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Short-Term, Efficient Sampling Strategies for Estimating Forest Moth Diversity Using Light Traps

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ABSTRACT Evaluation of species diversity for highly diverse taxa is extremely time-consuming and costly; thus, there is a need to develop efficient sampling strategies. We established a short-term, efficient sampling scheme to produce samples that represent a full-season sampling of moth assemblages with a high degree of seasonality. We sampled adult moths monthly for the duration of the moth flying season by using light traps in five forest stands in a cool-temperate region in central Hokkaido, northern Japan. From this sample, we generated various subsamples that reduced the sampling period and the number of traps per stand, and tested whether these subsamples provide estimates of species richness, abundance, and species turnover representative of those revealed by the whole season sample. Comparisons between the whole season sample and each subsample indicated that setting one light trap on a night in July and August, which shortened the sampling period to 25% and reduced sample size to 38%, was the most efficient sampling scheme to estimate abundance, species richness, and similarity in the whole season sample. The comparisons also suggest that it is efficient to use rarified species richness as a species richness estimator, and the Bray–Curtis index or Morisita’s $C_A$ for estimating species turnover between sites in moth assemblages.

KEY WORDS biodiversity, Lepidoptera, seasonality, species richness estimator, species turnover

Pervasive habitat loss and associated species extinction have emphasized the urgent need to evaluate species diversity. However, evaluating species diversity for highly diverse taxa, such as insects, is extremely time-consuming and costly because there are so many species in one area (Lawton et al. 1998). Given the limitations in resources available for intensive, long-term surveys, there is a need to develop efficient sampling methods for evaluating biodiversity (Ozaki et al. 2006).

Efficient sampling methods should reduce time and cost needed for sampling without causing significant information loss in species richness (number of species) at sites ($\alpha$ diversity) and species turnover (dissimilarity of species composition) between sites ($\beta$ diversity) (Jones and Eggleton 2000). Several species richness estimators (Colwell and Coddington 1994) and similarity indices (Chao et al. 2005) that substantially reduce undersampling bias have been developed to estimate species richness and turnover. These estimators and indices might provide tools to achieve reliable estimates of species richness and turnover from sampling schemes with reduced sample size (Brose 2002). Efficient sampling schemes should also consider patterns of seasonality because limitations in resources do not usually allow full-season sampling (Oliver and Beattie 1996).

Insects are one of the most hyperdiverse and critical components of terrestrial ecosystems (Odegaard 2000). Lepidoptera (mostly moths) is among the most speciose groups of insects and has important ecosystem functions as herbivores, detritivores, and pollinators (Schowalter et al. 1986, Summerville and Crist 2008). In addition to this impressive diversity, moths are easily collected with light traps that are widely recognized as the standard tool for sampling night-flying moths (Southwood and Henderson 2000). Although trap catches are influenced by weather, moon phase, and trap design (Yela and Holyoak 1997, Butler et al. 1999, Intachat and Woiwod 1999), light traps are useful tools to quantify moth communities if used with care (Kitching et al. 2000, Raimondo et al. 2004).

One constraint with using light traps is the processing cost associated with sorting and identifying individuals in the sample because light traps capture a huge number of moths (Thomas and Thomas 1994). Two studies examined optimal sampling hours within a night that reduced the catch without causing loss of information on moth communities (Thomas and Thomas 1994, Scalercio et al. 2009). However, few studies have examined the effect of sampling intensity...
Materials and Methods

Study Sites and Moth Sampling. Moth sampling was conducted in five forest stands in Sapporo, central Hokkaido, northern Japan (42° 53’–43° 00’ N, 141° 22–26’ E; 150–400 m above sea level). We selected two stands in deciduous broadleaved forests, two stands in Japanese larch (Larix kaempferi Carriere) plantations (42 and 47 yr old), and one stand in a Sachalin fir (Abies sachalinensis Masters) plantation (48 yr old). We chose these forest types because they are the most common forest types in the region. Study stands were always >10 ha and located >1 km apart. Stands in broadleaved forests are dominated by mizumara oak (Quercus crispula Blume), linden (Tilia japonica Simokai), painted maple (Acer pictum Thunb.), and cucumber tree (Magnolia obovata Thunb.). The coarser plantations have some naturally regenerated trees that include mizumara oak, linden, and Japanese elm (Ulmus davidiana variety japonica Nakai). Mean tree height in each stand ranges from 17 to 20 m. Forest understories are covered by dwarf bamboo [Sasa kurilensis (Rupr.) Makino et Shibata].

