

# Intensive Versus Long-Term Sampling to Assess Lepidopteran Diversity in a Southern Mixed Mesophytic Forest

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**ABSTRACT** As biodiversity loss increases through species extinction and habitat degradation, the need to catalog what remains becomes ever more important. The time and monetary limitations of long-term biodiversity surveys become a concern as demand for biodiversity studies rises. This study was initiated to compare the efficiency of an intensive sampling scheme with a relatively long-term sampling scheme. Richness and abundance of selected moth taxa were measured in a mixed mesophytic forest habitat during a period of 8 mo for the long-term collection. An equal number of samples was taken from the same habitat during 1 mo for the intensive collection. A total of 3,155 specimens representing 314 species of moths was collected in the long-term study compared with 4,198 specimens representing 261 species in the intensive study. Sorenson's index indicated 76% species overlap between the 2 studies. Based on total species richness for this habitat, the intensive collection recovered 15% less than the long-term collection and took about half the time. The total number of species identified from both collections was 362. Because many biodiversity assessments are currently conducted on a short-term basis, studies such as this can provide entomologists with rough estimates of the percentage of biodiversity collected using relatively rapid sampling schemes.

**KEY WORDS** Lepidoptera, biodiversity, conservation, species richness, diversity estimators

TODAY IT IS widely recognized that biodiversity is being lost rapidly on a global scale through degradation of ecosystems. As this decrease in diversity is observed through species extinction and habitat loss, cataloging what remains becomes increasingly critical. Yet as the demand increases for biodiversity studies, the time and monetary limitations of conducting thorough, long-term surveys become a concern. Additionally, commercial and bureaucratic pressures to produce quick results can limit time available to conduct such studies (Oliver and Beattie 1993).

Arthropod inventories can provide good indicators of habitat biodiversity because arthropods respond quickly to environmental changes, and are a highly diverse taxon (Longino 1994). Whether they are measured in terms of biomass, diversity, or ecological dominance, insects comprise a key component of terrestrial ecosystems and should be a major element in conservation planning and natural resource management (Fisher 1998). Lepidoptera, the 2nd largest order of insects, can yield large sample sizes and many species are easily identified. Thus, Lepidoptera are particularly appropriate for biodiversity surveys. Within the order, moths are relatively easy to survey because most species can be collected with simple light traps. Moths have the additional advantage in that they have been collected and studied extensively, thus providing a geographically comprehensive database founded on fairly reliable systematics (Holloway and Barlow 1992).

Coddington et al. (1991) suggested sampling methods should be fast, reliable, simple, and cheap. These recommendations are reflective of the urgency felt in many areas where species are being lost faster than they can be cataloged, as well as of the economic and resource limitations often faced by scientists. Although 1 of the advantages of sampling insects is their considerable richness, collecting insects also poses a problem because species diversity levels are high, estimated by Hawksworth and Mound (1991) to be between 6 and 80 million species worldwide. Additionally, our knowledge of structure and patterns of biodiversity at the landscape scale is lacking for such "megadiverse" groups as terrestrial arthropods, but it is at this landscape scale that conservation often operates (Coddington et al. 1996). Unfortunately, optimal sampling schemes have not been developed for biodiversity estimates. The goal of this study was to contrast 2 methods of sampling moth biodiversity in an area. One method involved conducting an intensive, short-term collection, and the other a relatively long-term collection. The 2 could then be compared to determine how representative they were of each other. The effects of sampling method on richness estimator performance were also examined.

## Materials and Methods

**Study Sites.** Two sites, located  $\approx 50$  km apart, were selected in West Feliciana Parish, in central Louisiana. The 1st site (Cabin) was in the Feliciana Preserve, a

privately owned undeveloped 61-ha tract of land 10 miles east of St. Francisville, LA, which was clear-cut  $\approx 40$  yr ago. The 2nd site (Magnolia Glen) was in the Tunica Hills Wildlife Management Area (WMA), 1,191 ha owned by the state of Louisiana. This area was reportedly cleared  $\approx 75$  yr ago, although probably only along the ridges, because there are trees estimated to be  $>200$  yr old in the ravines (R. Martin, personal communication).

