegypti* males can be released from the holding containers used as intermediate containers as described above. In some examples, the mosquitoes may be marked prior to release using, for example, a fluorescent spray technique. After a period of time following release (e.g., a few days), a number of mosquitoes can be
- 15 captured using one or more traps. Some portion of these captured mosquitoes may be among those previously released. The subset of these captured mosquitoes that were among those that were released can be determined. For example, mosquitoes that are marked using the fluorescent spray can be counted. Then, a property of an insect population can be determined using
- 20 information about, for example, the number of mosquitoes released, the number captured, the elapsed time since release, and so on. For instance, a geographic dispersion distance, recapture rate, or probability of daily survival can be computed using these or other derivative factors. These properties can be used to design effective IIT programs using conclusions relating to insect health, number of released insects required, estimates of the wild population size, and how insect population disperse in particular environments. The property can be determined more accurately
- 25 using the methods disclosed herein. For example, because the use of intermediate holding containers with sustenance provided can give the sedentary insects an opportunity to recover following shipment, the likelihood of survival following release can be enhanced and thus more closely approximate the behavior of insects in the wild.

- [0036] Turning next to FIG. 2, FIG. 2 shows an example flow diagram showing a process 200
- 30 for characterizing insect populations in a region for insect population monitoring and control, according to some examples of the present disclosure. In particular, process 200 describes an example implementation of an MRR technique that can be used following the process 100 described above with respect to FIG. 1. This process, and any other processes described herein, is

illustrated as a logical flow diagram, each operation of which represents a sequence of operations that can be implemented in hardware, computer instructions, or a combination thereof. In the context of computer instructions, the operations may represent computer-executable instructions stored on one or more non-transitory computer-readable storage media that, when executed by one more processors, perform the recited operations. Generally, computer-executable instructions include routines, programs, objects, components, data structures, and the like that perform particular functions or implement particular data types. The order in which the operations are described is not intended to be construed as a limitation, and any number of the described operations can be combined in any order and/or in parallel to implement the process.

10 [0037] Additionally, some, any, or all of the processes described herein may be performed under the control of one or more computer systems configured with specific executable instructions and may be implemented as code (e.g., executable instructions, one or more computer programs, or one or more applications) executing collectively on one or more processors, by hardware, or combinations thereof. As noted above, the code may be stored on a non-transitory computer-readable storage medium, for example, in the form of a computer program including a plurality of instructions executable by one or more processors.

[0038] At block 210, the operations include rearing a first number of insects. The first number of insects may have a unifying characteristic needed to determine the property in block 250 below. For example, the first number of insects may be *Wolbachia* male mosquitoes. However, process 200 can be used with wild-type mosquitoes or any other insect population of interest. For instance, wild-type *Ae. aegypti* mosquitoes can be reared beginning with a mixture of wild-collected eggs or eggs amplified in colony.

[0039] The reared insects can be reared over several generations to ensure suitable genetic diversity and stabilization of traits. The insects can be reared at a selected volume density. For example, *Wolbachia* male mosquitoes can be reared at a number density of approximately 2 larvae per milliliter. During rearing, the insects may be fed a specified diet.

[0040] Upon pupation, the insects may be sorted according to a specified trait. For example, *Wolbachia* male mosquitoes may be separated from females using a mechanical sieve. Later, as the males eclose into adults, any remaining females can be removed using an aspirator. The reared insects can be transferred into shipping or hand containers. In some examples, the reared insects may be transferred to an intermediate container and fed (e.g., a 10% sucrose solution) before release in block 220.

[0041] In some examples, a subset of the first number of insects may be marked. When insects are recaptured later, the marking can be used as a means to differentiate insects belonging to the first number of insects and wild insects. The number of recaptured insects belonging to the first number of insects, or a particular portion thereof, may be used to determine the property. For instance, a property such as insect recapture rate can be determined using markings, as described in detail below in FIG. 6.

[0042] Any suitable technique can be used for marking the insects. For example, some techniques for marking the insects include using fluorescent powders, *Wolbachia* trans-infection, ingestible dyes, immunomarking, trace elements, or stable isotopes to identify recaptured mosquitoes or other flying insects. Another approach involves application of a fluorescent marking using an aerosolizer and adhesive polymer to control the amount of marker applied and reduce any potential fitness costs of marking. This approach can be further combined with “DNA barcodes” to identify recaptured insects quickly in the field and expand the range of available markers beyond fluorescent colors alone. DNA barcoding can involve sequencing a region of a captured insect’s DNA to distinguish it from other species or intraspecies variants, allowing for accurate identification of recaptured insects.

[0043] One example method described herein may be used to apply fluorescent visible markers, to compare survival, dispersal, and recapture rates of packed and shipped *Wolbachia* male *Aedes aegypti* to locally-reared wild-type males. The method may involve transferring the first number of mosquitoes to a container. The container may include a port that can be connected and disconnected from a portable aerosolizer containing a mixture of a fluorescent dye and a polymer. For example, a fluorescent dye (e.g., a dye that fluoresces in the presence of ultraviolet light) such as a forensic dye containing metal-based inert, inorganic compounds can be used. Likewise, a polymer that binds the dye to the mosquito after a brief curing time can be used. The polymer may be, for example, a non-plasticized aqueous copolymer dispersion based on acrylic and methacrylic acid esters.

[0044] The portable aerosolizer may be, for example, a small, battery-operated, commercially available nebulizer for ease of use in the field. The aerosolizer (e.g., the nebulizer mouthpiece) can be connected to the port on the container. The aerosolizer can then be activated for a predetermined period of time to cause the mixture to adhere to the number of mosquitoes in the container. In one example, for a small hand-held container, the aerosolizer is turned on to its highest setting for about 30 seconds, discharging approximately 5 mL of mixture into the container. While the aerosolizer is activated, the container can be mechanically agitated (e.g.,

shaken) to cause the insects to move or fly about the container. In some cases, a too-short spray time (e.g., period of time that the aerosolizer is effectuated) may not result in sufficient adherence of the mixture to the insects and may need to be extended to obtain more complete coverage of the number of mosquitoes.

