Good Practice Guidelines
BUTTERFLY CANOPY TRAPPING

Corresponding author:
Mark E. Harrison

Corresponding author's e-mail:
mharrison@outrop.com

Citation: Purwanto, A., F. A. Harsanto, N. C. Marchant, P. R. Houlihan, K. Ross*, C. Tremlett and M. E. Harrison (2015) *Good Practice Guidelines: Butterfly Canopy Trapping.* Orangutan Tropical Peatland Project, Palangka Raya, Indonesia.
* née Brady.

Cover image: A male *Lexias pardalis* butterfly. Sarah Nolan/OuTrop

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E-mail: info@outrop.com
Website: www.outrop.com

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Acknowledgements

The methods described in this report were developed mostly in the Natural Laboratory for the Study of Peat-swamp Forest (NLPSF) by the researchers, staff and volunteers of OuTrop and CIMTROP, whom we thank for their hard work and dedication. Particular gratitude is extended to Abdul Azis, Nicholas Boyd, Alex Whiscombe, Robert Durgut, Erik Franks, Ivan Mendez, Sara Nolan and Tristam Allinson. We would like to thank the people and administrations of the University of Palangka Raya, Kereng Bangkerai, Kecamatan Sabangau and Kotamadya Palangka Raya, the University of Palangka Raya, the State Ministry of Research and Technology (RISTEK) and TSA-KALTENG for ongoing support. We are grateful to the US Fish and Wildlife Service Great Apes Conservation Fund, The Orangutan Project, Arcus Foundation, Rufford Small Grants for Nature, Wallace Global Fund and StOLT Foundation for financial support of our butterfly research; and to BioQuip for canopy trap donations. David Ehlers Smith provided the outline map for Borneo used in Figure 1, and Suzanne Turnock provided editorial and formatting assistance. OuTrop is affiliated with the Department of Geography at the University of Leicester, the College of Life and Environmental Sciences at the University of Exeter, and the Faculty of Humanities and Social Sciences at Oxford Brookes University, whom we thank for support.

Introduction

Good Practice Guidelines: Purpose and Use

OuTrop's Good Practice Guidelines (GPGs) draw upon our experience in the Sabangau Forest and other areas, to provide recommendations for people wishing to establish similar assessment or monitoring research in their own sites. We hope that this will help build capacity for these activities in Indonesia, therefore supporting conservation of its rich natural resources. We encourage people setting up similar research initiatives to always consider if any adaptations to our recommendations might be needed to suit the specific aims and limitations of their project. OuTrop are happy to discuss any questions and potential collaborations related to this. Revised versions of this document may be published on www.outrop.com from time to time, reflecting any new advances in the area. Good luck in your research!

Butterfly GPGs: Background

Fruit-feeding butterflies of the Nymphalidae family are commonly studied in tropical forests, both to increase our understanding of tropical forest ecology, and importantly also for conservation assessment and monitoring purposes. This is because they frequently show consistent and rapid responses to changes in habitat condition (Beccaloni and Gaston, 1995; McGeoch, 1998; Bonebrake *et al.*, 2010); can be systematically and relatively easily sampled in the field using fruit-baited traps (Austin and Riley, 1995; DeVries *et al.*, 1999; Hamer *et al.*, 2005; Houlihan *et al.*, 2013); and their morphology, distribution, natural history, and evolutionary relationships are well described, compared to other tropical insect guilds (Gilbert and Singer, 1975; D'Abrera, 1985; Kawahara and Breinholt, 2014). Consequently, research into fruit-feeding butterflies has been an important component of OuTrop's ongoing ecological monitoring programme development (Harrison *et al.*, 2012a, b).

Here, our overall aim is to provide tools to monitor the condition or "health" of tropical peat-swamp forests, and trends in this over time in relation to changes in human disturbance and conservation management.

To this end, working in the Sabangau Forest, we began experimenting with the use of fruit-baited canopy traps to sample this group in 2004 (Brady, 2004), conducted investigations to assess the impacts of canopy gaps on fruit-feeding butterflies (Houlihan *et al.*, 2013), produced an identification guide to the fruit-feeding butterflies of the Sabangau (Houlihan *et al.*, 2012), and in 2012 began an intensive and ongoing study involving monthly butterfly sampling in areas with varying levels of overall human disturbance (Marchant *et al.,* in prep). The specific aims of this current intensive study are to:

1. Identify those species or groups of species that show the most consistent and rapid responses to variations in forest disturbance, and thus are most suitable for use as an ecological disturbance indicator;
2. Identify any temporal (intra- or inter-annual) variations in fruit-feeding butterfly abundance that might confound the above assessments;
3. Develop and implement a simple system for monitoring and reporting on trends in abundance of ecological disturbance indicator species, which are easily interpretable by conservation managers; and
4. Advance our overall understanding of tropical butterfly and rainforest ecology, including ascertaining the influence of inter-annual fluctuations in climate on the butterfly community.

In these GPGs, we describe our methods for establishing baited canopy traps for fruit-feeding butterflies, and collecting and processing butterfly data. These have worked well in OuTrop's primary research sites in Sabangau (Figure 1), yielding useful insights into the impacts of human disturbance and "natural" stochastic factors (season, climate) on butterfly species richness and diversity, community composition and individual species' abundance (Brady, 2004; Houlihan *et al.*, 2013; Marchant *et al.,* in prep). With appropriate changes in bait, we have also successfully used these same general methods to study carrion-feeding butterflies in Sabangau (Tremlett, 2014). Consequently, the protocols described herein are suggested as a standard for the region, particularly in tropical peat-swamp forest.

