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SPECIAL FEATURE: NEON DESIGN

Design for mosquito abundance, diversity, and phenology sampling within the National Ecological Observatory Network

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Abstract. The National Ecological Observatory Network (NEON) intends to monitor mosquito populations across its broad geographical range of sites because of their prevalence in food webs, sensitivity to abiotic factors, and relevance for human health. We describe the design of mosquito population sampling in the context of NEON's long-term continental scale monitoring program, emphasizing the sampling design schedule, priorities, and collection methods. Freely available NEON data and associated field and laboratory samples, will increase our understanding of how mosquito abundance, demography, diversity, and phenology are responding to land use and climate change.

Key words: abundance; climate; Culicidae; diversity; global change; long-term monitoring; mosquito; phenology; Special Feature: NEON Design.

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Introduction

The National Ecological Observatory Network (NEON) is a continental-scale ecological observation platform being built to enhance understanding and forecasting of the ecological impacts of climate change, land use change, and invasive species. NEON is a collection of 60 sites distributed across the United States where standardized methods will be used for up to 30 years to collect data and samples of the physical and biological environment (Kao et al. 2012; A. Thorpe et al., *unpublished manuscript*). NEON is designed to enable users, including scientists, planners and policy makers, educators, and the general public, to address major questions in environmental sciences (NRC 2001, MEA 2005). NEON infrastructure and data are strategically aimed at those questions for which a coordinated national program of standardized observations is particularly effective. NEON's open access data and samples will enable users to map, understand, and predict the effects of human activities on ecosystems, and to understand and effectively address critical ecological questions. Detailed information on the overall NEON design can be found in the NEON Science Strategy document at www.neoninc.org.

Earth's environment is changing rapidly and NEON will provide essential data at the temporal and spatial scales to facilitate understanding, forecasts, and management of our changing biosphere (Keller et al. 2008, Schimel et al. 2011, Schimel and Keller 2015). NEON will collect data at 60 sites throughout the continental United States, Alaska, Hawaii, and Puerto Rico. For this purpose, NEON has delineated 20 ecoclimatic regions, termed domains (Hargrove and Hoffman 2004), that collectively span the range of climatic conditions and vegetative communities found within the NEON purview (Fig. 1). Each domain will include one core site (location fixed for 30 years) and two relocatable sites (location may be reassigned every 7–10 years over the 30-year lifespan of the Observatory). Core sites are/will be located in wild land areas to provide baseline measurements of the changing biotic and abiotic characteristics of associated domains, while the setting of relocatable sites may vary from wild lands to managed ecosystems. Site size varies considerably for both core (range from 11 km^2 to 214 km^2) and relocatable (5 km^2 to 50 km^2) sites. A broad array of measurements and samples will be collected at each site. The NEON Terrestrial Observation System (TOS) will complement other terrestrial as well as airborne and aquatic components of NEON (Kao et al. 2012; A. Thorpe et al., *unpublished manuscript*). Collocation of measurements associated with each component will facilitate the linking of data in cross-disciplinary analyses.

The NEON TOS will quantify the effects of climate change, land use, and biological invasions on terrestrial populations and processes by sampling key groups of organisms (sentinel taxa as well as causative agents of infectious disease) and biogeochemical cycling within air, land, and water systems (Kao et al. 2012; A. Thorpe et al., *unpublished manuscript*). Sentinel taxa were selected to include organisms with varying life spans and generation times, and wide geographic distributions, to allow 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 by sampling across major vegetation types in order to facilitate this inference when possible. Plot- and organism-scale measurements will also be coordinated with the larger scale airborne measurements, which will provide a set of synergistic biological data at the regional scale (Kampe et al. 2011). Details of these design elements and algorithms can be found in individual design documents available through the NEON website (www.neoninc.org). Among other objectives, NEON is charged with monitoring the responses of biodiversity and ecosystems to environmental change. Early in the conceptualization of NEON, a design committee (AIBSnews 2007) selected mosquitoes (Diptera: Culicidae) as a sentinel taxon for measurement.

Mosquitoes as a Sentinel Taxon

Mosquitoes are a diverse and widespread family of insects with aquatic larval and pupal forms and flying adults that have been extensively

