Pangaeus bilieatus (Hemiptera: Cydnidae) Behavioral Response to Light-Based Stimuli in the Laboratory[1]

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Abstract Peanut burrower bug, *Pangaeus bilineatus* (Say) (Hemiptera: Cydnidae), is a subterranean hemipteran pest of peanut, *Arachis hypogaea* L., in the southeastern United States. Light traps and pitfall traps are the only tools currently available for monitoring the insect's populations in the field. Optimizing light traps by using the most attractive light source for the pest while minimizing nontarget capture would enhance trap efficacy and could provide a more useful tool for integrated pest management. We evaluated the responses of male and female adult *P. bilineatus* to various wavelengths of light in a laboratory-based, two-choice bioassay. White compact fluorescent (CFL) bulbs (warm, bright, and white) attracted significantly more males and females than black CFL and black light-emitting diode bulbs. White CFL wavelengths attracted significantly more male and female adults in the laboratory and should be candidate bulbs to enhance *P. bilineatus* light trap efficacy in the field.

Key Words peanut burrower bug, light trap, wavelength, behavior, integrated pest management

Peanut burrower bug, *Pangaeus bilineatus* (Say) (Hemiptera: Cydnidae), is a hemipteran that is native to the United States: it spends much of its life in the soil and is an economically important pest of peanut, *Arachis hypogaea* L., in the southeastern United States (Aigner et al. 2021). It feeds directly on peanut seeds with piercing-sucking mouthparts, causing substantial reduction in quality and value (Aigner et al. 2021, Chapin and Thomas 2003, Chapin et al. 2006, Mbata et al. 2013). Its range in the United States covers most southern and eastern states (Aigner et al. 2021, Froeschner 1960). According to the USDA National Agriculture Statistics Services, Georgia produces approximately 50% of all peanuts grown in the United States (Aigner et al. 2021). Since 2010, the pest has been an annual economic concern for peanut farmers in Georgia, Alabama, Florida, and South Carolina, and given its cryptic nature and a lack of available management tools, it presents numerous management challenges. The cancellation of all food tolerances for chlorpyrifos in February 2022 (Hites 2021)—the only insecticide registered for use in peanut with proven efficacy against *P. bilineatus*—exacerbates these challenges and emphasizes the need for new and improved integrated pest management (IPM) tactics and strategies.

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Effective pest monitoring is the foundation of IPM because it provides information about the presence and abundance of pest populations that is required for accurate and timely management decisions. Light traps are relatively simple monitoring tools that are often used for studies on population dynamics of insect species that exhibit positive phototaxis, and light traps have been used to monitor the distribution and seasonal abundance of *P. bilineatus* in Texas and Georgia (Aigner et al. 2021, Highland and Lummus 1986). Although the efficacy of light traps for collecting *P. bilineatus* was first shown by Highland and Lummus (1986), there were no subsequent efforts to assess the insect's response to different wavelengths of light or to develop a more efficient light trap. Different insect orders are known to vary in their response to different wavelengths of light (Van Grunsven et al. 2014, Park and Lee 2017). Identifying a light source that is optimally attractive to the target insect while reducing unwanted bycatch would improve the quality of research data while lowering the costs associated with sorting large volumes of mixed species insect samples.

Given its cryptic, subterranean life history, directly assessing *P. bilineatus* populations in the field is difficult and light traps could be a more efficient and cost-effective monitoring tool for IPM decision-making than direct scouting. Currently, a lack of knowledge regarding economic injury levels and absence of thresholds for *P. bilineatus* in peanut, combined with only limited understanding of how trap capture relates to absolute pest abundance, reduces the utility of light traps for pest management decision-making in commercial production systems (Aigner et al. 2021). Nevertheless, light traps have played an important role in IPM programs for many insect pests (Kim et al. 2019) and traps with improved energy efficiency and catch efficiency could play an important role in future IPM for *P. bilineatus*.