A female Lexias pardalis *butterfly in Sabangau.Photo: Jessica Smallcombe/OuTrop.*

OuTrop are happy to advise and collaborate on butterfly studies in the region, and we encourage readers to contact us if they feel this would be beneficial to their programme. Note that these GPGs do not cover:

1. Methods for identifying butterflies; for this we refer readers to existing identification guides for the region (D'Abrera, 1985; Tsukada, 1991; Otsuka, 2001; and particularly Houlihan *et al.*, 2012 for readers working in peat-swamp forests).
2. Detailed discussion of data analysis, the needs for which may vary hugely between projects and for which many different options are available. General guidance on statistical analysis techniques is provided by numerous other authors (e.g., Zar, 1999; Magurran, 2004; Colwell, 2013; and with a butterfly focus: Hughes *et al.*, 1998; Marchant *et al.*, in prep).
3. Hand-netting sampling methods (Cleary and Genner, 2004; Vlašánek, 2013), which may be effective in many cases, but will under represent canopy species and are not very practical in peat-swamp forests such as Sabangau, owing to the dense undergrowth (OuTrop, unpublished data).

Finally, when using these methods for ecological monitoring purposes, it is very important to note that any changes to trap design or butterfly data collection protocols – even if to accommodate methodological "improvements" – may result in erroneous impressions of actual changes in the butterfly community that are in fact due simply to changes in methods. If it is necessary to change methods mid-way through a data collection, it is therefore imperative that these new methods are assessed and calibrated against the previous methods, such that any influence of method alterations can be accounted for in data analysis (Gardner, 2010).

Figure 1. Map showing the location of CIMTROP's Natural Laboratory of Peat-swamp Forest (LAHG on map: *Laboratoreum Alam Hutan Gambut*) and Kalampangan Zone research sites in Sabangau, Central Kalimantan, Indonesia.

Getting started

Study Design – Trap Numbers, Placement and Sampling Frequency

The optimal number and placement of butterfly traps required will vary substantially depending on the objectives of, and resources available for, any particular study. It is therefore impossible to provide over-arching recommendations that will accommodate the needs of all studies. The issues concerning study designs, sample independence and sample sizes for surveying fruit-feeding butterflies are the same as those for any ecological survey and we refer readers to the excellent treatments provided in numerous other texts for detailed consideration of these issues (e.g., Krebs, 1989; Sutherland, 1996; Gardner, 2010).

One key consideration when planning butterfly surveys is that most species' abundance can fluctuate markedly throughout the year, and even within single seasons or months (Hill *et al.*, 2003; Hamer *et al.*, 2005; Marchant *et al.*, in prep). As a result, a survey's timing may determine its findings to a potentially very large degree. In Sabangau, for example, we find that the populations of most fruit-feeding species peak in May, July and October, with low counts in December and February, June, August and September (Marchant *et al.*, in prep).

What is the Primary Aim of the Study?

This is important to define clearly from the outset, as it will heavily influence study design. Common aims and considerations relating to these are given below:

1. *Compiling a species list.* If this is the sole aim of the study, it will be desirable to sample in all different habitat types within an area, concentrate sampling in those parts of the study site with highest species diversity, have as high a

number of trap days as possible in each area to capture rare species, sample in different seasons, position traps at a variety of different heights, and consider trying a variety of different bait mixes and hand netting to maximise the number of different species caught. If this is not the sole or over-riding aim of the study, then it will be necessary to sample more systematically.

2. *Comparing the butterfly community and/or individual species' abundance between different areas/conditions (e.g. habitat types, disturbance categories).* Here, a stratified random study design will often be most appropriate, with equal sampling effort in each different condition and surveys in different conditions conducted (near) simultaneously, in order to reduce any potentially confounding effects of temporal (seasonal) variations in butterfly abundance or behaviour. Consideration should also be given as to whether it is necessary to achieve a complete sample of species in each condition (which will require a very high trapping effort), or whether an incomplete sub-sample of the community is sufficient (this will frequently be the case if wishing to compare indicators of species diversity or richness, or to detect differences in dominant species in the community, as in our project in Sabangau).

3. *Comparing the butterfly community and/or individual species' abundance over time within the same area (e.g., in a monitoring programme to establish the impacts of a conservation management activity).* In this case, it will be desirable to monitor traps in exactly the same locations and at the same height each time, to minimise any confounding effects of differences in (micro-) habitat between locations. It will also be desirable to ensure that surveys account for any potentially confounding temporal variations that may operate over a different time frame than that of interest (e.g., seasonal variations potentially obscuring the inter-annual variations of interest). As above, consideration should be given as to whether a complete sample of species is needed, or whether a sub-sample is adequate to achieve the programme aims.

4. *Assessing dispersal, longevity or ranging/territorial behaviour.* Here, it will be desirable to use mark-recapture methods to enable individual butterflies to be identified each time they are captured (Lewis, 2001; Fermon *et al.*, 2003; Vlašánek, 2013).

5. *Relating spatio-temporal variations in butterfly communities and/or individual species to variables relating to forest condition and/or some other taxa.* If so, then it will be necessary to ensure that the butterfly and non-butterfly components of the study are appropriately synchronised in space and time. For example, this might mean ensuring that butterfly canopy traps are positioned within tree plots used to assess forest structure; or that butterfly surveys are conducted at the same time each month as surveys of fruit availability or moth populations.

Other Considerations When Planning a Study

The following questions are also important to consider when designing your study:

1. *Is it necessary to survey butterflies at different canopy levels?* Butterfly species composition and abundance can vary in relation to canopy (trap) height (DeVries *et al.*, 1997; Fermon *et al.*, 2003; Houlihan *et al.*, 2013) and, thus, if aiming to obtain a complete species sample or a representative sub-sample of the overall canopy butterfly community, it will be necessary to employ traps at different canopy heights.

2. *Is including or excluding recaptures likely to lead to greatest bias in the data set?* Including data from multiple captures of the same individual can

create bias in data sets (Hughes *et al.*, 1998; Beck and Schulze, 2000; Marchant *et al.*, in prep), particularly if individuals or species show strong home ranging or "trap happy" behaviour, and the primary aim is to compare populations between different areas. Conversely, excluding recaptures can also create bias in data sets because the number of recaptures (and, hence, number of data points excluded) will increase over time and recapture probabilities may differ between species. The decision as to whether or not to include recaptures should therefore be made on a case-by-case basis, depending on the needs of the study.