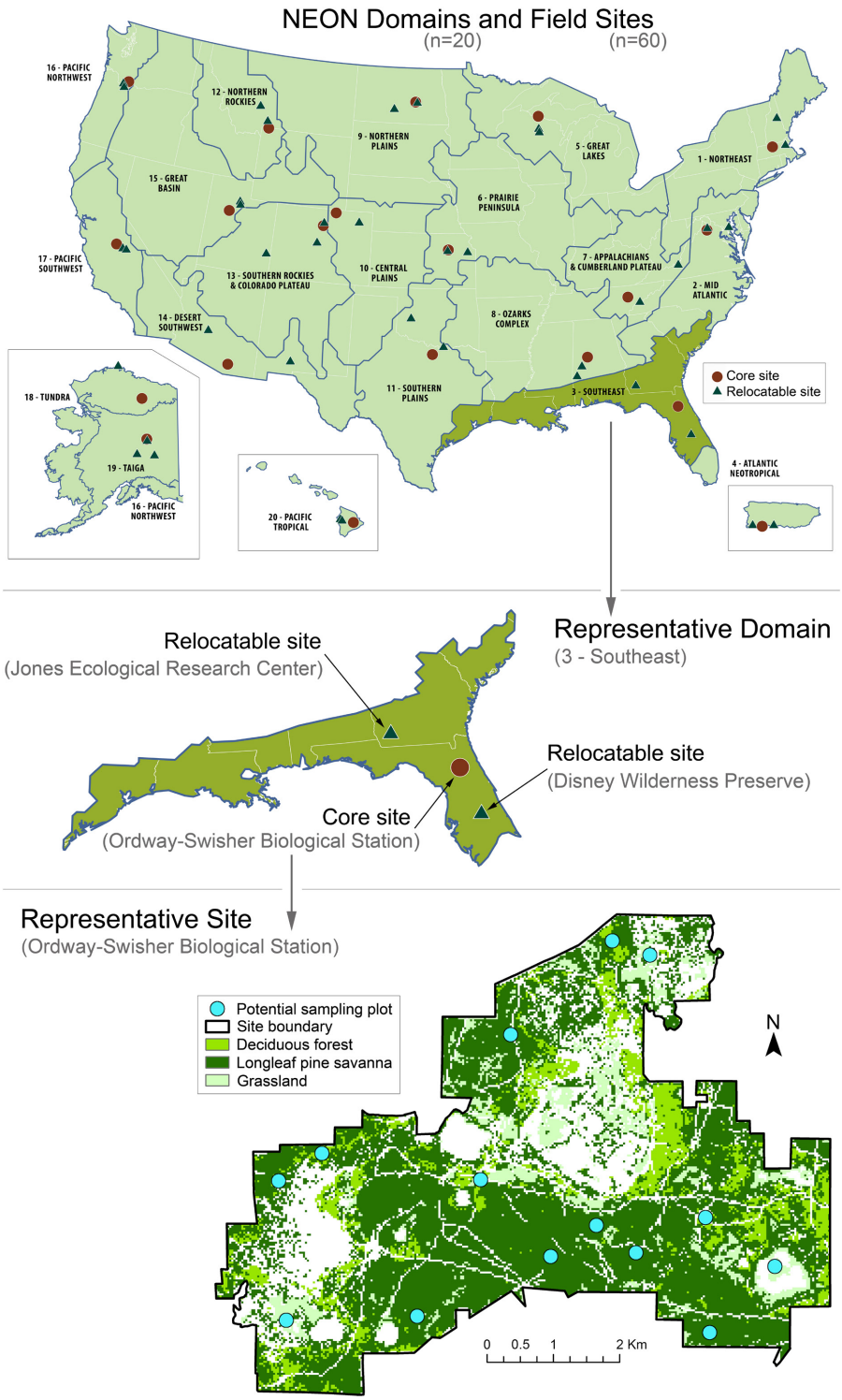

Fig. 1. Spatial hierarchy of NEON sampling scheme including 20 domains and 60 sites. A representative domain and its core and relocatable sites are highlighted as well as a representative site with potential sampling point locations spread across vegetation types. Reproduced with permission from Springer et al. 2015.

studied because of their ecological and epidemiological significance (Clements 1992, 1999, 2012, Service 1993, Spielman and D'Antonio 2001). As a dominant taxon in aquatic food webs, mosquitoes account for a sizable proportion of invertebrate biomass in aquatic systems and are a key food source for aquatic and terrestrial predators (e.g., fish, amphibians, spiders, birds). As a result, changes in mosquito populations (e.g., in response to land-use alteration or climate change) may have widespread impacts on ecosystems (Luck et al. 2003). Mosquitoes are excellent indicator species because they are sensitive to environmental variables such as temperature and precipitation (Gong et al. 2007, Morin and Comrie 2010). Even small changes in abiotic factors can influence development time and habitat availability with potentially cascading feedbacks on mosquito population density (Beck-Johnson et al. 2013).

Mosquitoes also are vectors for numerous parasites of humans, livestock, and wildlife. Mosquito-borne parasites will be measured by NEON, but are the subject of a different science design (Springer et al. 2015). Briefly, mosquitoes have been extensively studied to characterize and mitigate impacts of associated diseases. Most female mosquitoes take blood meals from vertebrates in order to provide protein for their developing eggs. Due to their potential impacts on human health, mosquito populations have and continue to be monitored and controlled by national, state, and local agencies. Mosquito-borne diseases can also influence the health of livestock [e.g., Eastern equine encephalitis (Kissling et al. 1954)] and wildlife populations [e.g., avian malaria (Van Riper et al. 1986), West Nile virus disease (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 such as seed dispersal, carrion scavenging, and insect regulation (LaDeau et al. 2008). As a compliment to the disease-related sampling objectives detailed in Springer et al. (2015), this manuscript focuses on measurements of mosquito abundance, diversity, and phenology that also motivate NEON mosquito sampling.

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 geographic distribution, demography, and seasonal phenology of mosquito species may be influenced by a variety of landscape-level drivers including climate, vegetation, and host availability (Buckner et al. 2010, Reisen 2010). Owing to their short generation times and high fecundity, mosquitoes generally 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. Variation in global climate is predicted to affect the distribution, demography, and seasonal phenology of many mosquitoes (Bradshaw et al. 2004, Morin and Comrie 2010, Beck-Johnson et al. 2013) and associated effects on disease transmission cycles have also been posited (Epstein et al. 1998). For example, as the climate warms, some mosquito populations are expanding their geographical ranges (Hongoh et al. 2011). Changing land use also could significantly affect mosquito species that are associated with humans or that thrive in human-modified environments. Mosquitoes are highly mobile and able to move into new areas as climatic conditions change, often aided by unintentional human transport (Lounibos 2002). Climate conditions influence not only mosquito distributions but also the life cycles and transmission of mosquito-borne pathogens (Gage et al. 2008). Higher temperatures below threshold levels can shorten the life cycle of both arboviruses 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 parasites they transmit into temperate areas.

