Light Trap Capture of Live Elenchus koebelei (Strepsiptera: Elenchidae)

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Light trap capture of live *Elenchus koebelei* (Strepsiptera: Elenchidae)

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Abstract

Strepsiptera are a small order of obligately endoparasitic insects. Adult females are neotenic and never leave their host, instead bearing motile young that seek out their own insect hosts to infect. Males eclose without killing their hosts. In their 4-h adult lifespan, they fly off to search for mating opportunities, assisted by unconventional eyes with few, but large, ommatidia. Such distinctive features make Strepsiptera interesting in their own right, but also offer an opportunity to better understand evolutionary innovation. Unfortunately, Strepsiptera also are minute, reclusive, and difficult to obtain, severely reducing the study thereof, especially species not infecting solitary bees or social wasps. Here we describe methods for the successful capture of a strepsipteran species. We placed an ultraviolet light trap among *Spartina alterniflora* Loisel (Poaceae) shoots to attract adult male *Elenchus koebelei* Pierce (Strepsiptera: Elenchidae) in salt marshes in the southeastern United States. In 72 d of sampling, 488 adult males were captured between 30 min before and 15 min after sunrise. None arrived more than 63 min before or 36 min after sunrise. The majority of *E. koebelei* were caught at wind speeds ranging from 0 to 10 km/h; however, a light breeze of about 1.5 km/h appears to be preferred. The highest daily catches occurred when the temperature was between 23 and 26 °C. No Strepsiptera were caught at temperatures below 17 °C. With 521 adult male *E. koebelei* caught in a single light trap, our results show this little-known parasite may be reliably obtained, enhancing opportunities for further study.

Key Words: temperature; time; wind speed; *Spartina alterniflora*; twisted-wing parasite; crepuscular

Resumen

Strepsiptera es una pequeña orden de insectos endoparasitarios obligados. Las hembras adultas son neoténicas y nunca abandonan a su hospedero, sino que tienen jóvenes móviles que buscan a sus propios insectos hospederos para infectar. Los machos eclosionan sin matar a su hospedero. En su vida adulta de 4 horas, vuelan para buscar oportunidades de apareamiento, asistidos por ojos no convencionales con pocos, pero grandes, ommatidios. Tales características distintas hacen que Strepsiptera sean de interés por ellos mismos, pero también ofrecen una oportunidad para comprender mejor la innovación evolutiva. Desafortunadamente, los estrepsípteros también son pequeños, solitarios y difíciles de obtener, lo que reduce drásticamente el estudio de los mismos, especialmente las especies que no infectan a las abejas solitarias o las avispas sociales. Aquí describimos métodos para la captura exitosa de una especie de Strepsiptera. Colocamos una trampa de luz ultravioleta entre los brotes de *Spartina alterniflora* Loisel (Cyperales: Poaceae) para atraer al macho adulto de *Elenchus koebelei* Pierce (Strepsiptera: Elenchidae) en las marismas del sureste de los Estados Unidos. En 72 días de muestreo, 488 machos adultos fueron capturados entre 30 minutos antes y 15 minutos después del amanecer. Ninguno llegó más de 63 minutos antes o 36 minutos después del amanecer. La mayoría de los *E. koebelei* fueron atrapados a velocidades de viento que van de 0 a 10 km /h; sin embargo, parece preferible una ligera brisa de aproximadamente 1.5 km/h. Las capturas diarias más altas ocurrieron cuando la temperatura estaba entre 23 y 26°C. No se capturaron los estrepsípteros a temperaturas inferiores a 17°C. Con 521 macho adultos de *E. koebelei* capturados en una sola trampa de luz, nuestros resultados muestran que este parásito poco conocido puede obtenerse de manera confiable, mejorando las oportunidades para un estudio posterior.

