Motivation

- Sampling of pathogen and vector species will provide insights into the changing ecology of a variety of tick-, mosquito-, and rodent-borne pathogens.

- The presence and abundance of vectors and associated pathogen species will be measured at as many as 60 sites for up to 30 years. A subset of samples will be tested for pathogens and archived for future analyses.

- The study designs have two principle objectives:
  1. Detect long-term changes in the seasonal mean or maximum of vector abundance or pathogen prevalence (right, upper)
  2. Characterize changes in aspects of the annual/seasonal cycles of vectors and pathogens (e.g., timing of seasonal onset and maximum, duration of cycle) (right, lower)

Ticks and tick-borne pathogens

- **Foci**: hard ticks (family Ixodidae). Six species are of particular interest: black-legged tick (*Ixodes scapularis*), western black-legged tick (*I. pacificus*), lone star tick (*Amblyomma americanum*), gulf coast tick (*A. maculatum*), Rocky Mountain wood tick (*Dermacentor andersoni*), and American dog tick (*D. variabilis*).

- **Field method(s)**: drag sampling at six plots per site. Adult, nymphal, and larval stages will be collected.

- **Schedule**: one sampling event at each site every 3-6 weeks annually between March and December.

- **Pathogen testing**: “next-gen” sequencing using 16S primers to detect bacterial pathogens (e.g., genera *Borrelia*, *Anaplasma*, *Francisella*, *Ehrlichia*, *Rickettsia*).
Mosquitoes and mosquito-borne pathogens

• **Foci:** utilize samples collected as part of mosquito abundance/diversity/phenology sampling (all species in family Culicidae). Pathogen testing will involve a variety of species, especially those in the genera *Culex* and *Aedes*

• **Field method(s):** each sampling event involves deploying a CDC CO₂ light trap for 40 hours (two nights and intervening day) at 10 plots per site

• **Schedule:** one sampling event every two weeks at core sites and every four weeks at relocatable sites year round when mosquitoes are present

• **Pathogen testing:** general screening to detect alphaviruses, bunyaviruses, and flaviviruses, with follow-up tests on virus-positive pools to identify pathogens

Rodents and rodent-borne pathogens

• **Foci:** utilize rodents in the family Cricetidae collected as part of small mammal abundance and diversity sampling. Pathogen testing will involve a variety of species, especially those in the genus *Peromyscus*

• **Field method(s):** each sampling event involves mark/recapture sampling using grids of 100 Sherman live traps deployed for three consecutive nights at 3-10 plots per site. Blood samples will be collected from Cricetid rodents using the retro-orbital or submandibular bleeding method

• **Schedule:** one sampling event every month at core sites and every other month at relocatable sites year round when sampling can be safely conducted

• **Pathogen testing:** ELISA-based testing to detect antibodies to hantaviruses and potentially arenaviruses

**Learn more**
The full science design will be available soon at [http://communities.neoninc.org/x/m4E-/](http://communities.neoninc.org/x/m4E-/)
Sampling protocols will be available soon through the NEON website at [www.neoninc.org](http://www.neoninc.org)