Here, we define the rationale and design for NEON's mosquito abundance, diversity, and phenology sampling. Mosquito sampling protocols (available at www.neoninc.org) are based on this design and therefore understanding the priorities that underlie the design will inform the use of NEON data. 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 surveillance by public health and vector control programs, and will enhance assessment of changes in mosquito abundance, diversity, and phenology in response to changes in climate, land-use practices, and other ecosystem drivers.

General Sampling Design Framework

Priorities, challenges, and considerations for mosquito abundance, diversity, and phenology sampling

Standardized, well established, and widely used sampling methods were selected to maximize comparability across time and among NEON sites and to facilitate integration of NEON data with those gathered by other mosquito collection programs. The following criteria contributed to NEON's mosquito sampling design: (1) high efficacy across a range of environmental settings; (2) ability to be implemented in a standardized manner across numerous sites collectively spanning a wide range of biotic and abiotic conditions such that data are comparable across time and space; (3) relatively simple sampling methods that can be performed consistently by disparate field crews over multiple years with minimal need for or chance of alteration; and (4) wide acceptance and use by the research community to increase the comparability of NEON data.

Mosquito sampling will be conducted regularly during the period of the year when mosquitoes are active, which varies by domain. This approach will generate a time series of abundance and diversity data to calculate phenological metrics (e.g., first appearance, peak abundance) at relevant timescales. Core sites will be sampled more frequently than relocatable sites. 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). In addition, core sites are in wild land areas and will provide baseline data to assess how ecological systems are changing through time. More intense sampling at core sites where domain staffs are based will facilitate the efficient collection of mosquito data by reducing travel time and sampling costs. Less frequent collecting at relocatable sites will expand the spatial extent of sampling within each domain.

At each site, collocation of mosquito sampling with other measurements made by NEON is prioritized to facilitate comparisons of different patterns and processes. Mosquito sampling will occur within the same vegetation types where other organismal and abiotic measurements are taken, and when possible, be collocated within the same Distributed Plots. Ideally, mosquito sampling would occur within or close to the plots where NEON measurements of plants, soils, and/or other organisms are made. One example of integrated measurements within NEON is the coordination of mosquito abundance and diversity sampling with mosquito-borne parasite measurements (Springer et al. 2015). These sampling efforts have been combined to balance the trade-offs between them. Abundance and diversity sampling aims to survey a broad cross-section of the assemblage of mosquitoes present at a site, while sampling for parasites targets particular vector species and requires as many individuals as possible for testing. Thus, the two designs differ in their foci and objectives and would be optimized using different sampling strategies. However, coordinating mosquito abundance and diversity sampling with sampling for mosquito-borne parasites can produce attractive efficiencies, including saving considerable time and money when the same mosquito samples are counted and identified as well as tested for parasites. The sampling approach 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 infection by parasites (Springer et al. 2015). Additional sampling efforts (e.g., more sampling points, additional sampling methods) may be implemented where the number of samples collected for mosquito abundance and diversity sampling is insufficient to achieve adequate statistical power for mosquito-borne parasite sampling. See Springer et al. (2015) for more specific information about such design modifications.

Sample Collection

Sampling methods

Mosquitoes will be sampled using Centers for Disease Control and Prevention (CDC)-CO_2 light traps, a standard and widely used method used by the CDC and numerous academic researchers, public health practitioners, and vector control agencies to sample mosquitoes and mosquito-borne parasites (Sudia and Chamberlain 1962). Although there are many variations on this basic trap, all use CO_2 to attract mosquitoes because CO_2 is a component of vertebrate breath that female mosquitoes use to locate hosts. The CO_2 attracts mosquitoes to the vicinity of the trap

and a fan pulls mosquitoes into a mesh bag for live storage until the trap is collected.

In order to test sampling design and determine the necessity of modifications to increase effectiveness in a wide variety of ecosystems and conditions, NEON staffs have deployed CDC-CO_2 light traps at more than 20 sites across the United States, from arctic to subtropical. Several logistical challenges associated with deploying traps in disparate environments have been addressed. For example, light bulbs, which are included as part of the CO_2 traps can result in a higher amount of bycatch (e.g., moths) compared to when lights are 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 NEON sampling. Some investigators have found comparable, or even greater catches in CO_2-baited CDC traps when the bulbs were removed (Herbert et al. 1972, Reisen et al. 1983). When necessary, aluminum foil will be wrapped around wires and battery leads to mitigate damage from grazing animals. When trees or other structures are not present, traps will be hung from sturdy shepherd's hooks. In addition, prototype sampling efforts have enabled the construction of a DNA barcode library (more details below), site-specific species lists, and a test dataset to use for optimizing NEON's data input and quality control processes.