5 [0045] Any suitable settings can be used in association with the aerosolizer. For instance, the aerosolizer can be configured to dispense a particle size of less than about 4 μm with a condensation rate of greater than about 0.2 ml/min. However, any combination of particle size, condensation rate, and other configuration parameters can be used in accordance with the particular application. For instance, some insects or environmental conditions may warrant
10 selection of varying combinations of dye, polymer, and aerosolizer settings.

[0046] The efficacy of the fluorescent marker approach can be verified using various methods to ensure that the numbers of subsequently recaptured insects accurately reflect the number of originally dyed insects. For example, verification techniques can be used to determine the coverage of the marker on all mosquitoes in containers following spray treatment, persistence of
15 the marker over time, the impact of the marker on male longevity, an estimate of the fraction of false positives or false negatives that may be collected upon recapture, and so on. In a typical example, the verification techniques can be used to confirm nearly complete coverage and persistence of fluorescence on mosquitoes out to several days post-marking. In another typical example, the verification techniques can be used to demonstrate no difference between marked
20 and unmarked populations in laboratory-conducted survival assays (e.g., a hazard ratio determined using a Cox proportional hazard model). In yet another typical example, the verification techniques can be used to show that no or a negligible amount of fluorescence is lost or transferred from storing unmarked and marked mosquitoes together.

[0047] For example, a collection of dyed insects treated with fluorescent spray as described
25 above can be prepared. Longevity (e.g., how long the insects live after application of the dye) can be estimated by counting and removing dead individuals periodically. Dead individuals can be screened for fluorescence and coverage estimated as the percent of insects with observable fluorescence under UV light out of total number of insects screened. Persistence can be evaluated by comparing the percentage of insects positive for fluorescence by day. The impact of marking
30 on male longevity can be estimated by releasing males sprayed with the fluorescent marker, or unsprayed control males, into medium- or large-sized holding containers and counting the number of dead individuals every 24 hours until death days for all individuals were recorded.

[0048] Similarly, whether marked insects inadvertently transfer marking to unmarked insects (e.g., “false positives”) or whether marked insects lose marking after being trapped (e.g., “false negatives”) can be estimated by releasing counted numbers of marked and unmarked males in an extra-large holding container overnight. Insects can then be collected from the extra-large
5 holding container, frozen for a period of time, and scored for fluorescence. For examples, rates of false positives or false negatives can be estimated by comparing numbers of fluorescent marked insects versus unmarked insects versus the initial number of released (marked and unmarked) insects before collection.

[0049] In an example of DNA barcoding, a collection of captured mosquitoes can be analyzed
10 using LAMP to identify the subset that are male *Wolbachia* mosquitoes. Following capture, the mosquitoes can be pre-sorted (e.g., to exclude females) and shipped in suitable containers to an analysis facility. Shipping methods may impact the quality of the sampled mosquitoes. For instance, shipping the mosquitoes while chilled or on ice can prevent the growth of mold, which can degrade the analysis. In another example, the shipping containers can be outfitted with silica
15 gel or cotton wool to inhibit microbial growth and preserve DNA without the need to ship on ice. Upon receipt at the analysis facility, DNA can be extracted from the mosquitoes all samples was extracted using a suitable suite of genomic DNA extraction and purification tools. The extracted DNA can be tested for the presence of *Wolbachia* using a LAMP assay involving the amplification of specific bacterial DNA sequences through isothermal amplification with certain
20 designated primers.

[0050] At block 220, the operations further include releasing the first number of insects at a first time. For example, the first number of insects can be reared in a first location as described above in block 210 and then shipped to a second, remote location using one or more shipping containers. Upon receipt, the first number of insects can be transferred to intermediate holding
25 containers prior to release as described above in process 100. Then, the first number of insects can be released substantially as described above in block 150.

[0051] The first number of insects may be released in a particular area or region. For example, the insects may be released in a particular neighborhood approximately 40 acres in size. The conditions upon release, such as temperature, humidity, and weather, may vary and may affect
30 the characterization of the insect population. In some cases, a portion of the insects may not survive the transition from rearing to release. The count of the first number of insects should be updated accordingly in the computations below.

[0052] At block 230, the operations further include, after a first predetermined period of time, capturing a second number of insects using one or more traps. The one or more traps may be, for example, cylindrical mosquito traps further including a fan and one or more attractant lures such as carbon dioxide, described in more detail with respect to FIG. 3 below. Other trap configurations may be similarly used.

[0053] The accuracy of some determined insect population characteristics may be improved if the deployed traps are serviced following release and during recapture. For example, the traps can be serviced approximately daily. Some deployments may require substantially more or less frequently than daily according to the particular application.

[0054] Data collection efficiency can be improved by applying unique labels or tracking devices to the traps. For example, trap location or collection date can be tracked using printed QR barcode labels scanned using suitable software and hardware components.

[0055] At block 240, the operations further include determining a first subset of the second number of insects that are also members of the first number of insects. For example, the first subset of the released insects can be determined using genetic markers, such as a DNA barcoding techniques or by counting fluorescent insects. For example, a DNA barcoding technique can be used to identify male mosquitoes that have been trans-infected with *Wolbachia* using a DNA barcoding technique such as loop-mediated isothermal amplification (“LAMP”). LAMP can be performed on a number of collected insects by first extracting DNA samples from each insect. Then, a subset of the insects with a particular genetic signature can be identified using a LAMP assay.

[0056] At block 250, the operations further include determining a property of an insect population based on at least one of the first number of insects, the second number of insects, the first subset, and the first predetermined period of time. The property may be, for example, a dispersion distance, a recapture rate, a probability of daily survival (“PDS”), a population size, or other property of an insect population that can be determined using counts of released and recaptured insects. Several examples of these determinations are described in detail below.

