HOW TO SAMPLE MOTH DIVERSITY EFFICIENTLY IN A SEASONAL ENVIRONMENT

BY

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Abstract — Here we consider how best to sample moth diversity in a seasonal environment by analyzing nightly data collected from 2011 to 2015 at a site in Clarke County, Georgia (latitude 33.8882°N, longitude 83.2973°W). We resample these nightly data, mining them to evaluate the effectiveness of sampling regimes based on lunar phase versus selected nights of the week, such as each weekend. We also show what proportion of taxa are not detected if particular months are not sampled. Overall the 1,825 nightly samples had 1,256 lepidopteran taxa. Resampling only nights of new moons (i.e., 13 samples/year; 3.6% of nightly effort), the most productive single night of the lunar cycle, yielded 600 taxa (48% of total). The least productive night of the lunar cycle, just before the full moon, yielded 455 taxa (36%). Resampling one night per week (i.e., 52 samples/year; 14% of nightly effort) yielded an average across the week of 876 taxa (70% of total), approximately the same as the 870 taxa recorded in an equivalent effort of 4-nights of sampling around each new moon. Excluding the four least diverse winter months and resampling nightly March through October yielded 1,243 taxa (99%). The most diverse month, May, had 760 taxa (61%). To standardize effort and facilitate cross-site comparisons, we recommend that participants sample on each new moon and if time and resources permit, on Saturday and/or Sunday morning.

Introduction — In partnership with John Douglass and the SLS, Discover Life's *Mothing* project (www.discoverlife.org/moth) is building a network of study sites to compare how moth communities differ geographically and respond to environmental factors, such as the local flora, weather conditions, and urbanization. This article is the third in a series of SLN articles in support of this joint venture. In the first, Pickering (2015) gave an overview of the project and its broader objectives, inviting SLS members to participate in various ways. In the second, Pickering (2016) presented nightly data on seasonal moth activity and considered how '*pupa banks*' and '*aposematic vs. cryptic coloration*' might help explain results. Here we present results on the diversity of lepidopterans across five years of nightly samples from a site in Georgia. We resample these data to compare the efficiency of different sampling regimes in terms of the number of taxa that we detected. At the SLS's annual meeting this coming October, we plan to give a training workshop to help participants establish and run sites.

Inventories

Inventorying the entire species richness of lepidopterans at a site is a difficult if not endless task, in part because the vagaries of hurricanes and lesser winds will continue to blow in rare, nonresidents from afar. Our species accumulation curve in Clarke County, Georgia, continues to increase after over six years and 2,100 nights of sampling lights (Pickering 2015, Fig. 3). As of May 2016, we have accumulated a total of 1,275 taxa. This total includes *Sphacelodes vulneraria* (Geometridae) and *Syngamia florella* (Crambidae), two species which we recorded for the first time after storms in 2016, possibly arriving from Central America and the Gulf Coast, respectively, where they typically occur.

Residents, migrants, and vagrants

Comparing biodiversity across sites in an ecologically meaningful way is a greater challenge than building a species list for each site because one must understand the relative abundance of each taxa over time and across sites. To compare sites and understand biotic interactions and ecological changes, we ultimately need to distinguish between breeding residents, regular migrants sampled passing through, and occasional vagrants. Other than their potential to act as colonists of a site or vectors of diseases, vagrants are probably of minimal ecological importance. Noting *S. vulneraria* in Clarke County's species list reveals little about the species' underlying biology, but knowing that only one specimen of this species was recorded in over 2,200 nights suggests that *S. vulneraria* is a vagrant.

Moth communities as bioindicators

Each lepidopteran species is restricted in its larval host specificity, typically, a limited number of plant or lichen taxa. By sampling resident moth species across sites, a relatively easy task because of their general attraction to lights, we plan to use moth inventories as an efficient means to compare the diversity and health of host communities. It is certainly easier to use lights to monitor moths that feed on lichens, for example, than it is to bash around in the forest and measure changes in lichen communities.

Standard sampling effort

The ability to standardize and analyze sampling effort across sites is a critical requirement to complete inventories, compare them across sites, and use the composition of taxa within them as bioindicators. A list of species from a site reflects both the site's diversity and sampling effort. Because either minimal sampling of a rich site or extensive sampling at a low diversity one could produce species lists of similar lengths, we must factor in sampling effort in understanding and comparing site differences.