CDC-CO_2 light traps arguably collect the greatest mosquito diversity of all common traps and are regularly used in mosquito-borne parasite surveillance (Sudia and Chamberlain 1962, Meyer et al. 1991, Service 1993), which maximizes comparability with other data sets. However, they have some limitations and may not provide comprehensive representations of the mosquito community structure or relative abundance (e.g., all mosquito species are not equally attracted to CDC-CO_2 traps) (Silver 2008). They target mainly host-seeking female mosquitoes and undersample blood-fed and gravid portions of the population. Pairing these traps with at least one additional trap type could increase the taxonomic breadth of sampling. Gravid traps are an attractive supplemental option because they attract some recently blood-fed female mosquitoes that are in the process of digesting blood and developing eggs (Reiter 1983), but the range of mosquito species they attract is limited (primarily *Culex pipiens* complex). Other logistical challenges associated with standardization and transport of the fetid water for attracting ovipositing mosquitoes also may limit their suitability for 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 parasite testing at some sites. Therefore, resting box and BG-sentinel traps may be used with CDC-CO_2 light traps to augment coverage of important vector species (see Springer et al. 2015 for more details).

Spatial distribution: Selecting sampling locations within sites

Mosquito trapping points, called Mosquito Points, will be chosen using a stratified random approach, maintaining stratification across dominant vegetation types (≥5% of total cover, using National Land Cover Database categories) at each site (D. T. Barnett et al., *unpublished manuscript*). Ten trapping points will be selected for long-term sampling such that the number of points per vegetation type is proportional to the percent cover at the site. Point locations will be constrained to fall within 30 m of roads in order to reduce travel time associated with each sampling event. Though constraining point locations may reduce the size of the geographic area of inference, the benefit of maintaining adequate sample size is deemed worth the cost. However, to maintain trap independence, traps will be placed a minimum of 300 meters apart, even if this reduces the total number of traps at small sites. After initial establishment, the location of trapping points will remain fixed for the duration of NEON sampling.

Temporal distribution: Format and frequency of sampling events

Mosquitoes exhibit diel activity patterns; some species are most active during crepuscular periods while others are most active during the day or night (Silver 2008). Traps are typically set in the late afternoon, and allowed to run through the night until the morning of 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 two consecutive nights for comparison with other mosquito monitoring efforts (e.g.,

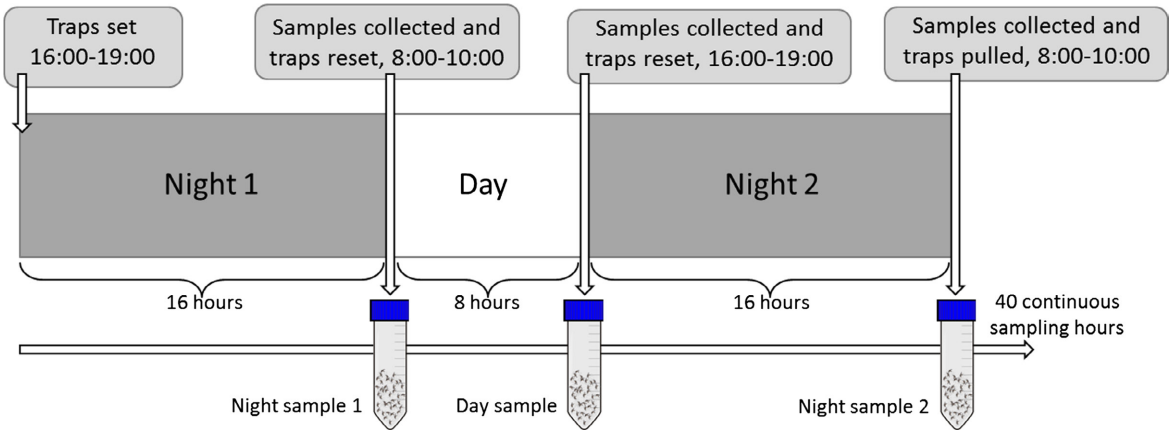

Fig. 2. Field season sample session, indicating timing of mosquito trap deployment and collection.

temporal data resolution at the "trap night" level) and also during the intervening day to sample the entire mosquito activity period and capture species that may be missed during overnight sampling. A single session of mosquito sampling will consist of three trapping periods—two consecutive trapping nights and the intervening day (Fig. 2). This 40-h sampling period will catch both day- and night-active mosquitoes, and thus maximize community representation by covering the full spectrum of mosquito activity. Mosquitoes will be sampled at the same frequency at all domains irrespective of local density. Sampling sessions will occur every other week at each core site and every off week at one of the relocatable sites (alternating between the two relocatable sites, resulting in a sampling rate of every fourth week at each relocatable site, Fig. 3). According to this design, one site is sampled every week in each domain.

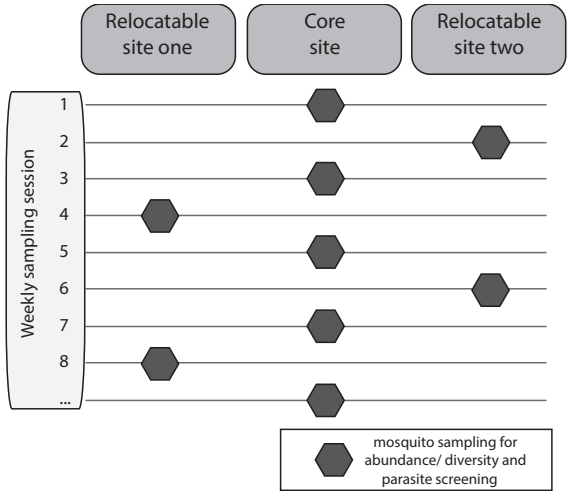

Fig. 3. Field season mosquito sampling schedule for a representative domain. Sampling occurs at one site each week, alternating between the core and relocatable sites. The number of weeks in the field season varies among domains.

