

# UNLOCKING THE



DEREK A. WOLLER,  
BERT FOQUET,  
SHELBY K. KILPATRICK,  
RYAN SELKING,  
CHARLES MAZEL,  
AND HOJUN SONG

# DARK: Harnessing Blue-Light Fluorescence to Illuminate Hidden Hexapods



Locating hexapods in the dark during field collecting with white light (usually as a headlamp) can be challenging, mainly due to insects' relatively small size and camouflaging abilities. Increasing the visual contrast between a specimen and its background can assist with searching, and one clever method to do this is via fluorescence, the optical process by which light is absorbed in one wavelength range and re-emitted in another, longer range. If a subject fluoresces and its surroundings do not, or if the surroundings fluoresce, but in a different color, the subject will often stand out like a beacon to human eyes.



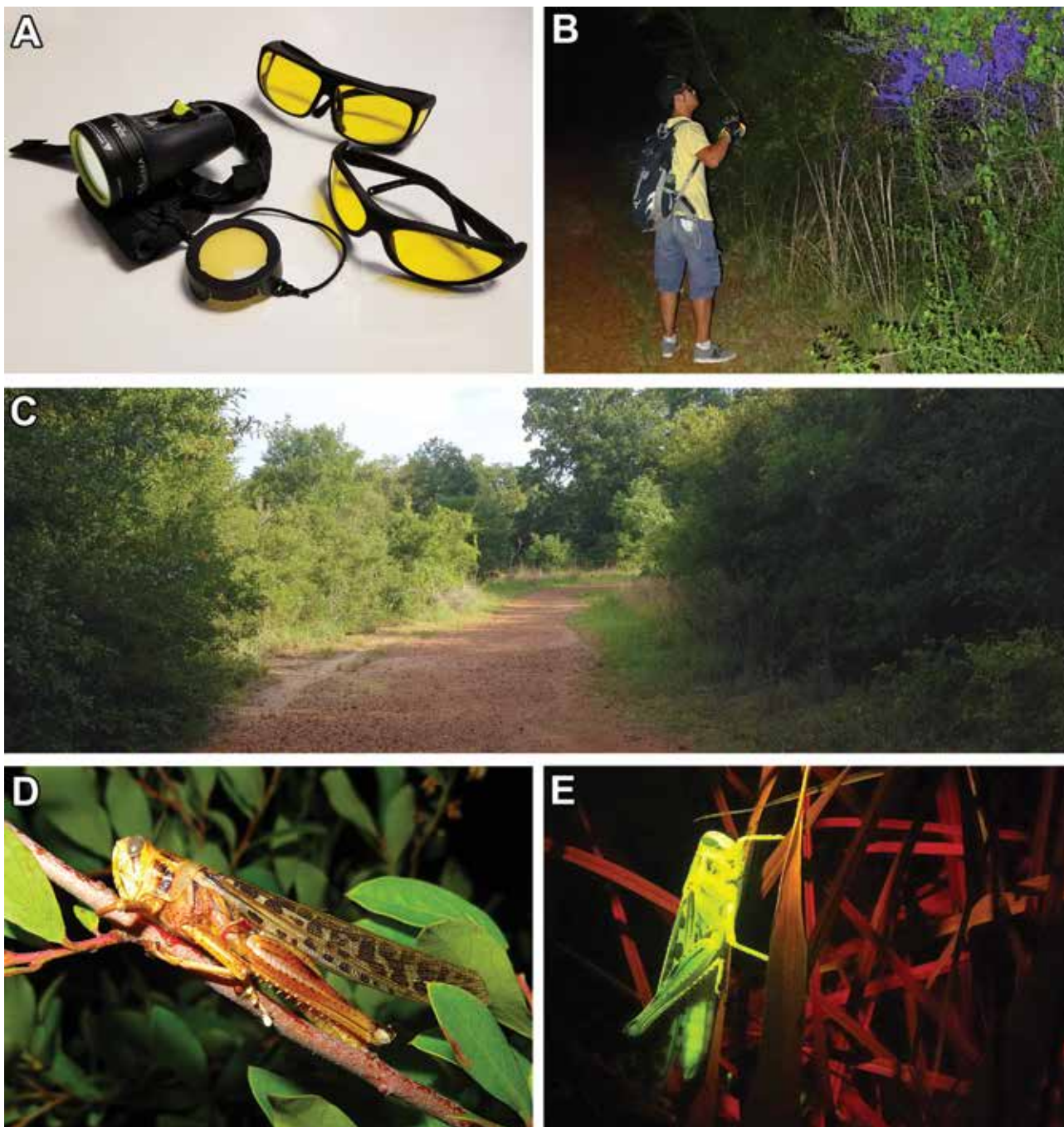


Fig 1. Field experiment at Lick Creek Park, College Station, Texas. A) Sola NIGHTSEA blue-light unit with white-light filter cover removed and barrier filter glasses: Models VG2 (upper, which fit over eyeglasses) and VG3 (lower). B) Participant using the blue-light unit to search for dead adult grasshoppers glued to plants along the trail. C) Experiment site along the Iron Bridge Trail. D) Example of a grasshopper specimen (lab-reared *Schistocerca* hybrids) glued to a shrub branch and imaged with white light. E) Another grasshopper specimen imaged with the blue-light unit through the lens of the barrier filter glasses. (Photo A by DAW; photos B, D, and E by BF; photo C by SKK.)

Fluorescence is used for a variety of entomological applications, such as mark-recapture sampling and detection of protein localization and function. Although fluorescence is not commonly employed for field collecting, when it is used, ultraviolet (UV) has been the wavelength of choice. Based on our many field and lab investigations, we propose that fluorescence excited by blue light (a technique commonly used in marine biology) should

also be strongly considered and could be used for the following functions:

- night collecting;
- natural history observations after dark;
- assistance with locating urban and agricultural pests in home gardens and crop fields (e.g., leaf miner damage fluoresces well);
- for general pest control (e.g., locating eggs, larvae, etc.); and
- as an enhancement tool for examining/

locating very faint, small-bodied orders (e.g., Protura, Diplura, and Zoraptera).

