TOS SCIENCE DESIGN FOR MOSQUITO ABUNDANCE, DIVERSITY, AND PHENOLOGY

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Change Record

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A	10/10/2014	ECO-02020	Initial release

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1 Description

1.1 Purpose

NEON design documents are required to define the scientific strategy leading to high-level protocols for NEON subsystem components, linking NEON Grand Challenges and science questions to specific measurements. Many NEON *in situ* measurements can be made in specific ways to enable continental-scale science rather than in ways that limit their use to more local or ecosystem-specific questions. NEON strives to make measurements in ways that enable continental-scale science to address the Grand Challenges. Design Documents flow from questions and goals defined in the NEON Science Strategy document, and inform the more detailed procedures described in Level 0 (L0; raw data) protocol and procedure documents, algorithm specifications, and Calibration/Validation (CalVal) and maintenance plans.

1.2 Scope

This document defines the rationale and requirements for mosquito abundance, diversity and phenology sampling in the NEON Science Design.

1.3 Acknowledgments

The design was reviewed by and refined with input from a technical working group consisting of researchers with relevant expertise. The mosquito technical working group includes Drs. Chris Barker, Roberto Barrera, Mark Blackmore, William Bradshaw, Desmond Foley, Howard Ginsberg, Mary Hayden, Chris Holzapfel, Steve Juliano, Laura Kramer, Shannon LaDeau, Chet Moore, Roger Nasci, Bill Reisen, and Harry Savage.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain information that shall be applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

AD[01]	NEON.DOC.000001	NEON Observatory Design
AD[02]	NEON.DOC.001282	Introduction to the TOS Science Designs
AD[03]	NEON.DOC.000913	TOS Science Design for Spatial Sampling Design
AD[04]	NEON.DOC.000911	TOS Science Design for Vectors and Pathogens
AD[05]	NEON.DOC.014015	Fundamental Sentinel Unit Bioarchive Facility Design

2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.014049	TOS Protocol and Procedure: Mosquito Sampling
RD[05]	NEON.DOC.001100	TOS Protocol and Procedure: Ground Beetle and Mosquito Specimen Processing

2.3 External References

External references contain information pertinent to this document, but are not NEON configuration-controlled. Examples include manuals, brochures, technical notes, and external websites.

ER [01]	
ER [02]	
ER [03]	

2.4 Acronyms

All acronyms used in this document are defined in RD[01].

3 INTRODUCTION

3.1 Overview of the Observatory

The National Ecological Observatory Network (NEON) is a continental-scale ecological observation platform for understanding and forecasting the impacts of climate change, land use change, and invasive species on ecology. NEON is designed to enable users, including scientists, planners and policy makers, educators, and the general public, to address the major areas in environmental sciences, known as the Grand Challenges (Fig. 1). NEON infrastructure and data products are strategically aimed at those aspects of the Grand Challenges for which a coordinated national program of standardized observations and experiments is particularly effective. The open access approach to the Observatory's data and information products will enable users to explore NEON data in order to map, understand, and predict the effects of humans on the earth and understand and effectively address critical ecological questions and issues. Detailed information on the NEON design can be found in AD[01], AD[02].

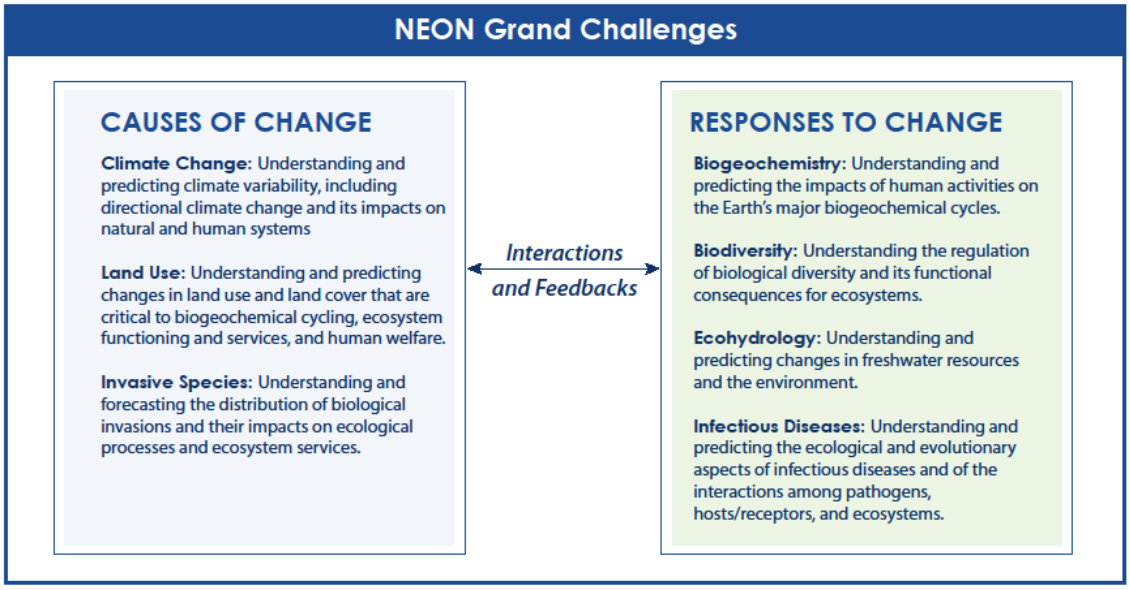

Figure 1. The seven Grand Challenges defined by the National Research Council (Committee on Grand Challenges in Environmental Sciences 2001)