Objective

Our goal here is to evaluate the efficiency of sampling regimes to inventory species as a function of effort. This task is complicated by the phase of the moon, which has been known for many years to affect the number of insects attracted to light (Williams 1936; Williams et al. 1956; Bowden & Church 1973; Yela & Holyoak 1997), and by the seasonality of species that fly at restricted times of the year, such as the geometrids *Erannis tiliaria* (Winter Moth)**,** *Phigalia denticulata* (Toothed Phigalia)*, Phigalia strigataria* (Small Phigalia), *Alsophila pometaria* (Fall Cankerworm Moth)**,** *Paleacrita vernata* (Spring Cankerworm Moth) and *Paleacrita merriccata* (White-spotted Cankerworm Moth)**,** which are winter specialists and typically only fly between November and March in Clarke County, Georgia.

Methods — Since February 2010, a team of 17 individuals has taken 194,000 photographs to document the activity of creatures attracted to lights at 275 Blue Heron Drive, Clarke County, Georgia (latitude 33.8882°N, longitude 83.2973°W). Pickering (2016) gives detailed methods of the research at this site, including descriptions of the site's vegetation, lights, photographic methods, image management, identification, and tabulation of taxa. Since 28 November 2010, we have sampled every night through May 2016, except for one night, 22 June 2012. Overall, we identified 99% of the lepidoptera photographed to taxa ('*valid species*', '*species group', or* '*morphospecies*'). All the data and associated images are online (see http://www.discoverlife.org/moth/report.html#GA_Clarke_Blue_Heron), including a species accumulation curve that is updated nightly.

Here we tabulate and resample the taxa in the 1,825 nightly samples taken in 2011 through 2015. We assigned each sample to a day of the week (Sunday, Monday, etc.) and a phase of the moon (day 0 through 29, with the new moon being 0; waxing halfmoon, 7; full moon, 14; waning half-moon, 21). Because lunar cycles do not align exactly to calendar days, the cycles end on either lunar day 28 or 29. In analyzing the data, we calculated the three days before the new moon and refer to them as lunar day -1, -2 and -3.

In each figure we graph the number of taxa resampled per year below the total accumulated across all five years. The yearly data show the variance.

Results & Discussion

Nightly samples across years — The 1,825 nightly samples yielded a 5-year total of 1,256 taxa, with individual years 2011-2015 totaling 812, 867, 920, 922, and 876, respectively. We use these values to evaluate resampling 13, 52, or 104 nights of effort per year based on the lunar phase versus days of the week.

Seasonal flight pattern — Fig. 1 presents the number of taxa recorded in each month for each of the five years and totals across all years. Overall, May was the most diverse month with a total 760 taxa (61% of 1,256 total; 8.5% of yearly effort). May had the highest monthly total in all years except 2012, when May's 404 taxa were slightly lower than June's 410 and August's 407. The four least diverse months were the winter months of November through February, which totalled 139, 84, 54, and 111 taxa, respectively. Nightly resampling of March through October, excluding the four least-diverse months, yielded 1,243 taxa (99% of total; 67% of yearly effort). Similarly, resampling the six months of April - September yielded 1,186 taxa (94% of total; 50% of yearly effort). For 33% of yearly effort, resampling the four months of March - June yielded 1,048 taxa (83% of total); April - July, 1,065 (85%); May - August, 1,058 (84%); June - September, 1,027 (82%). While this observed seasonal pattern will differ geographically across other latitudes and elevations, it suggests that once we determine a site's overall seasonal trend from the first year of sampling, it may be possible to discontinue sampling certain months, in this case November through February, and focus on months with more flight activity.