[0057] Some examples of determining the property of an insect population can be performed using a system of one or more computers configured to perform particular operations or actions by virtue of having software, firmware, hardware, or a combination of them installed on the system that in operation causes or cause the system to perform the actions. One or more computer programs can be configured to perform particular operations or actions by virtue of

including instructions that, when executed by data processing apparatus, cause the apparatus to perform the actions.

[0058] Turning next to FIG. 3, FIG. 3 shows an example flow diagram showing a process 300 for releasing and capturing insects for insect population monitoring and control, according to some examples of the present disclosure. In particular, process 300 describes an example implementation of an MRR technique that can be used in conjunction with the processes 100, 200 described above with respect to FIGs. 1 and 2, respectively. This process, and any other processes described herein, is illustrated as a logical flow diagram, each operation of which represents a sequence of operations. The order in which the operations are described is not intended to be construed as a limitation, and any number of the described operations can be combined in any order and/or in parallel to implement the process.

[0059] At block 310, the operations include identifying a release point locating in the region or area containing the insect population of concern. In some examples, the first number of insects may be released at a release point arranged in the center of a “trapping matrix.” The trapping matrix, or other similar arrangement, can include an arrangement of insect traps in the area surrounding the release point. The locations of the insect traps may be selected to effect a particular goal associated with the characterization of the insect population. For example, the trapping matrix trap locations may be selected to obtain even or uniform recapture of the insects in block 230 below to ensure a desired level of statistical confidence.

[0060] At block 320, the operations include determining one or more trap locations for the one or more traps, in which the one or more trap locations are selected based on a uniform trap location density and in which the one or more traps include one or more catch nets. The one or more traps may be cylindrical mosquito traps further including a fan and one or more attractant lures such as carbon dioxide. Other trap configurations may be used. For instance, commercially available insect traps can be used. In one example, Sentinel-2 traps manufactured by Biogents are used. The traps may be fitted with catch nets that can be removed and taken to a different location for insect counting. The use of carbon dioxide as an attractant can affect trap efficiency. For example, traps not using carbon dioxide may have a lower efficiency.

[0061] The one or more traps can be arranged in a trapping matrix. The trapping matrix may include, for example, concentric circles radiating out from the release point. The traps can be arranged approximately uniformly around each concentric circle. Maps of traps that show the

trapping matrix can be designed and processed for later analysis using suitable software such as the R “googleway” package.

[0062] At block 330, the operations include activating the one or more traps a predetermined period of time after releasing a number of insects at a first time. For example, for traps that include electric components (e.g., require batteries or electrical outlets), the traps may be activated after a period of time has elapsed. For example, to measure a property such as the recapture rate, a period of time between release and capture must elapse to allow the insect population to naturally vary after release. For instance, for male mosquito release as part of an IIT program, some or all of the traps may be activated 24 hours after release to allow the males to find natural harborage after release instead of using the traps as short-term harborage.

[0063] At block 340, the operations include, after another predetermined period of time, removing the one or more catch nets from the one or more traps. For example, the catch net can be removed and frozen for a period time (e.g., 2 hours) to kill any live insects in the catch net. The insects may then be sorted, counted, etc. using a light microscope according to suitable criteria such as when to species and sex. For example, considering male mosquitoes that are among the potentially marked population, marked mosquitoes can be scored as fluorescent or not using a UV flashlight in a dark room.

[0064] Turning next to FIG. 4, FIG. 4 shows an example flow diagram showing a process 400 for determining dispersal distance following insect release in a region for insect population monitoring and control, according to some examples of the present disclosure. In particular, process 400 describes an example implementation of an MRR technique that can be used in conjunction with the processes 100, 200 described above with respect to FIGs. 1 and 2, respectively. This process, and any other processes described herein, is illustrated as a logical flow diagram, each operation of which represents a sequence of operations that can be implemented in hardware, computer instructions, or a combination thereof. In the context of computer instructions, the operations may represent computer-executable instructions stored on one or more non-transitory computer-readable storage media that, when executed by one more processors, perform the recited operations. Generally, computer-executable instructions include routines, programs, objects, components, data structures, and the like that perform particular functions or implement particular data types. The order in which the operations are described is not intended to be construed as a limitation, and any number of the described operations can be combined in any order and/or in parallel to implement the process.

[0065] Additionally, some, any, or all of the processes described herein may be performed under the control of one or more computer systems configured with specific executable instructions and may be implemented as code (e.g., executable instructions, one or more computer programs, or one or more applications) executing collectively on one or more
5 processors, by hardware, or combinations thereof. As noted above, the code may be stored on a non-transitory computer-readable storage medium, for example, in the form of a computer program including a plurality of instructions executable by one or more processors.

[0066] At block 410, the operations include releasing a first number of insects at a release point. This block may proceed substantially as described above with respect to block 220.

10 [0067] At block 420, the operations further include, after a predetermined period of time, capturing a second number of insects using one or more traps. This block may proceed substantially as described above with respect to block 230.

[0068] At block 430, the operations further include, for each trap of the one or more traps, determining a distance between the release point and the trap. FIG. 5A shows an illustration of an
15 example of placement and geographical distribution 500 of insect traps 520 in an example region, according to some examples of the present disclosure. In distribution 500, release point 510 is shown in the center of a series of concentric circles 515 indicating the distance from the release point 510. For each trap 520, a distance 530 can be measured.

[0069] Distribution 500 also illustrates an example arrangement of the one or more traps. In
20 this example, the traps 520 were placed around the release point 510 based on a grid mapping system to ensure approximately even trap density. However, because this example region borders a body of water on the right side, trapping distance may be limited in that direction. As a result, additional traps may be placed on other side, skewing an otherwise uniform trap distribution. This example is provided to illustrate one example of roughly uniform trap density, but the local
25 trap density may vary from region to region depending on the particular geography, climate, and other circumstances of the region.