3.2 Components of the Observatory

There are five components of the Observatory, the Airborne Observation Platform (AOP), Terrestrial Instrument System (TIS), Aquatic Observation System (AOS), Aquatic Instrument System (AIS), and Terrestrial Observation System (TOS). Collocation of measurements associated with each of these components will allow for linkage and comparison of data products. For example, remote sensing data provided by the Airborne Observation Platform (AOP) will link diversity and productivity data collected on individual plants and stands by the Terrestrial Observation System (TOS) and flux data captured by instruments on the tower (TIS) to that of satellite-based remote sensing. For additional information on these systems, see Keller et al. (2008) and Schimel et al. (Schimel et al. 2011b).

3.3 The Terrestrial Observation System (TOS)

The NEON TOS will quantify the impacts of climate change, land use, and biological invasions on terrestrial populations and processes by sampling key groups of organisms (sentinel taxa), infectious disease, soil, and nutrient fluxes across system interfaces (air, land, and water) (AD[01], AD[02]). The sentinel taxa were selected to include organisms with varying life spans and generation times, and wide geographic distributions to allow for standardized comparisons across the continent. Many of the biological measurements will enable inference at regional and continental scales using statistical or process-based modeling approaches. The TOS sampling design captures heterogeneity representative of each site to facilitate this inference when possible. Plot and organism-scale measurements will also be coordinated with the larger-scale airborne measurements, which provide a set of synergistic biological data products at the regional scale. Details of these design elements and algorithms can be found in individual design documents available through the NEON website (www.NEONinc.org).

The standardization of protocols across all sites is key to the success of NEON (and its novelty) and must be maintained at all sites through time. Thus, although specific techniques may be required at some sites (e.g., due to different vegetation types), protocols have been developed to ensure data comparability. These details can also be found in individual design documents available through the NEON website (www.NEONinc.org).

The TOS Science Designs define the scientific strategies leading to high-level sampling designs for NEON sentinel taxa, terrestrial biogeochemistry, and infectious disease, linking NEON Grand Challenges and science questions to specific measurements (AD[02]). The TOS Spatial Sampling Design document describes the sampling design that collocates observations of the components of the TOS (AD[03]). TOS Science Design documents were developed following input from the scientific community, including module-specific Technical Working Groups, and the National Science Foundation (AD[02]). Science Designs will be reviewed periodically to ensure that the data collected by NEON are those best suited to meet the requirements of the observatory (AD[01]), are (to the extent possible) consistent with standards used by the scientific community, and fit within the scope of NEON. Additional information on the development and review process can be found in AD[02].

4 INTRODUCTION TO THE MOSQUITO ABUNDANCE, DIVERSITY, AND PHENOLOGY SAMPLING DESIGN

4.1 Background

4.1.1 Mosquitoes as a Sentinel Taxon

The Terrestrial Observation System (TOS) at NEON is charged with monitoring the responses of biodiversity and ecosystems to environmental change. A NEON design committee (AIBSnews 2007) selected mosquitoes (Diptera: Culicidae) as a focal taxon for measurement. Mosquitoes are a diverse and widespread family of insects with aquatic larval and pupal forms and flying adults that have been extensively studied because of their ecological and epidemiological significance. As a dominant taxon in aquatic food webs, mosquitoes compose a sizable proportion of invertebrate biomass in aquatic systems and act as a key food source for aquatic and terrestrial predators (e.g., fish, amphibians, spiders, birds). Mosquitoes also act as vectors for numerous parasites and pathogens of humans, livestock, and wildlife and their biology and ecology have been extensively studied to characterize and mitigate impacts of associated diseases. Most female mosquitoes collect blood meals from vertebrates in order to provide energy for their developing eggs. Due to their potential impacts on human health, mosquito populations have been and continue to be monitored by national, state and local agencies. Mosquito-borne diseases can also influence the health of livestock [e.g., Rift Valley Fever (Daubney and Hudson 1931), Eastern equine encephalitis (Kissling et al. 1954)] and wildlife populations [e.g., avian malaria (Van Riper III et al. 1986), West Nile Virus (Marra et al. 2004)]. For example, the emergence of West Nile virus in North America has resulted in widespread population declines of several common birds (e.g., crows, robins, wrens, chickadees, blue jays; LaDeau et al. 2007) with important potential consequences for ecosystem services like seed dispersal, carrion scavenging and insect regulation (LaDeau et al. 2008).