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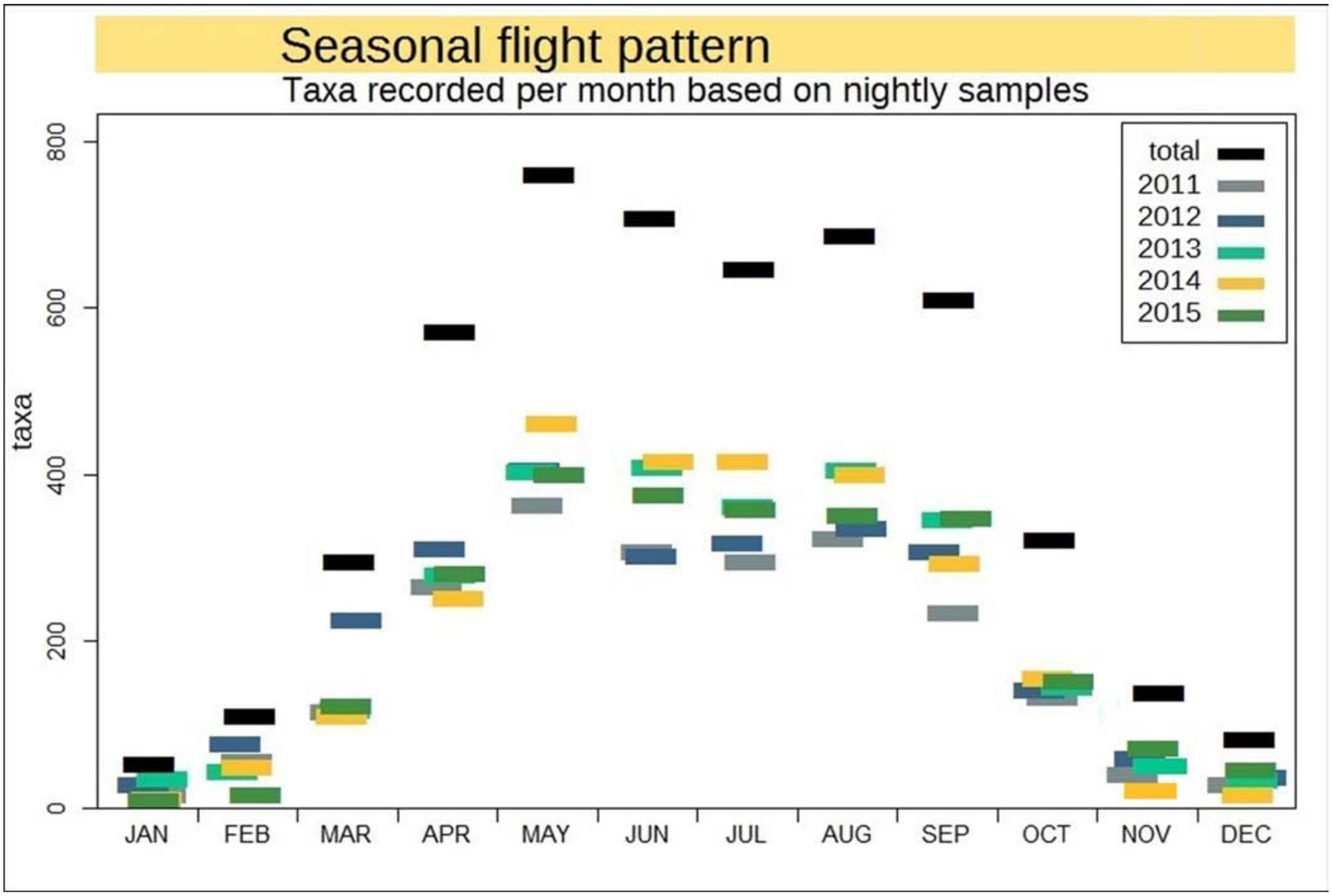

Fig. 1. SEASONAL FLIGHT PATTERN. Taxa recorded per month based on nightly samples. Blue Heron Drive, Clarke County, Georgia, 2011-2015.

Effort of 13 samples per year — Fig. 2 presents the number of taxa resampled for each day of the lunar cycle, where day-0 is the new moon. Of all the days in the lunar cycle, the new moon had the highest total across all years of 600 taxa (48% of total). However, there is inconsistency across the years in the day of the lunar cycle that had the highest number of taxa, being day-28 (251 taxa in 2011), day-0 (292 in 2012), day-1 and day-22 (304 in 2013), day-1 (311 in 2014) and day-24 (298 in 2015).