In practice, a specific study design is likely to reflect a balance between these considerations, plus the realities of access, available manpower and resources. OuTrop's own study design for ongoing fruit-feeding butterfly research in Sabangau is no exception to this. Here, we aim to assess spatial differences between areas of differing disturbance, trends within these areas over time, and to relate both of these to variations in forest "quality", as assessed through tree plots (Harrison *et al.*, 2010; Harsanto *et al.*, 2015) and litter-fall surveys (Harrison *et al.*, 2007; Harrison, 2013). Thus, in our Sabangau research:

- We conduct monthly surveys in each of two (formerly four) main disturbance types (Figure 2), with traps positioned systematically along transects inside our large tree plots and alongside our litter-fall traps. This allows for assessment of both intra- and inter-annual population trends.

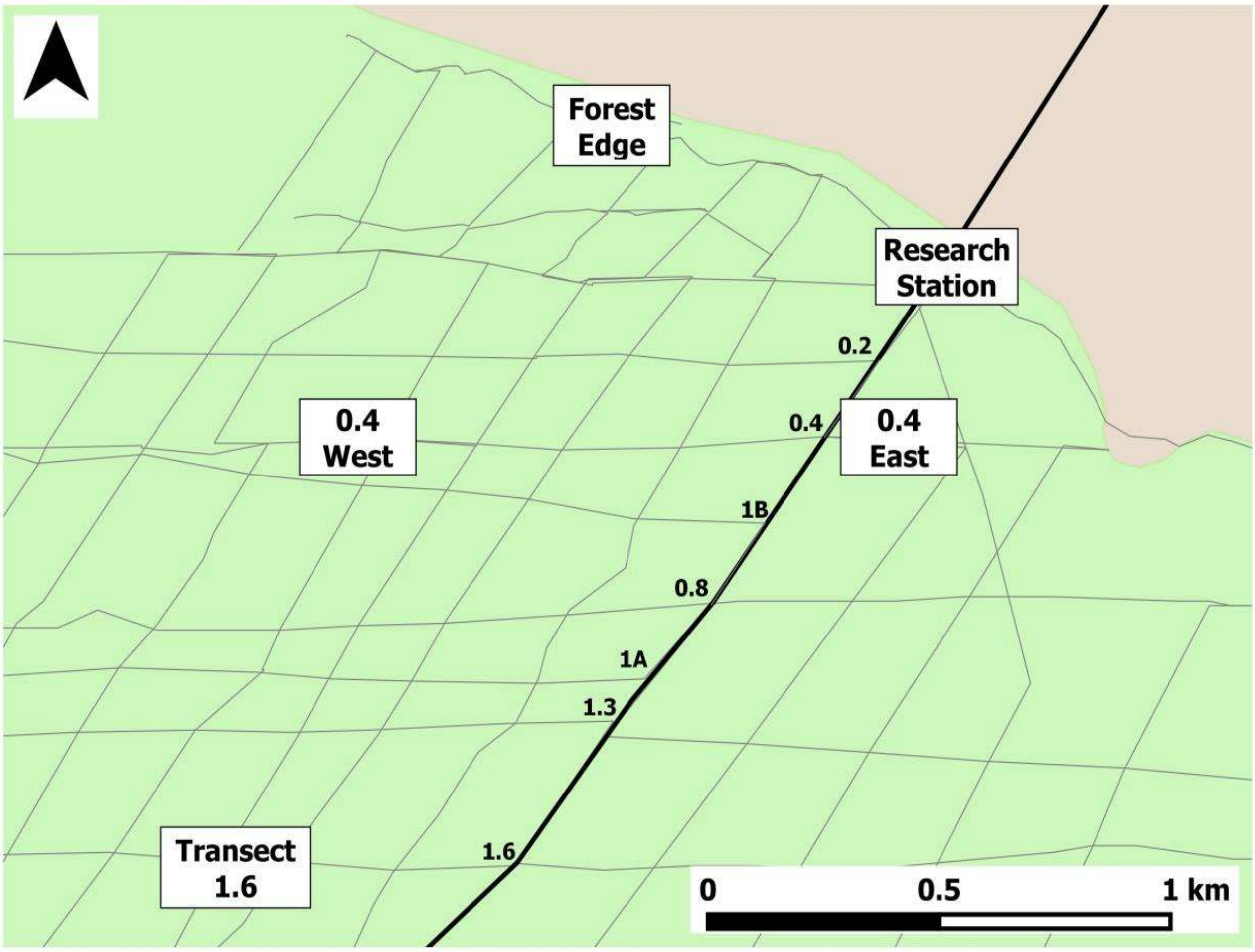

Figure 2. Positioning of OuTrop's butterfly transect locations in the Natural Laboratory of Peat-swamp Forest research station in Sabangau. Numbers along the bold transect indicate approximate distances in kilometres from the research station. Disturbance levels: Forest edge = forest edge; 0.4 East = most heavily logged between two old logging railways (site of ongoing long-term monitoring); 0.4 West = relatively less disturbed; Transect 1.6 = least disturbed (site of ongoing long-term monitoring).

Trap locations are fixed, with repeat surveys being conducted in the same locations each month.