Because of their sensitivity to environmental gradients and perturbations, mosquitoes represent an ideal sentinel taxon for evaluating the ecological effects of global change phenomena. The distribution of mosquito populations and seasonal phenology are influenced by many landscape factors including climate, vegetation and host availability (Buckner et al. 2010, Reisen 2010). Their short generation time and high fecundity allow mosquitoes to respond quickly to environmental change, but because of the group's high diversity and varied ecological niches, the nature and magnitude of these changes can differ markedly among species.

Changes in global climate are predicted to affect the distribution, demography, and seasonal phenology of many mosquitoes; associated effects on disease transmission cycles have also been posited (Epstein et al. 1998). For example, as the climate warms mosquito populations are expanding their geographical ranges (Hongoh et al. 2011) and transmission rates may increase, though climate is not the only driver of mosquito-borne diseases dynamics (Reiter 2001). Because of their frequent association with humans and ability to thrive in human-modified environments, mosquito ecology is also likely to be significantly affected by land use changes. Mosquitoes are highly mobile and able to move into new areas as climatic

conditions change, often aided by unintentional human transport (Lounibos 2002). Invasions of mosquitoes that carry human diseases from tropical into sub-tropical and temperate areas are more likely under warmer conditions. Climate conditions influence not only mosquito distributions but also the life cycles of pathogens and the transmission of disease (Gage et al. 2008). Higher temperatures can shorten the life cycle of viruses and mosquitoes, increase blood meal and oviposition rates and thereby the efficiency of transmission (Reisen 2010). Together, these climate effects may expand the biogeographic ranges of mosquitoes and the diseases they carry into temperate areas. Climate may also indirectly influence mosquitoes and disease dynamics by affecting the phenology of their hosts (Burkett-Cadena et al. 2011). In addition, mosquitoes have tremendous genetic variability and an extensive body of genetic information is available for these insects. NEON will measure mosquito populations across a broad spatial extent for the next 3 decades and will be able to detect changes in their distribution and phenology.

4.2 NEON's Contribution

Earth's environment is changing rapidly. Data at the temporal and spatial scales that NEON will provide are necessary to understand, forecast and manage our changing biosphere (Keller et al. 2008, Schimel et al. 2011a).

NEON's mosquito sampling will provide a cost effective and informative measure of a biological response to environmental, climate and land-use change. NEON sampling will augment state and local mosquito collection efforts and will enable us to characterize the response of mosquito populations, including abundance, phenology, and range to land use and other ecosystem drivers.

4.3 Purpose and Scope

This document defines the rationale and requirements for mosquito (Diptera: Culicidae) abundance, diversity and phenology in the NEON Science Design. Details about protocols including educational materials to conduct protocols, quality assurance and quality control or calibration and validation procedures are addressed in protocol specific documents.

5 SAMPLING FRAMEWORK

5.1 Science Requirements

This science design is based on Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

5.2 Data Products

Execution of the protocols that stem from this science design procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (RD[03]).

5.3 Priorities and Challenges for Mosquito Abundance, Diversity, and Phenology Sampling

Standardized, well established and widely used sampling methods were selected to maximize comparability across time and between domains within NEON and be comparable to other mosquito collection programs. To enhance utility to the scientific community, Center for Disease Control and Prevention (CDC)-CO_2 light traps, standard mosquito collection tools, were selected.

NEON's general strategy for the allocation of mosquito sampling effort is to sample core sites more frequently than relocatable sites. This mixed strategy of more focused attention to the core site will allow for the most efficient collection of mosquito data because sampling the core site requires far less travel time and can therefore be sampled at much lower cost. Core sites are prioritized because of the long-term nature of core site sampling (expected to continue for the entire 30-year lifespan on the observatory in contrast to relocatable sites, which are expected to be sampled for 7-10 years). Relocatable sites, which will be sampled less frequently than core sites, will serve to expand the spatial extent of inference.

Two primary constraints govern the spatial distribution of plots within a site. The first is collocation of measurements in order to promote comparability and the characterization of relationships between disparate measurements across the observatory. Second, sampling logistics limit the amount of travel time that can be allocated to checking mosquito traps. Due to these dual constrains, this design places traps within 30m of roads while following the stratified-random sampling design followed by other TOS protocols and co-locating the mosquito traps with distributed plots where possible.

6 SAMPLING DESIGN FOR MOSQUITO ABUNDANCE, DIVERSITY, AND PHENOLOGY

In keeping with NEON's broad mission, this design must be able to be deployed across a wide range of sites in a standardized way (e.g., methods, sampling frequencies) such that data are comparable across time and space. The design must be relatively uncomplicated so that it can be deployed consistently by disparate field crews over multiple years with minimal chance of alteration.

