Protocols and guidelines for measuring indices of abundance in firefly populations

Composite image of *Abscondita terminalis* flash patterns in Hong Kong, China (Photo by Yiu Vor).

2024

International Union for Conservation of Nature

Species Survival Commission

Firefly Specialist Group

IUCN SSC FIREFLY Specialist Group

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Published by the Firefly Specialist Group of the IUCN Species Survival Commission and the Xerces Society for Invertebrate Conservation.

Suggested Citation:

Yiu, V., W. F. A Jusoh, R. De Cock, R. Joyce, A. Walker, and C. Fallon. *Protocols and Guidelines for Measuring Indices of Abundance in Firefly Populations*. 2024. IUCN Species Survival Commission Firefly Specialist Group.

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Contents

Acknowledgments

The authors are indebted to the many researchers and students around the world whose contributions and findings have informed this document. In particular, we would like to thank Lynn Faust, Sandra García de Jesús, Raphaël Sarfati, Orit Peleg, and Owen Martin.

Introduction

This document is provided to guide researchers, land managers, and community scientists in measuring and monitoring indices of abundance in firefly populations. Anecdotal reports of fireflies abound, and there are worrying studies about the decline of insect populations and diversity around the world, but there is a lack of abundance data providing insight into population status and trends for the majority of firefly species and populations.

Establishing baseline abundance levels and monitoring trends in fireflies is important for understanding the extinction risk of species, identifying high priority species for conservation actions, and determining the impacts of different threats and conservation management practices and interventions, such as habitat restoration, artificial light mitigation, invasive species control, and prescribed fire.

The exact protocols to measure indices of firefly abundance will vary depending on the geographic region, climate, focal taxa, and research questions. This document presents multiple methods and discusses important considerations and best practices for each approach. These methods include **visual counts**, **photography of firefly flashes**, **3D videography**, and **non-lethal light lure traps** (Table 1). In addition, this document highlights some of the data-gaps that should be addressed in order to improve the reliability and effectiveness of the protocols.

Flight interception traps, Malaise traps, and sweep netting may be the most effective sampling methods for non-flashing, day active firefly species. However, these methods are not included in this document because they are non-selective and/or lethal. Best practices for monitoring insects using Malaise traps (Montgomery et al., 2021) also apply to fireflies.

Abundance monitoring versus presence-absence monitoring

While the focus of this document is index of abundance monitoring protocols, in some cases it may be more desirable to conduct presence-absence sampling (more accurately described as presence-*non-detection* or presence-presumed absence sampling), which tends to be less time and resource-intensive. Presence-absence sampling can provide information on the current distribution of a firefly species as well on whether populations persist at locations where they have been documented in the past. It can also provide an index of firefly biodiversity if presence-absence of more than one species is monitored.

This is a living document, and we expect these protocols to be refined over time as they are tested and as novel methods are developed.

What are fireflies?

Fireflies are one of the most popularly known and charismatic insect groups. Some authors use "glow-worms" in lieu of "fireflies" (Harvey, 1957; Ineichen & Rüttimann, 2012; McDermott, 1967; Okada, 1928). However, "glow-worms" sometimes also refer to the Keroplatidae (Diptera: Mycetophilidae) in the USA, Nearctic regions, Australia, and New-Zealand (De Cock, 2009). To prevent confusion, De Cock (2009) suggests specifying "lampyrid glow-worms" or "firefly glow-worms." Fireflies are also called “lightning bugs,” particularly in the eastern United States (Babu & Kannan, 2002; Fallon et al., 2021; Faust, 2017; Leconte, 1880).

Fireflies are soft-bodied beetles (Order Coleoptera), members of family **Lampyridae**, which are mostly luminous. Members of the closely-related families **Phengodidae** and **Rhagophthalmidae** are sometimes regarded as fireflies, because they share very similar luminescent behavior with the members of **Lampyridae**. Some members of the click beetles – about 100 species in the family **Elateridae** (Costa, 1975) and one species in family **Sinopyrophoridae** (Bi et al., 2019; Kusy et al., 2021) -- are also luminous. These five families of luminous beetles are collectively called **bioluminescent elateroid beetles** (Kusy et al., 2021). There are 2,419 species of Lampyridae, 258 species of Phengodidae and 53 species of Rhagophthalmidae listed on Integrated Taxonomical Informational System (retrieval in June 2024). We have spent relatively little effort on surveys, finding and describing new luminous beetle species, which may mean there are more unknown species than those currently described.

Based on their life-histories, fireflies can be grouped into three general types: daytime dark fireflies, glow-worm fireflies, and flashing-fireflies (Figure 1). In diurnal fireflies, adults are non-bioluminescent and primarily use pheromones for mate-finding. In glow-worm fireflies, flightless adult females use signaling glows to attract winged males. In flashing-fireflies, adult males and females communicate using species and temperature-dependent flash patterns.

Figure 1. Examples of firefly life-history types: (A) *Lucidota punctata*, a daytime dark firefly in Georgia, USA (© skitterbug CC BY). (B) An adult female glow-worm firefly, *Lampyris noctiluca*, in England (© Edward Bell CC BY). (C) *Abscondita terminalis*, a flashing firefly in Yunaan province, China (© 周瑜 CC BY-NC).

Definitions

Blink: see *flash*
Density: the number of individual organisms occupying a given area of habitat
Firefly: beetle in the family Lampyridae
Flash: a brief emission of light, usually shorter than 1 second
Flash cycle: the time between the start of one flash and the start of the next flash
Flash pattern: the pattern of light emissions from an adult firefly
Flash pattern interval: the time between the end of a flash pattern and the start of the next repetition of the flash pattern (also known as the flash pattern pause or the dark phase)
Flash period: (see flash cycle) the time between the start of one flash and the start of the next flash (Fig. 2)
Flicker: a modulated emission of light, repeatedly changing in brightness
Glow: a prolonged emission of light, especially longer than 1 second
Glow-worm firefly: species of Lampyridae in which flightless, larva-like females glow, while adult males and winged and may or may not be bioluminescent
Growing Degree Days (GDD): a unit used to track the accumulation of heat during the growing season in temperate and subtropical climates, calculated using daily high and low temperature measurements
Index of abundance: estimate of the relative population size of a species calculated from counts or observations per standardized unit of sampling effort
Pulse: a flash, especially as a unit with a flash pattern

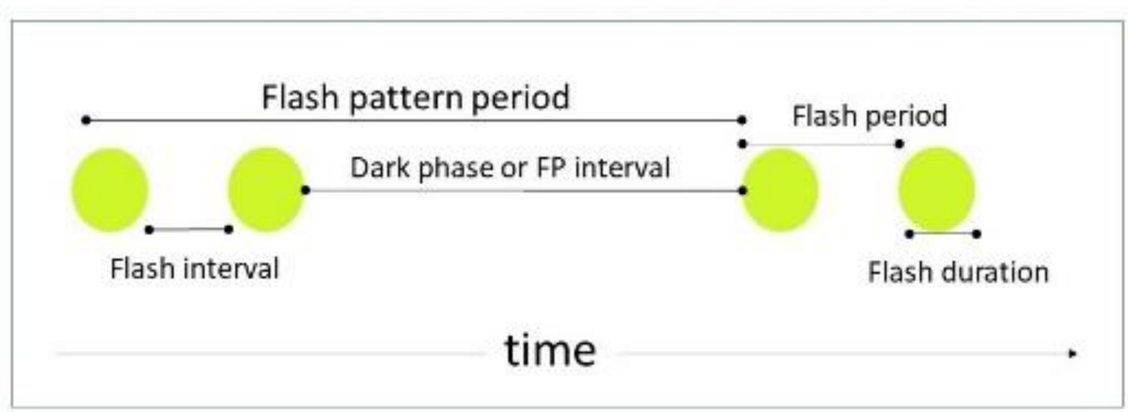

Figure 2. Diagram of a double-pulsed flash pattern, showing illustrating terminology used to describe and measure firefly flash patterns.

Necessary steps before monitoring firefly populations

Several actions should be taken before beginning to monitor firefly populations (Figure 3). These steps are crucial for monitoring to be effective, efficient, and useful.