We developed this list by harnessing blue-light fluorescence in portable form as a powerful flashlight to conduct a nocturnal field experiment to statistically investigate the efficacy of using blue-light fluorescence to locate grasshoppers. We also compiled observations from an extensive general investigation of the portable light's effects on all hexapod orders (and

some related organisms), which included examining preserved specimens from multiple insect collections, as well as live specimens in a variety of habitats worldwide.

### Shedding Light on Fluorescence

At the atomic level, fluorescence involves an electron being excited to a higher orbital by an incoming photon, losing a bit of energy, and then falling back to the ground state, thereby emitting a photon of lower energy than the original (Campbell and Dwek 1984). As mentioned, two common examples of light sources that stimulate (or excite) fluorescence are UV light (typically longwave UV, in the 360–400 nm range), which can be absorbed and re-emitted anywhere in the visible spectrum, and blue light (in the 440–470 nm range), which can be absorbed and re-emitted as greens, yellows, oranges, or reds. Thus, a specimen can become easier to see if it fluoresces while its background either does not fluoresce or emits a color different from the specimen.

For this reason, fluorescence in the form of UV is occasionally employed by entomologists who search for field specimens at night (e.g., Stahnke 1972, Moskowicz 2017), most often for scorpions, which are well known to fluoresce (Stahnke 1972, Gaffin et al. 2012). The technique has been more commonly used for field searching in other branches of science, notably geology (Schneider 2006) and marine biology. In the latter, blue-light fluorescence is routinely used for *in situ* exploration of reef surfaces for newly settled juvenile corals, which are on the order of a millimeter in size (Piniak et al. 2005, Baird et al. 2006, Johns et al. 2018). Interestingly, compared to conventional techniques, blue-light fluorescence has also been shown to be superior in the search for cryptic fish species (De Brauwer et al. 2017).

Although fluorescence techniques are not commonly used for entomological field collecting, they are used by entomologists for a variety of other tasks, including searching for dyes and dusts in/on mark-recapture specimens (methods reviewed in Hagler and Jackson 2001), exploring life-stage development (Sourakov 2017), as a taxonomic tool (Marek 2017), and even molecular-level applications, such as detecting protein localization and function (Valeur and Berberan-Santos 2012).

When fluorescence has been applied in entomology, it has typically involved the use of longwave UV light (in the vicinity of 365 nm). A common misconception across many disciplines is that fluorescence

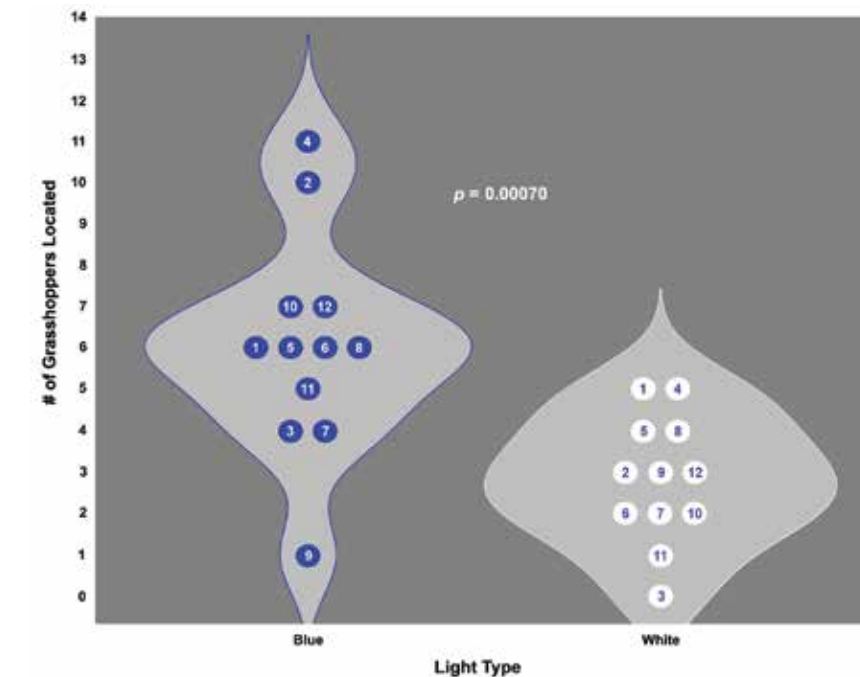


Fig. 2. Violin plots showing the range, frequency, and distribution of the fluorescence field-experiment results. Wide regions of each violin illustrate a higher-probability density of grasshoppers being located using the respective light type, whereas narrow regions represent lower-probability densities. Individual points within each violin are labeled with participant numbers indicated in Table 1. The mean number of grasshoppers located with each light type was significantly different:  $p = 0.00070$ .

requires the use of UV wavelengths. In some cases, UV may indeed be the best choice, but that is not always true. For example, in the marine environment, although many subjects fluoresce under UV light, blue light (in the vicinity of 450 nm) was generally superior for making more specimens fluoresce, and more brightly (Mazel, unpublished data). All of the marine studies cited previously used portable blue light sources as the tool for *in situ* fluorescence exploration. Note that when using blue light to excite fluorescence, it is necessary to wear complementary barrier filter glasses (yellow-lensed glasses, Fig. 1A) that block reflected excitation light and transmit the fluorescence.

### How We Unlocked the Dark

We conducted a nocturnal field experiment to determine the general efficacy of fluorescence, and we also carried out an extensive investigation of blue-light fluorescence (hereafter referred to as “fluorescence,” unless otherwise indicated) effects on all hexapod orders (and some related organisms) using preserved and live specimens, both indoors and in the field.