[0070] At block 440, the operations further include computing a statistical measure based on the distances. FIG. 5B shows an example histogram 550 of an example collection of dispersal distance measurements in an example region, according to some examples of the present
30 disclosure. Using a distribution such as the one visualized in histogram 550, a number of statistical measures can be computed. For example, the mean distance traveled (“MDT”) can be

calculated as using a correction factor to account for uneven distribution of traps across the release area. In another example, the median distance traveled can be similarly computed.

[0071] In some examples, the dispersal distances may vary among certain insect populations, which can provide indications that are useful for interpreting the collected data. For example, an asymmetry in the geographic distribution of dispersal distances may indicate an environmental effects such as a directional bias due to a persistent weather phenomenon (e.g., an easterly wind). The dispersal distances can be used to estimate parameters such as trap spacing or release point spacing to provide even coverage over an IIT treatment area.

[0072] Turning next to FIG. 6, FIG. 6 shows an example flow diagram showing a process 600 for determining a recapture rate following insect release in a region for insect population monitoring and control, according to some examples of the present disclosure. In particular, process 600 describes an example implementation of an MRR technique that can be used in conjunction with the processes 100, 200 described above with respect to FIGs. 1 and 2, respectively. This process, and any other processes described herein, is illustrated as a logical flow diagram, each operation of which represents a sequence of operations that can be implemented in hardware, computer instructions, or a combination thereof. In the context of computer instructions, the operations may represent computer-executable instructions stored on one or more non-transitory computer-readable storage media that, when executed by one more processors, perform the recited operations. Generally, computer-executable instructions include routines, programs, objects, components, data structures, and the like that perform particular functions or implement particular data types. The order in which the operations are described is not intended to be construed as a limitation, and any number of the described operations can be combined in any order and/or in parallel to implement the process.

[0073] Additionally, some, any, or all of the processes described herein may be performed under the control of one or more computer systems configured with specific executable instructions and may be implemented as code (e.g., executable instructions, one or more computer programs, or one or more applications) executing collectively on one or more processors, by hardware, or combinations thereof. As noted above, the code may be stored on a non-transitory computer-readable storage medium, for example, in the form of a computer program including a plurality of instructions executable by one or more processors.

[0074] At block 610, the operations include releasing a first number of insects at a release point. This block may proceed substantially as described above with respect to block 220.

[0075] At block 620, the operations further include, after a predetermined period of time, capturing a second number of insects using one or more traps. This block may proceed substantially as described above with respect to block 230.

[0076] At block 630, the operations include determining a subset of the captured second number of insects that are also members of the released first number of insects. The subset can be determined using any of the methods described above or a combination thereof. For example, insects can be marked using a fluorescent paint or screened for particular genetic signatures (e.g., DNA barcoding). Using redundant techniques can ensure that the resulting computed measurements are reliable, with one method for determining the subset acting as a check on the other. Additionally, using redundant techniques can be used to estimate the effect of the techniques. For example, the use of fluorescent dye as a marker may have determinantal effects on insect fitness. This hypothesis can be tested by using another method (e.g., DNA barcoding) as an alternative or redundant way to estimate the size of the subset.

[0077] At block 640, the operations include dividing the number of insects in the captured second subset by the number of insects in the released first number of insects to determine a recapture rate. The recapture rate can be defined generally as the proportion of the originally released insects that are successfully recaptured. Recapture rate can reflect the likelihood or effectiveness of recovering the released population. The recapture rate may be expressed using units of a number of insects per unit time or as a proportion of the population size.

[0078] Computation of the recapture rate can be performed using a system of one or more computers configured to perform particular operations or actions by virtue of having software, firmware, hardware, or a combination of them installed on the system that in operation causes or cause the system to perform the actions. One or more computer programs can be configured to perform particular operations or actions by virtue of including instructions that, when executed by data processing apparatus, cause the apparatus to perform the actions.

[0079] For example, the statistical significance (e.g., a p-value) can be evaluated using statistical tests such as the Kruskal-Wallis test to compare recapture rates under varying circumstances. Computed recapture rates can be adjusted for mortality and estimated using, for example, a nonlinear least squares regression configured to constrain recapture rates below 10%. In that case, a statistical package such as the “nls” function in R can be used in conjunction with the “port” method to constrain recapture estimates to be between 0 and 10%. These

configurations are optimized for fitting an adaptive nonlinear least squares algorithm where the parameters need to stay within certain bounds during optimization.

[0080] In a typical analysis involving recapture of male *Wolbachia* mosquitoes, recapture rates may range between about 1.4 - 7.5% in some examples. The recapture rates between disparate subsets can be used to obtain insights into the insect populations. For example, recapture rates between marked and unmarked insects can be compared to estimate the effects of marking on insect populations.

[0081] Turning next to FIG. 7, FIG. 7 shows an example flow diagram showing a process 700 for determining a probability of daily survival following insect release in a region for insect population monitoring and control, according to some examples of the present disclosure. In particular, process 700 describes an example implementation of an MRR technique that can be used in conjunction with the processes 100, 200 described above with respect to FIGs. 1 and 2, respectively. This process, and any other processes described herein, is illustrated as a logical flow diagram, each operation of which represents a sequence of operations that can be implemented in hardware, computer instructions, or a combination thereof. In the context of computer instructions, the operations may represent computer-executable instructions stored on one or more non-transitory computer-readable storage media that, when executed by one more processors, perform the recited operations. Generally, computer-executable instructions include routines, programs, objects, components, data structures, and the like that perform particular functions or implement particular data types. The order in which the operations are described is not intended to be construed as a limitation, and any number of the described operations can be combined in any order and/or in parallel to implement the process.