NEON is intended to be an integrated measurement system. Coordinating measurements between modules, e.g., mosquito abundance and diversity (the primary focus of this design) with pathogen presence and prevalence (AD[04]), is therefore a key component of the NEON design. A potential consequence of an integrated measurement design is sub-optimal sampling for individual modules at individual sites. Coordinating mosquito abundance and diversity sampling with sampling for mosquito-borne pathogens results in a number of attractive efficiencies, including saving considerable time and money because the same mosquito samples can be counted and identified as well as tested for pathogens. However, abundance and diversity sampling aims to survey a broad cross section of the mosquito community while pathogen testing targets particular vector species and requires as many individuals as possible for testing. Thus, the two modules differ fundamentally in their foci and objectives and would be optimized using different sampling strategies, resulting in a trade-off between data quality at the site level and comparability utilizing a combined sampling design. The sampling approach adopted and described here is optimized for mosquito abundance and diversity sampling by using taxonomically general sampling spread broadly across time and space. Collected mosquitoes will subsequently be tested for pathogen presence/prevalence and additional trapping for specific vector mosquito species will be implemented at certain sites based on sampling efficacy (AD[04]). In this document, 'mosquito sampling' is used to describe the sampling for the coordinated sampling activities for abundance and diversity and pathogen sampling, unless otherwise specified.

6.1 Sampling Methods

Mosquitoes will be sampled at NEON sites using CDC CO_2 light traps, a standard and widely used method used by the CDC and other mosquito researchers for public health monitoring for mosquito-borne diseases for a half-century (Sudia and Chamberlain 1962). Although there are many variations on this basic trap, all traps use CO_2 as bait because CO_2 is a component of vertebrate breath that mosquitoes use to locate hosts. The sublimating CO_2 attracts mosquitoes to the vicinity of the trap and a fan that pulls the mosquitoes into a net bag for live storage until the trap is collected by a field technician. Some traps include a light source, but light bulbs will be disconnected because, though they do help attract some mosquitoes, light also attracts a wide variety of bycatch organisms that complicate sample processing and ultimately reduce sample quality.

CDC CO_2 light traps arguably collect the greatest diversity of all common traps and are regularly used in mosquito-borne disease surveillance, thus maximizing comparability with other data sets (Sudia and Chamberlain 1962, Service 1993). However they have known limitations/biases (e.g., do not effectively

sample *Aedes* spp. or blood-fed mosquitoes generally targeted for pathogen testing) and ideally should be paired with at least one additional trap type to ensure taxonomically broad sampling. Gravid traps are an attractive option, in part because they effectively sample blood-fed mosquitoes (Reiter 1983), but logistic challenges associated with standardization and transport of the fetid water limit their usability within NEON. Resting box traps (Komar et al. 1995, 2011, Williams and Gingrich 2007, Burkett-Cadena et al. 2008) and BG-sentinel traps (Krockel et al. 2006, Meeraus et al. 2008) capture mosquitoes especially useful for pathogen testing at some sites. Therefore resting box and BG-sentinel traps may be implemented along with CDC CO_2 light traps to augment coverage of important vector species (see the TOS Science Design for Vectors and Pathogens (AD[04]) for more details).

Mosquitoes exhibit diel activity patterns; some species are most active during crepuscular periods while others are most active during the night (Silver 2008). Traps are typically set in the late afternoon, and allowed to run through the night until morning the following day. However, some mosquitoes are also day flyers (Hoel et al. 2009) and in order to maximize coverage of mosquito activity some daytime sampling will be conducted. NEON will trap during the night for comparison with other mosquito monitoring efforts and re-set the traps in the morning and trap during the day to sample the entire mosquito activity period and capture species that may be missed during evening sampling.

Mosquitoes exhibit strong seasonal abundance patterns. At many sites there may be months when mosquito activity is non-existent due to low temperatures. We will conduct spatially constrained low intensity sampling during the “off season” to monitor mosquito absence and will resume normal field season sampling upon the detection of mosquito presence (specific thresholds etc. described in section 6.2.2.5). Off season samples will be used to define the “shoulders” of the annual mosquito season at each site.

From 2010-2013, NEON prototyped CDC CO_2 light traps at 16 sites with broad geographical coverage (including sites in Massachusetts, New Hampshire, Michigan, North Dakota, Utah, Colorado, Virginia, Tennessee, Alabama and Florida). The methods detailed above were implemented in summer 2012 at the three sites in Domain 3, Jones Ecological Research Center, Ordway-Swisher Biological Station and Disney Wilderness Preserve. During the D3 prototype, mosquito traps were deployed for a total of 22 nights. On average, over 600 mosquitos were captured per trap and therefore 10 traps per site yielded over 6000 mosquitoes for a trapping night. Overall, a total of 141,892 mosquitoes from 43 different species were collected (Table 1).