**The Portable Blue Light.** The field experiment and all fluorescence surveys were undertaken using three blue-light

flashlights (Sola NIGHTSEA light, Light & Motion, Marina, CA, hereafter referred to as the “blue-light unit”) and multiple sets of barrier filter glasses (Fig. 1A: Models VG2 [fits over eyeglasses] and VG3, NIGHTSEA, Lexington, MA). The flashlights include multiple high-intensity royal blue light-emitting diodes (LEDs) that operate in two modes: spot (3 LEDs) and flood (6 LEDs). Within each of these modes, there are three settings: low (25% intensity), medium (50%), and high (100%), with the flood + high combination lasting the least amount of time and the spot + low lasting the longest. The light is designed for underwater use by scuba divers, taking advantage of the thermal conductivity of the surrounding water to prevent the light from overheating. Above water, the light can become too hot, at which point it will dim automatically to the lowest setting, but will continue working for several hours. We recommend using a glove for protection from this overheating effect during terrestrial use.

The light also comes with a cover, connected by a strap, made of a plastic material called a “remote phosphor.” This absorbs a large fraction of the blue light (Fig. 1B) and re-emits it in a broad range of wavelengths, resulting in white light (Fig. 1D). This can

**Table 1.** Fluorescence field experiment participant data, including the side of the trail each participant started on, the light color each participant started with first, the time each participant took to search for grasshoppers with each light color (5.00 min maximum), the number of specimens each participant located with each light color, and the difference between the number of specimens located with blue-light fluorescence versus white light.

| Participant # | Starting Side of the Trail | Starting Light Color | Blue Light Search Time (min) | Blue Light Specimens Located | White Light Search Time (min) | White Light Specimens Located | Blue vs. White Light Specimens Located |
|---------------|----------------------------|----------------------|------------------------------|------------------------------|-------------------------------|-------------------------------|--|
| 1             | Left                       | White                | 5.00                         | 6                            | 4.50                          | 5                             | 1                                      |
| 2             | Right                      | White                | 5.00                         | 10                           | 5.00                          | 3                             | 7                                      |
| 3             | Right                      | White                | 5.00                         | 4                            | 4.80                          | 0                             | 4                                      |
| 4             | Right                      | Blue                 | 5.00                         | 11                           | 4.50                          | 5                             | 6                                      |
| 5             | Left                       | White                | 5.00                         | 6                            | 4.58                          | 4                             | 2                                      |
| 6             | Left                       | Blue                 | 3.10                         | 6                            | 3.22                          | 2                             | 4                                      |
| 7             | Left                       | White                | 5.00                         | 4                            | 5.00                          | 2                             | 2                                      |
| 8             | Right                      | Blue                 | 5.00                         | 6                            | 5.00                          | 4                             | 2                                      |
| 9             | Right                      | Blue                 | 5.00                         | 1                            | 5.00                          | 3                             | -2                                     |
| 10            | Left                       | White                | 5.00                         | 7                            | 5.00                          | 2                             | 5                                      |
| 11            | Left                       | White                | 5.00                         | 5                            | 5.00                          | 1                             | 4                                      |
| 12            | Right                      | Blue                 | 4.75                         | 7                            | 5.00                          | 3                             | 4                                      |

be especially useful when a conventional headlamp or flashlight is not available. The remote phosphor also acts like a diffuser, spreading out the beam relatively further in both spot and flood modes. The battery is non-removable and can be recharged in about 2.5 hours. Optional accessories allow the light's underside screw-mount to be fitted to different types of handles.

**Testing Blue-Light Efficacy with New Eyes.** Our investigations suggested that it is far easier to find many types of hexapods, especially Polyneoptera (e.g., many grasshoppers and katydids), outside in the dark with blue light compared with a traditional white light. We decided to test this statistically with the assistance of 12 students (five male, seven female, and all from the Texas A&M University Entomology Department) who had never used the blue-light technology before. We devised a field experiment set up in a Texas park (Fig. 1B, C) to test the specific hypothesis that locating grasshoppers with fluorescence is easier than with white light. In brief, the experiment involved having each participant search for 30 freeze-killed grasshoppers (lab-reared *Schistocerca* hybrids) glued to plants (Fig. 1D, E) on one side of the trail using the blue-light unit, and then repeating the process on the other side with white light (same unit, but with its cover on). Participants counted as many grasshoppers as they could find in a five-minute timeframe and were not allowed to backtrack. Statistical

analyses were performed using R (R Core Team 2013), and results are reported in Table 1 and Fig. 2. For a detailed explanation of the experiment's setup and analyses, please see Supplemental Information (available at <https://doi.org/10.1093/ae/tmaa005>).

**Let the Extensive Blue-Light Surveys Begin!** Two types of hexapod fluorescence surveys (a survey of collections and field surveys) were conducted almost exclusively on adult specimens. Both were carried out primarily by DAW and BF, although unbiased second opinions and field assistance from others were occasionally requested. To survey the extent of fluorescence in museum specimens, we primarily utilized the breadth of holdings of the Texas A&M University Insect Collection (TAMU) in College Station, Texas. In a few cases, we also used DAW's personal collection, as well as the Arizona State University Hasbrouck Insect Collection (ASUHC) in Tempe, Arizona. Surveys occurred in complete darkness using the blue-light unit and barrier filter glasses, and were done through the medium of the specimen-containing unit (e.g., the glass of an insect drawer).

All known taxonomic orders of Hexapoda were included, with multiple suborders and families chosen for each (when possible). We reviewed numerous species/adult specimens within those taxonomic levels to capture a wide variety across the breadth of the subphylum.

Fluorescence effects were noted and recorded based on a back-to-back comparison of blue versus white light (Table 2).

