





CORE STANDARDIZED METHODS

FOR RAPID BIOLOGICAL FIELD ASSESSMENT




EDITED BY TROND H. LARSEN

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FRUIT-FEEDING BUTTERFLIES

Photo © Phil DeVries

A STANDARDIZED SAMPLING PROTOCOL FOR FRUIT-FEEDING BUTTERFLIES (NYMPHALIDAE)

Philip J. DeVries[1], Christopher A. Hamm[2] and James A. Fordyce[3]

Introduction

Butterflies are among the best-known insects in the world, and their great public appeal makes them a useful group for conservation inventories and monitoring. The Nymphalidae is the largest family of butterflies, and the feeding guild known as fruit-feeding nymphalids may comprise up to 50% of the nymphalid species richness in tropical forests (DeVries *et al*. 2012). One of the most salient characteristics of this group is that they can be sampled in a standardized manner to avoid human collector biases, thus facilitating comparisons of species richness, composition and abundance within and among habitat types. As such, standardized trap-sampling of fruit-feeding nymphalid butterflies has been shown to be an effective means for understanding tropical butterfly diversity in space and time, and for use in conservation efforts (DeVries and Walla 2001; Hill and Hamer 2004; Molleman *et al*. 2006; DeVries *et al*. 2012; Freitas *et al*. 2014). For these reasons, we propose focusing rapid, standardized sampling methods exclusively on fruit-feeding nymphalids, rather than on the entire butterfly community. There are many trap studies now being conducted, but most, however, are not directly comparable because they do not use consistent trap designs, sampling protocols or bait (see examples and citations in DeVries 1987, DeVries & Walla 2001, Batra 2006, Frietas *et al*. 2015). The sampling protocol provided here is based on more than 10 years of monthly sampling conducted in lowland Neotropical forests at Garza Cocha, Sucumbios Province, Ecuador and the Tirimbina Biological Reserve Heredia Province, Costa Rica that have been demonstrated to be directly comparable (DeVries & Walla 2001, DeVries *et al*. 2012).

Core Methods – The Trapping Protocol

Trap Construction – A completed trap is a cylinder 1 m tall and 37 cm in diameter with a closed top and open bottom (Fig 1). Two metal ring frames are sewn into the top and bottom, and the netting must completely close the top of the cylinder. A piece of transparent plastic sheeting can be placed on top of the cylinder to help keep rain out of the bait cup (optional, depending on sampling site, and rain frequency and intensity). The cylinder needs to be sewn such that the netting overlaps on the long axis by 2 cm leaving a 20 cm unsewn slit approximately 30 cm from the top to allow access to the trap interior. Suspended from the bottom ring of the cylinder is a 47-49 cm square trap base (3 mm durable

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plastic for wet habitats, 5 mm plywood for dry habitats) that hangs 6 cm below the opening of the cylinder (keeping this distance is important to minimize escapees). The diameter of the trap base needs to extend 5-6 cm beyond the cylinder diameter (this is important because it provides a landing platform). Holes are drilled on each side, and plastic cable ties or plastic cords can be used to attach the base to the trap. A small plastic bait cup is secured to the center of the base with a loop of thin, stiff wire that is passed through two holes drilled in the base. The wire is then pressed down into the mouth of the cup to keep the bait cup upright and centered on the base. The receptacle for the bait should have a volume of at least 200 ml (8 ounces), and just be tall enough to pass between the base and lower trap ring (6.5 cm maximum, not lower than 6 cm). Cheap, pliable plastic containers work well as they can be cut to size. A sufficient length of nylon cord needs to be secured to the bottom of the trap base to assist pulling canopy traps down from the canopy position. Looping it through the holes of the wire bait cup retainer works well.