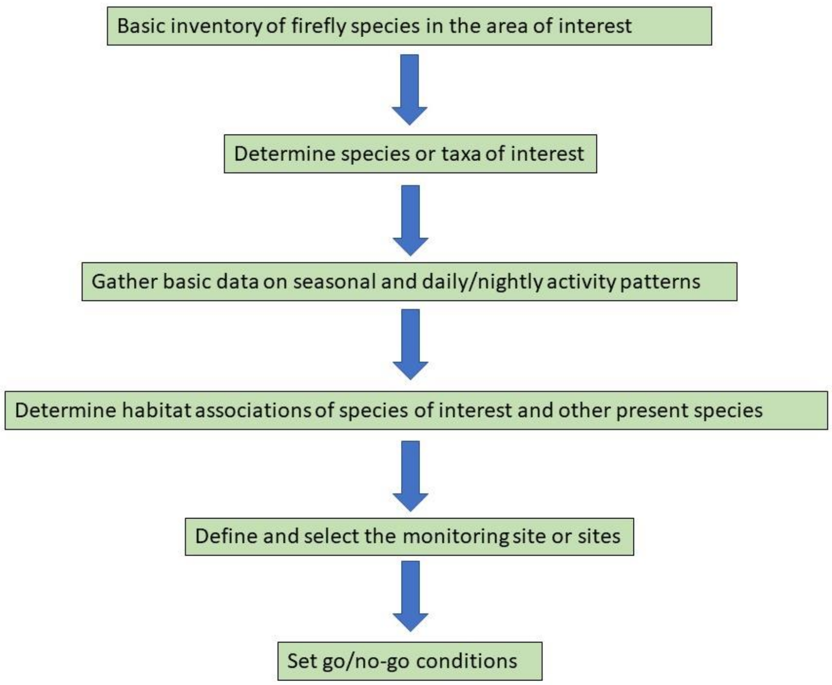

Figure 3. Flowchart of necessary steps before monitoring firefly population abundance. Some steps can occur concurrently.

Do a basic inventory of firefly species in the area of interest

Generate a species list or morphospecies list by reviewing species distributions in the literature and checking specimen collections. Conduct field surveys over the course of the growing season, using flash-pattern observations, hand netting, UV lights, and flight-intercept traps. Voucher specimens, dissections of genitalia, and examination by experts may be necessary.

Determine species or taxa of interest for monitoring

While it would be ideal to monitor the abundance of all firefly species in a given area, differences in phenology, nightly activity period, habitat associations, and life history make this difficult and very resource intensive. For this reason, it likely makes sense to focus resources on monitoring the abundance of species that are of conservation concern or that have particular cultural or economic significance, such as species at firefly tourism sites. Voucher specimens of focal species should be collected for confirming species identification.

For focal taxa that are flashing, the basic flash or glow behavior and flash pattern should be known and described. Flash pattern details would ideally include measurements in seconds of flash periods and flash pattern periods taken at a range of temperatures (see Figure 1). Simple methods for measuring and plotting this information are available in the literature (Iguchi, 2010; Lloyd, 2018) and may involve stopwatches or voice recorders. Another approach is to record digital video of firefly flash patterns and to analyze them using software, such as TiLia (Konno et al., 2016). The color of light emissions should also be described in either verbal (e.g. green, amber, yellow) or spectral (nanometers) terms.

Gather basic data on phenology and daily or nightly activity periods

For species of interest, record phenological data such as range of collection dates, average date of adult emergence or first adult activity, and average peak activity. In temperate regions, cumulative modified Growing Degree Days can be a helpful phenology tool to control for interannual differences in temperature (Faust & Weston, 2009).

> **Degree days**, a measure of the average daily temperature that has accumulated over the course of a growing season, is a tool that has been used for decades in agriculture to predict phenological occurrences such as the adult emergence of a given insect species or the germination of a given plant species. Compared to using day of year or date ranges, modified growing degree days (mGDD) may be more reliable predictors of adult firefly activity period, and are especially useful when planning firefly fieldwork at sites with different climates and at different latitudes.

It is also important to know the times of day when fireflies are most active and detectable. This is usually best expressed in terms of minutes after sunset. When comparing the behavior of species or populations in different seasons or latitudes, it may be helpful to use crep units (Nielsen, 1963), a unit of time equivalent to the length of civil twilight at a given location on a given date (Dreisig, 1975). For many species, highest activity will be during the first few hours after sunset, and for some dusk-displaying species this window may be quite short (Lloyd, 1966).

Record habitat associations of species of interest and other species present

In order to target monitoring efforts, it is helpful to know the habitat associations of focal firefly species. Do they appear to be habitat generalists or habitat specialists? Do they occur in wetlands or upland habitats? Creating basic maps of observation locations and habitat types at the site can help to identify appropriate monitoring sites and additional areas to inventory. It may also be helpful to make notes of microhabitats, as some species or life-stages may have very particular preferences.

Define and select the monitoring site or sites

The site(s) should:

a. Contain (or have the potential to contain) **appropriate habitat of the same type** for the focal species
b. Be **large enough** to allow for some spatial replication in sampling.
c. Be **small** and **accessible enough** to be effectively and safely sampled with available resources, including staff time
d. Be **separated by enough distance** to be reasonably confident that adult fireflies are not traveling between them. The appropriate distance will depend on whether the fireflies studied have roving or stationary behavior, with roving taxa requiring greater distances between sites. If it seems likely that adult fireflies are moving between two sites, those sites should be treated as replicates within a site rather than as independent sites. While some larger species may be capable of longer flights, 500 meters may be an appropriate minimum site-separation distance for many species.

e. Be expected to **remain accessible** for the foreseeable future, *if* the hope is to monitor the population over the long-term.

Set go/no-go conditions

Define the conditions under which monitoring can be safely and practically carried out. These conditions may vary depending on the site, target species, and risk management policies of the monitoring organization. In tropical or subtropical regions, suitable conditions for monitoring may differ significantly between species with flight seasons in rainy season versus dry season.

Example "go" conditions might be:

- Air temperature must be above 15° C/60° F.
- Relative humidity must be over 50%.
- Wind speed must not exceed Beaufort Force 3 (16 kph or 10 mph).
- Precipitation should be light enough to not noticeably affect firefly activity, endanger surveyors, or jeopardize camera equipment.
- There should not be audible thunder from lightning storms.

Monitoring protocols

Once you have taken the necessary steps to prepare, it is time to determine which monitoring protocol(s) you will use (Table 1). Regardless of the type, there are certain variables that should always be recorded (see Appendix I.).

Table 1. Overview of firefly abundance monitoring protocol types.

Protocol	Appropriate firefly groups
Visual counts using points, transects, quadrats, or plots	• Fireflies with flying, bioluminescent adults occurring at low to moderate densities that can be identified by flash pattern or are displaying in monospecific groups. • Fireflies with larvae or flightless females that are visually detectable and identifiable to species.
Long exposure photography	Fireflies with flying, bioluminescent adults that display in relatively concentrated areas. Particularly useful for species that display at high densities.
Digital 3D videography	Flashing fireflies occurring at moderate to high densities in concentrated areas or swarms.
Non-lethal light lure traps	Glow-worm fireflies, in which females are flightless and emit prolonged bioluminescent signals. This protocol targets flighted, mostly non-luminescent males to be counted and released.

Visual count protocols (point, transects, quadrats or plots)

Why use visual count methods (point, transects, quadrats or plots) to measure firefly index of abundance?

- These methods are non-invasive and non-destructive.

- These methods do not require specialized equipment or technology, and can be carried out by trained volunteers
- Firefly species that occur at relatively low densities, fly slowly, and flash frequently may be visually tracked and counted individually.
- In cases where individuals cannot be reliably tracked, flashes can be used as an indicator of the number of adult males, though this depends on various assumptions.
- Visual count protocols can also be used to estimate the abundance of lampyrid larvae in taxa where larvae are detectable and identifiable to species (for example in the case of *Lamprigera* spp. in Asia, *Micronaspis floridana* in the USA, or *Lampyris*, *Nyctophila*, *Luciola* and *Phosphaenus* spp. in Europe). Note that larval abundance data must be interpreted differently from data on adult firefly abundance.

What can visual count methods tell us about firefly populations?