Field surveys were also undertaken when it was as dark as possible, using the same equipment and basic procedures used for collection surveys (results incorporated into Table 2). Multiple habitat types were investigated across the U.S., as well as in Costa Rica, Ethiopia, and Mozambique. Observed taxa were varied (e.g., Figs. 3–5) and belonged to several of the same taxonomic families across numerous orders observed in the collection surveys. Polyneopterans, particularly those in the orders Orthoptera (e.g., Fig. 3G, H and Fig. 4A, B) and Phasmatodea (e.g., Fig. 4C, D), were frequently the focus of our investigations. For further details on how and where we conducted these surveys, please see Supplemental Information.

### Young Eyes Agree with Old Eyes

We hypothesized that fluorescence enhancement effects would make it easier to locate polyneopterans, particularly Orthoptera, during nocturnal field collecting, and our field experiment results validated our hypothesis. Overall, the 12 participants were able to locate more grasshoppers with fluorescence ( $p = 0.00070$ ; Table 1 and Fig. 2), although, interestingly, one participant was somehow able to find more with the white light. Moreover, each participant was able to spot at least one



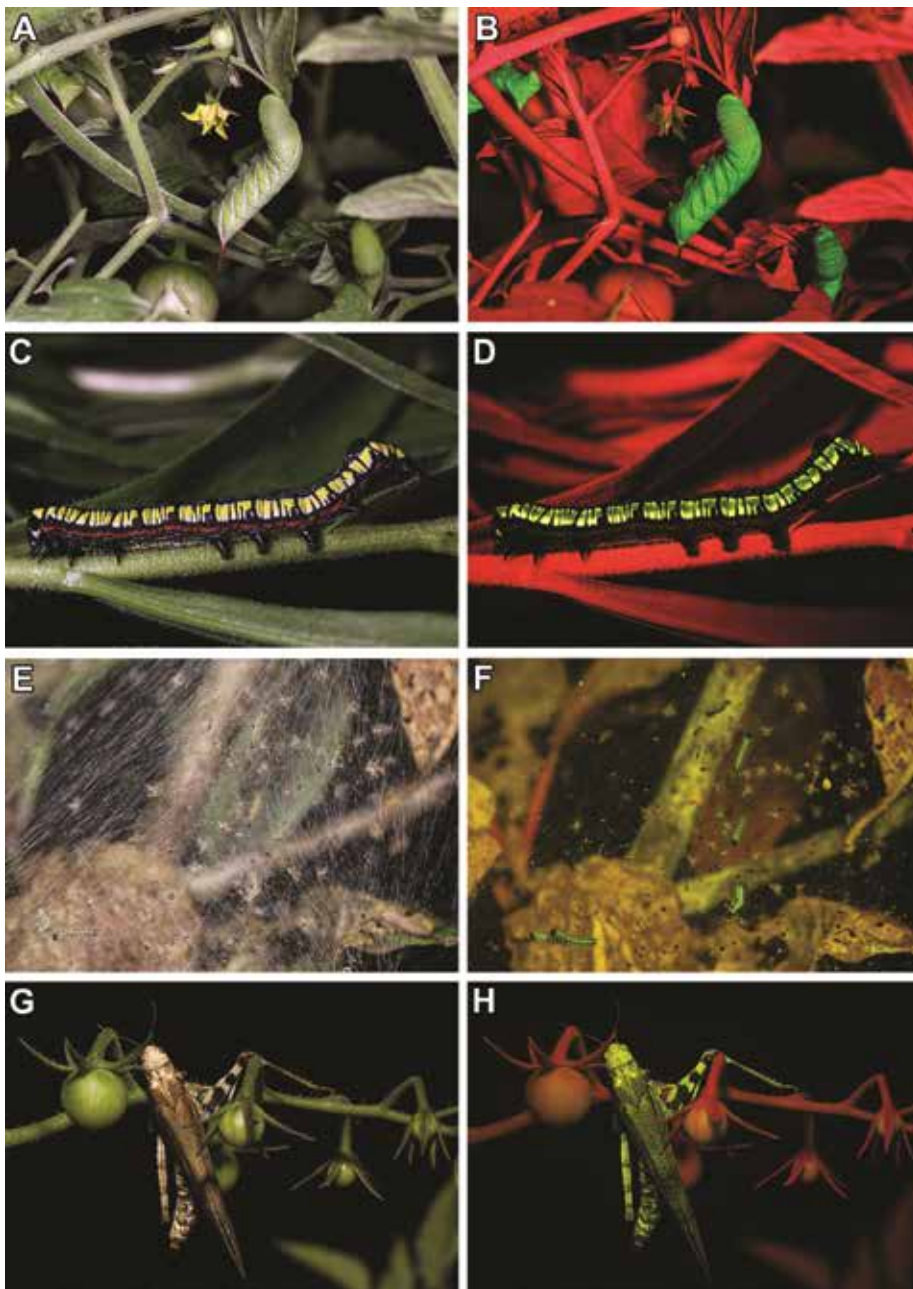


Fig. 3. Field images demonstrating effects of white light (left column) and blue-light fluorescence (right column). A and B) hornworm, *Manduca* sp. (Lepidoptera: Sphingidae); C and D) brown-hooded owlet, *Cucullia convexipennis* Grote & Robinson (Lepidoptera: Noctuidae); E and F) fall webworm, *Hyphantria cunea* (Drury) (Lepidoptera: Erebidae); G and H) Carolina grasshopper, *Dissosteira carolina* (Linnaeus) (Orthoptera: Acrididae). (Photos by CM)

grasshopper by using fluorescence, with a high of 11 spotted, whereas the highest number of grasshoppers found with white light was only five (Table 1 and Fig. 2). These findings suggested that five minutes was not enough time to spot all 30 grasshoppers hidden on each trail side, especially when each participant was not allowed to backtrack. This outcome did

not seem to be dependent on experience level, because the more experienced blue-light users who organized the experiment (four participants, data not shown) also participated in both trials and didn't fare much better. Although the experienced blue-light users spotted a higher average number of grasshoppers for both light types (predominantly blue) than the novice

participants, they still located no more than 10 specimens across both trials. Still, this experiment provides compelling evidence of the potential for fluorescence to enhance the ability to find hexapods (at least large grasshoppers, anyway) in darkness and likely speed up the process.