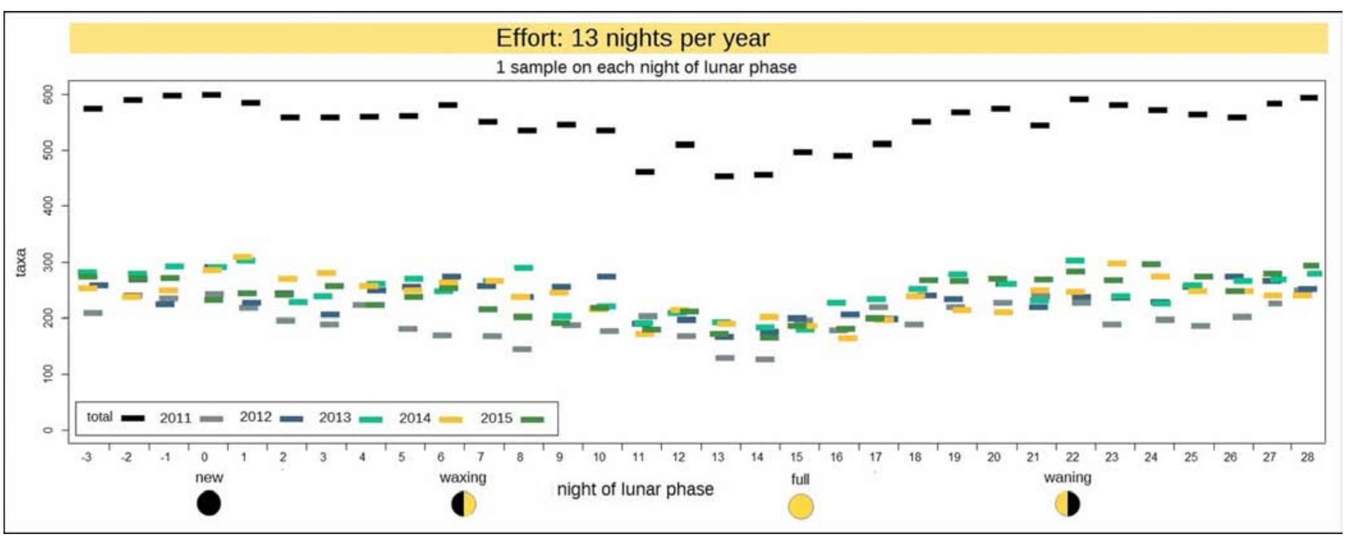

Fig. 2. EFFORT OF 13 NIGHTS/YEAR. Taxa recorded by resampling each night of lunar phase. Blue Heron Drive. Clarke County, Georgia, 2011-2015.

Fig. 2 clearly shows a depression of values observed immediately around the full-moon. Of the entire lunar cycle, the six days with the lowest totals were lunar day-12 through day-16, with values ranging from 455 (36% of total) to 511. The lowest total is on day-13, which is slightly above the full moon's value of 458 on day-14.

Although most of the days in the lunar cycle yield similar results, except for the nights around the full moon, we recommend sampling on each new moon in order to facilitate comparisons across sites.

<u>Effort of 52 samples per year</u> — Fig. 3 compares two regimes for sampling 52 nights per year based on the phase of the moon (on left) versus on a particular day of the week (on right). Here we resampled four nights around each new (day -1, 0, 1, 2), waxing (day 6, 7, 8, 9), full (day 13, 14, 15, 16) and waning moon (day 20, 21, 22, 23). Except for the samples around the full moon, the totals are similar across the other three lunar phases and the samples taken weekly. Resampling one night per week yielded an average across the week of 876 taxa (70% of total; ranging from 850 taxa on Tuesday to 905 on Sunday). This average is approximately the same as the total number of taxa resampled in an equivalent effort of 4-nights around each new (870 taxa), waxing (877 taxa), and waning (873 taxa) lunar phase. It exceeds the 4-night sample around each full moon (797 taxa; 63% of total).