The availability of low-cost, high-efficiency compact fluorescent (CFL) bulbs, and more recently light-emitting diodes (LEDs), has led to their use in newer light trap designs (Cohnstaedt et al. 2008). Despite potential benefits of LED technology, some studies have shown that LEDs are not as attractive to insects as incandescent or fluorescent light sources (Justice and Justice 2016, Kim et al. 2019). Herein, bioassays were conducted to assess the phototactic response of male and female adult *P. bilineatus* to various wavelengths of light emitted from CFL and LED bulbs in the laboratory and identify candidate light sources for future testing in the field.

Materials and Methods

Insect rearing. The experiment was conducted using insects from a laboratory colony of *P. bilineatus* maintained at the University of Georgia (UGA) in Tifton. The colony was established with nymphs and adults collected in November 2016 from a field in Terrell County, GA (31.7929°N, 84.3241°W). Adults were also collected from Emanuel Co., GA (32.60534°N, 82.26979°W) and added to the colony in December 2018. Both collection sites had been planted to peanut the summer before being made.

Plastic food storage containers (27.5 × 25.5 × 19.5 cm; FG631200CLR, Rubbermaid Commercial Products Inc., Winchester, VA) were filled with approximately 10 L of sandy loam soil collected at the UGA Lang-Rigdon Farm (31.511239°N, 83.549084°W) in Tifton. Containers were covered with a plastic lid. A single hole (16 × 8.5 cm) was cut into the center of each lid to provide ventilation, and cloth mesh screen (1.5 mm) was hot glued over the hole. Peanut burrower bugs in the laboratory colony were fed raw, untreated peanut seed (3–7% moisture content) and soil in rearing containers was wetted with approximately 100 ml of filtered tap water (model 56151-03, 3M Purification Inc., Meriden, CT) twice per week (every fourth day, or as needed). Soil moisture was maintained at 10–15% volumetric water content as averaged over four measurements per container with a FieldScout TDR 300 soil moisture probe (12.2-cm probe length; model 6430FS; Spectrum Technologies, Inc., Aurora, IL). Roughly 1 peanut seed/6.5 cm^2 was placed on the soil surface in each container. Containers were kept in an insect rearing room maintained at 28 $\pm$ 1.1°C and 55 $\pm$ 10% relative humidity (RH) under a photoperiod of 14:10 (L:D) h. WatchDog data loggers (1000/2000 Series, Spectrum Technologies, Inc., Aurora, IL) were used to monitor temperature and RH in the rearing room. Populations were allowed to grow in each container for approximately 1.5 mo before using adult peanut burrower bugs for bioassays. This ensured enough time for a new generation to appear so that healthy and relatively young adults were used for bioassays.

Three days before beginning bioassays, three rearing containers were moved into each of two controlled environment chambers (model I36LLVLC8, Percival Scientific Inc., Perry, IA). Adult *P. bilineatus* were collected most frequently in light traps in the field within 3 h of sunset (Aigner et al. 2021); therefore, the day (33 $\pm$ 1°C) and night (22 $\pm$ 1°C) temperatures and light setting (14:10 [L:D]) of the two environment chambers were adjusted and staggered such that the dark cycle began at either 1100 or 1700 h. Experiments conducted in the morning were conducted using peanut burrower bugs from the chamber with the dark cycle set to begin at 1100 h, and experiments conducted in the afternoon were conducted using peanut burrower bugs from the chamber with the dark cycle set to begin at 1700 h. In each case, 10 peanut burrower bugs were collected from containers in the environment chamber by using soft forceps; they were placed together in a Petri dish and allowed to rest for 10 min in a dark room before initiation of each replication of the experiment.