Visual count methods can provide an index of abundance of flashing adult male fireflies, glowing adult flightless female fireflies, or glowing larval fireflies. These indices do not correspond directly to absolute number of individuals in a population or to density of individuals in a given area, nor are they necessarily adequate for comparing population sizes between sites because of differences in detectability between sites or different weather conditions (affecting luminescent behavior) between observation dates. However, index of abundance values from visual count methods can provide insight into interannual and intergenerational trends at a given site, as well as changes in abundance during the flight period of a species.

Distance sampling is a method in which, in addition to counting individual organisms or organism signals (such as bird calls), surveyors record the distance at which each individual was detected. These data allow for the creation of a detection function that estimates the number of individuals that go undetected. This method has been used extensively for estimating densities of birds and butterflies, but has thus far been applied minimally to fireflies. The 3D reconstruction of firefly flashes from stereo videography holds promise for advancing distance sampling of fireflies, and experimentation with visual count distance sampling of flashing fireflies occurring at low densities is highly encouraged.

Visual Count Methods Assumptions and Pitfalls

Assumptions

- The probability that a male firefly will flash and the probability that an observer will detect that flashing individual is somewhat constant between sampling events (through time and distance).

Pitfalls

- Factors other than abundance of fireflies present within a sampling area can affect the activity and detectability of fireflies—moonlight, wind speed, vegetation and other visual obstacles, such as fog.
- Observers vary in their vision and experience level in detecting and counting fireflies, introducing observer bias.
- Multiple sympatric species and overlapping flash patterns may make it difficult to distinguish the flashes of different firefly species and different individuals.

- Surveys must be timed appropriately each evening, as it can be easy to miss the peak of activity if surveying begins too late or ends too early.

Visual count protocol instructions

Determine the dimensions of sampling units

The shape and size of the sampling unit for a visual count will depend on various factors, including **maximum distance at which the focal species can be detected at a site**; the size and shape of the focal habitat; the sensitivity of the habitat; which parts of the habitat can be safely accessed; the mobility and density of the target species; and the person power of the sampling team.

Less mobile species found at higher densities may be more conducive to stationary protocols and smaller quadrat or plot dimensions, whereas species that are more mobile and display at lower densities may be more conducive to transect methods and larger sampling units. If the sampling unit is small, it is advisable to include multiple replicates. See Table 2 for examples of sampling unit dimensions and rationales.

Determine placement of sampling units

For purposes of statistical inference, transects, plots, or points would ideally be placed randomly within the survey site using GIS software. However, this approach may not be possible because of sensitive habitats, surveyor safety considerations, or practical reasons such as monitoring efficiency. It may be more desirable to place sampling units systematically, such as at a given interval along a shoreline or field edge or in a grid. Once selected, the locations of transects, plots, or points should stay the same from year to year. Sampling locations should be photographed, mapped using GPS coordinates, and/or physically marked, such as with a ground stake. The method used for placement of sampling units (random, haphazard, systematic, discretion) should be described and documented.

Determine the minimum sampling effort time per evening

Establish the minimum duration of the nightly count period and the number of minutes spent actively searching or counting. Variables to consider include:

- The duration of nightly displays (this can range from about 20 minutes to multiple hours)
- The variability of peak nightly activity

Determine the within-season monitoring schedule

Establish the dates, or windows of dates, in which the counts will occur.

- Monitoring should occur within the **range of dates of known peak annual activity**. In temperate regions, this is often a 1-2 month window. Even in tropical regions where flashing adults of a given species may be active year-round, a peak in firefly activity often still occurs; for example, *Pteroptyx tener* monitored for three years in the Selangor River in Malaysia had peaks in index of abundance between June and August (Khoo et al., 2012).
- The date range **parameters and criteria should be consistent from year to year**. These may be date ranges, modified Growing Degree Day (mGDD) ranges, or a combination of date ranges and weather events (for example, first rains of the spring or rainy season). A wider sampling window

and a slightly higher sampling effort may be needed if date ranges are used, to account for interannual variability in weather/seasons.

- Visual count sampling nights should occur **frequently enough** to ensure that peak nights will be represented, and frequency will depend on the average length of peak display. In *Photinus carolinus*, for example, peak activity occurs over 2-5 nights (Faust & Weston, 2009). Species with brief adult flight seasons and seasonal peaks will require more frequent monitoring (<1 week between visits), while tropical and sub-tropical species with year-round or multi-month adult flight seasons may allow for longer sampling intervals (1-4 weeks).
- Number and frequency of sampling nights should be **realistic** and **sustainable** given staffing capacity, night-time working hours, fatigue, and safety considerations.

Conduct sampling plan

- To record data and tally firefly counts without the aid of artificial light, use a voice recorder or a clicker counter.
- If personnel levels allow, multiple surveyors can conduct counts simultaneously and totals can be averaged.
- For safety reasons, survey in groups, scout monitoring locations in the daylight to identify hazards, and use appropriate clothing and footwear to protect against thorns, biting insects, and venomous snakes.

See Table 2 for considerations, strategies and rationales when designing and implementing visual count schemes.

Interpreting flash counts

In cases where it is not feasible to visually track and count individual fireflies, the number of displaying fireflies in an area can be estimated based on the number of flashes seen during a given amount of time.

An estimate of the number of displaying fireflies in an area can be calculated using the following formula: **N= D/T*F**, where D is the duration of one flash cycle (also called the flash pattern period), T is sampling unit time, and F is the number of flash units detected during the sampling unit time.

It is very important to distinguish between counts of flashes and counts of individual fireflies when discussing and interpreting results.

Table 2. Table of considerations, strategies, and rationales from visual count studies and monitoring efforts

	Consideration	Strategy	Rationale & reference
Temporal	Monitoring season duration	Year-round	In Malaysia, *Pteroptyx tener* are active year-round, and peak activity varied from site to site and year to year (Khoo et al., 2012).
	Monitoring season duration	~5 months (late April-September)	At a subtropical site, Fu et al. (2006) observed two peaks in adult activity in *Luciola leii*, one in early May and another in August.

	Monitoring season duration	10 weeks	Surveys of *Photuris quadrifulgens* over a 10-week period captured the peak activity and produced a bell-curve of firefly abundance by week (Forrest & Eubanks, 1995).
	Monitoring season duration	~6 weeks	In *Photinus carolinus*, the time between male emergence and peak display is ~10-19 days, but the timing of this varies from season to season, and monitoring before emergence and after peak are necessary in order to ensure that peak abundance is captured (Faust & Weston, 2009).
	Sampling night frequency	3 nights per month, centered on the new moon	When monitoring a multivoltine species, Khoo et al. (2012) could account for variation from night to night and month to month.
	Sampling night frequency	One night/site/week	In taxa with adult display periods of ~6-8 weeks (*Photuris* sp. *Nipponluciola cruciata*, *Photuris quadrifulgens*) weekly sampling seems to capture peaks of activity (Forrest & Eubanks, 1995; Yang, 2012; Yuma, 2007).
	Sampling night frequency	Two nights/site/week	Suitable for pre-season reconnaissance (Faust & Weston, 2009) and species with seasonal peaks that last over 4 days.
	Sampling night frequency	Nightly	In order to not miss peak abundances, monitoring of species with short display seasons (e.g. *Photinus carolinus* and *Photuris frontalis*) should occur nightly.
Spatial	Monitoring site replication	7 sites	Khoo et al. (2012) monitored 7 sites along a multi-kilometer stretch of the Selangor River.
	Sampling unit dimensions: transect length	10-80 meters	This range of transect lengths can be used for fireflies at moderate densities, and transects this length can be sampled multiple times in an evening (Hagen et al., 2015; Lewis & Wang, 1991; Nada et al., 2023; Picchi et al., 2013).
	Sampling unit dimensions: Transect length	>1000 meters	Studies involving detection of female glow-worms or firefly larvae that glow continuously have used existing paths or roads as transects, covering distances of over 1 km (Atkins & Bell, 2016; Mbugua et al., 2020).
	Sampling unit dimensions: area	10-480 square meters	Sampling unit area may depend on the behavior of the species, the visibility in the habitat, and the density at which fireflies are occurring (Forrest & Eubanks, 1995; Lewis & Wang, 1991; Nada et al., 2023).