Bait – Traps are baited with locally obtained bananas that are first chopped into 2-3 cm pieces and mashed in a large container (that has a lid) by treading on the chopped bananas (wearing rubber boots is optional but useful). Approximately two large bananas are appropriate for each trap, but prepare 1.5 times the volume needed to initially bait all traps. This will be required for subsequent re-baiting during the sampling period. Depending on the source, bananas may have been sprayed with insecticides and fungicides and should either be peeled or washed prior to mashing. The mashed bananas should be allowed to ferment in the large container with the lid sealed for 48 hours prior to use. The day before trapping approximately 150-200 ml of banana mash is added to the bait receptacle in each trap such that the bait level is below the top of the receptacle. Sampling begins the next day. To keep the bait fresh, on day three of trapping add additional bait from the large container to the remaining bait in the receptacle.

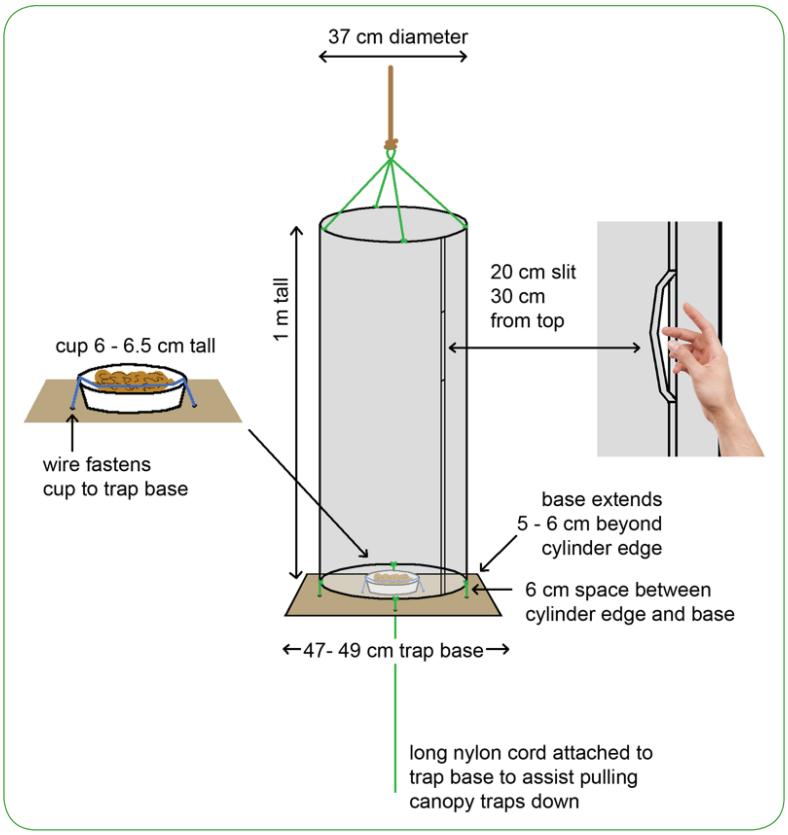

Figure 1 (A) Standardized butterfly trap design

Figure 1 (B)
Canopy trap ready to be deployed in lowland rainforest, Ecuador.

Materials and supplies

Equipment list per trap

- 1 m x 1.3 m of mesh material per trap – enough to make the cylinder and the top.
- Two rust-resistant metal rings – 37 cm diameter. These can be made from thick wire obtained at a local hardware store, and welded or taped into the correct diameter.
- 47-49 cm base plate made of 3 mm of durable plastic for wet habitats, or 5 mm plywood for dry habitats.
- 10 cable-ties – to affix base plate to trap. The space between trap bottom and bottom ring will dictate the length of cable-ties.
- 6.5 cm tall, 200 ml volume, plastic receptacle for bait (e.g., the cut base of a plastic water bottle works well).
- 0.5 m of flexible metal wire to affix bait receptacle to base plate.
- 70 m of nylon cord.
- BigShot line catapult – comes with cords and weights.
- Three 3 m poles for tripod construction when placing traps in open habitat, can be locally available materials (e.g. bamboo).
- One large bucket with a sealing lid for banana mash.
- Bananas, approximately two large bananas are required per trap to make the fermented banana mash. The total must be scaled to the size of the study, and additional mash to add during third day of sampling.

Other required equipment

- Indelible ink pens.
- Glassine envelopes: most specimens fit in size #1, large specimens will fit in size #2.
- Waterproof notebook for data entry (e.g., Rite-in-the-Rain).
- GPS device capable of accuracy within 10 m.
- Sealable plastic container for storing specimens.
- Silica gel or similar desiccant.
- Digital camera.
- Device to record minimum and maximum temperature and relative humidity.