Light sources. The relative spectral irradiance by wavelength of each light source was measured using a S2000 miniature fiber optic spectrometer and Ocean Optics SpectraSuite software (Ocean Optics, Inc., Dunedin, FL). Each measurement was taken from a single scan over an integration time of 0.1 s. The relative spectral irradiances by wavelength of each light source are shown in Fig. 1. Light sources used for the experiments included a black CFL (05645, Sunlite Mfg., Brooklyn NY), black LED (GVLA19BK, Wal Mart, Inc., Bentonville, AR), warm white CFL (EL/mdTQ 23W T2 5k, Signify NA Corp., Somerset, NJ), bright white CFL (EL/mdTQ 23W T2 2.7k, Signify NA Corp.), cool white CFL (EL/mdTQ 23W T2 4.1k, Signify NA Corp.), and an adjustable wavelength LED bulb (ASIN: B07DLSNNDS, Lumiman, Zhongshan, China). Specifications for each light bulb or bulb setting (in the case of the adjustable LED) are displayed in Table 1. The relative spectral irradiance and specifications of fluorescent circline light tubes (PN: GEL33774, General Electric Co., Cleveland, OH) previously used in the field are displayed in Table 1 and Fig. 1, respectively.

Apparatus. A light-based Y-tube apparatus (Fig. 2) was constructed from black foam core board (5-mm thickness; model PAC5554, Pacon Mfg., Leland, NC), clear polycarbonate tubing (19-mm inner diameter; 22-mm outer diameter; BULK-PT-PC-19, W.W. Grainger, Inc., Macon, GA), and polyvinyl chloride pipe

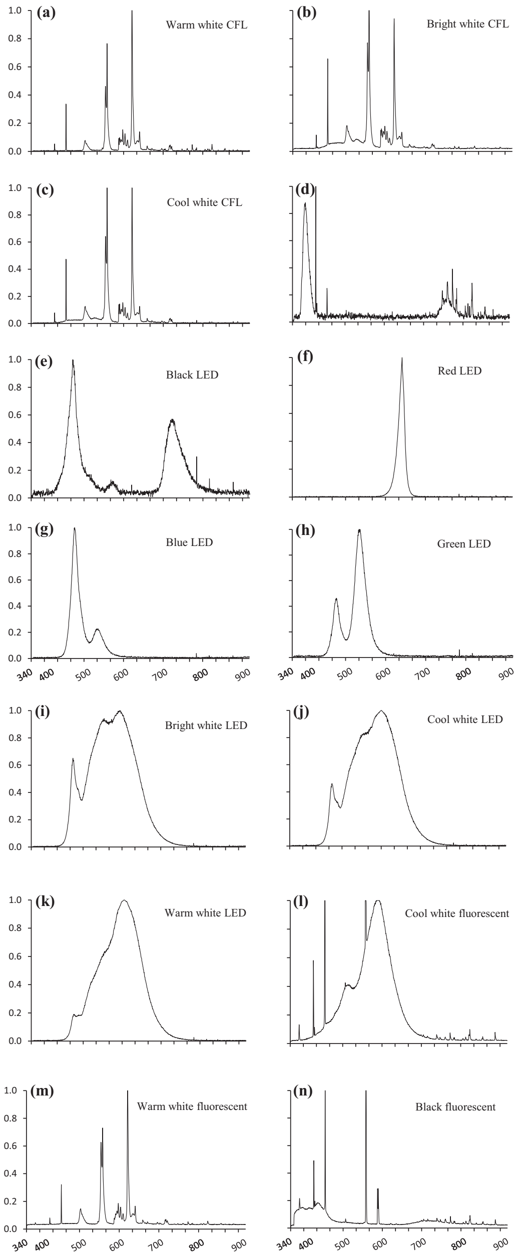

Fig. 1. Relative spectral irradiance (*y*; arbitrary units) by wavelength (*x*; nanometers) of light treatments used in *Pangaeus bilineatus* wavelength response bioassays. Treatments include (a) warm white compact fluorescent (CFL), (b) bright white CFL, (c) cool white CFL, (d) black CFL, (e) black light-emitting diode (LED), (f) red LED, (g) blue LED, (h) green LED,

Table 1. Specifications of light bulbs used in *Pangaeus bilineatus* wavelength response bioassays.