	Sampling unit dimensions: maximum detection distance	20-25 meters	Although flashing fireflies can sometimes be seen and counted from great distances (especially when occurring at low densities in open habitats), the distance from the viewer should not exceed 25 meters (Firebaugh & Haynes, 2016; Yiu, 2020). High abundance, short flash durations, short flash periods, and full-darkness conditions all require a shorter distance between the recorder and the fireflies.
	Sampling unit dimensions: radius of circular plot	10 meters	Firebaugh & Haynes (2016) marked off 10-meter radius circles with glow sticks.
Intensity of effort	Sampling effort per area	0.2-4 seconds of counting/square meter of sampled area	Sampling effort intensity ranged from 60 second count periods for a 10-meter radius circle (Firebaugh & Haynes, 2016) to a 120 second count period for a 9x3 meter rectangular plot (Lewis & Wang, 1991).
	Walking speed on transect	1-4 km/hour	This pace is slow enough to thoroughly count fireflies while fast enough to cover a sizeable area or to survey a short transect multiple times.
	Walking speed on transect	0.5 km/hour (20 minutes per 100 meters)	When counting glowing firefly larvae, a slow walking pace is most effective for increasing detectability because individual larvae may glow only once per minute (R. De Cock, pers. obs.).
	Observation bout duration for point counts or short transects	20-300 seconds	Factors that influence the duration of each counting bout include the density of fireflies, the size of the count area, and the flash pattern period of individuals.
Count precision	Data recording: Abundance bins	0, 1-5, 6-20, >20 (Firefly Watch)	While it is possible to obtain exact counts of firefly individuals at low numbers, the accuracy of counts decreases as abundance increases. Using abundance bins during data collection provides a balance between accuracy and precision. For later analysis, data can be converted from categorical to continuous data by using the lowest value within a bin (McNeil et al., 2024).
		1, 2-10, 11-50, >50 (*Firefly Atlas*)	
		1-5, 6-10, 11-25, 26-50, >50 (*Buscando Luciernagas*)	
		1-10, 11-20, 21-30, 31-40, >40 (L. Faust, pers. comm.)	

Photographic protocol

Why use long-exposure photography for monitoring flashing fireflies?

- Using digital photography methods reduces observer bias and the amount of training needed by observers and increases the efficiency of data collection in the field.
- The number of flashes captured in long exposure images is largely a function of the number of adult male fireflies displaying, which is an indicator of population density.
- At sites with large numbers of fireflies of the same species, such as in the case of synchronous fireflies (*Pteroptyx* spp.) in southeast Asia, digital photographic methods have been proven to be useful tools. See Figures 4 and 5 for examples of long-exposure images of flashing fireflies.

Figure 4. A long-exposure photograph of *Pteroptyx* fireflies along the Selangor River (from Khoo et al. 2014).

Figure 5. A 9-second exposure showing the flash patterns of *Abscondita terminalis* in China. (50 mm focal length, ISO 800, Aperture f/7.1). Photo by Yiu Vor.

What can long exposure photography tell us about populations of flashing firefly species?

Long exposure photography methods can provide an index of abundance of the number of flashing adult fireflies actively signaling during the time period sampled. Depending on the species, the adults counted may only include males. Flash rate of individual male fireflies is largely a factor of air temperature, so this is a crucial covariate to collect.

Long-exposure photography method assumptions and pitfalls

Assumptions

- The area visible in the camera's field of view is representative of the overall distribution of fireflies at a site.

Pitfalls

- Multiple sympatric species and overlapping flash patterns may make it difficult to distinguish the flashes of different firefly species.
- Space-use by flashing male fireflies can vary over the course of an evening and from season to season. Thus, incorporating some level of spatial replication is advisable.
- Detectability of flashes may vary depending on the camera being used, requiring extra calibration steps in order for valid comparisons between data gathered with different cameras.
- Post-processing of images may be time intensive and require training and skills in specialized software.

Long exposure photography protocol instructions

<u>Determine placement of cameras</u>

- Choose points that have **clear, unobstructed views** of areas of high firefly activity. Use a permanent ground marker such as a survey pin or piece of rebar to ensure that photos are taken from the same location each time.
- Height of the camera will be dictated by factors such as the following (see Figure 6 for an example camera and tripod set-up):
    a. The height of vegetation or other potential obstructions
    b. The height at which fireflies are displaying
    c. Comfortable heights at which to set tripods
- If possible, **position the camera to exclude reflective surfaces such as water or mud** and **avoid brighter patches of sky**, or in such a way that these areas can be cropped out of the image.
- Record and standardize the orientation of the camera using compass bearings.
- Record the GPS coordinates of the camera location or document its location in detail (e.g., distance from a nearby marker with compass bearing), in case the physical marker is lost or removed

Figure 6. Photo of camera and tripod set-up for *Pteroptyx tener* monitoring in Malaysia (image from Khoo et al., 2014).

<u>Determine the within-season monitoring schedule</u>

Establish the dates, or windows of dates, in which photo-monitoring will occur.

a) Monitoring should occur within the **range of dates of known peak annual activity**. In temperate regions, this is often a 1-2 month window (Faust, 2017). Even in tropical regions where flashing adults of a given species may be active year-round, a peak in firefly activity often still occurs; for example, *Pteroptyx tener* monitored for three years in the Selangor River in Malaysia had peaks in index of abundance between June and August (Khoo et al., 2012).
b) The date range **parameters and criteria should be consistent from year to year**. These may be date ranges, mGDD ranges, or a combination of date ranges and weather events (for example, first rains of the spring or rainy season). A wider sampling window and a slightly higher sampling effort may be needed if date ranges are used, to account for interannual variability in weather/seasons.
c) Photo-sampling nights should occur **frequently enough** to ensure that peak nights will be represented, and frequency will depend on the average length of peak display. In *Photinus carolinus*, for example, peak activity occurs over 2-5 nights (Faust & Weston, 2009). Species with brief adult flight seasons and seasonal peaks will require more frequent monitoring (<week), while tropical and sub-tropical species with year-round or multi-month adult flight seasons may allow for longer sampling intervals (1-4 weeks).
d) Number and frequency of photo-sampling nights should be **realistic** and **sustainable** given staffing capacity, night-time working hours, fatigue, and safety considerations.

Determine camera settings and image frequency and replication

Important camera settings to decide upon include ISO, aperture or f-stop, shutter speed, and lens focal length.

The number and spacing of photographic exposures taken will depend on factors such as the species-specific display time window, temporal variation in flash activity at multiple scales within an evening, and the battery life of the camera being used.

Image Curation and Processing

Noise reduction

Kirton et al. (2012) describe image processing methods using median filters and color separation of the green layer to facilitate the quantification of firefly flashes and reduce the incidence of false positives.

Quantifying firefly flashes in images

There are two main approaches to quantifying flashes from images. Particle analysis with software is used to automatically detect and count bright spots that meet a minimum size and brightness. For example, Kirton et al. (2012) used Olympus Soft Imaging Solutions analysis LS Research 2.8. A more labor-intensive approach is for individuals to count flashes in images manually or semi-manually. In some cases, this may be more accurate than using automated software, but it may introduce observer bias if multiple individuals are counting flashes in images. Various software products exist that facilitate the tallying of points identified as firefly flashes. Examples include DotDotGoose (free from the American Museum of Natural History), Adobe Photoshop (using the Count tool), and ImageJ. See Figure 7 and 8 for examples of semi-manual counts of flash units in long exposure images.

See Table 3 for considerations, strategies, and rationales when designing and implementing photographic index of abundance methods.