Trap Placement – In tropical forest and savanna habitats where tree canopies are at least 8-10 m, it is essential to place traps in the canopy because available evidence indicates that the canopy and understory butterfly communities are distinct (see rainforest studies cited in DeVries *et al* 2012; Freitas *et al* 2014; Fordyce & DeVries, unpublished; Brazilian Cerrado G. Freire Jr., pers. comm.). Canopy trap lines need to be shot over a tree limb with a line catapult such that the trap can be elevated and lowered easily from the ground without hitting other vegetation. This is important as it dictates what individual trees are selected to suspend the canopy traps. Canopy traps should be placed such that each trap is located within, or very close to the canopy of the individual tree selected. The 'Big Shot' brand catapult is very good for this purpose, or if necessary, it can serve as a model to build a similar apparatus from locally available materials. Understory traps are placed with a cord thrown over a convenient limb and suspended such that the trap base is 1 m above the forest floor. Traps need to be uniquely numbered and lettered for easy reference later (e.g., trap 10C, 10U, 5C, 5U, etc.).

To be consistent and comparable with published and future butterfly trap studies, each trapping station (consisting of a paired canopy and understory trap in forest, or single trap in open habitat) should be placed haphazardly within the area of each habitat type to be sampled. Trapping stations should be separated by at least 20 m (e.g., DeVries & Walla 2001). We use a haphazard design because the structure of a particular habitat often precludes using a strict randomization that makes trap placement difficult or impossible (e.g., presence of ravines, rivers, etc). The placement of a canopy trap in forest habitats depends on a suitable canopy tree. Tree selection is dictated by nearby vegetation (liana cover, mid-story palms and trees), inasmuch as not all trees will allow an easy line shot, or space to smoothly run traps up and down. Choosing an appropriate canopy tree will, in turn, determine the placement of the understory trap. In other words, common sense and habitat architecture should be used to facilitate trap placement.

In habitats where there is no forest canopy cover (e.g., grassland-like habitats), traps should be suspended by employing a tripod constructed of poles of sufficient length so the trap bottom is 1 m above the ground (to ensure comparability with forest traps).

For analytical purposes, each individual trap represents an independent sampling unit (i.e., canopy and understory traps of the same trapping station are separate sampling units).

Sampling Effort – In forested habitats a minimum of 5 stations should be established, each with a paired canopy and understory trap. In savanna-like habitats without high canopy cover, a minimum of 10 stations should be established. This maintains parity in minimum sampling effort across habitats.

Since it is not possible to sample the entire butterfly community during a rapid survey, it is important to understand the relationship between sampling effort and the number of species observed (Fig. 2). Sampling effort and observed species richness can be increased either by longer sampling duration or a greater number of traps. Figure 2 demonstrates the relative contribution of each approach to species richness. Given the time available for sampling at each survey site, as well as the availability of materials

and accessible area of the site to be sampled, this relationship can be used to determine how many trap stations should be established. Note that the standardized sampling protocol described herein allows for comparisons among sites with unequal sampling effort using standard rarefaction methods (Gotelli & Colwell 2001).