- These different transects are located at least 800 m away from each other (Figure 2), to reduce the risk of the butterflies moving between transects; greater spatial separation is not practical owing to access difficulties and the availability of spatially-corresponding tree plot and litter-fall data.
- Because our present goal is to obtain impressions of change within the overall fruit-feeding canopy butterfly community, we employ both low (trap opening at 1-2 m height) and high (7-10 m) traps, placed in exactly the same location and height in each month to minimise the potential for bias resulting from micro-habitat differences within the same disturbance level classification. Nylon cords are left in place between surveys to ensure that the locations remain consistent.
- Each month, we employ 10 traps in five low-high pairs at intervals of 50 m, all located within the plot/transect in each disturbance level (Figure 3), and leave traps open and in place for five consecutive days, yielding a total 50 trap days in each location each month.
- Monthly butterfly surveys (starting around 20th of each month) are timed to fall in between those for tree reproductive phenology (starting around 16th) and litter-fall surveys (starting on the penultimate day of each month).
- For our long-term monitoring, because we wish to avoid any potentially confounding influence of forest edge effects, all traps are located at least 300 m from the forest edge. Shorter-term studies have included sampling at and near to the forest edge, specifically to investigate the impacts of edges on the butterfly community.
- All recaptures are noted, allowing for assessment of dispersal, longevity and home ranging behaviour, plus potential inclusion of recapture data in temporal analyses; while also allowing the option of excluding these data if necessary for other analyses.

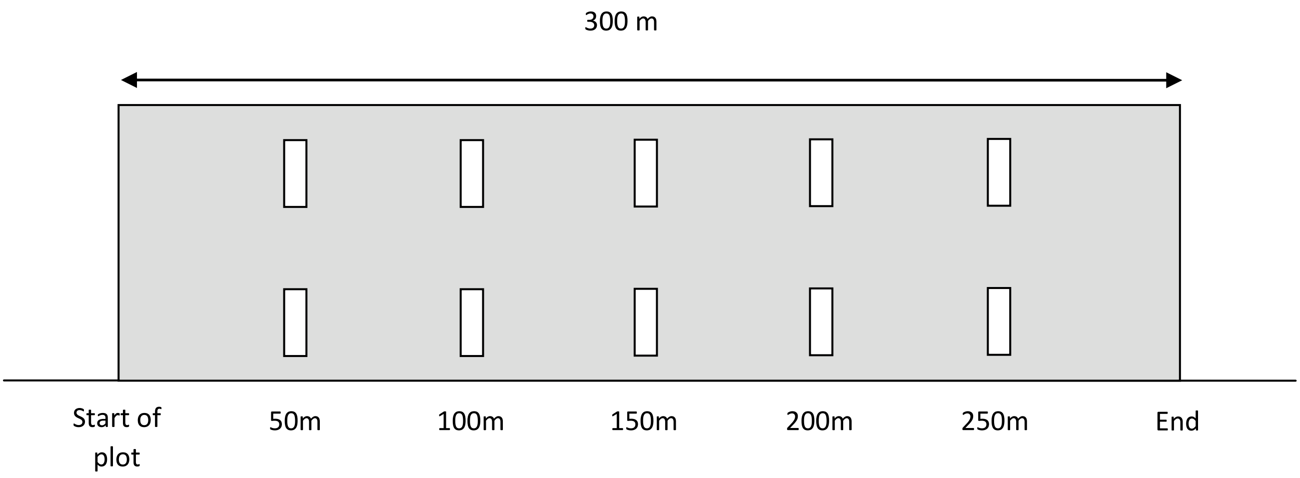

Figure 3. Positioning of OuTrop's low-high butterfly canopy trap pairs within 300 m-long tree plots used to monitor forest structure and tree reproductive phenology.

Trap Design

Trap designs for butterfly canopy trapping are now relatively standard (Austin and Riley, 1995; Daily and Ehrlich, 1995; Hughes *et al.*, 1998; DeVries *et al.*, 1999; Figure 4). These traps operate under the principle that butterflies will be attracted to the bait positioned on the plate affixed to the bottom of the trap and, because butterflies fly upwards upon take off, the butterflies will then become trapped in the net above. The traps are then lowered from their position in the canopy using the attached rope; following which butterflies can be individually removed, inspected and released unharmed; and then bait replaced and the trap hoisted back up into the canopy, if required.

Professionally-constructed butterfly canopy traps are now available from many commercial suppliers. In recent years, OuTrop have used traps kindly donated by BioQuip ('Pop-Up Butterfly Bait Trap, Tropical Type', model no. 1423). These have the advantage of being lightweight (310 g), very small when collapsed (21 x 5 cm), coming complete with a nylon carrying bag and thus being highly portable in the field. When purchasing professionally-made traps such as these, all necessary equipment and any necessary self-assembly instructions are generally always provided together with the trap.

Figure 4. A fully-assembled butterfly canopy trap in Sabangau. Photo: Nicholas Boyd/OuTrop.

Figure 5. Wing remains of predated butterflies in a canopy trap bait tray in Sabangau. Photo: Nicholas Marchant/OuTrop.

While professionally-designed traps are recommended, similarly effective traps can also be self-constructed using materials typically available in most towns in Kalimantan, provided the same general design criteria are met. Detailed equipment lists and assembly instructions for self-constructed traps are provided by Austin and Riley (1995), which is freely available online through the link provided in the References. OuTrop have successfully used Austin and Riley's (1995) Design #2 on numerous occasions, primarily owing to local equipment availability in Palangka Raya. Our capture results using home-made traps have been no different to those using professionally-constructed traps, with the main differences being that the professionally-constructed traps are easier to carry in the field owing to their collapsibility and that butterflies can be optionally removed through a purpose-made closable opening in the side netting.

Adaptations to Prevent Bait and Butterfly Predation

In some cases, other animals may interfere with sampling, either by consuming the bait or predating butterflies within the trap (Figure 5). We have observed bait to be taken by primates, squirrels (Figure 6), birds, beetles and ants; while butterflies have been predated by praying mantids (Mantodea, Figure 7) and bush-crickets / katydids (Tettigoniidae). The importance of this is likely to vary from site to site and over time, from insignificant to potentially very significant: in a recent study in Sabangau at the forest edge in the height of the dry season, over a third of traps showed some evidence of predation (e.g., disembodied wings found in the bait tray) and over 40% showed evidence of bait loss (Durgut, 2013). At other times and locations, however, we have found bait loss and predation to be relatively low (OuTrop, unpublished data).