### Illuminating Insights from Collection Surveys

We observed a great number of enhancement effects when we examined preserved specimens during our collection surveys (summarized in Table 2). The two most obvious were that lighter-colored or light-patterned (lighter equals brighter) and soft-bodied specimens typically displayed a greenish-yellow body coloration (e.g., Fig. 3A, B; Fig. 4A, B; and Fig. 5G, H); and that eyes stood out as white against very common black base-color (although often reddish in polyneopterans), a subtle effect that can be difficult to capture in images (e.g., Fig. 4C, D, E, and F). In some rare cases, metallic coloration (often green) can look darker, such as in Papilionidae (Lepidoptera) and Calliphoridae (Diptera). Conversely, metallic coloration may look brighter, as we found with some specimens of Curculionidae (Coleoptera).

In terms of occurrence and observed effects, nine of the 10 polyneopteran orders (Blattodea, Dermaptera, Grylloblattodea, Mantodea, Mantophasmatodea, Orthoptera [e.g., Fig. 1D, E; Fig. 3G, H; and Fig. 4A, B], Phasmatodea [e.g., Fig. 4C, D], Plecoptera, and Zoraptera) commonly displayed fluorescence effects that were principally obvious and bright, possibly because specimens in these orders are often lighter-colored and soft-bodied. Notably, specimens within these orders were also some of the largest to consistently demonstrate these effects. These effects were also commonly observed across their entire body, as opposed to in localized regions or specific patterns (e.g., eyespots in luna moths [*Actias luna* (Linnaeus), Lepidoptera: Saturniidae] or veins in the Arctiinae [Lepidoptera: Erebidae]). Embioptera was the sole polyneopteran order that did not display any effects at all, not even in live specimens. A similar pattern was observed in specimens of some of the other families of orders that otherwise displayed effects (e.g., Megaloptera: Sialidae, and Orthoptera: Myrmecophilidae [Table 2], and Coleoptera and Hymenoptera [Fig. 5C, D]). The only other orders that displayed zero effects were Raphidioptera

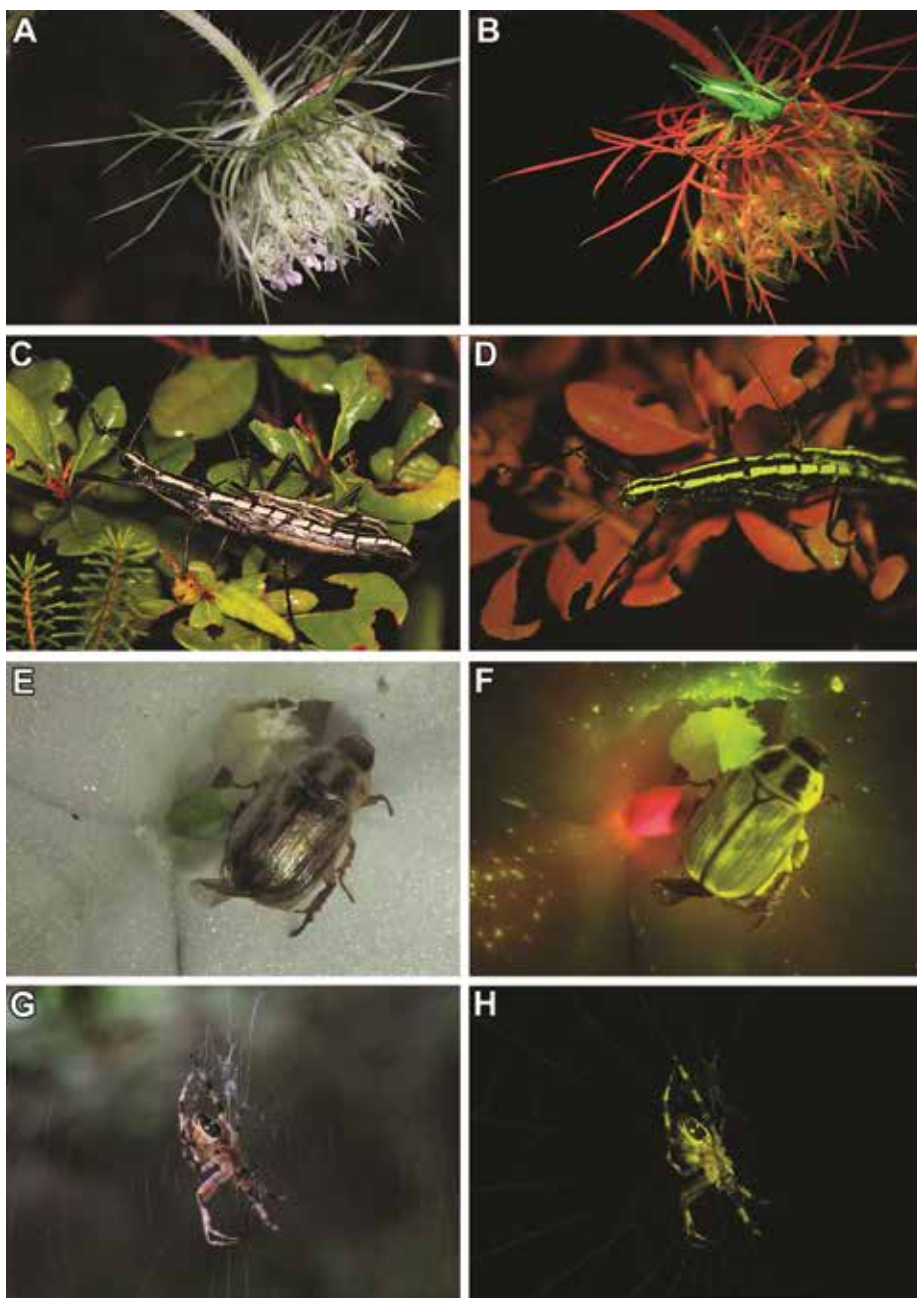


Fig. 4. Field images demonstrating effects of white light (left column) and blue-light fluorescence (right column): A and B) lesser meadow katydid, *Conocephalus* sp. (Orthoptera: Tettigoniidae); C and D) southern two-striped walkingstick, *Anisomorpha buprestoides* (Stoll) (Phasmatodea: Pseudophasmatidae); E and F) Asiatic beetle, *Exomala orientalis* (Waterhouse). (Coleoptera: Scarabaeidae); G and H) European garden spider, *Araneus diadematus* Clerck (Araneae: Araneidae). (All photos by CM except C by Rien Dekeyser)