Table 1. Data summary of prototype field mosquito collection from Domain 3 in the summer of 2012

Site	Total mosquito abundance	Number of sampling sessions conducted	Total number of trap nights	Plots per site	Average number of mosquitoes captured per trap	Average total abundance for one night of trapping at a site	Number of mosquito species recorded
Jones	30944	4	8	10	380	3868	22
Ordway	64390	4	8	8- 10	933	8049	26
Disney	46558	3	6	10	776	6651	31
Domain 3 total	**141892**	**11**	**22**		**682**	**6169**	**43**

Prototype sampling efforts have enabled the construction of a DNA barcode library, site-specific species lists (these lists will continue to be developed as part of characterization activities), and a test dataset to use for optimizing NEON's data ingest and quality control processes. In addition, logistical challenges associated with deploying traps in disparate environments have been addressed. Specific issues and lessons learned include:

- Light bulbs, which are included as part of the CO_2 traps were observed to result in a higher amount of bycatch (e.g., moths) compared to when lights were turned off. Because "cleaner" samples with less bycatch are much easier to process and result in better data quality, light bulbs will be disabled in the traps used in operations.
- Grazing livestock caused damage to traps at the Central Plains Experimental Range. To mitigate this damage, aluminum foil was wrapped around wires and battery leads. This technique will be useful at other grazed sites.
- At sites where trees are not available to hang mosquito traps (e.g. Central Plains Experimental Range, Sterling), NEON tested shepherd's hooks as an alternative structure from which to hang traps. Some models were found to be too thin and tended to break or bend or have too wide of a hook that the trap could swing off from. By trial and error, NEON identified a robust shepherd's hook that can accommodate mosquito traps at any sites where appropriate structures are not present.

6.2 Sample Processing

Minimal processing of mosquito samples will occur within each Domain lab; mosquitoes will be identified at external facilities. This approach will minimize identification errors due to the expertise needed to accurately identify mosquitos to species. NEON will also outsource all molecular, genetic, and pathogenic analyses of samples.

6.2.1 Sample Handling

After being live-trapped in the field, mosquitoes will be frozen, transferred to and stored in sample vials (dry) at -80°C until being sent to an identification and pathogen testing facility. These facilities will identify mosquitoes to species based on visual examination of external morphology. From among mosquitoes collected in each sampling event at each trap, either a set proportion or a fixed number of mosquitoes will be identified, based on catch rates during the first few years of operations. Initially, up to 200 mosquitoes will be identified and enumerated by species and sex from each trapping event. When more than 200 mosquitoes are collected, a representative subsample of ~200 individuals will be identified to estimate species composition. The uncounted proportion will be quantified to estimate abundance.

After identification, individuals of target species will be destructively tested for pathogens, as detailed in the TOS Science Design for Vectors and Pathogens (AD[04]). Ten individuals of each species will be removed before testing to serve as vouchers. These vouchers and all counted mosquitoes will be sent to an archive facility (AD[05]). The taxonomic identification of a subset of specimens will be checked via DNA barcoding to verify the stability of identifications over time and as a quality control measure.

6.2.2 DNA Barcoding

Identifying specimens using DNA barcoding requires a reference library. Prior to the start of formal sampling as many as possible of the mosquito species from each site will be collected to add to the DNA barcode library (Gibson et al. 2012). In every subsequent sampling year, up to 10 representative individuals of each species will be pointed, photographed and submitted for DNA barcoding (sequenced for the CO1 marker) (RD[05]). Mosquitoes that are rare, particularly difficult to identify or poorly represented in the archive will be prioritized for DNA barcoding. The specimens for this work are either field-collected during early sampling efforts or from museum archives. All assembled resources for each specimen - sequence data, photos, and other ecological information - can be accessed online. All of NEON's DNA-barcode data is freely available on the Barcode of Life Database (BOLD; http://www.barcodinglife.com/). These data contribute to the BOLD library are as a resource for the mosquito research community.

6.2.3 Archive

All identified specimens and any extracted DNA will be archived in existing facilities. Archiving plans, including details on accessing specimens for private investigation are detailed in the Fundamental Sentinel Unit Bioarchive Facility Design (AD[05]).

6.3 Spatial Distribution of Sampling

6.3.1 Selecting Plot Locations within Sites

Mosquito trapping plots will be chosen using a stratified random approach, maintaining stratification across dominant vegetation types (≥5% of total cover) at each site (AD[03]). Ten plots will be selected for long-term sampling such that the number of plots per vegetation type is proportional to the percent cover at the site. Plot locations will be constrained to fall within 30 m of roads in order to reduce travel time associated with each sampling event. Though constraining plot locations may reduce the statistical area of inference, the benefit of maintaining adequate sample size is deemed worth this cost. However, traps will be placed a minimum of 300 meters apart to maintain independence, even at the cost of reducing the total number of traps at small sites.