Temporal distribution: Field season vs. off-season sampling

Mosquitoes display seasonal abundance and activity patterns that vary among species and regions. At many NEON sites, there may be months when adult mosquitoes are not present or active due to low temperatures. During these periods (e.g., late fall through early spring at higher latitude sites), NEON mosquito sampling can be discontinued. 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 sites and among years within sites. 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 could frequently result in the start/end of sampling being mistimed because of interannual phenological variation. When mosquitoes are not active, rather than "field sampling" as described in the above section, NEON will employ

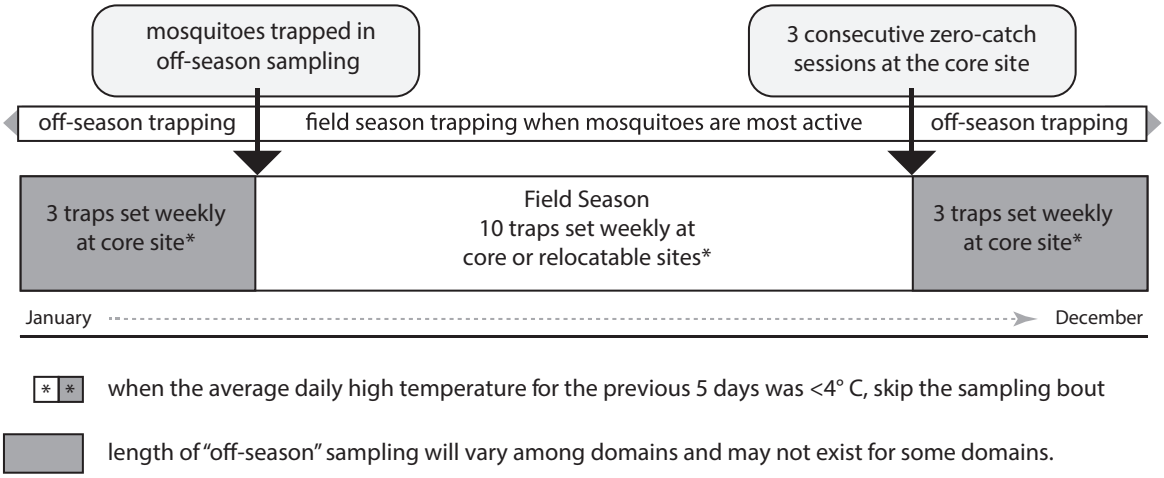

Fig. 4. Annual mosquito sampling timeline for a representative domain. Trapping occurs all year at the core site, with more traps during the field season (the warm part of the year when mosquitoes are most active).

"off-season" sampling to detect the resumption of mosquito activity (Fig. 4). Off-season sampling is spatially constrained low-intensity sampling to verify mosquito inactivity during the off season. 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 per week (see details below). Field season sampling will resume when flying mosquitoes are detected. Off-season samples will be used to define the "shoulders" of the annual mosquito season at each site. 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.

Within a domain, the end of the field season will occur following three consecutive zero-catch sampling sessions at the core site (Fig. 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 sampling at the core site). Note that field season sampling would still occur at the relocatable site during that 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 (three consecutive weeks of zero-catches at the core site, consisting of two field season, and one off-season sampling session), field season sampling at all three sites within the domain will stop. At this point, weekly off-season sampling at the core site will continue until a positive mosquito catch (e.g., in the spring of the following year). Neither catches nor zero-catches at relocatable sites have any effect on the

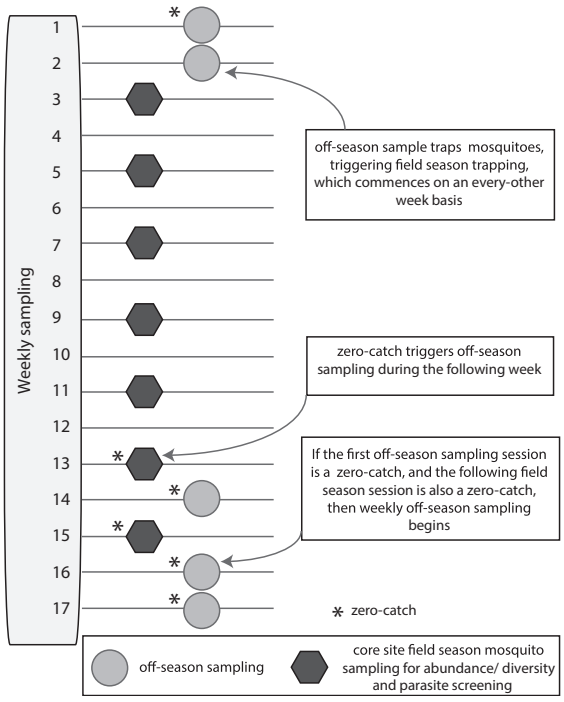

Fig. 5. An example schedule of mosquito sampling at a core site. The example begins with and ends with weekly off-season sampling at the core site and shows a brief field season. Sampling at relocatable sites is not shown but would occur in the even intervening weeks (4–14).

off-season/field-season transition. Though the core site may not necessarily be representative of relocatable sites in each domain, this aspect of the design is driven by logistical and financial constraints as opposed to ecological considerations. When at least one mosquito is collected during off-season sampling at the core site, field season sampling will resume (Fig. 5) at both the core site (10 traps every 2 weeks) and relocatable sites (up to 10 traps every 4 weeks).