and Strepsiptera, which did not show even a shift in eye coloration. In the cases of Embioptera and Strepsiptera, the most likely cause for this is because their bodies are entirely dark.

As an aside, we should note that none of the encountered preservation methods

(pinned/pointed, ethanol, and slide-mounted) or containing media (such as glass on drawers, vials, and coverslips) appeared to limit or alter fluorescence effects in visually detectable ways. In fact, for three orders (Diplura, Protura, and Zoraptera) that were all slide-mounted,

fluorescence greatly enhanced our ability to see the specimens, which were originally essentially invisible. This may be due to fluorescence alone, the mounting media/chemicals, or a combination; in short, more studies are needed.

### Field Surveys Mirror Collection Insights

Our general observations of the enhancement effects produced by fluorescence on live specimens during our field surveys mirror, in most cases, what we observed for preserved specimens, with the primary difference succinctly summarized as “more vivid.” This is particularly the case when hexapods are found on plants with an abundance of chlorophyll (such as large, fresh leaves), because the plants will fluoresce a brilliant red color, with greater contrast seemingly correlated with greater amounts of chlorophyll. This effect makes hexapods that fluoresce greenish-yellow (especially polyneopterans) stand out like beacons (e.g., Fig. 3A, B and Fig. 4A, B). Many specimens, however, were only slightly enhanced (e.g., Fig. 3E, F; Fig. 5A, B; and the beetle in Fig. 5G, H) or not at all (Fig. 5C–F).

In the field, proper contrast is often the key to spotting hexapods (and their relatives) more easily and rapidly, which is why a forest trail with mixed plant types on both sides was chosen for the field experiment (Fig. 1C). In fact, the lush rainforests in Costa Rica were one of the best areas in which we tested fluorescence, and many hexapods were located, especially cryptic katydids (Orthoptera: Tettigoniidae; e.g., Fig. 4A, B). This is in stark contrast to drier regions with more sparse vegetation, such as our sites in Texas, Arizona, Mozambique, and Ethiopia. Plants lacking high levels of chlorophyll (such as many grasses, dying/dead plants, and cacti) effectively look the same under both fluorescence and white light, meaning any hexapods (and their kin) found on these sorts of plants may only appear to be weakly fluorescing. In fact, if we re-did the collection survey, we would include a large fresh leaf for the sake of increasing the contrast level of relative fluorescence effects (as in Fig. 5G, H). Although we believe that our findings (Table 2) are sound, the inclusion of a known contrast comparison may have revealed further minor fluorescence effects and enhanced existing observations.

In terms of contrast enhancement, one exception we encountered was scorpions



**Table 2.** Blue-light fluorescence effects demonstrated by all known orders of Hexapoda observed during numerous collection and field investigations. Observations are organized by taxonomic order primarily according to the general top-to-bottom structure of the Hexapoda phylogenetic tree of Gullan and Cranston (2014). Enhancement effect is typically a greenish-yellow coloration, unless otherwise indicated, and listed in order of most common effect, which is directly correlated with the effect occurrence. Eye base-color is typically black, but also often reddish in some polyneopterans. Orders that lacked an enhancement effect entirely are identified in bold, and \* indicates a special note. A more detailed version of this table that includes families can be found in Supplemental Information, Table S1 (available at <https://doi.org/10.1093/ae/tmaa005>).