We collected moths using portable light traps developed by Okochi (2002). This trap consists of a rectangular cage (90 by 90 by 150 cm high) produced with half transparent white nylon netting and is equipped with a 6-W blacklight fluorescent tube powered by a 9-V alkaline dry battery. The cage has a horizontal slit (20 cm) on four sides through which moths may enter the cage to become trapped in the lower part, which has a funnel-type lid at the top to prevent moths from escaping.

We operated light traps on a night around the new moon in each month throughout the entire moth flying season, April–November, in 2005. Light traps also were operated in December, but no moths were caught. Trapping was not carried out on rainy or windy nights because these conditions can negatively affect light trap catches (Mizutani 1984, Choi 2008). On each sampling night, we set two light traps at a height of ~1.5 m at fixed locations that were 100 m apart in the center of each stand. Therefore, 80 trap nights (five stands × 8 mo × two traps) were accumulated in total. Traps remained lit throughout the nights, and early the next morning, captured moths were killed with ethyl acetate, and were frozen until identification. We identified 67–85% of sampling individuals per stand to species. Unidentified individuals were those with extensive wing wear or from microlepidopteran moths (e.g., Tortricidae). Voucher specimens from this study were deposited at Hokkaido Research Center, Forestry and Forest Products Research Institute.

Data Analyses. All sampling periods and traps in each stand were combined to yield a whole season sample. Then we split the whole season sample in several subsamples based on the following reduced sampling schemes. At first, we generated monthly subsamples from July to September to reduce sampling period. We used these months so that each subsample contained >5% of individuals in the whole season sample. However, none of the monthly subsamples were able to estimate species richness in the whole season sample, although the monthly subsample in August provided adequate estimates of abundance and similarity (data not shown). Therefore, we generated 2-month subsamples from every combination of 2 months from June to September, giving six combinations. Then for each 2-month subsample, we reduced sampling intensity by generating one-trap subsamples by randomly assigning two traps in each stand to two groups.

We calculated abundance and the following species richness estimators and similarity indices from the whole season sample and its subsamples. Abundance also was used to assess the processing cost for sorting and identifying moths in each sampling scheme. For species richness, we calculated observed species richness (Sobs), a rarefied species richness, and four nonparametric species richness estimators. Rarefied species richness is the expected number of species in a random sample of a given number of individuals using rarefaction (Hurlbert 1971). Rarefaction standardizes sample size to a common abundance level so that direct comparisons among samples can be made (Magurran 2004). A sample size of 100 individuals (S100) was chosen as the lowest number of individuals...
caught in each stand across the subsamples. Rarefied species richness represents the ranking of true species richness only when rarefaction curves do not intersect (Lande et al. 2000). So, we constructed sample-based rarefaction curves without replacement using trap nights as sampling units for the whole season sample and examined whether rarefaction curves for different stands intersected. These rarefaction curves were generated with Estimate S version 8.20 (Colwell 2005). We also calculated four nonparametric species richness estimators (Chao1, abundance-based coverage estimator for Chao1. We also set a cut off movement heterogeneity (Brose and Martinez 2004).

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Two-Month Subsamples. In total, 14,591 adult moths, consisting of 693 species in 29 families, were collected and identified in the five study stands. Of the total abundance, 36 and 40% were collected in July and August, respectively. Abundance in 2-mo subsamples were highest in July/August (76% of the total abundance) and lowest in June/September (16%) (Table 1). Abundance in June/July, June/August, July/August, and July/September were highly correlated ($r^2 > 0.8$) with that in the whole season (Table 2). The relationship between abundance in July/August and that in the whole season showed that although points were not on the diagonal line, the July/August subsample can estimate relative difference between stands in the whole season sample (Fig. 1).

For species richness estimators, all 2-mo subsamples underestimated the values in the whole season sample (Table 1). ACE had $r^2 > 0.7$ in June/August, July/August, and August + September subsamples (Table 2), but these high $r^2$ values were caused by an outlier exemplified in Fig. 1 in the July/August subsample.

Fig. 1. Relationships between values of each measure in 2-mo subsample in July + August and those in the whole season sample.

Results
Two light traps in each stand were randomly assigned into two groups, and mean $r^2$ values of the two groups are shown.