6.4 Temporal Distribution of Sampling

6.4.1 Sampling Session

A single session (called a "bout" in protocols) of mosquito sampling will consist of three CO_2 light trapping periods - two consecutive trapping nights and the intervening day (Figure 2) - at up to 10 plots per site (one trap per plot) during field sampling. This 40-hour sampling period will catch both day- and night-active mosquitoes and thus maximize community representation by covering the full spectrum of mosquito activity. Samples will collectively be used to characterize mosquito abundance, diversity and phenology at the site level. The three trapping events within a sample session will be kept separate to determine the unique species composition of day-time vs. night-time sampling. In addition, data at the resolution of a "trap night" is useful for comparison with other mosquito monitoring efforts.

Off-season sampling sessions will consist of one trap deployed at each of three distributed plots at the core site only for a single night (see details in section 6.4.4).

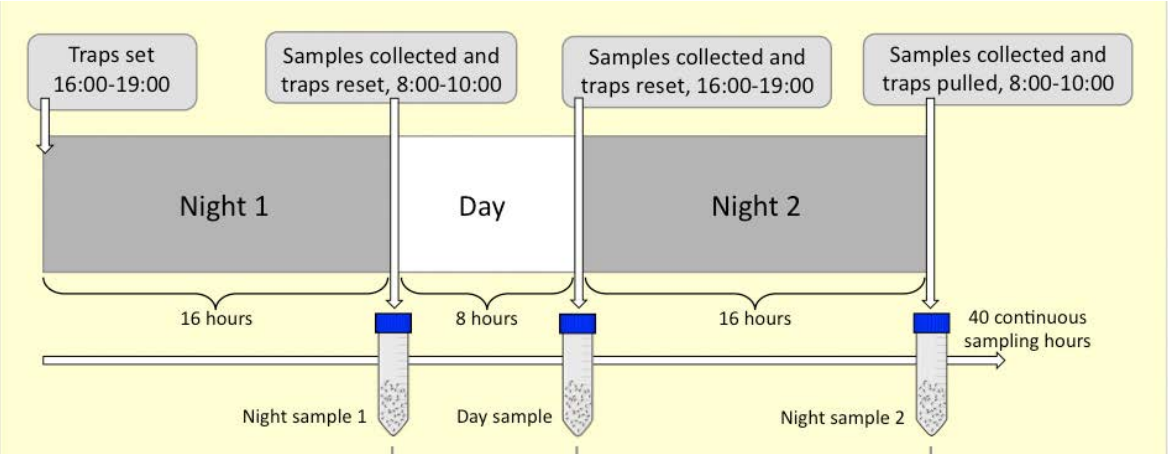

Figure 2. Field season sample session timing of mosquito trap deployment

Data will be reported at the trap level (i.e., each trap day/night by location within a site). In addition to mosquito abundance and diversity, mosquito phenology will be quantified. Phenology data (e.g., peak abundance, dormant time, first emergence) will be derived from abundance and diversity data collected during field sampling.

6.4.2 Temperature Threshold

Traps will only be deployed if the average daily high temperature for the previous 5 days was >4 C (Cossins and Bowler 1987). In cases where this criterion is not met, the presumed mosquito catch is zero and they will count as zero-catches for the purpose of triggering transitions between field season and off-season sampling. This threshold will be applied to both field season and off season sampling and if the threshold is not met the day prior to the first night of sampling during a sampling session, the entire sampling session will be canceled.

6.4.3 Field Season Sampling

Mosquitoes will be sampled using the same schedule at all domains where mosquitoes are present, irrespective of local density. During the field season, mosquito sampling sessions will occur every other week at the core site and every off week at one of the relocatable sites (alternating between the two relocatables, resulting in a sampling rate of every 4th week at each relocatable site) (Figure 3). According to this design, one site is sampled every week. The number of weeks in a field season varies among domains and the criteria for the beginning and end of the field season are detailed below (Section 6.2.2.5).