This habitat is primarily composed of mixed mesophytic hardwood forest dominated by magnolia (*Magnolia grandiflora* L.), holly (*Ilex opaca* Aiton), and beech (*Fagus grandifolia* Ehrhart) (Delcourt and Delcourt 1974). The forest also has a high diversity of small trees and shrubs, including dogwood (*Cornus florida* L.), winged elm (*Ulmus alata* Michaux), and ironwood (*Carpinus caroliniana* Walter). Indigenous understory herbs include Allegheny spurge (*Pachysandra procumbens* Michaux) and Indian pink (*Spigelia marilandica* L.) (McCook 1982). The Tunica Hills lie at the southern end of a narrow belt of forested, deep ravines that form the eastern border of the Mississippi River Valley between Baton Rouge and Memphis. This belt is bordered on the west by bottomland forest of the Mississippi River flood plain, and on the east by a drier pine-hardwood forest. Ravines that make up the hills in this area are not rock, but instead consist of loess soils that are made up of wind-blown sediments that originated in the Mississippi River flood plain. Sediments created these highly erodible hills, which eventually eroded into ravines that exist today (Delcourt and Delcourt 1974).

**Collection and Identification.** All specimens were collected with UV light traps. Each trap consisted of a 15-W UV light set within a funnel and baffle apparatus, which was placed atop a screened-in cage (2 by 1 by 1 m). Traps were operated with photo switches, and were powered by 12-V d/c batteries. Moths collected in traps were inspected in the morning. Commonly encountered species were counted and recorded on a data sheet in the field. Less common species were placed in cyanide jars and taken to the laboratory to be processed and identified. Among Lepidoptera targeted for species analysis were moth families traditionally included in the informal category known as macrolepidoptera, although some of the more readily identifiable families of microlepidoptera were also included. Species complexes that could be readily distinguished from all other species, but whose species could not be distinguished without genitalia mounts, were listed with species complex names separated by a slash. Most species groups, regardless of size, that required genitalia dissections or wing mounts for identification, were not included in the analysis.

Moths were identified using taxonomic literature, including general works such as Covell (1984), Holland (1968), the *Moths of America North of Mexico* series (Hodges et al. 1983 and references therein) and additional primary literature. Identifications were verified by comparison with specimens in the Louisiana State Arthropod Museum and the Mississippi Entomological Museum, and in consultation with Richard L. Brown (Mississippi Entomological Museum).

For the long-term collection, 2 UV light traps, 1 at the Cabin site and 1 in the Magnolia Glen site, were operated overnight twice a month from March to October 1995. This resulted in 32 collections (16 from each site) over an 8-mo period. For the intensive collection, 2 UV light traps located  $\approx 1$  km apart were operated in each of the 2 sites. At the Cabin site, 1 trap (C1) was located alongside a artificial lake by a forest, with a ravine  $\approx 10$  m deep on the backside of the dam. The 2nd trap (C2) was located in a forest by a ravine  $\approx 10$  m deep. At the Magnolia Glen sites, 1 trap (MG1) was located on a level surface within the forest, and the 2nd trap (MG2), also in the forest, was by a ravine  $\approx 20$  m deep. The 4 traps were run for 2 consecutive nights per week, 4 wk in a row, between May and June 1995. This resulted in the same number of collections taken from this habitat, 32 (16 from each site, 8 from each trap), in the span of 1 mo instead of 8. For the intensive collection, moths were collected and identified using the method described above, with the exception that on the 1st collection day of each week all trapped moths were killed to prevent any released moths from returning the following night and thus being counted twice. On 2 dates of the study, 15 May and 1 June, the intensive and long-term collection dates overlapped, and data from the same traps were used for both collections. In other words, 2 of the 32 samples recorded for each survey were identical for the long-term and intensive collection.