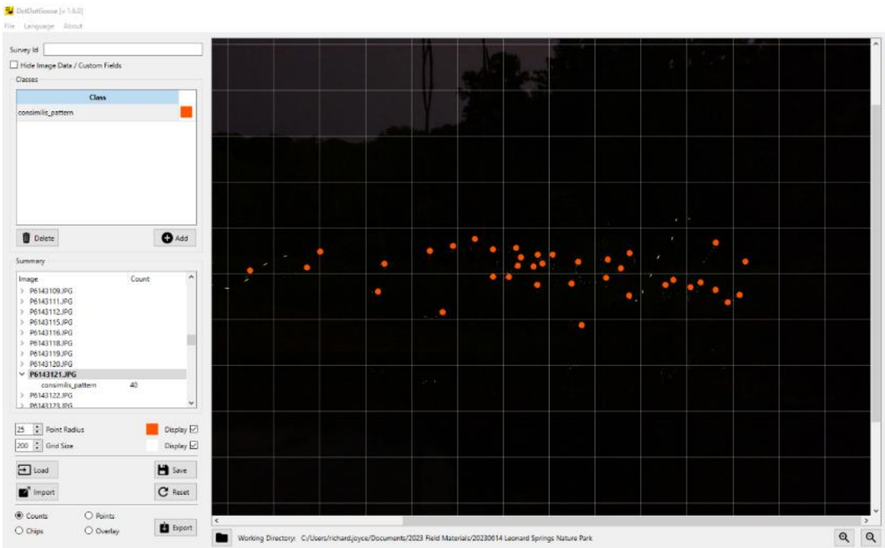

Figure 7. Using DotDotGoose software to count flash patterns of *Photinus consimilis* in a composite photo made up of 15 8-second exposures. (30 mm focal length, ISO 400; Aperture F2.3). Assuming a flash pattern period of 9 seconds, a constant rate of flash patterns, and a closed system (no fireflies enter or exit the frame during the exposure) the number of displaying adults detected by the camera is N=9/120 x 37=2.78.

Figure 8. Thirty five flashes of *Abscondita terminalis* shown on a long exposure photo (9s exposure, aperture f/5.6, ISO 6400, 85mm focal length). Knowing that the flash pattern period (flash cycle) for *Abscondita terminalis* was 3.4 seconds, we can calculate the number of displaying individuals using the following formula: N= 3.4/9 x 35 = 13. Photo by Yiu Vor.

Table 3. Table of considerations, strategies, and rationales when designing photographic monitoring protocols for your site and species.

	Consideration	**Strategy**	**Rationale & reference**
Temporal	Monitoring season duration	Year-round, 3 nights per month	In Malaysia, *Pteroptyx tener* are active year-round, and peak activity varied from site to site and year to year (Khoo et al., 2012).
	Monitoring season duration	~5 months (late April-September)	At a subtropical site, Fu et al. (2006) observed two peaks in adult activity in *Luciola leii*, one in early May and another in August.
	Monitoring season duration	10 weeks	Surveys of *Photuris quadrifulgens* over a 10-week period captured the peak activity and produced a bell-curve of firefly abundance by week (Forrest & Eubanks, 1995).
	Monitoring season duration	~6 weeks	In *Photinus carolinus*, the time between male emergence and peak display is ~10-19 days, but the timing of this varies from season to season, and monitoring before emergence and after peak are necessary in order to ensure that peak abundance is captured (Faust & Weston, 2009).
	Monitoring season duration	4 weeks	Four weeks is the duration of the peak flight of *Photinus palaciosi* in Nanacamilpa, Mexico. Sampling did not take place during the full moon (S. García De Jesús, pers. comm., 2024).
	Sampling night frequency	3 nights per month, centered on the new moon	When monitoring a multivoltine species, Khoo et al. (2012) could account for variation from night to night and month to month.
	Sampling night frequency	Weekly (per site)	In taxa with adult display periods of ~6-8 weeks (*Photuris* sp. *Nipponluciola cruciata*, *Photuris quadrifulgens*), weekly sampling seems to capture peaks of activity (Forrest & Eubanks, 1995; Yang, 2012; Yuma, 2007).
	Sampling night frequency	Two nights/site/week	Suitable for pre-season reconnaissance (Faust & Weston, 2009) and species with seasonal peaks that last >3 days.
	Sampling night frequency	Two nights/site/week	Sampling multiple nights per week reduced the possibility of missing peak flight in *Photinus palaciosi* (S. García De Jesús, pers. comm.).
	Sampling night frequency	Nightly	In order to not miss peak abundances, monitoring of species with short display seasons (e.g. *Photinus carolinus* and *Photuris frontalis*) should occur nightly.

Spatial	Site replication	7 sites	Khoo et al. (2012) monitored 7 sites along a stretch of the Selangor River.
	Site replication	6 plots on one farm	Yang (2012)
	Site replication	2 sites	Two photography stations were used to record *Photinus palaciosi* activity at a firefly viewing site (García De Jesús, pers. comm.).
	Site scale	Stretches of river bank ranging in length from 85-369 meters	Monitoring site scale in Khoo et al. (2012) was determined by the widest span of display trees visible from 1-2 camera tripod set-ups.
	Site scale	An approximately 20 x 21 meter plot (420 m^2 or 0.042 hectares)	Yang (2012) used a plot size that could be captured within the field of view of the camera while not failing to detect flashes at greater distances. The camera was tilted slightly down to restrict the field of view.
	Site scale	A plot 20 meters wide and 30 meters deep.	The camera's field of view was able to record *Photinus palaciosi* flashes within an open area bordered by denser vegetation. The camera lens was focused to capture flashes at the furthest distance (S. García De Jesús, pers. comm.).
Camera placement	Distance	Camera is set up ~50-250 meters from treetop firefly displays	Khoo et al. (2012) were able to photograph full views of riverside display trees at considerable distances.
	Distance	Camera is set up at edge of 20 x 21 plot	Yang (2012) found that flashes were detectable by a Nikon Coolpix p600 camera at least to 20 meters, but became increasingly difficult to distinguish from other light spots in the image, such as reflections and artificial light.
	Distance	Camera is set up at edge of 20m x 30m plot.	(García De Jesús, pers. comm.)
	Distance	360 GoPro cameras placed in the midst or at the edge of firefly swarms.	Sarfati et al. (2020) have found that 10-15 meters is the approximate distance at which detection of flashes drops off.
Camera settings	Focal length	70-200 mm	Khoo et al. (2012) used high focal lengths for high magnification to photograph firefly display trees at a distance of 50-250 meters.
	Focal length	28mm-70 mm	A shorter focal length allows a wider field of view and a brighter image when photographing non-congregating fireflies at close or moderate distances.

	Focal length	18 mm	A short focal length was used to monitor *Photinus palaciosi* in an open area of 20 m x 30 m (García De Jesús, pers. comm.)
	ISO/sensor sensitivity	ISO 3200 equivalent	Higher ISO settings are used in order to pick up firefly flashes at a distance (Kirton et al., 2012).
	ISO/sensor sensitivity	ISO 400-12,800	Yiu (pers. comm.) recommends this range. ISO must be high enough to detect flashes but not so high as to degrade image quality or add excessive noise.
	ISO/sensor sensitivity	Increase from ISO 100 at 19:30 to ISO 3600 at 21:00.	Increasing ISO as conditions darken allows for optimization of successful flash capture and image quality (García De Jesús, pers. comm.)
	Exposure time	8 seconds	Yang (2012) took 8-second exposures, which was about the length of the flash cycle of *Photinus pyralis* and three times longer than the flash cycle of the *Photuris* species being photographed.
	Exposure time	1 second	Flash intervals for *Photinus palaciosi* were 0.94-0.96 seconds. Thus, a 1-second exposure time minimized double-counting of displaying individuals (García De Jesús, pers. comm.).
	Exposure time	0.5 second	*Pteroptyx tener* males have a flash interval of about 0.27 seconds, so shutter speeds of 0.5 seconds capture 1-2 flashes of each active male (Kirton et al., 2012)
	Exposure time	<30 second exposure time	Exposure times longer than 30 seconds will likely result in a bright background, making firefly flashes less visible.
	Lens aperture	F 2.8	Kirton et al. (2012) used f 2.8.
	Lens aperture	F 2.8- f/8	Yiu (pers. comm.) recommends an intermediate aperture, which balances the light reaching the sensor with depth of field.
	Lens aperture	F 3.5	(García De Jesús, pers. comm.).
Sampling intensity	Image capture: within-evening frequency	1 photo every 30 seconds (120 photos per hour) until the battery runs out (~2 hours)	Yang (2012) found changes in number of flashes per photo per second over the course of the evening.
	Image capture: within-evening frequency and replication	3 images/site/night, with about 60 seconds between exposures	Kirton et al. (2012) found that averaging the number of flashes of three image replicates reduced the effect of short-term variation.