A variety of potential solutions have been trialled in an attempt to prevent these problems. The effectiveness of these solutions may vary between locations and across seasons, so it is recommended to experiment with these beforehand if predation and/or bait loss are identified as potentially problematic at a research site. Potential solutions include (Austin and Riley, 1995; Durgut, 2013):

1. Applying petroleum jelly to the rope above the trap to repel ants and other small crawling animals;
2. Using a length of fishing line between the rope and trap attachment;
3. Attaching a washing-up bowl to the rope ca. 1 m above the trap (found to be relatively ineffective in Sabangau);
4. Attaching an aluminium cone to the rope ca. 1 m above the trap. (found to be relatively effective in Sabangau); and
5. Fixing a chicken-wire mesh (ca. 1 cm grid) over the top of the bait, such that bait can be reached by a butterfly proboscis, but not by other animals (effective in Sabangau, particularly when combined with #4 above).

Figure 6. A *Callosciurus notatus* squirrel attempting to raid a home-made butterfly canopy trap in Sabangau. Photo: Ivan Mendez/OuTrop.

Figure 7. A praying mantis inside one of our butterfly traps. Photo: Nicholas Marchant/OuTrop.

Bait

A variety of different fruit-based baits can be used to attract butterflies, and each potential mix is likely to differ in its effectiveness among butterfly species, sites and potentially even seasons. It may therefore be desirable to experiment with different bait mixes at the beginning of a research programme, to establish which is most useful for the needs of that particular programme. Regardless of the exact bait mix selected, it is vital to keep the bait mix constant once a monitoring programme has begun, as changes to the bait mix may result in changes in captures that might introduce bias to the dataset and lead to erroneous conclusions concerning changes in the butterfly community (cf. Gardner, 2010). All fruit-based baits work under the principle that rotting fruit ferments, leading to alcohol production, which is the olfactory attraction for butterflies (Hughes *et al.*, 1998). Attraction can often therefore be increased by adding sugar (to encourage fermentation) and/or alcohol to the bait mix.

In Sabangau, we employ the following bait mix for one trap (Figure 8), with measurements adjustable based on the number of traps used:

1. Take two small over-ripe / fermenting bananas (N.B. bananas in Central Kalimantan are typically very small; lower numbers may be needed in other areas);
2. Mash with two tablespoons of sugar;
3. Stir in two tablespoons of the local grape-based liquor, Malaga (12% ABV).

This bait mix is recommended for general fruit-feeding butterfly trapping in Kalimantan's tropical peat-swamp forests, owing to the large number of species and individuals it attracts (Houlihan *et al.*, 2012, 2013), plus the widespread availability of the necessary ingredients. It is normally easiest to make enough bait mixture for all traps to be sampled that day in one batch, and then to carry a single tub into the field and measure out equal amounts of the mixture into each

Figure 8. Bait mixture ingredients for fruit-feeding butterfly traps. Photo: F. A. Harsanto/OuTrop.

trap using a spoon. Bait may be made up either the night before or on the morning of surveys; most important is to remain consistent across sites and time periods surveyed.

Because capture rates decline with increasing bait age, it is advisable where possible to replace/replenish/stir bait every few hours (Hughes *et al.*, 1998). In most cases, including during our own research, changing bait with such frequency will be impractical and thus daily bait changes are recommended. In Sabangau, it is normally necessary to replenish the bait each day by stirring and adding two tablespoons of the above mixture. Further, we recommend making fresh supplies of bait mixture each day, rather than making up mixtures for multiple days' sampling at once.

Sampling of butterflies with other dietary preferences requires other types of bait; e.g. a prawn paste and shrimp mix for carrion-feeding butterflies (Hamer *et al.*, 2006). In Sabangau, we have previously used a prawn paste-only bait for this purpose (Tremlett, 2014). This worked well and has the advantage that the prawn paste is in easily carried and dispensable tubes, therefore eliminating the need to carry pots of stinking fish or chicken-based baits around in the field.

Setting up Traps in the Field

Equipment Needed

A team of at least two people is required to set up traps, with more people being required if large numbers of traps need to be carried and especially if multiple trap set-up groups are to be deployed. The following equipment will be needed:

1. The canopy trap/s
2. Nylon cord of around 2-3 mm thickness. Cord length will need to be at least double the desired height of the trap (see point #7 in Procedure for Setting up Individual Traps). Raffia should not be used to hang traps, as it frays over time and often gets stuck when trying to raise/lower traps
3. Slingshot
4. Lead fishing weight
5. Fishing line of a minimum length of three times the desired maximum height of the traps.
6. Material for tagging, cut into tags of ca. 4 x 10 cm
7. Permanent marker
8. Nails and hammer
9. Densiometer.

Procedure for Setting up Individual Traps

1. Carry packed traps out to pre-defined trap locations, opening up and attaching the rope to the top of the trap at the trap location.
2. Number the trap according to a standardised system, using the tagging material and permanent marker. Tags may be written before entering the field, which minimises problems associated with markers drying out or not working properly in hot or wet conditions. Affix the tag either to the trap using fishing line, or to a nearby tree using the hammer and nail.
3. For each trap number, record the trap location (both using GPS and position along any forest transect), trap height, date set and other desired variables, for which we suggest the following will frequently be useful:
   a. Trap height – this should be measured from the base of the bait tray. Height can be determined by measuring a length of taut raffia between the bottom of the trap and the ground.
   b. Canopy cover – standing directly underneath the trap, look through the densitometer at head height and estimate the percentage of the view that is obscured by the canopy. A more refined estimate can be obtained through estimating the percentage canopy cover within different height classes (e.g. 0-5, 6-10, 11-15, 16-20 m, etc.; note that the sum of different height classes can exceed the overall densitometer reading if different canopy layers overlap). Through this process, it is possible to determine the main canopy height and the position of the trap relative

to different canopy layers, which might influence the number and species of butterflies captured.

c. Basic habitat description – a simple categorical or text description of the habitat for reference purposes (note habitat type and level of disturbance, including any large tree falls). We restrict this to the 5-m radius surrounding the trap.
d. Ground cover – percentage cover of living material, dead wood and bare ground / leaf litter within a defined area below the trap (e.g. 2 m diameter).