[0082] Additionally, some, any, or all of the processes described herein may be performed under the control of one or more computer systems configured with specific executable instructions and may be implemented as code (e.g., executable instructions, one or more computer programs, or one or more applications) executing collectively on one or more processors, by hardware, or combinations thereof. As noted above, the code may be stored on a non-transitory computer-readable storage medium, for example, in the form of a computer program including a plurality of instructions executable by one or more processors.

[0083] At block 710, the operations include, after predetermined periods of time after releasing a number of insects, capturing a number of insects using one or more traps. This block may proceed substantially as described above with respect to block 220 and 230.

[0084] At block 720, the operations further include, for each captured number of insects, determining a subset of the number of captured insects that are also members of the number of released insects. This block may proceed substantially as described above with respect to block 240.

- 5 [0085] At block 730, the operations include, for each captured number of insects, determining a recapture rate by dividing the number of insects in the subset by the number of released insects. This block may proceed substantially as described above with respect to block 640.

[0086] At block 740, the operations include adjusting the determined recapture rates based on an estimated mortality rate and the predetermined periods of times. For example, the recapture
10 rates computed at block 730 can be divided by an estimated survival probability factor to yield a corrected measure that accounts for the expected loss of individuals over time.

[0087] At block 750, the operations include computing the probability of daily survival using the determined adjusted recapture rates. In some examples, PDS can be estimated by fitting an exponential decay model or other suitable function to the adjusted recapture proportion. In the
15 exponential decay example, the fit can be transformed into a linear function using the natural logarithm and fit using a linear regression to this plot. The slope of the regression line can correspond to the natural logarithm of the PDS value. FIG. 8 shows an example graph 800 of adjusted recapture proportion versus time for use in determining PDS, according to some examples of the present disclosure. In a typical PDS example involving release male mosquitoes,
20 most released males will not survive more than a few days after release.

[0088] PDS and estimated recapture rates adjusted for mortality can be estimated using the nonlinear least squares regression from statistical computing packages (e.g., the R “nls” package), modified to constrain recapture rates below 10%. Survival data can be further analyzed using, for example, Cox proportional hazard models in the R “survival” package. Fitting models
25 to field data can be influenced by unexpected variables (e.g. weather, ant damage, etc.), resulting in a poor model fit. Some models may affect PDS estimates through assumptions such as a closed system in which insects can only escape through capture or death. Some models can account for migration outside of the trapping area to capture insect survival over longer dispersal distances that are not accounted for in the model. PDS can be used to derive the average insect
30 life expectancy, defined as $\frac{1}{-\ln(PDS)}$.

[0089] Computation of the probability of daily survival can be performed using a system of one or more computers configured to perform particular operations or actions by virtue of having software, firmware, hardware, or a combination of them installed on the system that in operation causes or cause the system to perform the actions. One or more computer programs can be
5 configured to perform particular operations or actions by virtue of including instructions that, when executed by data processing apparatus, cause the apparatus to perform the actions.

[0090] Turning next to FIG. 9, FIG. 9 shows an example flow diagram showing a process 900 for determining a population size estimate following insect release in a region for insect population monitoring and control, according to some examples of the present disclosure. In
10 particular, process 900 describes an example implementation of an MRR technique that can be used in conjunction with the processes 100, 200 described above with respect to FIGs. 1 and 2, respectively. This process, and any other processes described herein, is illustrated as a logical flow diagram, each operation of which represents a sequence of operations that can be implemented in hardware, computer instructions, or a combination thereof. In the context of
15 computer instructions, the operations may represent computer-executable instructions stored on one or more non-transitory computer-readable storage media that, when executed by one more processors, perform the recited operations. Generally, computer-executable instructions include routines, programs, objects, components, data structures, and the like that perform particular functions or implement particular data types. The order in which the operations are described is
20 not intended to be construed as a limitation, and any number of the described operations can be combined in any order and/or in parallel to implement the process.

[0091] Additionally, some, any, or all of the processes described herein may be performed under the control of one or more computer systems configured with specific executable instructions and may be implemented as code (e.g., executable instructions, one or more
25 computer programs, or one or more applications) executing collectively on one or more processors, by hardware, or combinations thereof. As noted above, the code may be stored on a non-transitory computer-readable storage medium, for example, in the form of a computer program including a plurality of instructions executable by one or more processors.

[0092] At block 910, the operations include releasing a first number of insects at a release
30 point. This block may proceed substantially as described above with respect to block 220.

[0093] At block 920, the operations further include, after a predetermined period of time, capturing a second number of insects using one or more traps. This block may proceed substantially as described above with respect to block 230.

5 [0094] At block 930, the operations further include determining a subset of the captured second number of insects that are also members of the released first number of insects. For example, the subset may include insects marked with a fluorescent spray. This block may proceed substantially as described above with respect to block 630.

10 [0095] At block 940, the operations further include determining a recapture rate of the second number of insects that are also members of the released first number of insects by dividing the number of captured insects in the subset by the first number of insects released. This block may proceed substantially as described above with respect to block 640.

15 [0096] At block 950, the operations further include computing a population size estimate of the insects that are not members of the number of released insects using the recapture rate and a size of the first number of released insects. For example, a Lincoln-Peterson Index can be determined to estimate the population size. The Lincoln-Peterson Index is a method used to estimate the population size of a species in a given area in an MRR context. The Lincoln-Peterson Index can be computed by dividing the number of marked individuals released by the recapture rate computed in block 940. Alternatively, the Lincoln-Peterson Index can be computed by multiplying the first number of released insects by the second number of captured insects and
20 dividing this product by the number of insects in the subset.

[0097] The population size estimate can be used estimating numbers of insects needed to achieve sufficient overflooding ratios in an IIT release program. A typical IIT program may require a target of ~10:1 released trans-infected insects to wild-type insects to sufficiently control a large wild population. In some examples, the effectiveness of the IIT program can be improved
25 through source reduction work ahead of releases.

