Mosquito and Ground Beetle sampling plan

Both ground beetles and mosquitoes will be sampled at distributed plots, which are spread across vegetation types at every NEON site.

**Ground Beetle Sampling Goals**
- Ground beetle diversity sampling will be used to help us understand how the distribution of ground beetles changes in different habitats and ecosystems over time.
- Ground beetle species are found in each of the NEON domains, are easy to sample, and changes in their numbers can indicate significant changes in the local ecological community.

**Key Aspects of Ground Beetle Sampling Design**
- NEON will conduct biweekly sampling over the course of the growing season (roughly May – October)
- NEON will collect ground beetle specimens using 4 pitfall traps at each of 10 distributed plots per site (40 traps)
- Each sampling bout occurs for 14 consecutive days and requires a pair of field technicians to access plots on foot
- Pitfall cups are buried flush in the ground and shaded by flat covers to keep out sun, rain and vertebrates

**How Does Pitfall Sampling Work?**
Pitfalls will be deployed to capture beetles for sampling. To set up a pitfall trap, a field technician digs a small, shallow pit (approximately 7 cm deep and 11 cm wide). Two shallow bowls are nested within one another into the pit. The bowls are filled with preservative and covered with a 20 cm-wide cover that is nailed into the ground. There is a small gap between the cover and the ground for insects to enter the trap. Insects that fall into the trap are euthanized and preserved.
Mosquito Sampling

Mosquito sampling will focus on how the diversity of mosquito communities, and the demography and phenology of associated populations, will change in different habitats and ecosystems over time. Mosquitoes are easy to sample and their populations are sensitive to environmental change. Mosquitoes are found in each of the NEON domains.

Key Aspects of Mosquito Sampling

• NEON will conduct mosquito sampling every two weeks at core sites and every four weeks at relocatable sites during the period of the year when mosquitoes are actively flying. A system of off-season sampling will be implemented during the remaining months to detect mosquito emergence.

• At each site, sampling will take place at 10 points, each located within 30m or a road.

• During a sampling bout, field technicians will set one CO₂ light trap at each plot. Traps will be deployed for two nights and the intervening day and samples retrieved the morning after the night they were set or the evening of the morning they were set.

How Does CO₂ Light Trap Sampling Work?

CO₂ light trap sampling involves using CO₂ emitting traps to attract and capture mosquitoes. A trap is made of a small insulated cooler, a rain cover, a fan and light assembly (lights will not be used) and a catch cup. The insulated cooler is loaded with solid carbon dioxide pellets (also called dry ice). Carbon dioxide is a byproduct of animal respiration that attracts mosquitoes. The trap is hung from a tree, post or other structure. Once the trap is deployed, mosquitoes are drawn into the trap’s catch cup by a fan. After the technician collects samples from the trap, the mosquitoes are identified to species and some are tested for pathogens.

DNA barcoding

A subset of all the ground beetles and mosquitoes collected at each site will be submitted for DNA barcoding each year. DNA barcoding is a taxonomic method that uses a short genetic marker (mitochondrial gene, COI) to identify species. NEON is using DNA barcoding to establish a library of ground beetles and mosquitoes at our sites to aid in specimen identification. Along with expert morphological identifications, this will ensure consistency of identifications across many technicians at different sites for 30 years of data collection. All of NEON’s barcode data are publicly available.