4. Traps should be located at least 1-2 m from any nearby branches / foliage, to minimise the risk of disturbance and bait predation from other forest fauna, and traps colliding with trees/branches during high winds.
5. The fishing line (attached to the cord) should be fired over high branches using a slingshot and lead weight. Once over the desired branch, pull the fishing line to bring the nylon cord over. For lower branches, it may be possible to throw the lead-weighted rope over the branch, and/or to substitute the lead weight for a piece of wood from the forest. Great care should be taken when doing this, particularly if using sling shots and lead weights, which could hit people and cause injury.
6. Add the appropriate amount of bait mixture to the trap's bait plate.
7. Attach a cord to the bottom of the bait tray before raising it. This can help to lower the trap if the upper cord gets stuck.
8. Particularly for higher traps, it can often be useful to create a loop similar to a flag pole (Figure 9). The cord should be looped through a root or other tying point on the ground, then tied to the bottom of the trap and pulled tight. It is usually necessary to attach a cord to the bottom of the trap so that it can be pulled down. This can be advantageous for high traps, because (a) they frequently experience greater movement due to stronger winds and tree swaying; and (b) the length of rope necessary to hoist a trap into the canopy is usually heavier than the trap itself, which may prevent the trap from descending when checking butterflies, thus requiring repeated rope shaking to dislodge the trap and resulting in butterflies escaping.
9. Hoist the trap up to the required height using the pre-measured raffia as a guide (Figure 10; see point #3a) and secure the ground end of the rope to a solid nearby tree. Saplings, roots and lianas are best avoided. The rope should be pulled quite tight to prevent the trap from swinging during high winds.
10. In Sabangau, many of our traps are located in tree reproductive phenology plots and/or near litter-fall traps, and in cases such as this it is important to avoid major tree shaking/canopy disturbance while erecting traps, as this may dislodge fruit and leaves.
11. If conducting repeat monitoring in the same locations, remove the trap but leave the string attached, so traps can be easily re-erected.

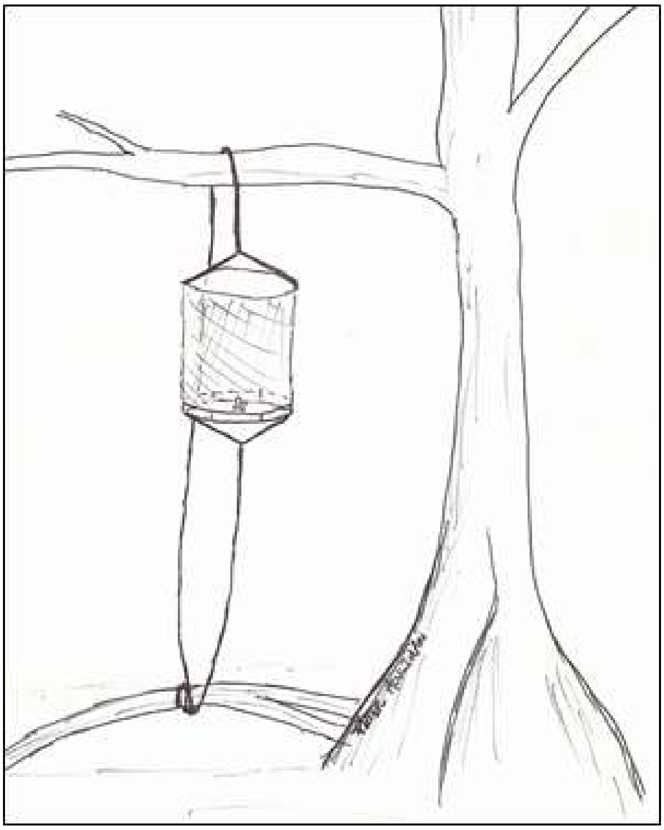

Figure 9. Diagram of the "flag-pole" system for attaching canopy traps (see point #8).

Figure 10. An open, baited, “high” butterfly canopy trap in Sabangau. Photo: Andrew Walmsley/OuTrop.

Trap Maintenance

Especially in longer-term studies, traps will suffer damage through animal disturbance, collisions during high winds, exposure to rain and strong sunlight, rusting of metal materials, etc. It is therefore important to check each trap for damage before setting up and at each subsequent field check. Many problems can be easily fixed in the field through carrying spare supplies when checking traps, and it is therefore advisable to always carry the following when checking traps:

1. Sewing needles
2. Fishing line
3. Nylon cord and raffia
4. A sling shot, weight and fishing line, for re-hanging fallen traps
5. A small amount of spare trap netting
6. Tagging material
7. Permanent marker
8. Nails
9. Hammer

More serious problems, such as very large net tears or snapped metal supports, may require traps to be brought back to camp for repair or to be replaced. It is therefore advisable to carry at least one spare trap when conducting surveys, which can be used to immediately replace a completely broken trap if needed.

Data collection, storage and analysis

Daily Data Collection Schedule

As noted above, once set up, baited and opened, traps should be checked at minimum once daily. Traps should be set up and baited at least 24 hours before data can be collected. During these checks, butterflies should be sampled through the procedure outlined below, new bait added, traps checked for any maintenance problems and repairs conducted as necessary.

Butterfly activity in Borneo tends to peak at around midday (1100-1400h, see Hughes *et al.*, 1998), and so we check all traps between 1000 and 1500h. It is advisable to conduct a brief pilot study to establish peak daily activity periods in any new site and adjust daily sampling times accordingly, before embarking on intensive sampling.

Recording data in the Sabangau Forest Photo: Andrew Walmsley/OuTrop.