	Bulb Type	Wattage	Lumens	K (color temperature)
a	Warm white CFL	23	1600	2700
b	Bright white CFL	23	1600	5000
c	Cool white CFL	23	1600	4100
d	Black CFL	20	15	2700
e	Black LED	7.5	800	2700
f	Red LED	7.5	800	5000
g	Blue LED	7.5	800	5000
h	Green LED	7.5	800	5000
i	Bright white LED	7.5	800	5000
j	Cool white LED	7.5	800	4100
k	Warm white LED	7.5	800	2700
l	Cool white fluorescent	22	1100	4100
m	Warm white fluorescent	22	1400	3000
n	Black circline fluorescent	22	NA	NA

CFL = compact fluorescent; LED = light-emitting diode; NA = not available.

fittings and was adapted from the design described by Leskey et al. (2015). A fixture was constructed to allow light from two bulbs to be simultaneously, yet independently, directed through two gooseneck fiber optic cable attachments into one or the other side of the light-based Y-tube (Fig. 3).

Experimental design. All experiments were conducted in the dark in a 3.7-m^2 windowless room, with a temperature of 23.8 ± 1°C and a RH of 37.8 ± 4.3%, at the UGA Peanut Entomology Laboratory in Tifton.

Each treatment replicate of the two-choice bioassay paired one of the experimental bulbs with either a black CFL or black LED light bulb. For each replicate, 10 peanut burrower bugs were placed in the release chamber of the Y-tube and given 30 min to make a choice. A 1-cm-diameter hole at the lower surface of each polycarbonate tube located 14 cm from the release chamber allowed peanut burrower bugs that had made a choice to fall from the tube into a collection chamber. After 30 min, the Y-tube cover was removed, the number of peanut burrower bugs in the collection chambers

←

(i) bright white LED, (j) cool white LED, and (k) warm white LED. Relative spectral irradiance of circline fluorescent light tubes currently used in the field include (l) cool white fluorescent, (m) warm white fluorescent, and (n) black fluorescent.