Other than ACE, $S_{100}$ and Chao1 had $r^2 > 0.8$ in the July + August subsample (Table 2). Figure 1 showed that the July + August subsample can estimate relative difference in $S_{100}$ and Chao1 between stands in the whole season sample. Values of similarity indices did not differ largely between 2-mo subsamples and the whole season sample (Table 1). Similarity indices had high $r^2$ values in most of the 2-mo subsamples (Table 2). Especially, the Bray–Curtis index had the highest $r^2$ among the three similarity indices except for in the July + September subsample. Among the subsamples, the Bray–Curtis index and $C_0$ had the largest $r^2$ in July + August subsample. The relationship between values of similarity indices in July + August and those in the whole season showed that points were close to the diagonal line (Fig. 1), indicating that all similarity indices in the July + August subsample produced unbiased estimates of the whole season sample. These results from 2-mo subsamples indicate that July + August is the optimal two-month sampling period to estimate abundance, species richness and similarity in the whole season sample. However, this sampling scheme only reduced the abundance to 24%, and >2,000 moths on average were captured in each stand (Table 1). Therefore, we generated one-trap subsamples to reduce sampling intensity.

One-Trap Subsamples. Although abundance was reduced to half of the 2-mo subsamples, $r^2$ of abundance were still $>0.7$ in June + July, June + August, July + August, and July + September subsamples (Table 3). The relationship between abundance in July + August and that in the whole season showed that the one-trap subsample in July + August can estimate relative difference between stands in the whole season sample (Fig. 2).

For species richness estimators, values of $S_{obs}$ and nonparametric estimators decreased greatly, whereas values of $S_{100}$ decreased only slightly when the number of traps in each stand was reduced from two to one (Tables 1 and 4). ACE in August + September had the highest $r^2$ among the species richness estimators (Table 3), but this high $r^2$ was due to an outlier. Other than ACE, $S_{100}$ in July + August had $r^2 > 0.8$ and the relationship between $S_{obs}$ in this subsample and that in the whole season sample shows that the July + August subsample can estimate relative difference between stands in the whole season sample (Fig. 2). Because $r^2$ of species richness estimators were <0.65 in the other subsamples, $S_{100}$ in July + August was the only estimator that adequately estimated species richness in the whole season.

Values of similarity indices decreased only slightly when the number of traps in each stand was reduced from two to one (Tables 1 and 4). Although the Bray–Curtis index and $C_A$ still had high $r^2$ in most of the one-trap subsamples, $r^2$ of adjusted Sørensen decreased substantially (Tables 2 and 3). Among the subsamples, the Bray–Curtis index and $C_A$ had the largest $r^2$ in the July + August subsample. The relationship between values of similarity indices in the July + August subsample and those in the whole season sample shows that $C_A$ produced almost unbiased estimates of the whole season sample, but values of the Bray–Curtis index were slightly underestimated (Fig. 2). In summary, when we reduced the number of traps in each stand from two to one, we found that the July + August subsample, which shortened the sampling period to 25% and reduced sample size to 38%, can estimate abundance, species richness, and similarity in the whole season sample.

When we grouped moth species according to their larval feeding plants or voltinism, the one-trap subsample in July + August can estimate abundance, species richness and similarity in the whole season sample in most of the groups (Table 5): $r^2$ of abundance and $S_{100}$ were >0.7 except for woody plant feeders, whereas the Bray–Curtis index and $C_A$ had $r^2 > 0.8$ for all the groups.

Rarefaction. Sample-based rarefaction curves for the whole season sample showed that although species richness increased steadily with the accumulated number of individuals, rarefaction curves in five stands did not intersect each other across the range of our sampling efforts (Fig. 3). Furthermore, values of the four nonparametric estimators changed with the accumulated number of individuals and did not reach stable asymptotes in any of the five stands (Fig. 4).

Discussion

Efficient Sampling Scheme. Seasonality is by far the most important variable affecting community composition of forest Lepidoptera in temperate regions (Summerville and Crist 2003, Sayama et al. 2011). The current study clearly reveals that even in the moth assemblages with high seasonality, short-term sampling can provide values of abundance, species richness, and turnover representative of the whole season sampling if we choose the appropriate sampling periods and diversity indices. In particular, sampling both in July and August was the optimal period for estimating abundance, species richness, and similarity. This is consistent with the previous findings that choosing the peak season of moth emergence maximizes sampling efficiency (Sparrow