**Statistical Analyses.** Species accumulation curves and quantitative species richness estimators were generated using EstimateS Richness Estimator Program version 5.0.1 (Colwell 1997). Results of 5 richness estimators were compared. The first 3, incidence-based coverage estimator (Lee and Chao 1994), Chao1 (abundance-based) (Chao 1984) and Chao2 (incidence-based) (Chao 1987), estimate population size based on capture-recapture data. The 4th estimator, 2nd-order jackknife (Heltshe and Forrester 1983), is based on numbers of uniques and duplicates and number of quadrants sampled (Chazdon et al. 1997). The Michaelis-Menten equation, originally developed to analyze conversion rates in enzyme kinetics (Raajmakers 1987), uses maximum likelihood to estimate parameters and their variances. One hundred randomizations and 10 abundance classes were used in the analyses. A  $z$ -test was calculated using the results from 1st-order jackknife data provided by EstimateS (Heltshe and Forrester 1983). The  $z$ -test was selected because information on the distribution of the richness statistics was lacking. Thus, as an appeal to the Central Limit Theorem, the assumption is made that the data are approaching a normal distribution. It is recognized that an artifact of running traps for consecutive nights during the intensive collection is that data points were correlated, but the assumption is that the same community was still sampled in both collections. The following 3 indices of alpha (within habitat) diversity were also computed: Fisher alpha (Rosenzweig 1995), Shannon Weiner index, and Simpson's index (Magurran 1988 and original references therein).

Table 1. Richness, abundance, and percentage recovery of moths by site and by trap

	Cabin			Magnolia Glen			Intensive only					
	Long-term	Intensive	Total	Long-term	Intensive	Total	C1	C2	Total	MG1	MG2	Total
Abundance	1,165	1,664	2,829	1,989	2,534	4,523	725	939	1,664	1,302	1,232	2,534
Richness	216	200	271	259	211	306	131	165	200	172	166	211
% species recovered	80	74	—	85	69	—	66	83	—	82	79	—

## Results

A total of 6,870 moths was identified in this study, 3,154 from the long-term collection and 4,198 from the intensive. Altogether, 362 species were identified, 314 from the long-term study representing 228 genera and 21 families, and 261 species from the intensive representing 196 genera and 20 families. By site, 2,289 moths and 271 species were recovered from the Cabin traps and 4,523 moths and 306 species were collected from the Magnolia Glen traps (Table 1). Moths from 13 superfamilies were identified. The majority of the moths identified were macrolepidoptera, with the exception of the superfamily Pyraloidea, which ranked 3rd in number of species. Total abundance in the intensive and long-term collections add up to >6,870, the total number of moths collected and identified in the study, because the same moths from 2 nights (15 May and 1 June) were recorded for both collections.

EstimateS species richness estimator predictions for the long-term data ranged from a high of 428 for the 2nd-order jackknife to a low of 346 for the Michaelis-Menten means (Table 2; Fig. 1). The estimators were also calculated for the intensive data, and ranged from a high of 339 species for the 2nd order jackknife to a low of 275 for the Michaelis-Menten means (Table 2).

A rank abundance plot for the long-term collection indicated that species distribution roughly followed a log normal pattern (Fig. 2). As is often observed with this type of distribution, the majority of species, 60%, were of intermediate abundance (11–100 individuals per species), 27% were uncommon (1–10 individuals per species), and 13% were very abundant (>100 individuals per species). Rank abundance for the in-

tensive study had a similar distribution, but with a greater percentage (32%) of species falling within the very abundant category.

Sorenson's index of species overlap (Magurran 1988) revealed that 76% of the species of moths collected in the long-term study were also recovered in the intensive collection. Sorenson's quantitative index, which takes abundance into account, indicated 86% overlap. By site, there was 69% overlap of species between the long-term and intensive collections for the Cabin site, and 71% overlap for the Magnolia Glen Site. By traps, there was 65% overlap between the Cabin traps (C1 and C2) during the intensive collection, and 75% overlap between Magnolia Glen traps (MG1 and MG2) during the intensive collection. The  $z$ -test revealed that the intensive and long-term collections were significantly different at  $\alpha = 0.05$  ( $z = 5.02$ ,  $z_{critical} = 1.96$ ).