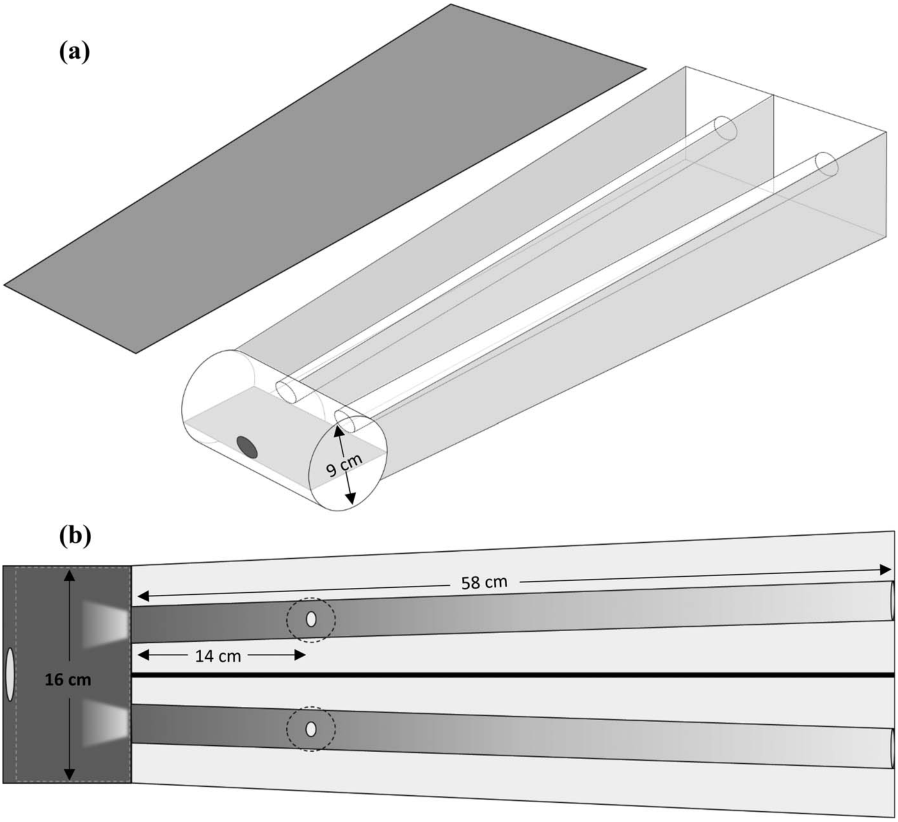

Fig. 2. Illustrations (not to scale) of test chamber used for *Pangaeus bilineatus* wavelength response bioassays: (a) oblique view of test chamber with lid and (b) overhead view of test chamber. Holes were drilled into the bottom of each treatment tube (14 cm from stage area) to trap peanut burrower bugs after they made a choice.

was recorded and then the bugs were discarded. The relative location of the two bulbs was reversed after each replicate to control for potential directional bias. Some individuals did not make a choice after 30 min, and each treatment was replicated until at least 30 peanut burrower bugs made a choice.

Statistical analysis. Data were analyzed in R 4.0 (R Core Team 2020). A χ^2 goodness-of-fit test and tests of independence were used to identify treatment differences of count proportions within sex and between sex, respectively. All analyses were conducted with a significance level of 0.05.

Results and Discussion

Significantly more males chose bright white LED light when tested against black CFL light (χ^2 [1, $n = 37$] $= 4.57$, $P = 0.0326$), and males chose black CFL light

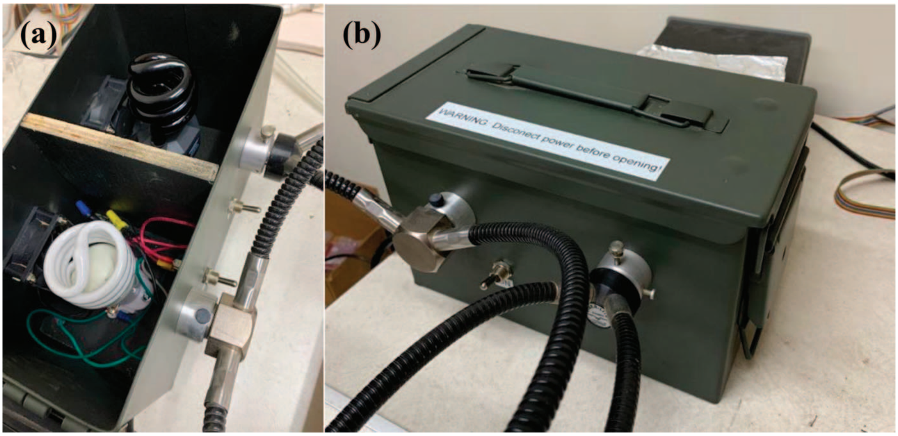

Fig. 3. Interior (a) and exterior (b) of dual light fixture used for *Pangaeus bilineatus* wavelength response bioassays.

significantly more than green LED light (χ^2 [1, n = 33] = 6.82, P = 0.0090; Table 2). In assays that included the black LED bulb, significantly more males chose warm white CFL (χ^2 [1, n = 50] = 6.48, P = 0.0109), cool white CFL (χ^2 [1, n = 32] = 4.50, P = 0.0339), and red LED light (χ^2 [1, n = 30] = 4.80, P = 0.0285; Table 2). No other statistically significant differences in male response were observed.

In assays comparing black CFL light with cool white CFL light, significantly more females chose cool white CFL light (χ^2 [1, n = 31] = 3.90, P = 0.0482; Table 2). In assays that included black LED light, significantly more females chose warm white CFL (χ^2 [1, n = 35) = 4.83, P = 0.0280) and cool white LED (χ^2 [1, n = 34] = 7.53, P = 0.0061; Table 2) lights. No other statistically significant differences in female response were observed.