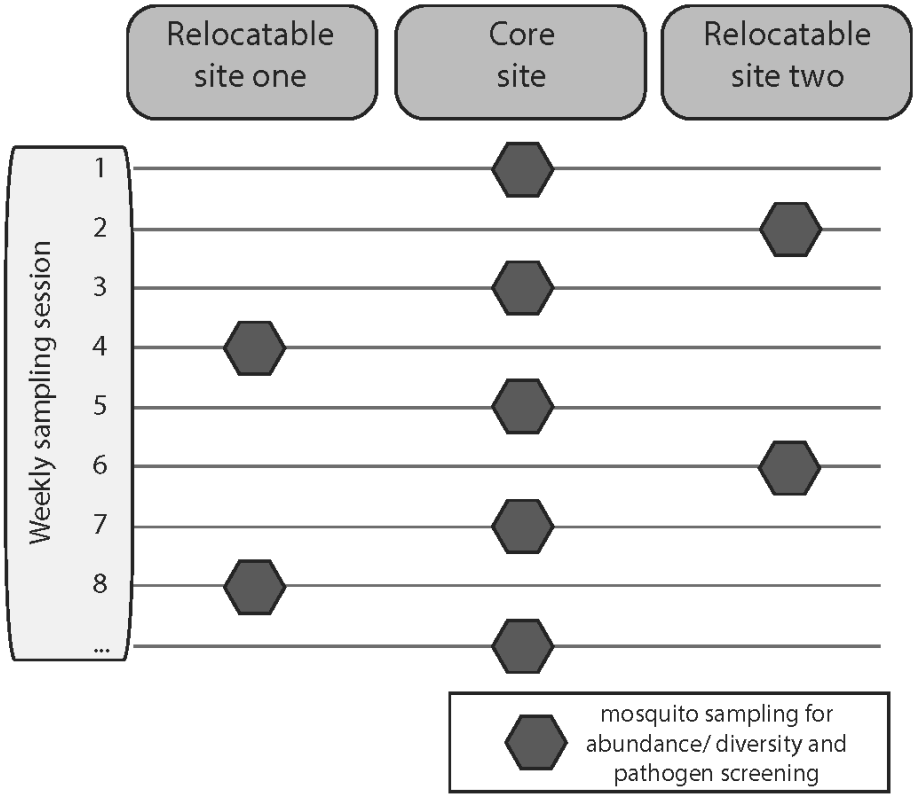

Figure 3. Field season mosquito sampling schedule for a representative domain. Sampling occurs at one site each week, alternating between the core and relocatable sites. The number of weeks in the field season varies among domains.

This mixed strategy of more focused attention to the core site prioritizes the creation of a long-term data set at core sites and will allow for the most efficient collection of mosquito data because the core site is usually much closer to the domain lab. In addition, the core site will be the indicator site for off-season sampling.

6.4.4 Seasonal Timing

Mosquitoes display seasonal abundance and activity patterns that vary among species and regions. Most of these patterns exhibit some general level of consistency. For example, at many NEON sites, mosquitoes are absent during part of each year where winter months are associated with unfavorable climatic conditions and mosquitoes re-emerge in early/mid spring. At seasonal sites “field season” mosquito sampling is unnecessary during the winter and will be suspended until mosquitoes become active in the spring. This strategy requires that sampling be stopped and restarted in parallel with seasonal mosquito activity patterns at a site, an endeavor complicated by the fact that the precise timing of these seasonal events can vary considerably among years. While logistically attractive, an approach that uses a fixed calendar date to determine when to stop and restart “field season” sampling

each year is unacceptable because it will frequently result in the start/end of sampling being mistimed because of interannual phenological variation. As an alternative, NEON will employ an "off-season" sampling plan distinct from those employed during field season sampling in order to track mosquito activity (Figure 4).

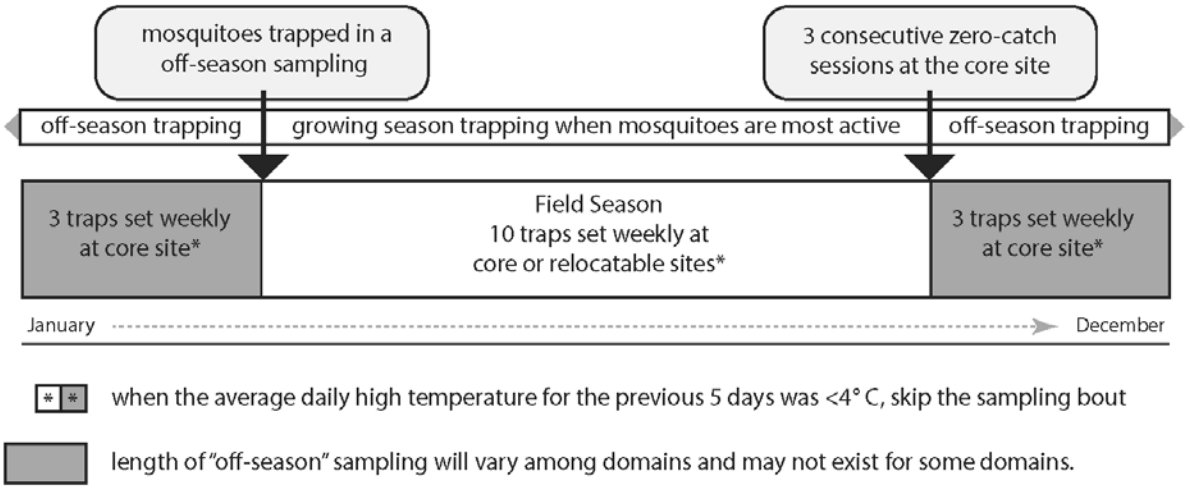

Figure 4. Annual mosquito sampling timeline for a representative domain. Trapping occurs all year at the core site, with more traps during the warm part of the year when mosquitoes are most active. During the field season, both the core and relocatable