Palabras Clave: temperatura; hora; velocidad del viento; *Spartina alterniflora*; parásito de alas retorcidas; crepuscular

The order Strepsiptera consists entirely of obligate parasites of other insects [Strepsiptera: New Latin, from Greek strepsi- = "twisted" and pteron = "wing"]. The family Elenchidae parasitizes planthoppers (Homoptera: Delphacidae). Adult females have larviform bodies lacking wings, legs, and eyes, and never leave their host insect. They are neotenic, giving birth to motile male and female offspring that crawl and spring on plants and animals seeking suitable insect hosts to enter. Adult males have the eponymous "twisted" wings of this order. In search of mating opportunities, males emerge from their hosts and fly with the aid of unique eyes resembling tiny blackberries (Fig. 1). The male inseminates a portal on the cephalothorax of the female (Kathirithamby 1989), which in the case of *Xenos peckii* Kirby (Strepsiptera: Xenidae) is known to be protruded further forth from the living wasp host and inflated to increase perceptibility at times when males are apt to fly (Hrabar et al. 2014). The vast majority of knowledge about Strepsiptera pertains to diurnal species in the genus *Xenos* that infect *Polistes* (Hymenoptera: Vespidae) wasps. This paper introduces a black light technique to capture the crepuscular strepsipteran *Elenchus koebelei* Pierce (Strepsiptera: Elenchidae). Insights into *E. koebelei* natural history and physiology based on the technique also are presented.

Typically, Strepsiptera mate only once (Hughes-Schrader 1924; Kathirithamby 1989), with evidence of the male dying very soon after (Kathirithamby 1989). Many specifics of the life history of *E. koebelei* remain unknown, but based on *E. tenuicornis* Kirby (Strepsiptera: Elenchidae), it is expected in summer, when development times are shortest, that after several weeks the female gives birth to about 1,500

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Fig. 1. Above: Image of an adult male *Elenchus koebelei* standing on an anesthetic stage. Note its bifurcated antennae, black tapioca-like eyes, modified forewing that forms a haltere (only 1 of the pair is visible), silver sheen hindwings (iridescent in color images), and extensive thorax. Below: An *E. koebelei* positioned above a penny for size comparison. [When closing in on a calling female, *E. koebelei* fly upright with the abdomen tip turned under (Muir 1906).] Adjacent are 3 pictures of visibly stylopized planthoppers. Pupating male *E. koebelei* bulge from the sides of their hosts. The arrows indicate puparia.

(Hassan 1939; Kathirithamby 1989) motile first-instar larvae that crawl out of her "brood canal" to find new hosts. The brood canal is an initially sealed passage between the female parasite and her cast-off but partially encompassing exuviae, leading to the outside world. The male ruptures this in the act of mating. Eclosed male Strepsiptera live for only a few h (Hubbard 1892; Cook 2014). "Calling" adult virgin females release a mate-attracting pheromone (Cvačka et al. 2012; Tolasch et al. 2012). Insects parasitized by Strepsiptera are referred to as "stylopized."

Elenchus koebelei are minute Strepsiptera (< 1.5 mm) that infect *Prokelisia marginata* Van Duzee 1897 and *P. dolus* Wilson 1982 (Hemiptera: Delphacidae), planthoppers which in turn feed on *Spartina alterniflora* Loisel (Poaceae) in the subtropical salt marshes of southeastern United States (Khalaf 1968; Stiling et al. 1991a). In summer in northern Florida, the planthopper hosts take about 6 wk to mature (Stiling et al. 1991b), and there are at least 5 overlapping generations per yr (Stiling et al. 1991b; Denno 1994). Studying strepsipteran eyes requires live specimens, which necessitated the development of new capture techniques. However, because the majority of Strepsiptera are known from light trap by-catch (Green 1902; Meadows 1967; Shepard 1979), similar methods can be used to catch virtually any known crepuscular or nocturnal Strepsiptera.