| Order                 | Curation Method       | Effect Occurrence | General Enhancement Effect(s)   |
|-----------------------|-----------------------|-------------------|---|
| Collembola            | slide-mounted         | common            | enhances faintly to obviously   |
| Protura               | slide-mounted         | common            | enhances faintly; essentially invisible originally  |
| Diplura               | slide-mounted         | common            | essentially invisible originally and then greatly enhanced  |
| Archaeognatha         | ethanol               | common            | enhances obviously  |
| Zygentoma             | pinned/ethanol        | common            | enhances obviously  |
| Ephemeroptera         | ethanol               | common            | enhances lighter coloration: lighter = brighter   |
| Odonata               | pinned                | common            | eyes appear whitish; enhances obviously overall, but some only faintly  |
| Plecoptera            | pinned/ethanol        | rare              | faintly enhances lighter coloration; eyes appear whitish, but very faintly  |
| Dermoptera            | pinned                | occasional        | enhances lighter coloration and patterns, particularly tarsi and some antennal segments in some cases: lighter = brighter   |
| Zoraptera             | slide-mounted         | common            | enhances greatly; essentially invisible originally  |
| Orthoptera            | pinned                | none to common    | eyes often appear whitish; enhances at least some anatomy obviously in most (lighter=brighter), although darker exoskeletons are generally fainter or not enhanced at all |
| <b>Embioptera</b>     | <b>ethanol</b>        | <b>none</b>       | <b>none; *all surveyed representatives with dark exoskeleton</b>  |
| Phasmatodea           | pinned                | common            | enhances obviously; lighter = brighter  |
| Grylloblattodea       | ethanol               | common            | enhances, but relatively faintly; *only had access to two specimens of a single species (rare in collections)   |
| Mantophasmatodea      | pinned                | unknown           | enhances faintly; *only had access to a single specimen (rare in collections)   |
| Mantodea              | pinned                | common            | enhances lighter coloration; effect on eyes (sometimes appearing whitish) similar to body   |
| Blattodea             | pinned/ethanol        | none to common    | often enhances lighter coloration, often tegmina in general, but sometimes only or not at all   |
| Psocodea              | slide-mounted         | common            | enhances faintly, with lighter specimens the faintest   |
| Thysanoptera          | pinned/ethanol        | none/common       | enhances faintly or not at all  |
| Hemiptera             | pinned                | rare/common       | eyes sometimes appear whitish; enhances lighter coloration and patterns in some cases   |
| <b>Rhaphidioptera</b> | <b>pinned</b>         | <b>none</b>       | <b>none</b>   |
| Megaloptera           | pinned                | none/common       | eyes sometimes appear whitish; occasionally faintly enhances lighter patterns, especially on thorax and wings, or not at all  |
| Neuroptera            | pinned/ethanol        | none/common       | eyes appear whitish; enhances lighter coloration, but sometimes only faintly or not at all  |
| Coleoptera            | pinned                | rare to common    | eyes sometimes appear whitish; enhances lighter coloration and patterns; metallic coloration can look black or brighter   |
| <b>Strepsiptera</b>   | <b>pinned/ethanol</b> | <b>none</b>       | <b>none; *all surveyed representatives with dark exoskeleton</b>  |
| Diptera               | pinned                | none to common    | eyes sometimes appear whitish; occasionally enhances lighter coloration and patterns or not at all  |
| Mecoptera             | pinned                | none/occasional   | enhances faintly or not at all  |
| Siphonaptera          | slide-mounted         | occasional        | faintly enhances lighter coloration, mostly abdomens  |
| Trichoptera           | pinned/ethanol        | common            | eyes appear whitish, but can be difficult to see due to relatively small size   |
| Lepidoptera           | pinned                | common            | eyes appear whitish; often enhances lighter coloration and patterns, plus veins sometimes   |
| Hymenoptera           | pinned                | rare to common    | eyes sometimes appear whitish; occasionally enhances lighter coloration and patterns or not at all  |



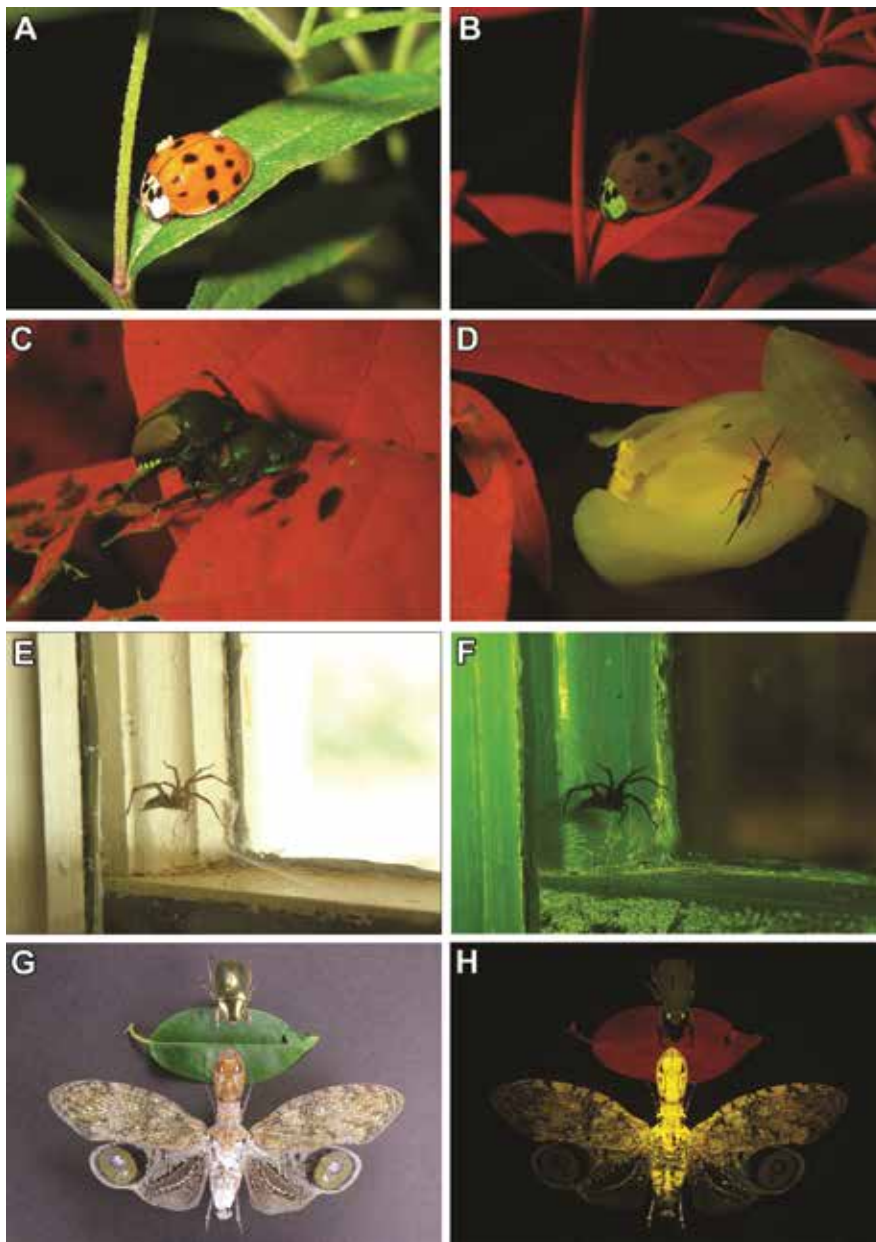


Fig. 5. Field and preserved specimen images demonstrating effects of white light (WL) and blue light (BL). A (WL) and B (BL): Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae); C (BL): Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae); D (BL): Braconidae (Hymenoptera); E (WL) and F (BL): unidentified arachnid; G (WL) and H (BL): preserved specimens with leaf for contrast of *Chrysina aurigans* (Rothchild & Jordan) (Coleoptera: Scarabaeidae) (top) and peanut bug, *Fulgora latermaria* (Linnaeus) (bottom) (Hemiptera: Fulgoridae). (Photos by CM)