Field Data Collection

Equipment Needed

1. Binoculars
2. Butterfly hand net
3. Butterfly identification guide (e.g., D'Abrera, 1985; Otsuka, 2001; Houlihan *et al.*, 2012), kept at the research base for easy reference – most guides are too large and unwieldy to carry in the field.
4. For field use, butterfly identification plates are recommended (cf. Appendix A). These should ideally be constructed bespoke, based upon the species known to occur in the site/s being surveyed.
5. Camera, preferably with macro lens
6. Vernier calliper
7. Permanent marker
8. Bait mixture (inside plastic box)
9. Spoon
10. Invertebrate sample envelopes (if collecting samples)
11. Field data sheets, pencil, clipboard and rain-proof cover
12. Trap repair kits (see Trap Maintenance Section)

Procedure

The following procedure should be followed each time a trap is checked:

1. Approach the trap slowly and inspect it at a distance using binoculars. Try to count and identify all butterflies within the trap; this is important to try and account for any butterflies that may escape during the checking process. Also look for butterflies perched on the outside of the trap or on the ground underneath, and record these as incidental observations.
2. One surveyor should lower the trap quickly but carefully to the ground. As soon as the cord is untied the butterflies may start to fly around and may escape, so it is important to move quickly. When the trap is reachable, the second surveyor should cover the bottom of the trap with a hand net and grasp the fabric in the middle of the trap, in between the lowest butterfly and the trap opening.
3. Carefully remove each butterfly from the trap one by one, taking care not to disturb other butterflies or allow them to escape. While exact data collection needs will vary from study to study, collect the following data are recorded for each butterfly, which we recommend will be useful for most researchers:
   a. Identification, to species where possible. With practice, this can be achieved relatively easily in the field for most species, using an appropriate identification guide for assistance where necessary (e.g. (D'Abrera, 1985; Otsuka, 2001; Houlihan *et al.*, 2012). If you find a species new to you or are unsure of the identification, take a photograph of both the upper and lower wing, for double checking with an identification guide and/or relevant expert (this is a good habit even if you are confident of your identifications). Pay particular attention to cryptic and species-rich genera such as *Tanaecia* and *Charaxes*.
   b. Measure the length of the forewing (shoulder to tip of wing), and overall body length (top of head to end of abdomen) using a vernier calliper.
   c. Sex. For sexually dimorphic species, this can be determined through differences in wing colours/patterns and sometimes through variations in body size. For sexually monomorphic species, try to determine whether the butterfly is male or female by inspecting the abdomen. See field guides (D'Abrera, 1985; Otsuka, 2001; Houlihan *et al.*, 2012) for guidance on butterfly sexing.
   d. The condition / age of the butterfly, estimated using a four-point scale:
      i. Newly emerged, colours very bright, undamaged
      ii. Wing scales slightly faded
      iii. Wing scales faded, occasionally with some minor wing tears
      iv. Butterfly appears old and is unlikely to survive much longer. Wing scales very faded and may have damage to wings

4. If monitoring recaptures, all butterflies caught in traps should be marked on the underside of their left forewing using a standardised numbering system and permanent marker. In Sabangau, we assign unique letters to each species and then assign sequential numbers to each captured individual from the species, according to codes listed in our Field Butterfly ID Plates (Appendix A). For example, using this scheme, butterfly "A8" would be the eighth individual of *Lexias pardalis* captured. If a butterfly is re-captured in a later survey, the unique code for the butterfly should then be marked in the 'recapture' column on the data sheet.
5. If compiling a site butterfly reference collection and you find a species that is not included in this, then you should kill the butterfly by pressing its thorax between your thumb and index finger. Place the butterfly (with wings open) in a wax-paper envelope, and label the envelope with the species name, date of capture, location of capture and collector's name, before adding to the collection. Note that, with the advance of digital photography, physical specimen collections are increasingly being replaced by photographic libraries. OuTrop does not keep a specimen reference collection and maintains only a photographic library, which has the advantages of being easily sharable to aid verification of identifications by external experts, plus not requiring butterflies to be killed and not requiring long-term storage of physical specimens, which can be challenging in a tropical environment, where mould, etc., easily develops.
6. Try to determine and record whether the bait has been disturbed in the previous day (e.g. by a bird or squirrel), and whether there are any signs of butterfly predation, such as disembodied wings or legs on the bait try.
7. Note whether there have been any significant weather events since the traps were last inspected; e.g., heavy rain, strong winds.
8. Carefully remove and release insects of any other groups from the trap, taking particular care with stinging bees and wasps.
9. If keeping the trap open the next day, use the spoon to replace/replenish the bait mixture and turn / mix old, dry bait from traps. Ensure that the trap netting is properly aligned and then hoist back up into position using the rope, tying this back in place when complete.
10. If not keeping traps open the next day, then untie the ropes and re-package the traps, taking care to discard all bait beforehand to avoid old bait becoming impregnated into the netting, and smelling or damaging the net.

Collecting Weather Data

On both short-term (daily, particularly through strong storms) and longer-term (monthly, seasonal, inter-annual) time scales, butterfly activity and abundance (both real and captured in traps) is influenced by weather (Grøtan *et al.*, 2012; Valtonen *et al.*, 2013). It is generally therefore advisable to record weather data during any butterfly trapping period. In Sabangau, we collect weather data twice daily, using a thermometer and rain gauge located at the NLPSF research camp. This type of data collection is easy to incorporate in most studies. Where this is not possible, weather data may be freely available for nearby localities online through websites like www.wunderground.com, which also provides data on variables such as relative humidity, wind speed and visibility.

Data Storage and Analysis

Data should be immediately recorded in the field, using either bespoke paper data sheets (see next section for examples used in Sabangau), a palm pad, tablet or other mobile data input device. Even during the dry season, it is always strongly advisable to carry a water-proof cover or bag to prevent damage to data from rain, or even data sheets tearing as a result of movements within your field pack while walking through the forest.

Upon return to the research base, data should be entered or backed up to computer as soon as practicable. Data are generally most easily stored in an Excel or Access database. The main advantage of Excel is that it is easier to use for those with limited computer abilities; whereas the main advantages of Access are that it helps keep data more neatly organised and reduces the probability of data input errors.