Materials and Methods

It was expected that *E. koebelei* would be attracted to ultraviolet electromagnetic radiation from earlier work on *X. peckii* Strepsiptera (λ_{max} = 346 nm) (James et al. 2016). Accordingly, we designed and constructed portable light sources from a pair of 15 watt T8 UV lamps (diam: 2.54 cm; 1 in). One was a black light (BL); the other black light blue (BLB), using General Electric 35884 and 35885 GE F15T8 fluorescent tubes, respectively (45.72 cm; 18 in) (General Electric Corp., Fairfield, Connecticut, USA). Later, when collecting at Wakulla Beach, Florida, we replaced the original black light blue with a longer GE 10531 F40BLB, a 40 watt T12 black light blue (dia.: 3.81 cm; 1.5 in; length: 121.92 cm; 4 ft). All GE black lights have peak emittance at 368 nm (General Electric 2017), but black light lamps provide more visible light. This makes collecting easier, but may not be as attractive to Strepsiptera. General Electric T12 rapid start ballasts were used with all of the lamps. This likely increased the UV output of the T8 bulbs, at the anticipated cost of reduced lifespan. To provide waterproofing, silicon sealed PVC caps were placed over the wiring at the ends of each of the lamps. The lights were hung vertically atop a white sheet draped over a frame of PVC tubing, as shown in Figure 2. Batteries and ballast were protected by a plastic bucket that could rest on the ground at low tide, or be suspended from the trap by wire at high tide. Electrical contacts were soaked in undiluted white vinegar to remove saltwater-induced corrosion. A toothbrush also was used to help clean heavily corroded surfaces. By attaching shorter legs, the base of the frame could sit beneath the canopy of *S. alterniflora*. In the less dense *Spartina* of Wakulla Beach, this coupled with wetting the trap base greatly enhanced *E. koebelei* catch.

A Taylor 9840N Instant Read Digital Thermometer (Taylor, Oak Brook, Illinois, USA) was used to take local temperature measurements. Beginning in late 2015, a Kestrel 5500 Weather Meter (KestrelMeters.com, Minneapolis, Minnesota, USA) was used to record local wind speed, temperature, and humidity.

The custom light trap was used to collect *E. koebelei* Strepsiptera, where sweep netting in stands of *S. alterniflora* had revealed substantial numbers of stylopized *Prokelisia marginata* or *P. dolus* planthoppers. Strepsiptera were collected in late Aug through Sep, and a few d in Oct and Nov in 2013; in 2014, only in Oct; and in 2015, in Jul through early Aug (Table 1). The main collection sites were the north branch of Guana Tolomato Matanzas National Estuarine Research Reserve, also known as GTM Research Reserve (GTM) (30.0702°N, 81.3447°W), in St. John's County, Florida, just off the Guana River near the Atlantic Coast, and Wakulla Beach (WB) (30.1050°N, 84.2616°W) in Wakulla County, Florida, on the Gulf of Mexico (Fig. 3).

Elenchus koebelei males that landed on the light trap were captured and placed into vials via an aspirator or a moistened fine-tipped paintbrush. After returning from a field site, author James identified species under a dissecting microscope by immobilizing captured insects with a FlyStuff Flypad (10.1 × 14 cm; 4.0 × 5.5 in) (Genesee Scientific Corp., San Diego, California, USA) connected to a CO_2 beer regulator paired with a paintball CO_2 tank. Use of a portable CO_2 cylinder (590 or 710 ml; 20 or 24 oz) allowed sample processing in unconventional laboratory settings. Tanks can be refilled inexpensively at scuba diving or paintball shops.

Sunrise is the most relevant timing event for animals that are active around dawn (matinal). Measuring time relative to sunrise allows capture times to be compared at different dates within a field season,