## Discussion

The most significant finding of this study is the similarity in moth catch between the intensive collection and the long-term. A quantitative way to assess this is to contrast the number of species in each collection with the total number of species recovered from this habitat. The long-term collection recovered 314 species out of a total of 362 identified when combining both studies, totaling 87%. The intensive collection recovered 261 species, 72% of the total, or 15% of that recovered during the long-term collection. By site, the intensive collection was 6% short of the total number of species recovered during the long-term collection for the Cabin site, whereas there was a 16% difference between collections for the Magnolia Glen sites (Table 1).

A gross time analysis was conducted to obtain an estimate of the amount of time invested in each collection. When time spent traveling to sites and collecting was taken into consideration, approximately twice the amount of time was required to conduct the long-term collection than the intensive. The long-term collection required 16 separate 20-h trips, resulting in a total of 320 h. The intensive collection involved four 2-d trips that lasted  $\approx 44$  h each, totaling 176 h. Thus, for 55% of the time spent conducting the intensive collection, it was only 15% short of what was obtained in the long-term.

The number of moths trapped during the 1-mo collection was greater than that recovered in the 8-mo collection. This was most likely a result of the time of year in which the intensive collection was conducted.

Table 2. Summary values, richness estimates, and diversity indices for long-term and intensive collections

Value	Long-term	Intensive
No. species	314	261
No. singletons	78	56
No. doubletons	35	41
No. species unique to study	95	47
Species richness estimates		
Chao1	401	299
Chao2	394	307
ICE	375	308
Michaelis-Menten means	346	275
Second-order jackknife	428	339
Alpha diversity indices		
Fisher Alpha	87.00	62.00
Shannon-Wiener	4.93	4.44
Simpson	78.53	39.82