Significant differences in phototaxis by sex were observed for comparisons of black LED light against cool white (χ^2 [1, n = 73] = 5.50, P = 0.0191; Table 3) and warm white LED (χ^2 [1, n = 68] = 4.60, P = 0.0320; Table 3) lights. Males were more likely to choose black LED light, whereas females were more likely to choose warm white and cool white LED lights (Fig. 4; Table 3). The reason for these disparities is unknown, but reports of sex-based differences in phototaxis within an insect species are rare. Nevertheless, sexual dimorphism of insect eye structure with receptors of different spectral sensitivity is not uncommon (Briscoe and Chittka 2001).

Previous studies reported capture of *P. bilineatus* adults in light traps equipped with fluorescent bulbs producing white and black light (Abney and Aigner 2018, Highland and Lummus 1986), and black lights have been the standard of many insect trap designs for decades (Harding et al. 1966). Conventional fluorescent black light bulbs used in traps for research studies on *P. bilineatus* in Georgia resulted in collections with high biomass of nontarget species (B.L.A., pers. obs.). This nontarget bycatch decreases overall efficiency and increases the cost of research by increasing processing time. Concerns have also been raised about the indiscriminate attraction of insects to light sources in the environment and the potential negative ecological effects that this might have (Nabli et al. 1999). A

Table 2. Results of χ^2 goodness-of-fit tests to identify significant differences of treatment decisions within sex of *Pangaeus bilineatus* for each treatment combination tested.

Bulb Type		Male				Female			
		χ^2	*P*	*n*	df	χ^2	*P*	*n*	df
Black CFL	WW CFL	0.50	0.4795	32	1	0.03	0.8618	33	1
	CW CFL	1.48	0.2230	33	1	3.90	**0.0482***	31	1
	BW CFL	0.53	0.4652	30	1	1.20	0.2733	30	1
	BW LED	4.57	**0.0326**	37	1	0.00	1.00	30	1
	WW LED	2.13	0.1441	30	1	0.81	0.3692	31	1
	CW LED	1.20	0.2733	30	1	0.50	0.4795	32	1
	Blue LED	3.67	0.0555	33	1	1.20	0.2733	30	1
	Green LED	6.82	**0.0090**	33	1	0.03	0.8618	33	1
	Red LED	2.61	0.1060	31	1	0.53	0.4652	30	1
Black LED	Black CFL	0.13	0.7150	30	1	0.29	0.5900	31	1
	WW CFL	6.48	**0.0109**	50	1	4.83	**0.0280**	35	1
	CW CFL	4.50	**0.0339**	32	1	3.00	0.0833	27	1
	BW CFL	1.68	0.1944	38	1	1.88	0.1701	34	1
	BW LED	3.67	0.0555	33	1	0.29	0.5900	31	1
	WW LED	3.79	0.0516	38	1	2.13	0.1441	30	1
	CW LED	0.64	0.4200	39	1	7.53	**0.0061**	34	1
	Blue LED	3.46	0.0623	35	1	0.13	0.7150	30	1
	Green LED	0.12	0.7316	34	1	0.13	0.7237	32	1
	Red LED	4.80	**0.0285**	30	1	0.00	1.00	34	1
	Black LED	0.00	1.00	30	1	0.00	1.00	22	1

CFL = compact fluorescent; WW = warm white; CW = cool white; BW = bright white; LED = light-emitting diode.

* Bold values indicate significant *P* value ($<$0.05).

significant reduction in the abundance and diversity of nontarget insects collected in light traps was observed in Georgia when conventional white fluorescent light bulbs were used in place of conventional black fluorescent light bulbs in *P. bilineatus* population dynamics research (B.L.A., pers. obs.). Although *P. bilineatus* was collected in traps with either bulb, the relative phototactic response of the target insect to the two light sources is unknown.

The increased energy efficiency of CFL and LED bulbs compared with that of incandescent and conventional fluorescent bulbs could significantly decrease energy costs,

Table 3. Results of χ^2 tests of independence to identify significant differences of treatment decisions between sex of *Pangaeus bilineatus* for each treatment combination tested.