[0098] FIG. 10 illustrates a simplified block diagram depicting an example device 1000 for implementing one or more example methods of the description, according to some examples of the present disclosure. The computing device 1000 includes a processor 1010 which is in communication with the memory 1020 and other components of the computing device 1000
30 using one or more communications buses 1002. The processor 1010 is configured to execute processor-executable instructions stored in the memory 1020 to perform the techniques described herein, such as part or all of the example processes 400, 600, 700, and 900 described. The

computing device 1000, in this example, also includes one or more user input devices 1070, such as a keyboard, mouse, touchscreen, microphone, etc., to accept user input. The computing device 1000 also includes a display to provide visual output to a user.

[0099] The computing device 1000 can include or be connected to one or more storage devices 1030 that provides non-volatile storage for the computing device 1000. The storage devices 1030 can store system or application programs and data used by the computing device 1000. The storage devices 1030 might also store other programs and data not specifically identified in this description.

[0100] The computing device 1000 also includes a communications interface 1040. In some examples, the communications interface 1040 may enable communications using one or more networks, including a LAN; WAN, such as the Internet; metropolitan area network (“MAN”); point-to-point or peer-to-peer connection; etc. Communication with other devices may be accomplished using any suitable networking protocol. For example, one suitable networking protocol may include the Internet Protocol (“IP”), Transmission Control Protocol (“TCP”), User Datagram Protocol (“UDP”), or combinations thereof, such as TCP/IP or UDP/IP.

[0101] While some examples of methods and systems are described in terms of software executing on various machines, the methods and systems may also be implemented as specifically configured hardware, such as field-programmable gate array (“FPGA”) specifically to execute the various methods. For example, examples may be implemented in digital electronic circuitry, or in computer hardware, firmware, software, or in a combination thereof. In one example, a device may include a processor or processors. The processor includes a computer-readable medium, such as a random access memory (“RAM”) coupled to the processor. The processor executes processor-executable program instructions stored in memory, such as executing one or more computer programs. Such processors may include a microprocessor, a digital signal processor (“DSP”), an application-specific integrated circuit (“ASIC”), field programmable gate arrays (“FPGAs”), and state machines. Such processors may further include programmable electronic devices such as PLCs, programmable interrupt controllers (“PICs”), programmable logic devices (“PLDs”), programmable read-only memories (“PROMs”), electronically programmable read-only memories (“EPROMs” or “EEPROMs”), or other similar devices.

[0102] Such processors may include, or may be in communication with, media, for example computer-readable storage media, that may store instructions that, when executed by the

processor, can cause the processor to perform the steps described as carried out, or assisted, by a processor. Examples of computer-readable media may include, but are not limited to, an electronic, optical, magnetic, or other storage device that may provide a processor, such as the processor in a web server, with computer-readable instructions. Other examples of media
5 include, but are not limited to, a floppy disk, CD-ROM, magnetic disk, memory chip, ROM, RAM, ASIC, configured processor, all optical media, all magnetic tape or other magnetic media, or any other medium from which a computer processor can read. The processor, and the processing, described may be in one or more structures, and may be dispersed through one or more structures. The processor may include code executable to carry out one or more of the
10 methods (or parts of methods) discussed above with respect to Figures 4, 6, 7, and 9.

[0103] While the present subject matter has been described in detail with respect to specific embodiments thereof, it will be appreciated that those skilled in the art, upon attaining an understanding of the foregoing may readily produce alterations to, variations of, and equivalents to such embodiments. Accordingly, it should be understood that the present disclosure has been
15 presented for purposes of example rather than limitation, and does not preclude inclusion of such modifications, variations, and/or additions to the present subject matter as would be readily apparent to one of ordinary skill in the art. Indeed, the methods and systems described herein may be embodied in a variety of other forms; furthermore, various omissions, substitutions and changes in the form of the methods and systems described herein may be made without departing
20 from the spirit of the present disclosure. The accompanying claims and their equivalents are intended to cover such forms or modifications as would fall within the scope and spirit of the present disclosure.

[0104] Unless specifically stated otherwise, it is appreciated that throughout this specification discussions utilizing terms such as “processing,” “computing,” “calculating,” “determining,” and
25 “identifying” or the like refer to actions or processes of a computing device, such as one or more computers or a similar electronic computing device or devices, that manipulate or transform data represented as physical electronic or magnetic quantities within memories, registers, or other information storage devices, transmission devices, or display devices of the computing platform.

[0105] The system or systems discussed herein are not limited to any particular hardware
30 architecture or configuration. A computing device can include any suitable arrangement of components that provide a result conditioned on one or more inputs. Suitable computing devices include multipurpose microprocessor-based computing systems accessing stored software that programs or configures the computing system from a general purpose computing apparatus to a

specialized computing apparatus implementing one or more embodiments of the present subject matter. Any suitable programming, scripting, or other type of language or combinations of languages may be used to implement the teachings contained herein in software to be used in programming or configuring a computing device.

- 5 [0106] Embodiments of the methods disclosed herein may be performed in the operation of such computing devices. The order of the blocks presented in the examples above can be varied—for example, blocks can be re-ordered, combined, and/or broken into sub-blocks. Certain blocks or processes can be performed in parallel.

- [0107] Conditional language used herein, such as, among others, “can,” “could,” “might,”
10 “may,” “e.g.,” and the like, unless specifically stated otherwise, or otherwise understood within the context as used, is generally intended to convey that certain examples include, while other examples do not include, certain features, elements, and/or steps. Thus, such conditional language is not generally intended to imply that features, elements and/or steps are in any way required for one or more examples or that one or more examples necessarily include logic for
15 deciding, with or without author input or prompting, whether these features, elements, and/or steps are included or are to be performed in any particular example.