	Image capture: Within-evening frequency and replication	A sequence of four photos, with 30 seconds between each exposure, repeated at 10-minute intervals. This produced 40-76 photos per night.	The total flashes from each 4-photo sequence were averaged. The 10-minute pause between sequences was intended to reduce the likelihood of recording the exact same set of individual fireflies (García De Jesús, pers. comm.).
Image analysis	Image format	RAW files (6000 pixels wide by 4000 pixels tall)	Using RAW files offers the most options for analyzing the information captured by the camera sensor (S. García De Jesús, pers. comm. 2024).
	Minimum particle size	2 pixels	Kirton et al. (2012) found that a minimum particle size of two pixels captured the majority of firefly flashes and reduced false detections from reflections or patches of light sky.
	Brightness thresholds	36-255 of green layer	This brightness threshold maximized detection of *Pteroptyx tener* flashes while reducing false detections from bright spots such as from the sky or water (Kirton et al., 2012).
	Brightness thresholds	150-255	García De Jesús et al. (pers. comm. 2024) used gray-scale brightness thresholds for tallying flash units of *Photinus palaciosi*.

Stereo 360-degree videography monitoring protocol

Why use stereo 360-degree videography?

- Recently developed methods (Martin et al., 2023; Sarfati et al., 2020; Sarfati & Peleg, 2021) allow for the processing and analysis of video recorded with relatively low-cost equipment and field protocols.
- Analytical methods allow for the three-dimensional reconstruction of firefly flash patterns and the identification of flight trajectories of individual fireflies.

What can stereo 360-degree videography tell us about firefly populations?

- Relative and absolute density of displaying male fireflies within a three-dimensional area during a given period of time.
- Quantitative metrics of flash patterns (inter-flash gap, flash duration, number of flashes in pattern).

Stereo 360-degree videography monitoring protocol instructions

Many of the same considerations in Table 3 also apply to the design of monitoring schemes that use stereo 360-degree videography. The following provides a brief overview of the steps used for recording stereo 360-degree videos of firefly flash patterns for monitoring purposes.

Configure GoPro Max Settings

a) Recording mode should be 360-degree mode.
b) Set resolution and frame rate to 5.6K|30 (horizontal resolution of 5600 pixels and frame rate of 30 frames per second).
c) Set ISO minimum to 6400.

Place cameras

a) Two 360 GoPro Max cameras should be placed as close to the center of firefly swarms as possible, with minimal obstructing vegetation.
b) Cameras can be supported on tripods of equal height or on surfaces such as boardwalk railings.
c) The two cameras should be 1-2 meters (3-6 ft) apart, with their main lenses facing in the same direction.
d) Recording location should be as free from sources of artificial light as possible.

Record video

a) Shortly before the anticipated start of fireflies flashing, press the record button on both cameras.
b) Stand free of the recording area and minimize the use of artificial light, limiting use to dim, red-filtered light.
c) Retrieve cameras either after the firefly flashing display has stopped or once the camera batteries are drained, whichever comes first.

Collect and record contextual data

Collect data about the recording event such as the GPS coordinates of camera location, the date, the start time of the recording, the sunset time, air temperature, camera height above the ground, and distance between the cameras.

Process and analyze firefly footage

Footage can be processed using methods described by Sarfati and Peleg (2021) and Martin et al (2023), resulting in a spreadsheet of flashes with x, y,z, and t values, where x, y, and z are the spatial coordinates and t is time. Additional processing can connect flashes into flash trajectories.

Glow-worm firefly lure trap monitoring protocol

Why use glowing lure traps?

- Glowing lure traps could be a useful tool for monitoring a variety of firefly genera (and similar bioluminescent beetles) on multiple continents (see Table 4).

- They target adult male glow-worm fireflies. Because in multiple taxa (*Lampyris*, *Phausis reticulata*, *Microphotus dilatatus*) females often stop glowing as soon as they have mated (Cicero, 1981; De Cock et al., 2014; Sivinski et al., 1998; Tyler, 2002), detection probability of females via visual surveys is likely lower and more variable compared to detection of adult males with lure traps. Furthermore, in many glow-worm firefly species the adults fly without luminescence, so trapping them is the best way to get counts and estimates of adult abundances.
- Compared to visual surveys for adult females, there is little to no observer bias due to varying experiences level and vision of different observers.
- Sampling scope (zone of influence) and intensity (time sampling) may be higher than with visual searches, leading to higher efficiency.

Table 4. Examples of glow-worm firefly genera that could be sampled using glowing lure traps. Genera in bold have been successfully sampled with light lure traps.

Region	**Example Genera**
Europe	***Lampyris, Nyctophila, Lamprohiza***
North America	*Microphotus*, ***Phausis***, *Pleotomodes*, *Pleotomus*, *Prolutacea*
Asia	***Lampyris****, Pyrocoelia, Lamprigera, Diaphanes*, *Oculogryphus*, *Rhagophthalmus* (Rhagophthalmidae)
Africa	*Afrodiaphanes, Diaphanes*, ***Lampyris***, *Pelania*

What can glowing lure traps tell us about glow-worm populations?

- Overall timing and peak of the adult male flight period
- Index of abundance of adult male glow-worms
- Occurrence/non-detection in areas sampled

Trapping assumptions and potential pitfalls

- This approach depends on the **quick**, **confident**, and **non-lethal identification** of males in the field. In areas with multiple species in which males are very similar, it may not be possible to measure species-level indices of abundance without collecting voucher specimens during sampling.
- This approach assumes that the **attractiveness of glow lure traps to adult male glow-worms is stable through time and space**. However, it is possible or likely that when adult females peak in abundance/density, probability of attraction and capture is lower, given the number of glows that may be present in the area. A similar phenomenon has been shown with pan traps, with traps capturing fewer bees when there are abundant floral resources nearby (Baum & Wallen, 2011; Westerberg et al., 2021). Because it is suspected that in some species of adult male glow-worms find females in part by following pheromones emitted by females (De Cock et al., 2014), the presence of females may make glow lure traps less attractive.
- It assumes that **retention rate is high and stable**—that is, adult males caught in traps mostly stay in traps, and the probability of males escaping varies little between traps, sites, and individual glow-worms.

- While the glow-lure trap method is intended to be non-lethal, heavy trapping effort **may have negative impacts** on population through **incidental mortality of captured males** and **reduced reproductive success of both males and females**.
- See Appendix II for a preliminary data to gather on glow-worm firefly populations before conducting monitoring.

Glow-worm lure trap protocol instructions

<u>Design and build the glow lure traps</u>.

- Inexpensive traps can be made by cutting off the top of a two-liter plastic beverage bottle and inverting the tapered portion so as to form a funnel (De Cock et al., 2014, Figure 9). In species with glowing males (e.g. *Phausis reticulata*, *Lamprohiza splendidula*) painting the surfaces of the bottle with a dark matte paint may prevent glows of trapped males from attracting additional males to the outside of the trap. Applying a coat of polytetrafluoroethylene (Teflon) to the inside of the trap will make it harder for fireflies to escape (De Cock et al., 2014). Placing threads across the opening of the trap can also help with retaining trapped male glow-worms. Finally, placing moist leaves inside the traps is a precaution to improve survival and retention of trapped males (De Cock, pers. comm.)

- Generally, yellow or lime-green (~550 nm) lights are effective for most species (De Cock et al., 2014; De Cock, 2014; De Cock & Guzmán-Álvarez, 2013), but *Diaphanes* in Rwanda were attracted to a trap with a red (630nm) light (Pacheco et al., 2016). *Lampyris* males are lured more effectively and from greater distances by brighter lures (De Cock, pers. comm), while *Lamprohiza splendidula* and *Phausis reticulata* prefer dimmer light intensities (De Cock & Guzmán-Álvarez, 2013). Light intensity of LEDs can be adjusted using resistors or color neutral photography filters. Light source options include 1) Tritium (betalights), 2) battery operated or solar powered LEDs, or 3) single-use, 20 mm glowsticks. See Table 5 for light source considerations.
- Designs should be tested to ensure that the traps are effective at capturing and retaining the target species.
- Include contingency plans in case of technologies changing or products going out of stock.