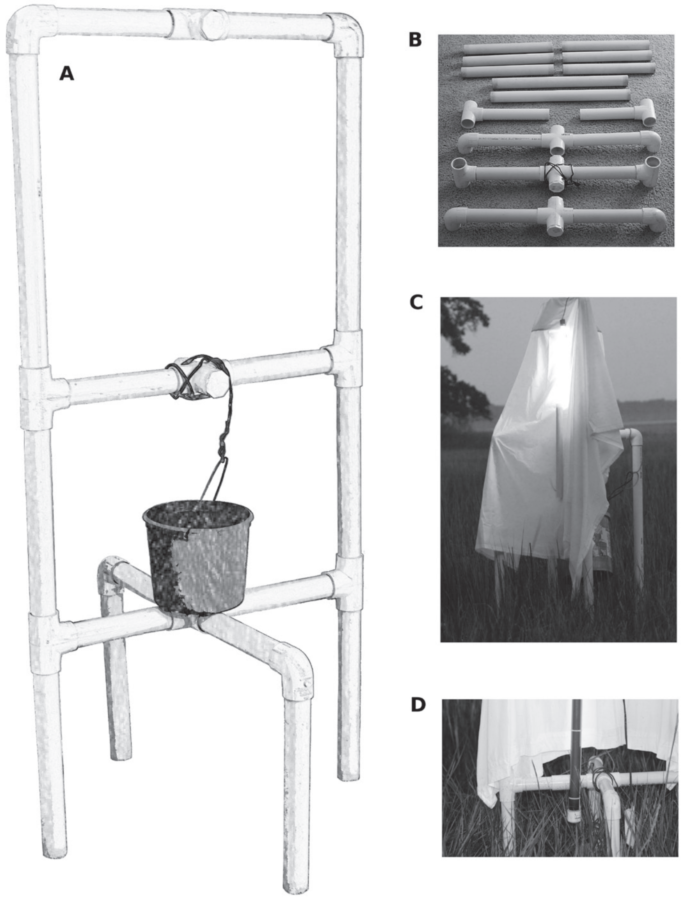

Fig. 2. The light trap. A. Schematic of the PVC skeleton. Electronics were placed in the bucket, which could be suspended from the trap at high tide. B. PVC parts. Some parts were cemented together for strength and ease of construction. C. Trap with sheet and lights in place. In taller grass, longer trap legs can be used to help provide a crease into which attracted Strepsiptera can fly, walk, or fall. This reduces specimen loss through desiccation or drop-off into grass. In short grass it may be better to wet the trap base and use a long ultraviolet light. D. The base of the trap when used with the long light.

between different field seasons, and between separate sites. Although the length of twilight can change dramatically depending on latitude and the time of yr, in northern Florida the duration of civil twilight—the time before sunrise when artificial illumination is not necessary to clearly distinguish terrestrial objects—remained between 23 and 27 min through summer and fall, with an average length of 24:44 (min:s). The duration of nautical twilight—a phase of twilight preceding civil twilight, when artificial lighting is necessary for humans to see acceptably on moonless mornings, but the horizon is still distinct—over the same time period ranged between 27 and 33 min, with an average of 29:20 (min:s). Astronomical twilight ranged from 27 to 35 min, and averaged 28:53 (min:s).These averages correspond to the twilight times labeled in Figures 4 and 5.

Variation in the start and duration of collection times is documented in Table 2. Effective start and duration times were determined in 2013. That knowledge was exploited in subsequent seasons to reduce effort and increase catch. During each season an early start time and long duration was revisited, which reaffirmed the soundness of the more abbreviated schedules (Fig. 4). Due to processing limitations in 2015, the site often was abandoned even though additional Strepsiptera still could have been collected.

SPECIES IDENTIFICATION

There are several ways to identify adult male *E. koebelei* in the field, even if one has never seen a living Strepsiptera before. First, adult Strepsiptera are not skillful walkers. Because of this and their general frenetic nature, energetic adult males tend to use their wings to assist them in walking. The most telltale sign of an *E. koebelei* is the arc their vibrating wings sweep out. It is difficult to describe, but utterly unlike any other flapping pattern. Once seen, it is immediately identifiable. Second, when less energetic, *E. koebelei* often walk with their wings held together above the thorax. However, the wings swing from side-to-side when they step because their legs are specialized for grasping onto a female. Third, although *E. koebelei* are tiny, often their bifurcated antennae can be recognized. When walking, they are extended forward with the branches separated, as with *E. tenuicornis* (Hassan 1939). Initially, one may find it helpful to use a magnifying glass to assist with in-field identification, but after a few sightings it is normally much more efficient to proceed with the naked eye.

Results

We captured 521 adult male *Elenchus koebelei* using the mobile light trap: 284 from the Guana Tolomato Matanzas Research Reserve on the Atlantic, and 237 from Wakulla Beach on the Gulf of Mexico. These Strepsiptera were caught primarily in mid-Jul through Oct (Table 1). Male elenchids have a short adult lifespan of about 4 h (Cook 2014). Although some Strepsiptera were processed in the field, the remaining live specimens were vigorous at least 2 h after capture, allowing sufficient time for processing or experimentation in the lab.