		Male versus Female			
Bulb Type		χ^2	*P*	*n*	df
Black CFL	WW CFL	0.0180	0.8934	65	1
	CW CFL	0.1115	0.7384	64	1
	BW CFL	0.00	1.00	60	1
	BW LED	1.4577	0.2273	67	1
	WW LED	2.0068	0.1566	61	1
	CW LED	0.0017	0.9669	62	1
	Blue LED	0.0823	0.7742	63	1
	Green LED	3.1096	0.0778	66	1
	Red LED	0.1334	0.7149	61	1
Black LED	Black CFL	0.00	1.00	61	1
	WW CFL	0.00	1.00	85	1
	CW CFL	3.18e−31	1.00	59	1
	BW CFL	0.00	1.00	72	1
	BW LED	2.1936	0.1386	64	1
	WW LED	4.5982	**0.0320***	68	1
	CW LED	5.4951	**0.0191**	73	1
	Blue LED	1.6765	0.1954	65	1
	Green LED	0.0607	0.8055	66	1
	Red LED	1.8788	0.1705	65	1
	Black LED	0.00	1.00	52	1

CFL = compact fluorescent; WW = warm white; CW = cool white; BW = bright white; LED = light-emitting diode.
* Bold values indicate significant *P* value ($<$0.05).

extend run times, and facilitate the use of light traps at remote locations. Nevertheless, this will only be possible if the more efficient bulbs are adequately attractive to the target pest. The results presented herein show that *P. bilineatus* exhibits positive phototaxis to ultraviolet (UV) and white light from CFL and LED sources. Overall, there were only minor differences in the attractiveness of paired light sources, but white light from LEDs or CFLs was generally more attractive than the UV light sources. This suggests that white lights could be expected to collect similar (or greater) numbers of *P. bilineatus* in the field while potentially reducing nontarget captures compared with UV light. Field studies should be conducted to validate the findings reported herein.

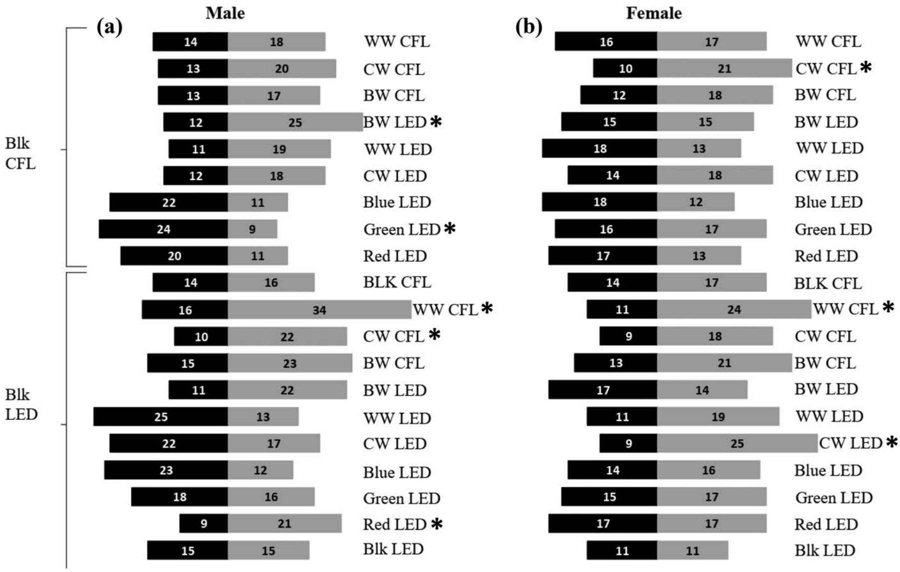

Fig. 4. Counts of *Pangaeus bilineatus* male (a) and female (b) responses to wavelength choice bioassays. All treatments were tested against either black compact fluorescent (CFL) or black light-emitting diode (LED) light bulbs. An asterisk (*) denotes significant differences in χ^2 results.

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