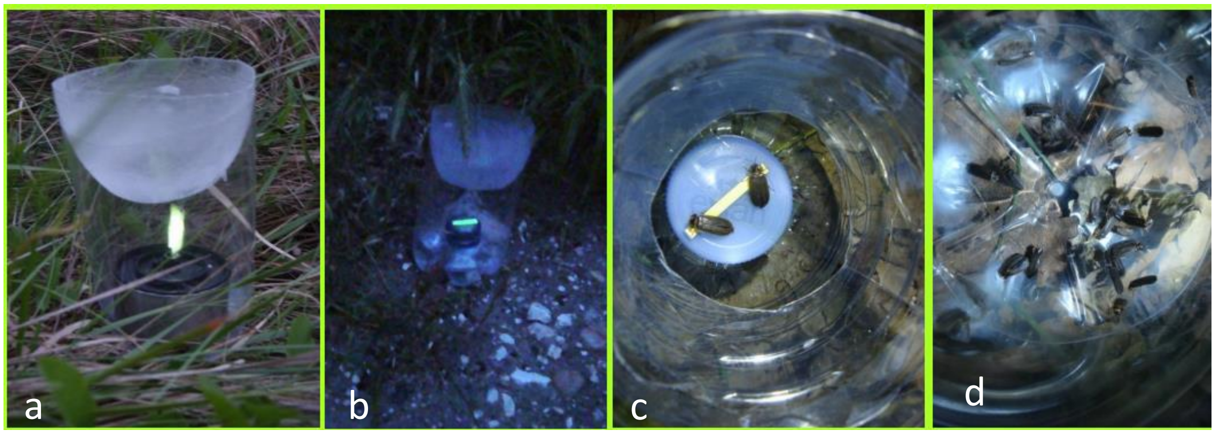

Figure 9. a) Plastic PET-bottle trap with solar-powered LED as a lure; b) Plastic PET-bottle trap with green betalight lure; c*) Lamprorhiza delarouzei* males in PET-bottle trap with green betalight; d) Final catch after 2 hours of *Lamprorhiza delarouzei* males in green betalight PET-bottle trap. Images from De Cock (2014). In order to prevent males from landing on the bottle sides, it is recommended to use non-transparent bottles or to paint or cover the bottle sides.

Design the glow lure trap layout.

- Depending on the terrain and configuration of the habitat, traps could be laid out in a **linear transect**, an **X-shaped array** or a **circular array**. See Figure 10 for an example of a transect array and Figure 11 for X-shaped array example. Studies monitoring other taxonomic groups of insects using pitfall traps, pan traps, and light traps may provide insights about appropriate layouts (Montgomery et al., 2021).
- For traps to be independent sampling units, they should be **far enough apart** that a male glow-worm will only see one lure trap at once. A study of *Lampyris noctiluca* in Finland found that individual glow lure traps separated by at least 100 meters caught more adult males than glow lure traps arranged in clusters of four, each separated by 50 cm (Lehtonen & Kaitala, 2020). Some studies have used a 100-meter spacing along a transect (De Cock & Guzmán-Álvarez, 2013). Studies of *Phausis reticulata* suggest that flying males can only visually detect females and female-like glows from a distance of one meter or less, suggesting that trap spacings of as little as two meters may be acceptable, but bright LEDs may be detectable to male *Lampyris noctiluca* from tens of meters away (De Cock pers. comm.)
- Trap stations of 3-4 traps, arranged 1-2 meters apart, may also be used.

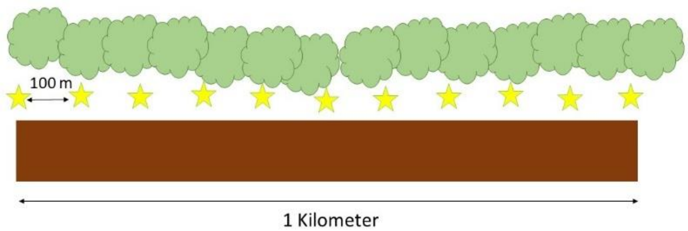

Figure 10. Example transect array of glow lure traps for linear habitats such as trail-sides, riparian areas, or hedgerows. Eleven glow-lure traps are placed along a 1,000 meter transect, with 100 meters between traps.

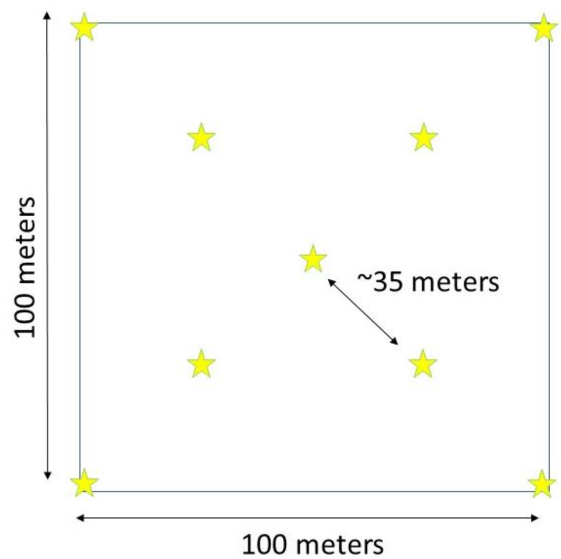

Figure 11. Example array of 9 glow lure traps arranged as in two perpendicular lines within a 1-hectare (100 meter x 100 meter) plot.

<u>Design the within-season monitoring schedule:</u>

a. Trap nights should **span the possible adult male flight period** of the target species at the monitoring site. For example, the blue ghost firefly's flight period in North Carolina spans four months (April-July) and has two peaks; *Lampyris noctiluca* in the United Kingdom has an adult season from June to August, but usually with a strong peak of male activity around mid to late June. Length of trapping period will depend on both length of flight period and variability of flight period, which in turn depends on weather conditions, with warm springs leading to earlier peaks and cold spring seasons leading to later peaks. See Table 6 for an example within-season monitoring calendar.
b. The date range **parameters and criteria should be consistent from year to year**. These may be date ranges, mGDD ranges, or a combination of date ranges and weather events (for example, first rains of the spring or rainy season). A wider sampling window and a

slightly higher sampling effort may be needed if date ranges are used, to account for interannual variability in weather/seasons.

c. Trapping nights should occur **frequently enough** to ensure that peak nights will be captured, and frequency will depend on the average length of peak display.
d. Number and frequency of trap nights should be **realistic** and **sustainable** given staffing capacity, night-time working hours, fatigue, and safety considerations.

Determine the within-evening sampling schedule

a. Before the season begins create a table with sampling dates, sunset times, and civil twilight times. (See Table 7 for an example within-evening monitoring schedule.)
b. Traps should be deployed before it is fully dark.
c. Trap time should be long enough to cover the peak level of evening activity. Two hours is likely sufficient. If precipitation or other forms of trap disturbance are not expected, traps can be checked early in the morning (before sun exposure can overheat the traps), but retrieval time should be recorded.

Execute sampling plan

- Work in pairs or groups for safety.
- Deploy traps in the configuration decided upon, adjusting trap placement as needed so that they are not obscured by vegetation.
- Check traps to count, identify and record captured male fireflies, recording the time that each trap was checked.
- See Appendix III for an example data-sheet for glow-worm firefly trapping.
- Release fireflies unharmed at the location where they were captured once all of the traps have been checked.

Table 5. Glow-lure trap protocol considerations, strategies, and rationales.