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 for triggering transitions between field season and off-season sampling. This threshold will apply 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.

Sample Processing

Taxonomic identification based on morphology

Minimal processing of mosquito samples by NEON field technicians will occur within each domain laboratory. NEON will outsource all molecular, genetic, and pathogenic analyses of samples. After being live-trapped, mosquitoes will be either frozen in the field or immediately upon arrival at the domain laboratory. Frozen mosquitoes will be transferred into labeled cryovials, stored at −80°C and shipped on dry ice to external facilities for taxonomic identification. Samples generated from the two nights and one day of trapping within a sample session will be kept separate to determine the unique species composition of day-time vs. night-time sampling. Mosquitoes will be identified to species based on visual examination of external morphology. Up to 200 mosquitoes will be identified and enumerated by species and sex from each trap collection. When more than 200 mosquitoes are collected, a random subsample of ~200 individuals will be identified to estimate species composition and the abundance of the remaining mosquitoes.

Parasite testing and archiving

After identification and enumeration at the trap level, samples from the same sample session at each site will be pooled by species for parasite testing and archiving. Individuals of target species will be destructively tested for parasites (as detailed in Springer et al. 2015). Prior to selecting mosquitoes for parasite testing, 10 individuals of each species per domain per year will be removed from samples and pointed to serve as vouchers. All identified mosquitoes not selected for pathogen testing or voucher preparation will be sent to an archive facility. If resources permit, any remaining unidentified mosquitoes will also be archived. Samples will remain frozen throughout sample processing and storage so that the quality of mosquito samples for mosquito-borne parasite testing is not compromised.

DNA Sequence Identification Methods

DNA barcoding (sequencing of the CO1 marker) will be used to verify the consistency of species identifications over time and as a quality control measure on data from taxonomic identification facilities. Identifying specimens using DNA sequencing requires a reference library. Prior to the start of formal sampling, as many mosquito species as possible from each NEON site will be collected and sequenced to expand the library (Gibson et al. 2012). The specimens for this work are either field-collected during early sampling efforts or from museum archives. In every subsequent sampling year, up to 10 representative individuals of each species collected in every domain will be pointed, photographed, and submitted for DNA sequencing. Species of mosquitoes that are locally rare, particularly difficult to identify or poorly represented in the archive will be prioritized for DNA barcoding. Pointed specimens, each missing a leg that was removed for a tissue sample, will be archived at museum facilities and available for loan. All assembled resources for each specimen—sequence data, photos, and other ecological information—will be accessible online. All of NEON's DNA-barcode data are freely available on the Barcode of Life Database (BOLD; http://www.barcodinglife.com/). These data contribute to the BOLD library and are a resource for the research community.

Data Reporting

Mosquito samples will be used collectively to characterize mosquito abundance, diversity,

and phenology at the site level. Each trapping event will generate a trapping report that includes the locations, times, and dates when traps were set/collected at each site and all associated field metadata. Abundance data will be reported by species and sex at the trap level for each trapping event (i.e., three events per sample session). Diversity metrics will be reported at the trap and site level for each sampling session. In addition, species-level mosquito phenology data (e.g., first detection, peak abundance, senescence, dormant time) will be derived from abundance and diversity data collected during field sampling. Summary data at larger temporal and spatial scales will also be available as well as sampling locations, surrounding vegetation type, and associated metadata. Testing of mosquitoes for parasites will result in additional data as described in Springer et al. (2015). All NEON data will be freely available via an online portal (data.neoninc.org). NEON will apply quality assurance and control algorithms on all data before posting to the portal and will report associated error metrics with raw and processed data.

The following data generated by mosquito sampling will be made available through the NEON online data portal:

(1) At the spatiotemporal scales of the sampling event (a single trap, either day or night at a particular trapping point):
   - Mosquito abundance: the numbers of mosquitoes collected, by species/sex combination
   - Sampling effort: the duration of trap deployment in hours/minutes
   - Mosquito diversity: Shannon and Simpson diversity indices
(2) At the spatiotemporal scale of the entire site/domain and season:
   - Mosquito abundance: the number of mosquitoes collected, by species/sex combination
   - Mosquito diversity: Shannon and Simpson diversity indices and species occurrence lists
   - Mosquito phenology metrics (e.g., first detection, peak abundance)
(3) The type(s), number(s), and availability of archived samples. These will include: pointed type specimens and whole untested mosquitoes.

A subset of identified mosquitoes will also be tested for parasites as detailed in Springer et al. (2015).

Opportunities

Given NEON's open-access data policy, this mosquito sampling design should enable the testing of a variety of hypotheses and broad spatiotemporal comparisons within and among sites. The large number and unknown nature of these potential questions complicates decisions regarding sampling effort. In general, the proposed sampling design reflects a balance between resource availability within the NEON project and local logistical constraints at each field site. Data generated from this design can be used along with other NEON data or combined with independent research by the scientific community. Below are a number of potential uses for NEON mosquito data.