Data analysis needs will differ from project to project, and so we do not attempt to provide guidelines on specific data analysis techniques herein. For this, we refer readers to the numerous other texts that provide in-depth discussions on data analysis (Hughes *et al.*, 1998; McGeoch, 1998; Zar, 1999; Magurran, 2004; ; Colwell, 2013; Marchant *et al.*, in prep). OuTrop are happy to discuss database needs and data analysis with any researchers interested in developing butterfly trapping research at their site.

Sabangau Field Data Sheets

Canopy Trap Data Sheet

Used for recorded site characteristics around the trap location at the beginning of the study and, if desired,at regular intervals thereafter.

"General habitat description" may include notes on, for example, whether there is a closed canopy, if the site is at the forest edge/in an open burnt area, if peat-swamp or heath forest, etc.

CANOPY TRAP DATA SHEET

Date: Observers: Site name:

					Canopy cover						Ground cover (% in 2 m radius)			
Trap #	Transect Location	GPS waypoint	General habitat description (5m radius)	Trap height (m)	0-5 m	6-10 m	11-15 m	16-20 m	20+ m	Overall	Living material	Dead wood	Bare ground / leaf litter	Other (descibe)

Butterfly Captures Data Sheet

This sheet is used to record data for each butterfly caught in the traps. Notes:

- “Recap?” indicates whether the butterfly is a recapture
- “Code” = the unique code assigned to each individual captured (see Field Data Collection Procedure point #4).
- “M/F” = male or female
- “Condition/Wings” is classified according to the categories under point #3d in the Field Data Collection Procedure section.
- “Predation signs” indicates whether there are any signs of butterfly predation in the traps (Y/N; record any details in “Comments”)
- The number “big” and “small” moths caught in the trap, if any, are recorded in the “Moths” column.
- “Comments” is for any other pertinent observations.

BUTTERFLY CAPTURES DATA SHEET

Date:	Any significant weather since traps were last checked?
Observers:	

Transect	Trap #	Time	Recap ?	Code	*Species*	M/F	Condition		Forewing Length (mm)	Body Length (mm)	Bait missing ?	Predation signs?	Moth		Escaped before marking	Comments
							Colour	Wings					big	small		

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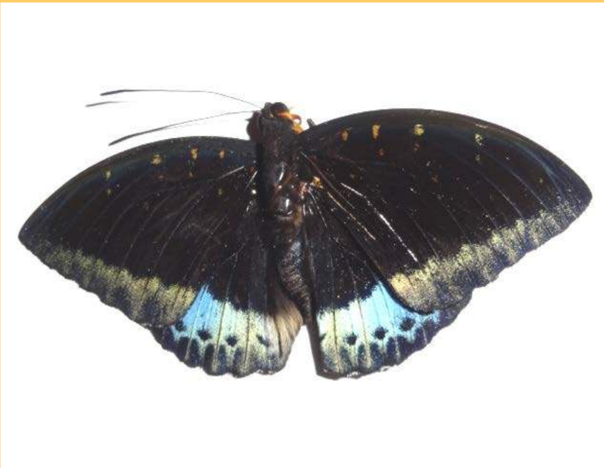
Appendix A

Example Sabangau Field Butterfly Identification Sheet

It is recommended that field ID sheets are laminated to prevent damage from rain and moving around in bags while in the field. Sheets should include all species (regularly) found in an area, are intended as a quick reference guide for those with a reasonable pre-existing acquaintance with an area's butterflies and should be used in conjunction with detailed field guides (D'Abrera, 1985; Tsukada, 1991; Otsuka, 2001; Houlihan *et al.*, 2012).

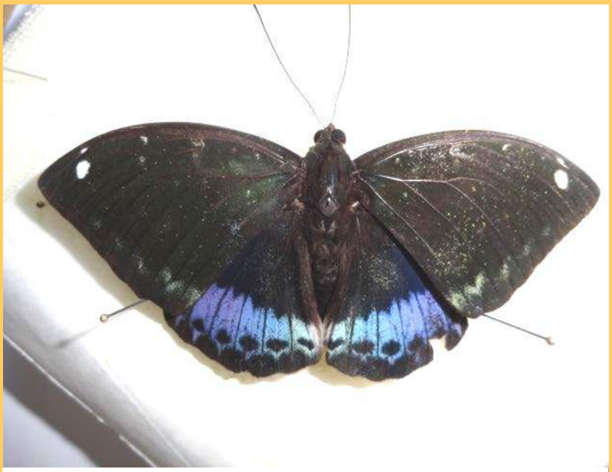
A



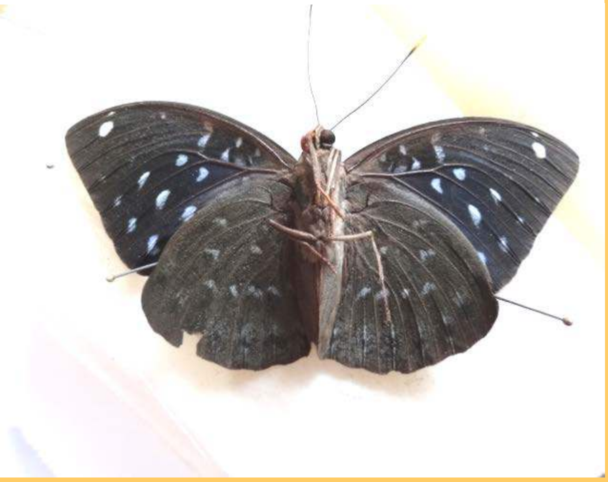
Lexias pardalis Male

Lexias pardalis Female

U



Lexias cyanipardus Male



Lexias cyanipardus Female

The Orangutan Tropical Peatland Project
E-mail: info@outrop.com

www.outrop.com | @outrop | facebook.com/outrop