In 2014, we were available to trap *E. koebelei* only from Oct to Nov, the latter portion of the field season in a yr in which cool temperatures arrived early. During the 3-yr study, no Strepsiptera were captured on 14 mornings (Fig. 6; Table 1), 11 of which occurred in 2014. Nonetheless, that yr was indispensable for determining the lowest temperature at which *E. koebelei* eclose (Fig. 7).

In 21 field days at Wakulla Beach, the number of live captures per day had a minimum of 0, a lower quartile of 0, a median of 1,

Table 1. Sampling of adult male *Elenchus koebelei* occurred in late Aug through Sep and on a few days in Oct and Nov in 2013, only in Oct in 2014, and from mid-Jul to mid-Aug in 2015. Wakulla Beach (WB) is on the Gulf Coast of Florida, nearly due south of Tallahassee. Guana Tolomato Matanzas National Estuarine Research Reserve (GTM) is on the Atlantic Coast of Florida, due east of Wakulla Beach.

Site	Year	Sampling days	Male *Elenchus koebelei* caught	Average caught per sampling day	Average caught live per sampling day
GTM	2013	20	120	6.00	5.25
	2014	11	11	1.00	1.00
	2015	20	153	7.65	4.55
WB	2013	17	236	13.94	13.24
	2014	4	0	0.00	0.00