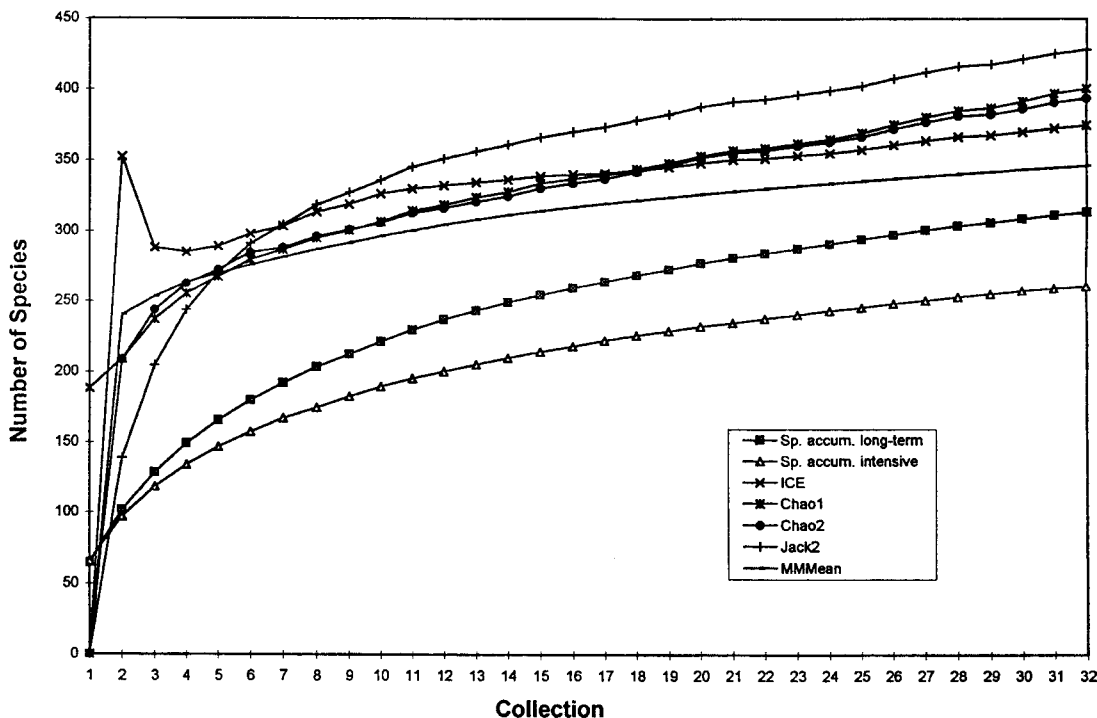


Fig. 1. Species accumulation curve for long-term and intensive collections and richness estimates for long-term collection.

To maximize catches during the intensive collection, late spring was selected because greatest moth abundance was expected at that time. As a result, large numbers of various species of seasonally abundant moths were collected. For example, the 10 most common moths collected from the intensive study (Table 3) occurred at twice the densities as those from the long-term (Table 4).

Species diversity and abundance data from the long-term collection shows effects of seasonality (Fig. 3). Moth abundance peaked between May and June, when the intensive collection was conducted. Moth richness rose in late June and was relatively high throughout August. If the goal was to maximize collections during the intensive study, the preferred period would have been late June, when there was not only a peak in abundance, but also an increase in the number of species present. Because these data represent a single year, replicates over several years are required before consistent patterns can be documented. However, the high level of similarity between intensive and long-term collections, despite potential seasonal bias, is encouraging. Choosing the best time of year to sample allows a large percentage of the diversity in an area to be cataloged in a relatively short time.

Because the number of species identified in the long-term collection was greater than that predicted by the estimators for the intensive, it is clear that the estimators were not accurate predictors of species richness in the case of the intensive collection. It is

possible that this is due in part to the high numbers of prolific species collected during the intensive study. Repeat catches of high numbers of the same species may have erroneously influenced the estimators by indicating the intensive collection had approached capturing the "total" number of species in the habitat. Thus, the predictions were too low. Additionally, the estimators assume data points are independent, and this was not the case for the intensive collections. As traps were run on consecutive nights, samples from these pairs of nights were almost certainly correlated.

A greater number of singletons (species represented by only 1 individual) was expected to be found in the intensive study than in the long-term, because the intensive collection encompassed a shorter sampling period, and therefore was more likely to capture fewer representatives of most species. However, a larger number of singletons was detected in the long-term study (Table 2). Additionally, of the 362 species identified in this study, 95 were only recovered in the long-term collection and 47 were unique to the intensive (Table 2). This high number of species collected exclusively during the intensive collection was unexpected. One explanation is that it is simply a result of adding more traps. The very nature of adding additional traps, which were  $\approx 1$  km from the original long-term traps, expanded the habitat breadth of the sampling and attracted a slightly different subset of species. Thus, it is a reflection of microhabitat differences between traps. This difference was more evident between the C1 and C2 traps, with 17% differ-