	Consideration	**Strategy**	**Rationale & reference**
Temporal	Trap night frequency	Every 1-2 weeks	Must be frequent enough to not miss near-peak abundance but spaced out enough to be sustainable within the season and across years. Weekly trapping may be a practical frequency from a logistical standpoint for species with longer seasons.
	Trap night frequency	Every 2-3 days	In *Lampyris,* the male adult flight period is just 2-3 weeks with a strong but short peak of 2-3 days (Van den Broeck et al., 2021, R. De Cock pers. obs). After conducting nightly trapping of *Lampyris noctiluca* in Switzerland, Riesen et al. (2011) proposed a trapping interval of 2-3 days to lessen impacts on the population.
	Length of nightly trap deployment	2 hours	The peak of adult male activity in some glow-worm species occurs within the first few hours of darkness (Faust, 2017).
	Length of nightly trap deployment	30 minutes	Tyler (2011) deployed an array of 11 lures spaced 5 meters apart for 30 minutes on each sampling night.
Lures	Light source	Battery-powered LED	Inexpensive and easy to obtain; non-radioactive (unlike Tritium). Intensity is adjustable with resistors.
	Light source	Tritium (light source)	Weather-proof, long-lasting, don't need batteries.
	Light source	20 mm glow sticks	Inexpensive and easy to obtain.
	Light source	Solar-powered LED	Relatively inexpensive and easy to obtain; reusable.
Spatial	Number of traps in an array	10-15	Number of traps will depend on the scale of the monitoring site and the available time and staff to deploy and retrieve traps.
	Arrangement of traps	Two transects forming an X within a square plot	This arrangement has been used in bee pan-trapping and may work for glow-worm fireflies.
	Arrangement of traps	Linear or meandering transect	Deployment and retrieval along linear transects or existing trails or roads may be more safe, practical, and efficient in certain habitats.
	Distance between traps	5-100 meters	It is best to space out the traps enough that they do not compete with each other for males (Lehtonen & Kaitala, 2020). The appropriate distance may vary depending on the species and the habitat.

	Precise trap placement	Visible spots unobstructed by vegetation	Females of glow-worm firefly species such as *Microphotus* often signal from exposed perches (Cicero, 1981). Traps should be visible to males flying nearby. Tyler (2011) opportunistically placed lures on top of ant hills to enhance visibility to *Lampyris noctiluca*.

Table 6. Example glow-lure trapping season calendar

Date	**Activity or context**
April 28	Earliest recorded adult activity
April 30	Reconnaissance visit/trapping
May 1-May 3	First trap night window
May 5	Full moon
May 8-10	Second trap night window
May 15-17	Third trap night window
May 22-24	Fourth trap night window
May 29	End of season reconnaissance

Table 7. Example within-evening glow-lure trapping schedule

Time	**Action**
Pre-sunset	Arrive at survey site, prepare traps, set traps
Civil dusk (end of civil twilight; usually 20-30 minutes after sunset)	Effective start of trapping time
2 hours after civil dusk	Earliest time to check and retrieve traps
Dawn or early morning of following day	Alternative time to check traps.

Mark-recapture methods

By marking individuals and then examining proportions of marked individuals and new individuals in later captures or detections, population size estimates can be made.

Mark-recapture methods have been applied to adult *Luciola lateralis* (Koji et al., 2012) and larval *Luciola parvula* in Japan, *Phosphaenus hemipterus* glow-worm fireflies in Belgium (De Cock & Matthysen, 2005), adult *Nipponluciola cruciata* in Japan, *Photinus pyralis* and *Photuris versicolor* in the United States (Firebaugh & Haynes, 2016), adult male *Lampyris noctiluca* (Riesen et al., 2011); adult *Photinus corruscus* (Rooney & Lewis, 2000); and adult male *Pyractomena lucifera* in the United States (Buschman, 1984). Non-toxic paint, gel pens, and fluorescent powder can be used to mark individual fireflies

These methods are generally too labor and resource intensive for long-term or large-scale application, but they can help to inform and validate other monitoring methods. For example, population estimates from mark-and-recapture studies can be compared with visual count data, and mark-and-recapture can provide insight into lifespans and survival rates of adult individuals.

Data management and curation

Firefly abundance data should be backed up with digital and hard copies of raw data. We recommend that, when possible, data fields of digital data use Darwin Core terminology in order to facilitate data aggregation and collaboration. Data can be stored in a relational database or in individual tables. It may be desirable to have separate tables for site data and sampling event data, with unique identifiers to join data as needed.

Analyzing and interpreting abundance data

In order to use firefly abundance data to answer specific questions and make inferences, you should consult a statistician to ensure that statistical methods are appropriate for your data set, sample size, and questions.

A few statistical tools useful for analyzing abundance data include the following:

- Generalized additive mixed models (GAMMS) (Gardiner & Didham, 2020)
- Random forest algorithms (McNeil et al., 2024)
- Population curve models (Soulsby & Thomas, 2012).

Appendix I: Metadata to collect along with abundance data

Category	Variable	Darwin Core header(s)	Visual count	Photo	Video	Light lure trap
Site information	GPS coordinates of site	decimalLatitude; decimalLongitude	X	X	X	X
	GPS coordinates of camera, plot centers, transect starts, or traps	decimalLatitude; decimalLongitude	X	X	X	X
	Location description	locality	X	X	X	X
	Country	country	X	X	X	X
	State or Province	stateProvince	X	X	X	X
	Habitat	habitat	X	X	X	X
	Photos showing habitat	NA	X	X	X	X
Event information	Date or date range of sampling	eventDate	X	X	X	X
	Time(s) of sampling event	eventTime	X	X	X	X
	Recorder, surveyor, observer	recordedBy	X			
Weather and sky conditions	Wind during sampling (Beaufort Scale)	NA	X	X	X	X
	Air temperature	NA	X	X	X	X
	Precipitation	NA	X	X	X	X
	Humidity	NA	X	X	X	X
	Cloud cover	NA	X	X	X	X
	Lunar phase or lunar illumination (Śmielak, 2023)	NA	X	X	X	X
	Light pollution	NA	X	X	X	X
Sampling methods and equipment	Exposure time	NA		X		
	ISO	NA		X	X	
	Camera focal length	NA		X	X	
	Frame rate	NA			X	
	Image resolution	NA		X	X	
	Trap type	NA				X
	Light source, brightness and wavelength	NA				X
	Time spent sampling	NA	X	X	X	X

Appendix II. Preliminary Data to Collect on Glow-worm Populations Prior to Monitoring

Few glow-worm firefly species have been studied closely enough that effective monitoring programs can be carried out. To monitor populations, an understanding of the basic ecology and life history of a species or a population is first needed. Below are some of the important steps recommended to collect key data on species of interest.

Phenological timing

- Sample across the season, ideally capturing the start and end of detectable female displays and male flight, and use weather data to determine predictors of activity, such as growing degree days, precipitation patterns, light cycles.

Nightly behavior patterns

- What time of night do glowworms become active? Do females continue glowing after mating?

Attractiveness of glow lure wavelengths

- Trial multiple wavelengths (colors) and intensities (brightnesses) of light to determine which are most effective for attracting the species of interest.

Variables affecting catch rates

- What is the best spacing for trap layout (i.e. how far do males travel to find females, and how close do they have to be to see females)?
- What does transect count for females look like for the species of interest?
- Does moon phase impact catch rates?
- In which habitats are the highest numbers of males caught? (E.g. open areas, along vegetation margins or in sheltered valleys.)

Trap retention

- Compare multiple trap designs and trap-checking intervals to determine what percentage of adult male glow-worm fireflies that enter a trap will remain in the trap after a given amount of time.

Appendix III: Example Data-sheet for male glow-worm trapping

Monitoring project info

Monitoring coordinator:	Names of field personnel:

General Location

Name of Refuge/Park/Management Area/Other conservation land unit:		
County/County equivalent:	State/Province:	Country:

Monitoring Site

Site Name:	Elevation:	
Latitude:	Longitude:	Geodetic Datum:

Date/Time/Weather: Start of trap night

Time	Day	Month	Year	Temperature	Wind	Humidity	Precipitation	Moonlight	Cloud Cover

Date/Time/Weather: End of trap night

Time	Day	Month	Year	Temperature	Wind	Humidity	Precipitation	Moonlight	Cloud Cover

Nearest Weather Station:

Trap-night notes

Trapping results

Site name	Trap array ID	Trap/light type	Species	Number caught	Time deployed	Time checked
Shady Woods	A	Yellow LED	*L. noctiluca*	0		
Shady Woods	A	Green LED	*L. noctiluca*	5		
Shady Woods	A	Green glowstick	*L. noctiluca*	3		
Shady Woods	B	Yellow LED	*L. noctiluca*	1		
Shady Woods	B	Green LED	*L. noctiluca*	0		
Shady Woods	B	Green glowstick	*L. noctiluca*	3		
Shady Woods	C	Yellow LED	*L. noctiluca*	7		
Shady Woods	C	Green LED	*L. noctiluca*	2		
Shady Woods	C	Green glowstick	*L. noctiluca*	0		

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