- [0108] Disjunctive language such as the phrase “at least one of X, Y, or Z,” unless specifically stated otherwise, is otherwise understood within the context as used in general to present that an item, term, etc., may be either X, Y, or Z, or any combination thereof (e.g., X, Y, and/or Z).
20 Thus, such disjunctive language is not generally intended to, and should not, imply that certain examples require at least one of X, at least one of Y, or at least one of Z to each be present.

- [0109] Use herein of the word “or” is intended to cover inclusive and exclusive OR conditions. In other words, A or B or C includes any or all of the following alternative combinations as appropriate for a particular usage: A alone; B alone; C alone; A and B only; A and C only; B and
25 C only; and all three of A and B and C.

- [0110] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the disclosed examples (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “including,” “having,” and the like are
30 synonymous and are used inclusively, in an open-ended fashion, and do not exclude additional elements, features, acts, operations, and so forth. Also, the term “or” is used in its inclusive sense (and not in its exclusive sense) so that when used, for example, to connect a list of elements, the

term “or” means one, some, or all of the elements in the list. The use of “adapted to” or “configured to” herein is meant as open and inclusive language that does not foreclose devices adapted to or configured to perform additional tasks or steps. The term “connected” is to be construed as partly or wholly contained within, attached to, or joined together, even if there is something intervening. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. Additionally, the use of “based on” is meant to be open and inclusive, in that a process, step, calculation, or other action “based on” one or more recited conditions or values may, in practice, be based on additional conditions or values beyond those recited. Similarly, the use of “based at least in part on” is meant to be open and inclusive, in that a process, step, calculation, or other action “based at least in part on” one or more recited conditions or values may, in practice, be based on additional conditions or values beyond those recited. Headings, lists, and numbering included herein are for ease of explanation only and are not meant to be limiting.

[0111] The various features and processes described above may be used independently of one another, or may be combined in various ways. All possible combinations and sub-combinations are intended to fall within the scope of the present disclosure. In addition, certain method or process blocks may be omitted in some implementations. The methods and processes described herein are also not limited to any particular sequence, and the blocks or states relating thereto can be performed in other sequences that are appropriate. For example, described blocks or states may be performed in an order other than that specifically disclosed, or multiple blocks or states may be combined in a single block or state. The example blocks or states may be performed in serial, in parallel, or in some other manner. Blocks or states may be added to or removed from the disclosed examples. Similarly, the example systems and components described herein may be configured differently than described. For example, elements may be added to, removed from, or rearranged compared to the disclosed examples.

[0112] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0113] As used below, any reference to a series of examples is to be understood as a reference to each of those examples disjunctively (e.g., “Examples 1-4” is to be understood as “Examples 1, 2, 3, or 4”).

[0114] Example 1 is a method for releasing shipped insects, comprising: receiving one or more shipping containers for transporting insects, each shipping container containing a plurality of insects; for each shipping container of the one or more shipping containers, preparing one or more holding containers by: transferring the plurality of insects to a respective holding container
5 at a first time; and providing sustenance for the plurality of insects at the first time; and releasing a first plurality of insects by releasing the plurality of insects contained in each of the one or more holding containers a predetermined amount of time after the first time.

[0115] Example 2 is the method of any one or more of the preceding or following example(s), wherein the insects are male mosquitoes.

10 [0116] Example 3 is the method of any one or more of the preceding or following example(s), wherein the insects include first male mosquitoes that have been infected with a bacteria.

[0117] Example 4 is the method of any one or more of the preceding or following example(s), wherein the male mosquitoes are adult mosquitoes.

15 [0118] Example 5 is the method of any one or more of the preceding or following example(s), wherein the sustenance comprises a sucrose solution.

[0119] Example 6 is the method of any one or more of the preceding or following example(s), wherein the plurality of insects contained in each of the one or more shipping containers are in at least one of a compressed state or a chilled state.

20 [0120] Example 7 is the method of any one or more of the preceding or following example(s), wherein each of the one or more holding containers is sized to permit the plurality of insects to move about the holding container.

[0121] Example 8 is the method of any one or more of the preceding or following example(s), wherein each of the one or more holding containers is a substantially cylindrical prism or a substantially rectangular box.

25 [0122] Example 9 is the method of any one or more of the preceding or following example(s), wherein each of the one or more holding containers is a substantially cylindrical prism, comprising a proximal end and a distal end, wherein the proximal end is capped and the distal end is configured to mate with a shipping container of the one or more shipping containers to enable the insects to transfer from the shipping container to the holding container.

[0123] Example 10 is the method of any one or more of the preceding or following example(s), wherein the predetermined amount of time corresponds to a recovery period for the insects.

[0124] Example 11 is the method of any one or more of the preceding or following example(s), wherein the predetermined amount of time is between 12 and 24 hours.

5 [0125] Example 12 is the method of any one or more of the preceding or following example(s), wherein the one or more shipping containers are adapted for transportation according to at least one of road travel, rail travel, air travel, or sea travel.

[0126] Example 13 is the method of any one or more of the preceding or following example(s), wherein the one or more holding containers are adapted for release of the insects using at least
10 one of a hand-release method, a vehicle release method, or a passive release method.

[0127] Example 14 is the method of any one or more of the preceding or following example(s), wherein: the one or more holding containers are adapted for release of the insects using a hand-release method; and each of the one or more holding containers is a substantially cylindrical prism or a substantially rectangular box.

15 [0128] Example 15 is the method of any one or more of the preceding or following example(s), further comprising: after a first predetermined period of time, capturing a second plurality of insects using one or more traps; determining a first subset of the second plurality of insects that are also members of the first plurality of insects; and determining a property of an insect population based on at least one of the first plurality of insects, the second plurality of insects,
20 the first subset, and the first predetermined period of time.

[0129] Example 16 is the method of any one or more of the preceding or following example(s), further comprising marking a second subset of the first plurality of insects and wherein the determining the property is further based on the second subset.