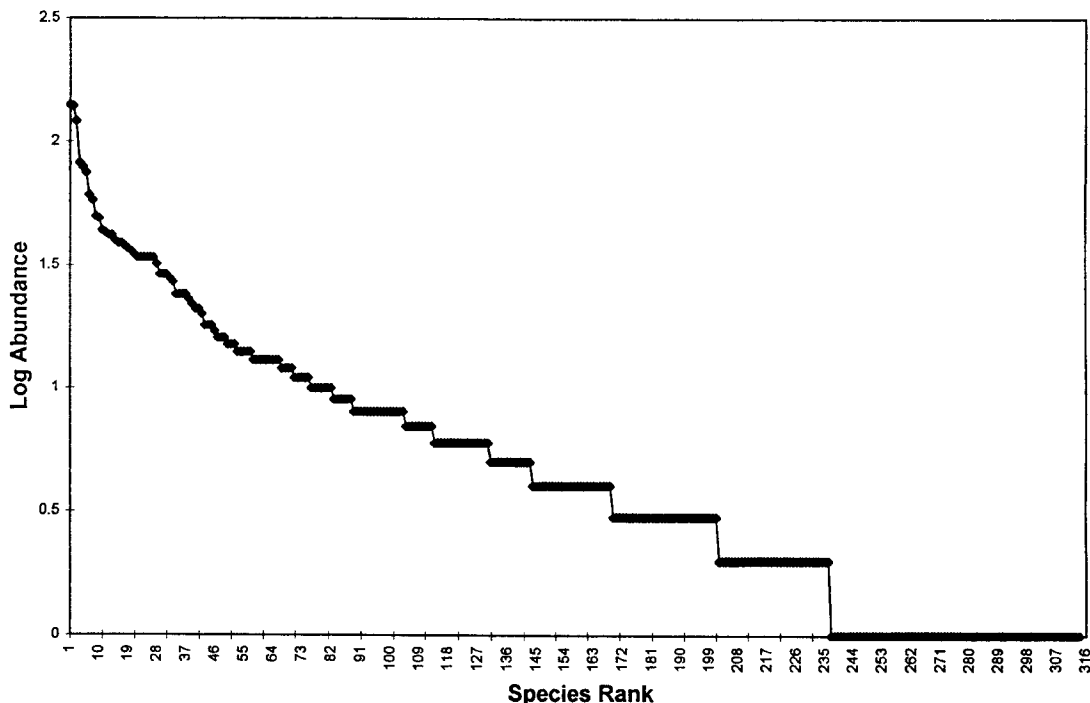


Fig. 2. Rank abundance curve for long-term collection.

ence in species recovery (Table 1) and 65% overlap of species, whereas there was only 3% difference between MG1 and MG2 in species recovery, with 75% overlap of species.

An important question raised by this study is the applicability of the results. For example, how would these data compare with a study involving a different order of insects, or if one looked at moths in a different ecosystem? Carlton and Robison (1999) reported little seasonal variation of species among beetles in forest litter, although abundance fluctuated. Knowing this, if one were to optimize a sampling protocol for forest litter-dwelling beetles, it would be advantageous to focus more on variation in abundance rather than richness.

Another limitation of this study is that collections were only made for 1 season. Based on the seasonality data gathered, late June appears to be the most effective time in which to sample. However, seasonal fluctua-

tions in diversity and abundance are likely to vary from year to year. Sampling for 1 season alone undoubtedly resulted in the omission of numerous species of moths that were not encountered during the year this study was conducted. Species accumulation curves for both collections were still increasing when all 32 samples had been taken from each (Fig. 1), indicating that new species were still being added. However, similarity between traps within sites during the intensive collection, and between sites when comparing the intensive and long-term collections, was consistently high. This suggests that reasonable comparisons can be made between collections for the year in which the study was conducted. This study provides a baseline of data upon which future insect surveys in this habitat may be developed.

Insect inventories can provide a tremendous amount of information about natural systems. Their

Table 3. Ten most abundant moths from intensive collection

Species	Abundance
<i>Baileya ophthalmica</i> (Guenée)	327
<i>Hypoprepia fucosa</i> Hübner	303
<i>Halysidota tessellaris</i> (J. E. Smith)- <i>harrisii</i> (Walsh)	274
<i>Clemensia albata</i> Packard	203
<i>Prochoerodes transversata</i> (Drury)	135
<i>Pero honestaria</i> (Walker)/ <i>hubneraria</i> (Guenée)	111
<i>Malacosoma americanum</i> (F.)	98
<i>Samea multiplicalis</i> (Guenée)	85
<i>Malacosoma dissiria</i> Hübner	82
<i>Eulithis diversilineata</i> (Hübner)	79
Total	1,697