| Table 3. | $r^2$ values obtained from the linear regressions of values of each measure in one-trap subsamples on those in the whole season sample |
|----------------------|---------------------------------------------------------------------------------|
| Sampling period       | June + July | June + Aug. | June + Sept. | July + Aug. | Aug. + Sept. |
| Abundance             | 0.54        | 0.70        | 0.00         | 0.86        | 0.56         | 0.51         |
| Species richness estimator | S_0      | 0.33        | 0.36        | 0.20        | 0.44        | 0.46         | 0.39         |
|                      | S_0       | 0.64        | 0.13        | 0.44        | 0.81        | 0.49         | 0.08         |
|                      | Chao1     | 0.31        | 0.48        | 0.08        | 0.20        | 0.10         | 0.63         |
|                      | ACE       | 0.37        | 0.55        | 0.40        | 0.39        | 0.04         | 0.92         |
|                      | Jack1     | 0.09        | 0.06        | 0.27        | 0.05        | 0.06         | 0.21         |
|                      | Jack2     | 0.25        | 0.19        | 0.31        | 0.18        | 0.10         | 0.39         |
| Similarity index     | Bray–Curtis | 0.74       | 0.73        | 0.55        | 0.93        | 0.52         | 0.38         |
|                      | Adjusted  | 0.76        | 0.07        | 0.27        | 0.44        | 0.63         | 0.12         |
|                      | Sørensen  | 0.72        | 0.12        | 0.19        | 0.86        | 0.70         | 0.15         |

Two light traps in each stand were randomly assigned into two groups, and mean $r^2$ values of the two groups are shown.
et al. 1994, Landau et al. 1999). It is therefore critical to obtain the information about the annual activity cycles to determine the optimal sampling period. In July and August, however, >2,000 moths were captured in each stand, and so there is a huge processing cost to sort and identify these individuals. Efficient sampling schemes should reduce both the time invested in collection and the processing cost after collection (Oliver and Beattie 1996, Lawton et al. 1998). In this sense, it is efficient to reduce the number of traps in each stand from two to one because we can estimate abundance, species richness and similarity in the whole season sample even with one trap per stand. This sampling scheme performed well for most of the moth groups classified based on larval feeding plants and voltinism. These findings suggest that setting one light trap on a night in July and August is the most efficient sampling scheme to produce samples that are representative of the whole-season sampling.

Fig. 2. Relationships between values of each measure in one-trap subsample in July + August and those in the whole season sample. Two light traps in each stand were randomly assigned into two groups, represented here with different symbols.


**Table 4. Mean (SD) of species richness estimators and similarity indices in each stand obtained from one-trap subsamples**

| Sampling period | June + July | June + Aug. | June + Sept. | July + Aug. | July + Sept. | Aug. + Sept. |
|-----------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Species richness estimator | | | | | | |
| $S_{obs}$ | 118.60 (19.91) | 134.10 (15.43) | 68.50 (11.93) | 187.90 (21.08) | 138.80 (15.91) | 145.10 (15.57) |
| $S_{100}$ | 49.79 (6.19) | 51.00 (4.98) | 44.90 (5.32) | 56.70 (4.38) | 50.70 (6.16) | 50.47 (3.76) |
| Chao1 | 175.34 (36.45) | 206.51 (28.35) | 129.10 (35.14) | 265.56 (19.76) | 216.50 (36.08) | 224.46 (40.64) |
| ACE | 194.33 (47.56) | 234.37 (43.19) | 168.02 (41.58) | 270.38 (21.91) | 243.33 (52.30) | 244.29 (47.85) |
| Jack1 | 168.00 (25.03) | 191.59 (18.97) | 105.29 (17.64) | 256.53 (21.92) | 198.39 (19.75) | 205.30 (19.39) |
| Jack2 | 195.72 (35.73) | 226.53 (20.80) | 130.82 (23.67) | 295.27 (21.11) | 234.61 (24.85) | 241.75 (24.85) |
| Similarity index | | | | | | |
| Bray–Curtis | 0.29 (0.09) | 0.36 (0.09) | 0.27 (0.08) | 0.34 (0.08) | 0.28 (0.08) | 0.37 (0.16) |
| Adjusted Sørensen | 0.70 (0.15) | 0.80 (0.07) | 0.70 (0.13) | 0.77 (0.11) | 0.70 (0.14) | 0.80 (0.08) |
| Morisita’s $C_s$ | 0.29 (0.18) | 0.41 (0.17) | 0.35 (0.21) | 0.31 (0.14) | 0.31 (0.18) | 0.38 (0.16) |

Two light traps in each stand were randomly assigned into two groups, and means of the two groups are shown.