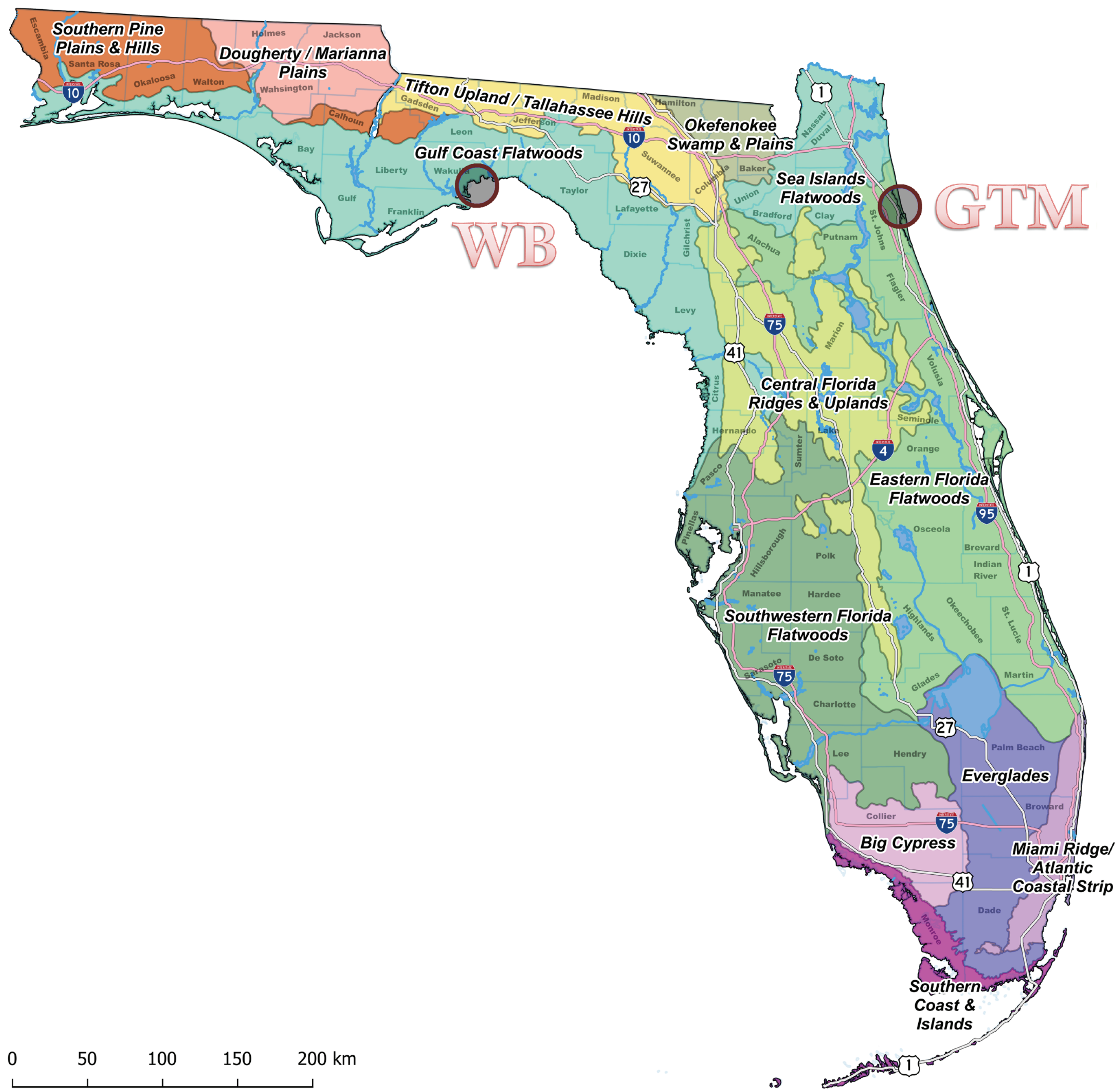

Fig. 3. Collection sites: The north branch of the Guana Tolomato Matanzas National Estuarine Research Reserve in Saint John's County, near Florida's Atlantic Coast, and Wakulla Beach, on the Gulf Coast in Wakulla County. [Produced with assistance from Eco-Regions of Florida. Level IV Ecoregions graphic developed by the Watershed Monitoring Section, Division of Environmental Assessment and Restoration, Florida Department of Environmental Protection, Tallahassee, Florida. Sourced from Griffith et al. (2001). Adapted with permission.]

a third quartile of 15, a maximum of 60, and a total live catch of 225, with an approximately 62% chance of catching at least 1 live Strepsiptera each morning. However, without including results from 2014, the lower quartile, median, and third quartile improve to 1, 3, and 18, respectively, and the chance of catching a live male rises to 13 out of 17, about 76%. In 51 field days at Guana Tolomato Matanzas Research Reserve, the minimum number of *E. koebelei* captured live was 0, the lower quartile was 1, the median 3, the third quartile 6, the maximum was 23, and the total live catch was 207, with a 45 out of 51 (about 88%) chance of capturing at least 1 live eclosed male each morning. Without 2014, the lower quartile, median, and third quartile improve to 2, 3.5, and 6, respectively, with a 39 out of 40 (97.5%) chance of catching a live male on any sampling day. These values are based on the 432 *E. koebelei* that were collected alive (Strepsiptera can quickly dry out on hot ultraviolet lights and die just before collection), including those caught at unrecorded times.

Most (488 out of 521, about 94%) *E. koebelei* arrived at the apparatus between 30 min before and 15 min after sunrise, as shown in Figure 5. None were captured more than 63 min before sunrise, or