[0130] Example 17 is the method of any one or more of the preceding or following example(s),
25 wherein marking the first plurality of insects comprises: transferring the first plurality of insects to a marking container, the marking container including a port removably connected to an aerosolizer containing a mixture comprising a fluorescent dye and a polymer; and effectuating the aerosolizer for a predetermined period of time to cause the mixture to adhere to the first plurality of insects.

30 [0131] Example 18 is the method of any one or more of the preceding or following example(s), wherein capturing the second plurality of insects using the one or more traps comprises:

identifying a release point; determining one or more trap locations for the one or more traps, wherein the one or more trap locations are selected based on a uniform trap location density and wherein the one or more traps comprise one or more catch nets; activating the one or more traps a second predetermined period of time after releasing the first plurality of insects at the first time; 5 and after a third predetermined period of time, removing the one or more catch nets from the one or more traps.

[0132] Example 19 is the method of any one or more of the preceding or following example(s), wherein the property is one of a dispersal distance, a recapture rate, a probability of daily survival, or a population size estimate.

10 **[0133]** Example 20 is a computer-implemented method, comprising: receiving first information about a first plurality of released insects, including a first count of the first plurality of released insects, wherein: the first plurality of released insects are released from one or more holding containers a first predetermined amount of time after a first time; the first plurality of released insects are transferred from one or more shipping containers for transporting insects to 15 the one or more holding containers at the first time, the first time corresponding to receipt of the one or more shipping containers; and the one or more holding containers each include sustenance for the first plurality of released insects; after a second predetermined period of time, receiving second information about a second plurality of captured insects, including a second count of the second plurality of captured insects, wherein a portion of the second plurality is included in the 20 first plurality; determining a third count of the portion; and determining a property of an insect population based on at least one of the first count, the second count, the third count, and the second predetermined period of time.

CLAIMS

What is claimed is:

1. A method for releasing shipped insects, comprising:
receiving one or more shipping containers for transporting insects, each shipping container containing a plurality of insects;
for each shipping container of the one or more shipping containers, preparing one or more holding containers by:
transferring the plurality of insects to a respective holding container at a first time;
and
providing sustenance for the plurality of insects at the first time; and
releasing a first plurality of insects by releasing the plurality of insects contained in each of the one or more holding containers a predetermined amount of time after the first time.
2. The method of claim 1, wherein the insects are male mosquitoes.
3. The method of claim 2, wherein the insects include first male mosquitoes that have been infected with a bacteria.
4. The method of claim 2, wherein the male mosquitoes are adult mosquitoes.
5. The method of claim 1, wherein the sustenance comprises a sucrose solution.
6. The method of claim 1, wherein the plurality of insects contained in each of the one or more shipping containers are in at least one of a compressed state or a chilled state.
7. The method of claim 1, wherein each of the one or more holding containers is sized to permit the plurality of insects to move about the holding container.

8. The method of claim 7, wherein each of the one or more holding containers is a substantially cylindrical prism or a substantially rectangular box.
9. The method of claim 8, wherein each of the one or more holding containers is a substantially cylindrical prism, comprising a proximal end and a distal end, wherein the proximal end is capped and the distal end is configured to mate with a shipping container of the one or more shipping containers to enable the insects to transfer from the shipping container to the holding container.
10. The method of claim 1, wherein the predetermined amount of time corresponds to a recovery period for the insects.
11. The method of claim 10, wherein the predetermined amount of time is between 12 and 24 hours.
12. The method of claim 1, wherein the one or more shipping containers are adapted for transportation according to at least one of road travel, rail travel, air travel, or sea travel.
13. The method of claim 1, wherein the one or more holding containers are adapted for release of the insects using at least one of a hand-release method, a vehicle release method, or a passive release method.
14. The method of claim 10, wherein:
 - the one or more holding containers are adapted for release of the insects using a hand-release method; and
 - each of the one or more holding containers is a substantially cylindrical prism or a substantially rectangular box.
15. The method of claim 1, further comprising:
 - after a first predetermined period of time, capturing a second plurality of insects using one or more traps;

determining a first subset of the second plurality of insects that are also members of the first plurality of insects; and

determining a property of an insect population based on at least one of the first plurality of insects, the second plurality of insects, the first subset, and the first predetermined period of time.

16. The method of claim 15, further comprising marking a second subset of the first plurality of insects and wherein the determining the property is further based on the second subset.

17. The method of claim 16, wherein marking the first plurality of insects comprises:
transferring the first plurality of insects to a marking container, the marking container including a port removably connected to an aerosolizer containing a mixture comprising a fluorescent dye and a polymer; and
effectuating the aerosolizer for a predetermined period of time to cause the mixture to adhere to the first plurality of insects.

18. The method of claim 15, wherein capturing the second plurality of insects using the one or more traps comprises:
identifying a release point;
determining one or more trap locations for the one or more traps, wherein the one or more trap locations are selected based on a uniform trap location density and wherein the one or more traps comprise one or more catch nets;
activating the one or more traps a second predetermined period of time after releasing the first plurality of insects at the first time; and
after a third predetermined period of time, removing the one or more catch nets from the one or more traps.

19. The method of claim 15, wherein the property is one of a dispersal distance, a recapture rate, a probability of daily survival, or a population size estimate.

20. A computer-implemented method, comprising:

receiving first information about a first plurality of released insects, including a first count of the first plurality of released insects, wherein:

the first plurality of released insects are released from one or more holding containers a first predetermined amount of time after a first time;

the first plurality of released insects are transferred from one or more shipping containers for transporting insects to the one or more holding containers at the first time, the first time corresponding to receipt of the one or more shipping containers; and

the one or more holding containers each include sustenance for the first plurality of released insects;

after a second predetermined period of time, receiving second information about a second plurality of captured insects, including a second count of the second plurality of captured insects, wherein a portion of the second plurality is included in the first plurality;

determining a third count of the portion; and

determining a property of an insect population based on at least one of the first count, the second count, the third count, and the second predetermined period of time.