Efficiency of Species Richness Estimators. Because time and money are always in short supply, we need to accurately predict species richness in an assemblage by using as small a sample as possible (Magurran 2004). Previous studies demonstrate that nonparametric species richness estimators are most promising in estimating species richness in highly diverse communities (Colwell and Coddington 1994, Gotelli and Colwell 2001). In the current study, however, nonparametric estimators can estimate values in the whole season sample only in 2-mo subsamples, suggesting that they do not work well with reduced sample size. In addition, values of any nonparametric estimators did not achieve stable asymptotes across the range of our sampling efforts, indicating that nonparametric estimators do not estimate true species richness even from the whole season sample. Values of nonparametric estimators have not reached stable asymptotes in previous studies on moth assemblages using light traps (Landau et al. 1999, O’Hara 2005, Summerville and Crist 2005). From these results, Summerville and Crist (2005) emphasize that nonparametric estimators should only be used to provide a minimum approximation for predicting species richness. Therefore, nonparametric estimators seem to be useless in estimating species richness of moth assemblages because they neither estimate true species richness in the assemblages nor estimate values in the whole season sampling from reduced sampling schemes.

In contrast, we were able to estimate $S_{100}$ in the whole season sample from the one-trap subsample in July + August. This is probably because $S_{100}$ is more resistant to undersampling than nonparametric estimators, which is indicated by the smaller decrease in $S_{100}$ than the nonparametric estimators when the number of traps in each stand was reduced from two to one. Furthermore, sample-based rarefaction curves did not intercept each other, suggesting that $S_{100}$ represents the ranking of true species richness (Lande et al. 2000). Therefore, it is efficient to use rarefied species richness to detect ranking of species richness in moth assemblages. Brehm et al. (2003) also suggests that rarefied species richness is a suitable measure of local diversity in moth assemblages.

Efficiency of Similarity Indices. Compared with the species richness estimators, similarity indices used in the current study were highly useful in estimating species turnover from reduced sampling schemes. Values of similarity indices decreased only slightly when the number of traps in each stand was reduced from two to one. This is probably because within-stand diversity is much smaller than between-stand diversity in forest moth community composition (Summerville et al. 2003).

Among the similarity indices, the Bray–Curtis index had the highest $r^2$ in most of the subsamples. Although the Bray–Curtis index yielded slightly underestimated values in the one-trap subsample in July + August, the

![Graph showing species richness vs. number of individuals for different plant types.]
high $r^2$ was sufficient to estimate the relative difference between stands. Because the Bray–Curtis index has been shown to be one of the most effective measures of species similarity (McCune and Grace 2002), it is preferred to use this index in moth assemblages. $C_A$ also had high $r^2$ values in most of the subsamples. $C_A$ produced unbiased estimates in the one-trap subsample in July + August, indicating that this index has smaller undersampling bias than the Bray–Curtis index. $C_A$ has a major advantage in that it is virtually independent of sample size (Morisita 1959). In this respect, $C_A$ is more robust than the well-known Morisita–Horn index (Wolda 1981). Therefore, $C_A$ should be used to estimate similarity in sampling schemes with small sample size although this index is known to be sensitive to abundance of dominant species (Wolda 1981).

In contrast, $r^2$ of the adjusted Sorensen index, which was developed to reduce undersampling bias, was lower than those of the other two indices. This might be because the adjusted Sorensen index is sensitive to the difference in sampling period in assemblages with high seasonality. However, values of this index decreased when the number of traps in each stand was reduced from two to one, suggesting that this index is still affected by sample size. Chao et al. (2005) indicates that some bias remains in this index, especially under severe undersampling and for highly dissimilar samples, which might be the case in the samples from moth assemblages.

Problems in Reduced Sampling Scheme. Any attempts to reduce sampling periods and processing effort inevitably would result in some loss of information. There is generally a tradeoff between sampling efforts and the number of species collected (Balmford et al. 1996). For example, the short-term sampling scheme recommended in the current study missed up to 40% of the species collected in the whole season sample. These species include those emerging only in
spring or in autumn, especially some geometrid winter moths that occur only in November (Sayama et al. 2011). If any of these species have particular conservation concern, occurrence period of the species with conservation concern should be covered within the sampling period. Thus, the practical approach to yield the optimal sampling scheme depends on compromise between time and resources available and conservation objectives.

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