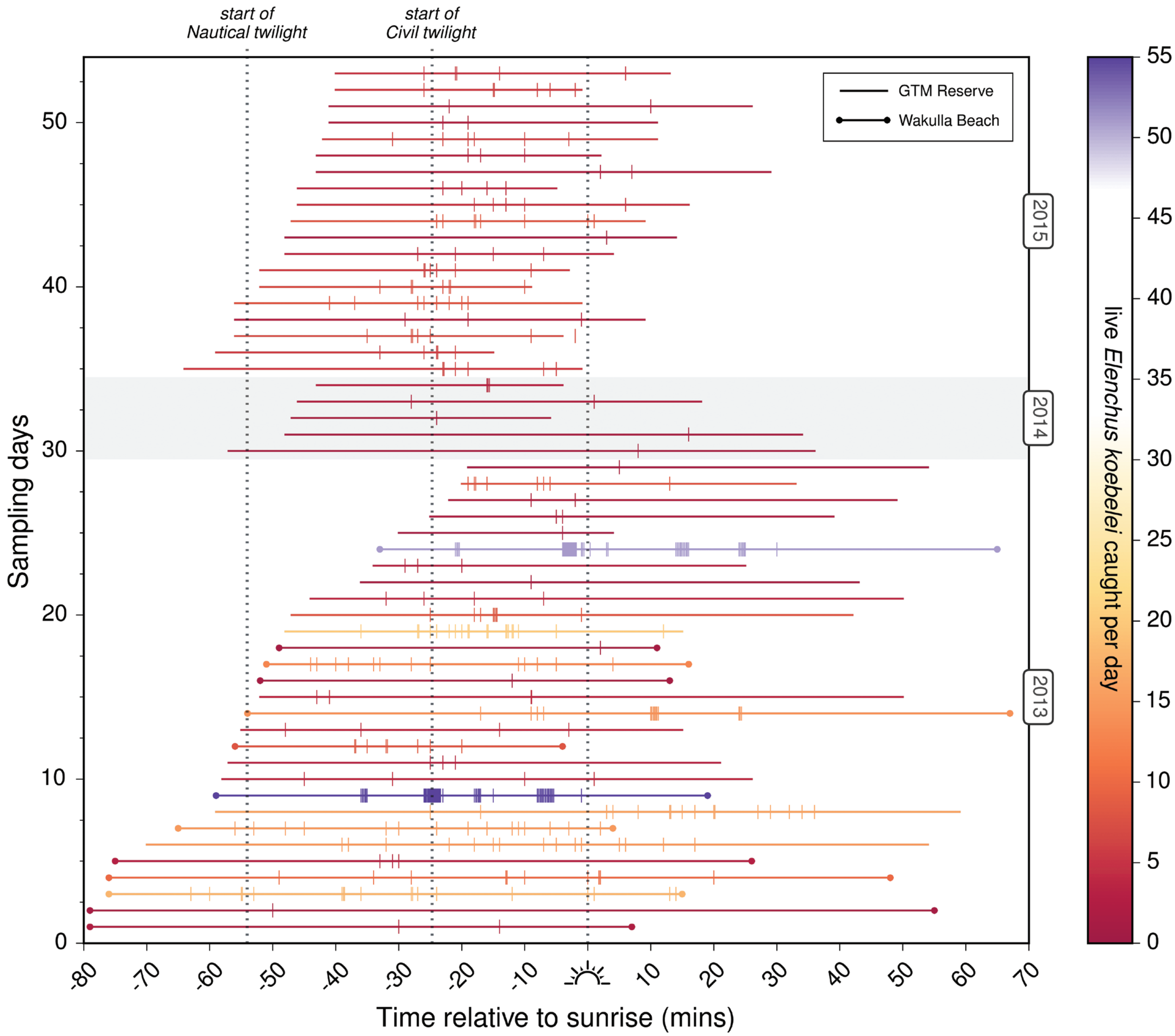

Fig. 4. Successful sampling days, grouped by year and ordered by start time. The figure displays the delay between the beginning of sampling and the first live capture, the time between live captures, and the time after the last live capture until disbanding of daily sampling. Seven captures were made during astronomical twilight, all of which occurred at Wakulla Beach: 5 on one morning, and 2 on another. There were no sampling days with live catches between 24 and 50 at either site.

36 min thereafter (Supplemental data). On average, the light trap was operational beginning 49 min before sunrise until 21 min after sunrise.

Apart from finding an area with visibly stylopized planthoppers, the most important determinants of sampling success are temperature (500 out of 521, about 96%, were caught at temperatures ranging from 21.7–26.1 °C; 71–79 °F), wind speed (485 out of 521, about 93%, were caught at wind speeds from 0–11.3 km/h; 0–7 mph), and timing (488 out of 521, about 94%, were captured from 30 min before to 15 min after sunrise).

Discussion

Having live Strepsiptera allows for experimentation and detailed investigation. We used a portable light trap to provide reliable access to live adult male *E. koebelei* during the species' mating season. Because Strepsiptera are known to be caught in light traps, the same general approach should be applicable to any Strepsiptera that flies in low light. However, one must first determine where they are and when they are active.