(1) Due to the wide variety of data collected at each NEON site, NEON data on mosquito populations (e.g., local abundance and diversity) can be related to landscape factors and abiotic conditions. Data from 60 NEON sites across North America broaden the spatial extent of these relationships and allow the effects of factors like drought indices, soil moisture, and rain events on mosquito populations to be quantified across a broad geographical area. In addition to a broad spatial extent, NEON sampling will continue for at least 30 years, enabling detection of potential longer term effects of phenomenon like the El Niño-Southern Oscillation, climate change, and habitat succession on mosquito abundance and diversity. Such long-term sampling will provide time-series datasets for modeling relationships between mosquito ecology and a range of environmental factors.
(2) While covering broad spatial and temporal scales, NEON data on mosquito populations will be recorded to the species level. This will enable tracking of the spatial distribution

(e.g., range expansion or other distributional changes) and seasonality of mosquito species of interest. For example, the spread and arrival of invasive species could be detected.

(3) In addition to species-level identification, mosquito populations will be sampled frequently throughout the growing season, producing fine scale time series data allowing for changes in phenology within sites or across landscape-scale gradients to be quantified. Due to the short generation times of mosquitoes, even small shifts in emergence times could have large implications for demographics, abundance, and disease transmission.

(4) In addition to data on mosquito abundance, diversity, and phenology, mosquitoes will be screened for parasites (Springer et al. 2015), illuminating the relationships between mosquito ecology (e.g., local density, phenology) and the dynamics of mosquito-borne parasites. In combination with other NEON data (e.g., bird surveys at each site), connections between the population dynamics of mosquitoes and their hosts could be elucidated. This may in turn help to explain changes in zoonotic transmission ecology. For example, NEON data could be used to compare mosquito abundance and phenology with the nesting, migration phenology, and abundance of their avian enzootic host populations.

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Literature Cited

AIBSnews. 2007. NEON design 2007. BioScience 57:198–200.

Beck-Johnson, L. M., W. A. Nelson, K. P. Paaijmans, A. F. Read, M. B. Thomas and O. N. Bjørnstad. 2013. The effect of temperature on Anopheles mosquito population dynamics and the potential for malaria transmission. PLoS ONE 8. http://dx.doi.org/10.1371/journal.pone.0079276

Bradshaw, W. E., P. A. Zani, and C. M. Holzapfel. 2004. Adaptation to temperate climates. Evolution 58:1748–1762.

Buckner, E. A., M. S. Blackmore, S. W. Golladay, and A. P. Covich. 2010. Weather and landscape factors associated with adult mosquito abundance in southwestern Georgia, U.S.A. Journal of Vector Ecology 36:269–278.

Burkett-Cadena, N. D., M. D. Eubanks, and T. R. Unnasch. 2008. Preference of female mosquitoes for natural and artificial resting sites. Journal of the American Mosquito Control Association 24:228–235.

Clements, A. N. 1992. The biology of mosquitoes. Vol. I. Development, nutrition and reproduction. CAB International, Wallingford, UK.

Clements, A. N. 1999. The biology of mosquitoes. Vol. 2. Sensory reception and behaviour. CAB International, Wallingford, UK.

Clements, A. N. 2012. The Biology of mosquitoes, Volume 3 transmission of viruses and interactions with bacteria. CAB International, Wallingford, UK.

Cossins, A. R., and K. Bowler. 1987. Temperature biology of animals. Chapman & Hall, New York, New York, USA.

Epstein, P. R., H. F. Diaz, S. Elias, G. Grabherr, N. E. Graham, W. J. M. Martens, E. Mosley-Thompson, and J. Susskind. 1998. Biological and physical signs of climate change: focus on mosquito-borne diseases. Bulletin of the American Meteorological Society 79:409–417.

Gage, K. L., T. R. Burkot, R. J. Eisen, and E. B. Hayes. 2008. Climate and vectorborne diseases. American Journal of Preventive Medicine 35:436–450.

Gibson, C. M., R. H. Kao, K. K. Blevins, and P. D. Travers. 2012. Integrative taxonomy for continental-scale terrestrial insect observations. PLoS ONE 7:e37528.

Gong, H., A. Degaetano and L. C. Harrington. 2007. A climate based mosquito population model. Proceedings of the World Congress on Engineering and Computer Science, San Francisco, USA, October 24–27, 2007. http://www.iaeng.org/publication/WCECS2007/WCECS2007_pp673-676.pdf.

Hargrove, W. W., and F. M. Hoffman. 2004. Potential of multivariate quantitative methods for delineation and visualization of ecoregions. Environmental Management 34:S39–S60.

Herbert, E. W., R. P. Meyer, and P. G. Turbes. 1972. A comparison of mosquito catches with CDC light traps and CO2-baited traps in the Republic of Vietnam. Mosquito News 32:212–214.

Hoel, D. F., D. L. Kline, and S. A. Allan. 2009. Evaluation of six mosquito traps for collection of *Aedes albopictus* and associated mosquito species in a suburban setting in north central Florida. Journal Of The American Mosquito Control Association 25:47–57.

Hongoh, V., L. Berrang-Ford, M. E. Scott, and L. R. Lindsay. 2011. Expanding geographical distribution of the mosquito, *Culex pipiens*, in Canada under climate change. Applied Geography 33:53–62.

Kampe, T. U., J. McCorkel, L. Hamlin, R. O. Green, K. S. Krause and B. R. Johnson. 2011. Progress in the development of airborne remote sensing instrumentation for the National Ecological Observatory Network. Page 81560A *in* W. Gao, T. J. Jackson, J. Wang, and N.-B. Chang, editors. Proceedings of the society of photographic instrumentation engineers, San Diego, California, September 15, 2011. International Society for Optics and Photonics. http://dx.doi.org/10.1117/12.892572

Kao, R. H., et al. 2012. NEON terrestrial field observations: designing continental-scale, standardized sampling. Ecosphere 3:1–17.