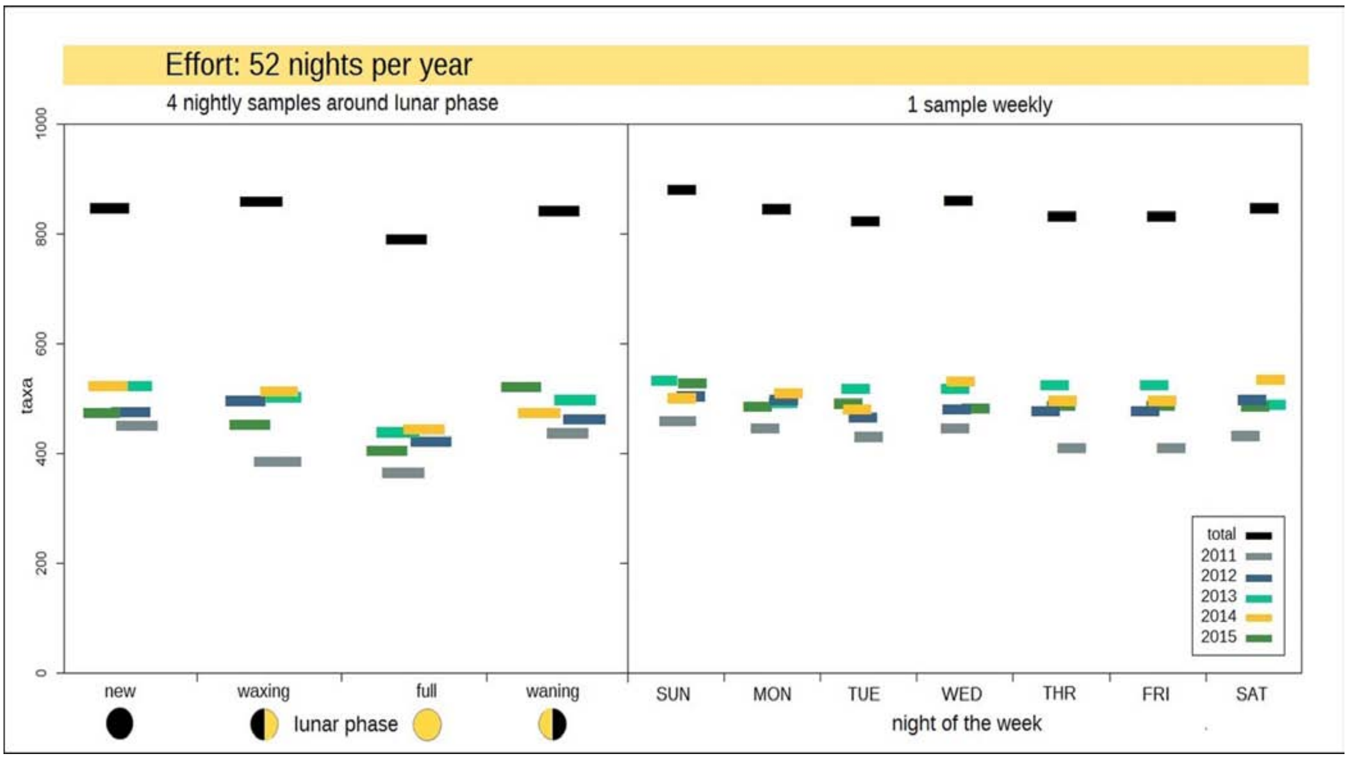

Fig. 3. EFFORT OF 52 NIGHTS/YEAR. Taxa recorded by resampling 4-nights around the new, waxing, full, and waning moons in comparison to sampling one night per week. Blue Heron Drive, Clarke County, Georgia, 2011-2015.

Thus, if effort is increased to 52 nightly samples annually, we recommend sampling one night per week, ideally on a weekend morning in order to facilitate cross-site comparison by doing it as much as possible on the same nights across sites. While we can safely assume that the moths are oblivious to the particular day of the week, human participants could exhibit observer bias, having good and bad nights of the week.

<u>Effort of 104 samples per year</u> — Fig. 4 compares two regimes for sampling 104 times per year based on lunar phase (on left) versus 2 nightly samples per week (on right). The lunar phases represent 8-nights around the new moon (days -3, -2, -1, 0, 1, 2, 3, 4), waxing half-moon (days 4, 5, 6, 7, 8, 9, 10, 11), full moon (days 11, 12, 13, 14, 15, 16, 17, 18), and waning half-moon (days 18, 19, 20, 21, 22, 23, 24, 25), with respective total taxa values of 1007 (80% of total), 1006 (80%), 972 (77%), and 1020 (81%). These values are less than 1,024 taxa (82% of total) average of resampling the 21 possible pairs of nights (range of 1,002 through 1,044). The right side of the figure also shows the values of resampling three specific pairs of weekend nights.