(Arachnida: Scorpiones) in Arizona, which fluoresced brilliantly no matter their background, as might be expected given their fluorescing properties, unique among Arthropoda (Stahnke 1972; Gaffin et al. 2012). Other arachnids we encountered demonstrated mixed effects, ranging from weak effects (e.g., Fig. 4G, H) to no

effect (e.g., Fig. 5E, F), and more investigations are needed to begin summarizing them. The other exception was specimens of a sand-treader cricket (Orthoptera: Rhaphidophoridae: *Ammobaenetes* sp.) observed on the white sand dunes of Monahans Sandhills State Park, Texas. Most interestingly, the sand-treader

crickets were essentially camouflaged on the sand of the dunes, but fluorescence highlighted them well, presumably due to their overall light body coloration.

### Comparisons of Blue-Light Fluorescence to UV Fluorescence

Although the differences were not explicitly tested in this study, we undertook some cursory fluorescence effect comparisons between blue light and the more well-known UV light in the form of a black light (both a weaker headlamp version and a more powerful type that required the use of glasses to protect eyesight) with various adult grasshoppers in both field and lab settings. In a nutshell, the fluorescence effects of blue light on the grasshoppers far outshone those of the UV lights, but the latter did demonstrate some comparatively weak effects. Further studies are suggested, but we think that blue light will demonstrate greater effects in most comparisons. We base this hypothesis largely on our current experiences with blue light, our many years of using black lights for locating scorpions in the wild (to most observers, scorpions appear brighter under blue light), and the caterpillar images found in Moskowitz (2017), which appear to be much weaker compared to the fluorescence effects under blue light that we have observed in caterpillars (as in Figs. 3A–F), although the same species were not examined.

### Ruminations and Reflections

As discovered through our extensive testing of fluorescence via the blue-light unit and based on feedback from participants in the experiment, the blue-light unit can take some visual adjustment to use, primarily because even when set on its highest/widest setting, the beam can be relatively narrow compared to a more traditional flashlight or headlamp. The fluorescence effects can also be a bit disorienting as your brain adjusts to its new way of seeing, compounded by the use of the barrier filter glasses. This is especially true when examining inanimate objects (particularly indoors), many of which fluoresce in unique ways: water sometimes appears to be white, and the ability to reveal poor paint jobs (especially in hotel rooms!) is seemingly magical. We think it would be beneficial to attach the blue-light unit to a helmet or incorporate the technology into a more traditional headlamp model. Overall, though, many participants

in the experiment stated that they were impressed with the blue-light unit's abilities and were excited to try fluorescence with other taxa beyond grasshoppers.

Based on the results of our field experiment, combined with our extensive collection and field surveys, we are comfortable extending the concept of increased ease and speed of collecting using blue-light fluorescence to many other untested hexapod taxa. This should especially be the case for the overwhelming majority of other polyneopterans, because fluorescence produced positive fluorescence enhancement effects in some way for most of the specimens we examined. cursory testing showed that various eggs and larvae, especially white ones, fluoresce well, suggesting valuable applications in detecting insect pests in field crops and home gardens.

As a final note of potential interest, we discovered that green net bags (specifically, those from BioQuip Products, Inc.) fluoresced red using the blue-light unit, making it very easy to find many hexapods caught in the net without having to switch to a white light. We have little doubt that many more unique uses will be found for blue-light fluorescence by entomologists (e.g., examining immature hexapods, because most are relatively soft-bodied and typically lighter in color) and we strongly encourage everyone to try it themselves. The results for your favorite group may surprise and excite you!

### Acknowledgments

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## WE HAVE LITTLE DOUBT THAT MANY MORE UNIQUE USES WILL BE FOUND FOR BLUE-LIGHT FLUORESCENCE BY ENTOMOLOGISTS.

12 experiment participants: Charluz M. Arocho Rosario, Jeffrey Barbosa, Elaine Chu, Anthony Cormier, Andrew J. Graf, Drew Hopkins, Emily F. Knight, Tiffany Le-Ngoc, Richelle Marquess, Brian Rich, Tammy L. Starr, and Bri Trejo. This work was partly supported by the U.S. Department of Agriculture (Hatch Grant TEX0-1-6584 to HS).

### Conflict of Interest

CM is the founder and President of NIGHTSEA, from which all the lights and barrier filter glasses used in this study were purchased, but only through the independent initiative of DAW. DAW also invited CM to be an author, given his unique expertise on fluorescence. CM's specific contributions to this manuscript were writing descriptions of fluorescence and its applications, editing the overview of fluorescence equipment, generating ideas for future research, and taking almost all the photographs in Figs. 3–5. CM did not participate in the design, execution, or analysis of the field or collection studies.

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**Derek A. Woller**, Chandler, AZ 85249; **Bert Foquet**, Song Laboratory of Insect Systematics and Evolution (Department of Entomology, Texas A&M University, Minnie Belle Heep Center, Room 412, Campus MS 2475, College Station, TX 77843-2475); **Shelby Kerrin Kilpatrick**, Department of Entomology, Pennsylvania State University, 501 ASI Building, University Park, PA16802; **Ryan Selking**, Pesticide Education Program, Pennsylvania State University, 220 Special Services Building, University Park, PA, 16802; **Charles Mazel**, NIGHTSEA, 235 Bedford St., Lexington, MA 02420; and **Hojun Song**, Song Laboratory of Insect Systematics and Evolution.

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