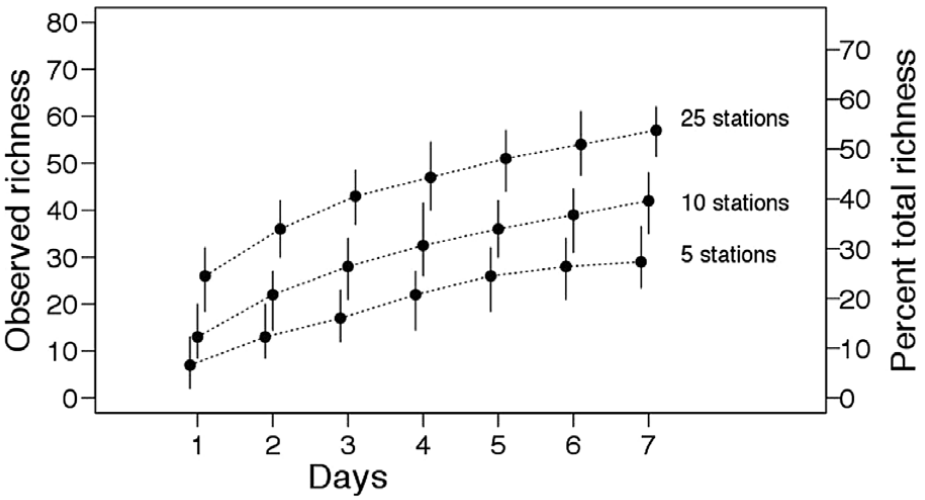

Figure 2
Null expectation of richness captured as a function of sampling effort based on empirical capture probabilities obtained at Reserva Biologica La Tirimbina, Sarapiqui, Costa Rica. Bars indicate 95% highest density interval based on 1000 simulated data sets. Total species richness observed over the course of this 5 year study was 102 species (DeVries et al. 2012). Y-axis indicates raw richness, Z-axis indicates percentage of total richness.

Data Collection and Management – On trapping days each trap needs to be checked at least once a day, sometimes twice, depending on daily capture abundance. In some areas, certain seasons or months may show high species abundances that require checking the traps more frequently. All individual butterflies should be removed, killed, placed in individual glassine envelopes and the relevant data written directly on the envelope with indelible pen (locality, trap number and vertical position, date, etc.). A minimum of two researchers is needed to check the traps at least once (sometimes twice) a day. One person is responsible for removing and processing sampled individuals, while the other records envelope data for each individual butterfly into a field notebook (example below).

Example of field notebook data taken during sampling:

Name	Position	Station ID	Date	Location
Archaeoprepona demophon	Canopy	1	1 jan	Tirimbina
Hamadryas februa	Understory	2	1 jan	Tirimbina

After initiating trap sampling in a new area there will be an initial period when the researchers will need to learn to identify the genera and species in their samples. In areas where field guides are unavailable researchers should make up temporary names for recording in the field notebook (e.g., large orange spot, brown 2 eyes). Eventually the samples will be determined to species by a specialist, at which time the temporary field names in the notebook can be modified.

Specimens should be deposited into an appropriate and curated repository, such as a museum or natural history collection. Data from individual specimens should be digitized as soon as possible and stored in multiple locations. Ideally, the data are stored in some type of database (e.g., SQL), a format that allows for easy hosting and dissemination of data. Data collected by this method should be given a DOI and made publicly available as soon as possible and placed under a Creative Commons license that allows free use as long as proper attribution is given. These data can be hosted free of charge on sites such as FigShare.

Conservation Implications – This sampling protocol provides a standardized method for assessing the species diversity of a butterfly feeding guild. In tropical forests fruit-feeding nymphalid butterflies show fluctuations in abundance and richness, and respond to disturbance (e.g., DeVries, Murray & Lande 1999; Hill & Hamer 2004; Molleman *et al*. 2006; Bossart & Opuni-Frimpong 2009). Using these standardized methods makes it relatively easy to compare results among sites, to understand community-level changes over time, and to evaluate fluctuations in rare and common species within and among sampling sites. For these reasons fruit-feeding nymphalids have great potential as a group for revealing critical patterns for conservation monitoring.

Limitations – All sampling methods have limitations, trade-offs, and biases. Based on the systems that we know well (lowland rain forest), fruit-feeding butterfly richness and abundance are idiosyncratic across time and do not necessarily reflect seasonal trends (Fig. 3; Table 1), thus complicating comparisons where long-term data are not available. For example, Table 1 shows monthly pairwise (dis)similarity of butterfly communities at Reserva Biologica La Tirimbina, Costa Rica. Each month is roughly equally similar to all other months, and there are no obvious seasonal (or temporal) autocorrelations in community composition. This might be advantageous, in that there is no obvious “best time of the year” to assess these communities. However, it also exposes the weakness of short-term studies to capture community composition, as many less-common species will not be detected (see also Fig. 2). Thus, reliable estimates of species richness and records of species occurrence might require long-term trap data, and comparisons among short-term studies should be conducted with a keen awareness of these limitations. Furthermore, testing fruit-feeding butterflies for seasonal effects will be required for other habitats such as savanna, grasslands, paramo and wetlands where there are no data currently available.