Keller, M., D. S. Schimel, W. W. Hargrove, and F. M. Hoffman. 2008. A continental strategy for the national ecological observatory network. Frontiers in Ecology and the Environment 6:282–284.

Kissling, R. E., R. W. Chamberlain, M. E. Eidson, R. K. Sikes, and M. A. Bucca. 1954. Studies on the North American arthropod-borne encephalitides. 2. Eastern Equine Encephalites in Horses. American Journal of Hygiene 60:237–250.

Komar, N., R. J. Pollack, and A. Spielman. 1995. A nestble fiber pot for sampling resting mosquitoes. Journal of the American Mosquito Control Association 11:463–467.

Komar, N., B. J. Biggerstaff, N. A. Panella and R. J. Kent Crockett. 2011. The Centers for Disease Control and Prevention Resting Trap: A Novel Device for Collecting Resting Mosquitoes.

Krockel, U., A. Rose, A. E. Eiras, and M. Geier. 2006. New tools for surveillance of adult yellow fever mosquitoes: comparison of trap catches with human landing rates in an urban environment. Journal of the American Mosquito Control Association 22:229–238.

LaDeau, S. L., A. M. Kilpatrick, and P. P. Marra. 2007. West Nile virus emergence and large-scale declines of North American bird populations. Nature 447:710–713.

LaDeau, S. L., P. P. Marra, A. M. Kilpatrick, and C. A. Calder. 2008. West nile virus revisited: consequences for North American Ecology. BioScience 58:937–946.

Lounibos, L. P. 2002. Invations by insect vectors of human disease. Annual Review of Entomology 47:233–266.

Luck, G. W., G. C. Daily, and P. R. Ehrlich. 2003. Population diversity and ecosystem services. Trends in Ecology & Evolution 18:331–336.

Marra, P. P., S. Griffing, C. Caffrey, A. M. Kilpatrick, R. McLean, C. Brand, E. M. I. Saito, A. P. Dupuis, L. Kramer, and R. Novak. 2004. West nile virus and wildlife. BioScience 54:393–402.

MEA. 2005. Ecosystems and human well-being?: Current State and Trends / Millennium Ecosystem Assessment. Page 155 *in* R. Hassan, R. Scholes, and N. Ash, editors. World health. Island Press, Washington, District of Columbia, USA.

Meeraus, W. H., J. S. Armistead, and J. R. Arias. 2008. Field comparison of novel and gold standard traps for collecting *Aedes albopictus* in Northern Virginia. Journal of the American Mosquito Control Association 24:244–248.

Meyer, R. P., W. K. Reisen, and M. M. Milby. 1991. Influence of vegetation on carbon dioxide trap effectiveness for sampling mosquitoes in the Sierra Nevada foothills of Kern County, California. Journal of the American Mosquito Control Association 7:471–475.

Morin, C. W., and A. C. Comrie. 2010. Modeled response of the West Nile virus vector *Culex quinquefasciatus* to changing climate using the dynamic mosquito simulation model. International Journal of Biometeorology 54:517–529.

NRC. 2001. Grand challenges in environmental sciences. National Academies Press, Washington, D.C., USA.

Reisen, W. K. 2010. Landscape epidemiology of vector-borne diseases. Annual Review of Entomology 55:461–483.

Reisen, W. K., M. M. Milby, W. C. Reeves, R. P. Meyer, and M. E. Bock. 1983. Population ecology of *Culex*

tarsalis (Diptera: Culicidae) in a foothill environment of Kern county, California: temporal changes in female relative abundance, reproductive status, and survivorship. Annals of the Entomological Society of America 76:800–808.

Reiter, P. 1983. A portable, battery-powered trap for collecting gravid culex mosquitoes. Mosquito News 43:496–498.

Schimel, D. and M. Keller. 2015. Big questions, big science: meeting the challenges of global ecology. Oecologia 177:925–934.

Schimel, D., M. Keller, S. Berukoff, R. Kao, H. Loescher, H. Powell, T. Kampe, D. Moore and W. Gram. 2011. 2011 Science Strategy: enabling continental-scale ecological forcasting. NEON.

Service, M. W. 1993. Mosquito ecology, field sampling methods. Elsevier Applied Science, New York, New York, USA.

Silver, J. B. 2008. Mosquito ecology. Springer Netherlands, Dordrecht.

Spielman, A. and M. D'Antonio. 2001. Mosquito. A natural history of our most persistent and deadly foe. Hyperion, New York, New York, USA.

Springer, Y. P., et al. 2015. Continental-scale surveillance of infectious agents: Tick-, mosquito-, and rodent-borne parasite sampling designs for the National Ecological Observatory Network (NEON). Ecosphere 7. http://dx.doi.org/10.1002/ecs2.1271

Sudia, W. D. and R. W. Chamberlain. 1962. Battery-operated light trap, an improved model. Mosquito New 1:126–129.

Van Riper, C. III, S. G. Van Riper, M. L. Goff and M. Laird. 1986. The epizootiology and ecological significance of malaria in hawaiian land birds. Ecological Monographs 56:327–344.

Williams, G. M., and J. B. Gingrich. 2007. Comparison of light traps, gravid traps, and resting boxes for West Nile virus surveillance. Journal of Vector Ecology: Journal of the Society for Vector Ecology 32:285–291.