Table 4. Ten most abundant moths from long-term collection

Species	Abundance
<i>Hypagyrtis unipuncta</i> (Haworth)	141
<i>Baileya ophthalmica</i> (Guenée)	139
<i>Halysidota tessellaris</i> (J. E. Smith)- <i>harrisii</i> (Walsh)	121
<i>Clemensia albata</i> Packard	82
<i>Hypoprepia fucosa</i> Hübner	79
<i>Euclaea delphinii</i> (Boisduval)	58
<i>Prochoerodes transversata</i> (Drury)	50
<i>Cosmosoma myrodora</i> Dyar	49
<i>Isochaetes beutenmuelleri</i> (Henry Edwards)	43
<i>Pantographa limata</i> (Grote & Robinson)	42
Total	804

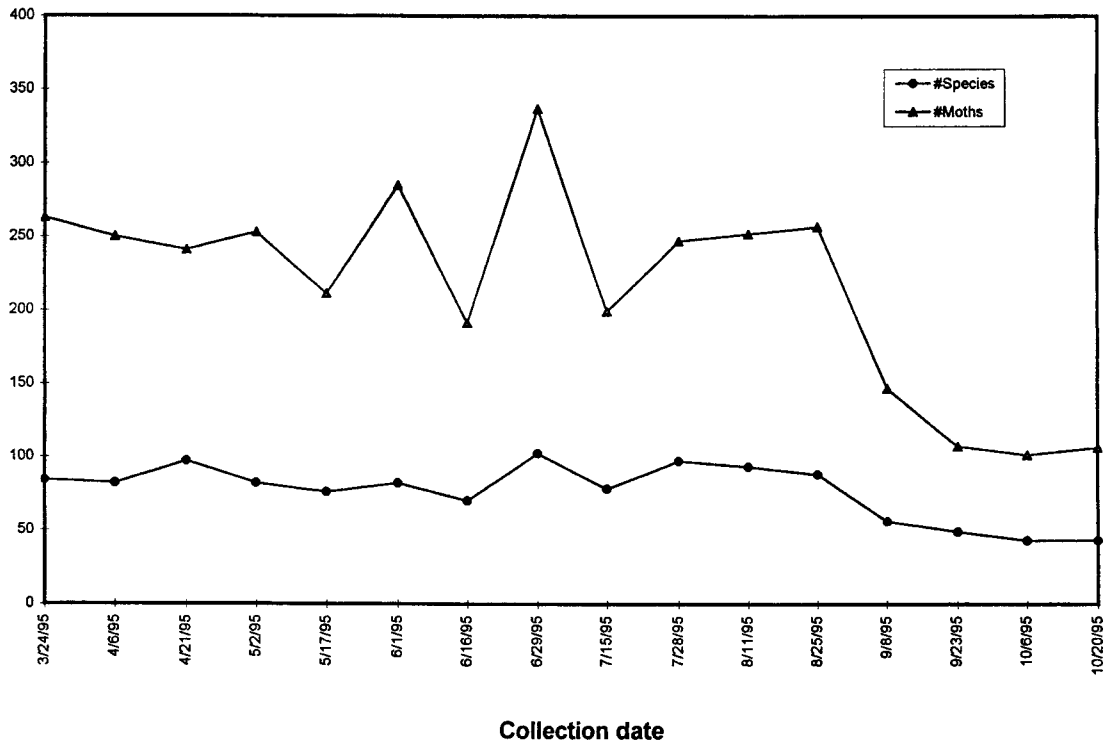


Fig. 3. Seasonal abundance and species richness of moths from long-term collection.

ubiquity, abundance, and diversity make insects ideal indicators of changes in an ecosystem (Oliver and Beattie 1993). Variation in species richness at the landscape scale is a critical component in conservation planning and natural resource management (Coddington et al. 1996). One of the biggest obstacles entomologists face today is cataloging this tremendous source of information, which can often be overwhelming. This study has demonstrated that a considerable proportion of the diversity in an area that would be collected in a relatively long-term study can be recovered in a short, intensive sampling survey. Thus, choosing indicator groups, such as a subset of moths, and extrapolating from them, may be the direction that will have to be taken if we are to effectively assess our rapidly changing ecosystems and thus understand how to better preserve and manage them.

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