While trap-sampling only fruit-feeding butterflies using this standardized protocol provides comparable data across multiple sites, it will not capture nectar-feeding species in the Nymphalidae or other butterfly families. Opportunistic collecting with a hand net should therefore be done to complement trap-sampling. Such hand-collecting will contribute to our understanding of diversity at the site, but due to the biases associated with this method, data cannot be compared across different sites. Alternatively one could conduct Pollard transects (Pollard & Yates 1993), but there are serious drawbacks with this method in tropical habitats that have high richness and low abundance, or where the butterfly fauna is poorly known (Hamm 2013). Moreover, transect-based survey methods cannot account for potential vertical stratification of butterfly communities in lowland tropical forests.

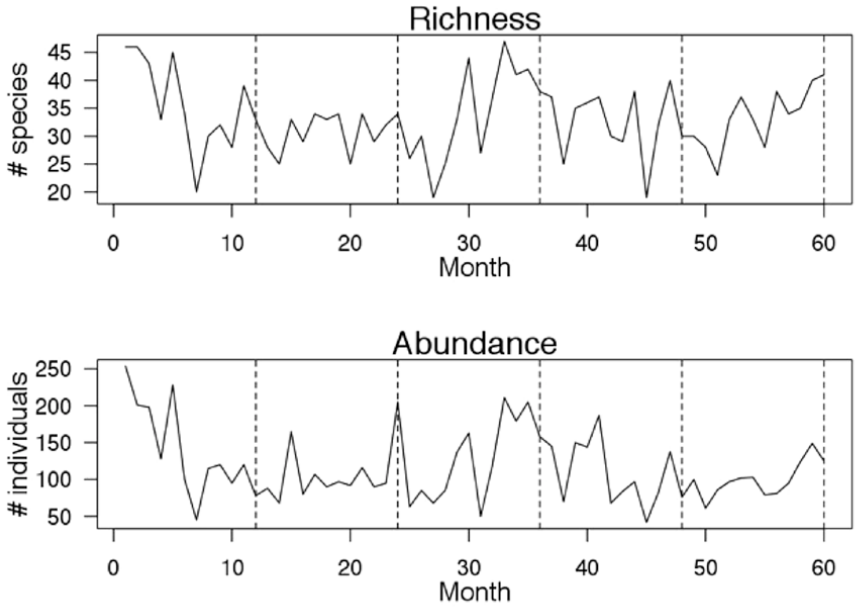

Figure 3
Richness and abundance over time for five year trap study at Reserva Biologica La Tirimbina, Sarapiqui, Costa Rica. Although richness and abundance are idiosyncractic (i.e., showing no seasonality), richness and abundance are highly correlated (r = 0.8).

TABLE 1: Monthly pairwise Jaccard (dis)similarity matrix of butterfly communities based on richness (incidence) data.

Note the idiosyncratic nature of monthly comparisons – monthly comparisons at the same site range between 0.23 and 0.40, with no obvious temporal trend.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Jan	0.00											
Feb	0.37	0										
Mar	0.36	0.33	0									
Apr	0.31	0.33	0.28	0								
May	0.31	0.38	0.35	0.25	0							
Jun	0.38	0.35	0.32	0.32	0.34	0						
Jul	0.34	0.36	0.35	0.25	0.32	0.23	0					
Aug	0.34	0.40	0.39	0.35	0.32	0.37	0.31	0				
Sep	0.33	0.35	0.34	0.27	0.27	0.29	0.25	0.25	0			
Oct	0.30	0.39	0.32	0.33	0.31	0.31	0.24	0.38	0.26	0		
Nov	0.32	0.36	0.36	0.33	0.36	0.29	0.27	0.36	0.29	0.30	0	
Dec	0.28	0.30	0.35	0.30	0.24	0.32	0.28	0.28	0.29	0.33	0.33	0

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