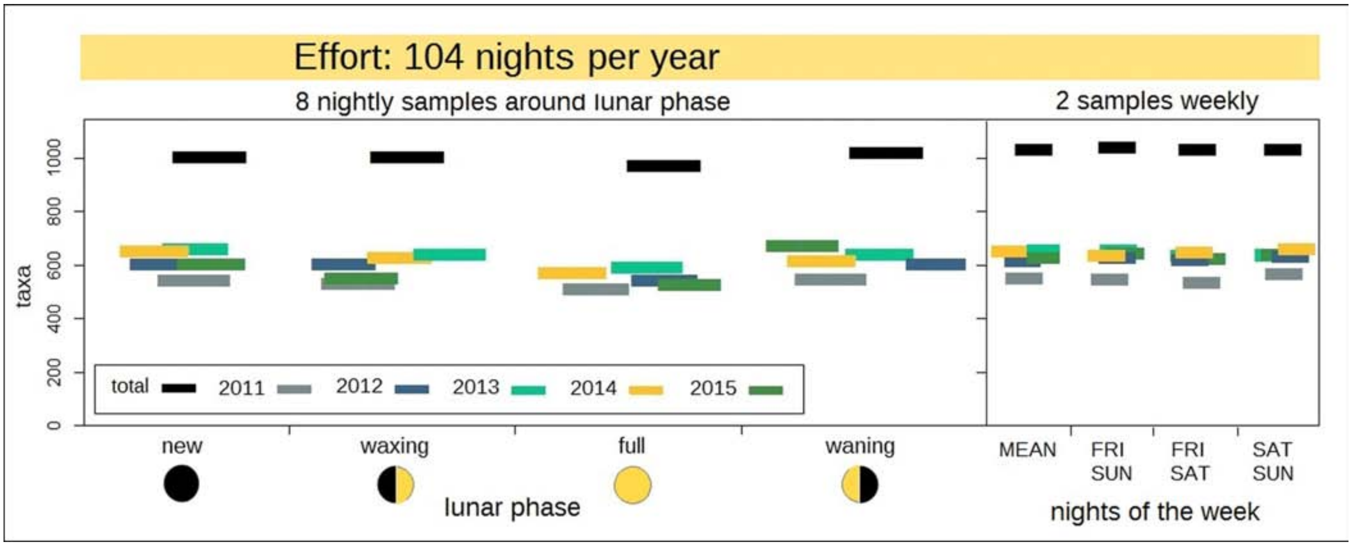

Fig. 4. EFFORT OF 104 NIGHTS/YEAR. Taxa recorded by resampling 8-nights around the new, waxing, full, and waning moons in comparison to sampling two nights per week. Blue Heron Drive, Clarke County, Georgia, 2011-2015.

Conclusions

Based on the above results, we recommend that the most efficient means to document the moths at a site is to sample on each new moon for a total of 13 samples per year. If winter samples do not yield any moths in the first year, then sampling new moons in these months can be skipped in subsequent years.

If time and resources permit, we recommend additional sampling. Our results show that sampling four days around each new moon yields the same overall diversity as sampling one day each week, i.e., 52 nights per year in both regimes. Despite the similarity in results, we recommend sampling each week rather than around each new moon, as weekly sampling gives more seasonal data across years and makes it easier to determine the annual number of flights per species.

If enthusiasm really strikes, as we hope it does, add a second day per week to increase species accumulation, and a third, …

For all practical purposes, because species accumulation curves for flying insects never level off, there is a need to develop the means to compare only resident species across sites. With the exception of locally rare taxa and ones that tend not to be attracted to lights, resident taxa accumulate rapidly in the first year of sampling. In the next article in this series, we will compare the accumulation of moths at two sites in Clarke County, introducing a series of metrics based on occurrence patterns to determine residents from vagrants.

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