Within a domain, the end of the regular sampling season will occur following three consecutive zero-catch sampling sessions at the core site (Figure 5). A field season zero-catch at the core site (no mosquitoes caught in all 10 traps) will trigger off-season sampling at the core site the following week (the intervening week before the next field season session at the core site). Note that field season sampling still occurs at the relocatable site during this intervening week. If the off-season sample is a zero-catch and the following sampling week at the core site is also a zero-catch (3 consecutive weeks of zero-catches at the core site, consisting of 2 field season sessions and 1 off season session), all field season sampling at the core and relocatable sites will stop and weekly off-season sampling at the core site will continue until a positive mosquito catch triggers the resumption of field season sampling (e.g., in the spring of the following year). Zero-catches at relocatables have no effect on the off-season/field-season transition.

Off-season sampling will continue once per week until at least one mosquito is collected. This will initiate the resumption of field season sampling (Figure 5), including both the core site (10 traps every 2 weeks) and relocatable sites (10 traps every 4 weeks).

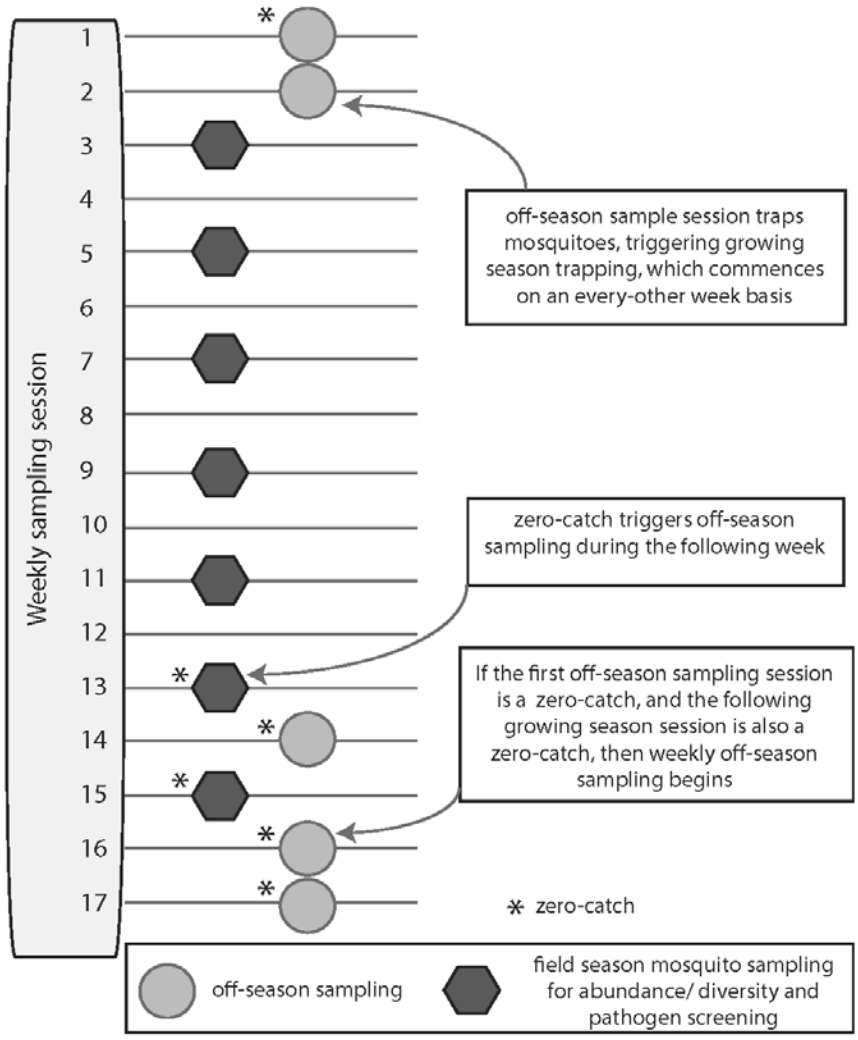

Figure 5. An example of mosquito sampling at a core site. The example begins with and ends with weekly off-season sampling and shows a brief field season. Sampling at relocatable sites is not shown but would occur in the intervening weeks (4-14).

6.5 Logistics and Adaptability

Once a set of long-term mosquito sampling plots is selected, NEON will generally maintain a fixed plot design. In some cases results may point to a plot being dropped in favor of sampling at a new and potentially more productive or informative location. Decisions about if and when to change the sampling design will be made in consultation with an expert review committee, the Assistant Director of Terrestrial Ecology, and Observatory Director.

7 REFERENCES

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