SUITABLE HABITAT

Prerequisites to catching *E. koebelei* are persistent stands of *S. alterniflora*, the larger, the better, and an abundance of *Prokelisia marginata* or *P. dolus* planthoppers, or both at once. It is also very important that sweep netting yield positive results prior to attempting light trap collection. Before realizing the primacy of this qualification, we sampled without success at a few waterfront locations in Franklin and Wakulla counties, Florida in 2013. Sweep netting also helps one to know where to position a light trap. However, given the presence of visibly stylopized planthoppers, there is every reason to expect live

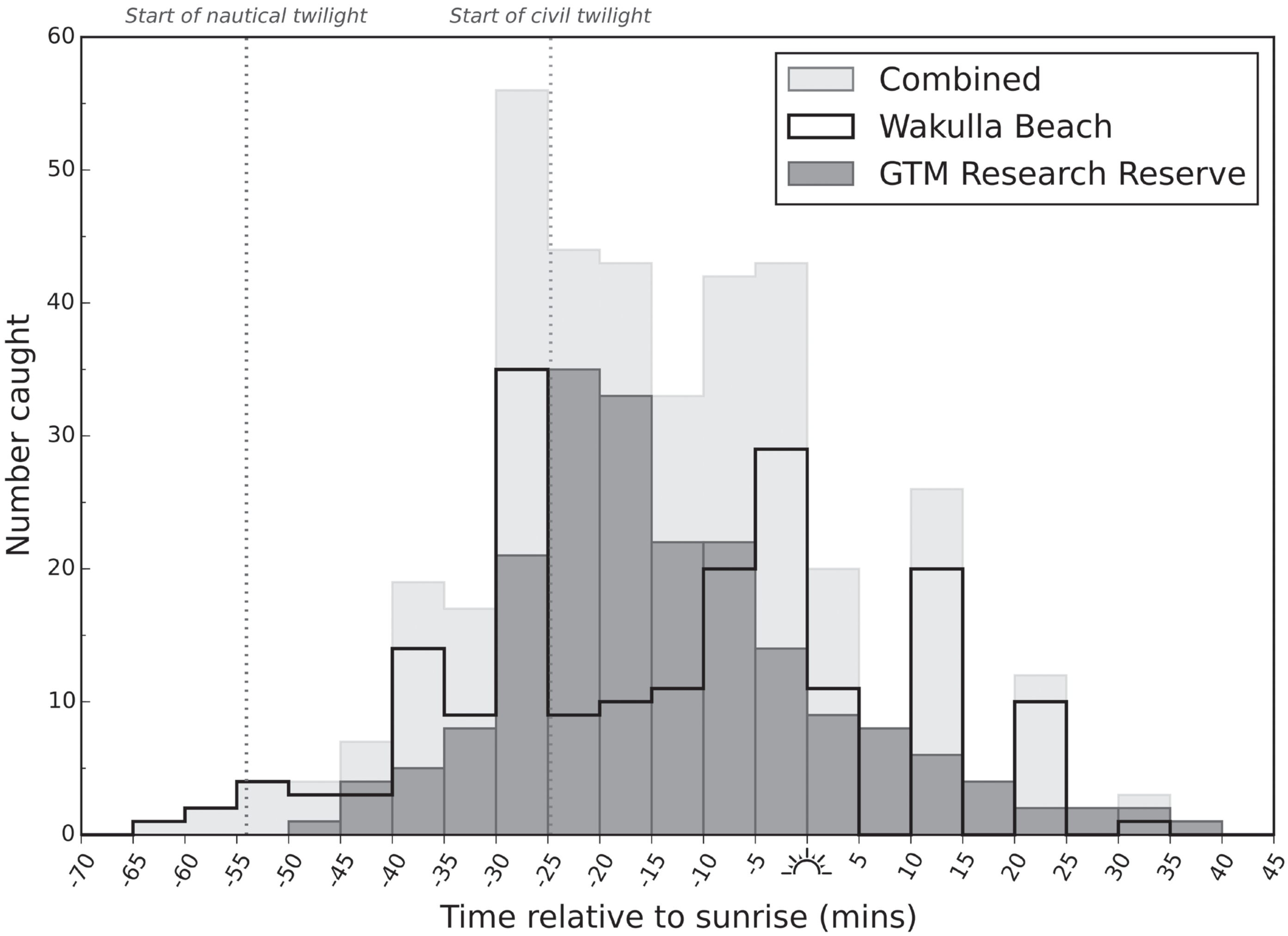

Fig. 5. Live *Elenchus koebelei* males caught over a 3-year period plotted against minutes relative to sunrise. Most eclosed males were caught between 30 min before sunrise and sunrise itself. None were caught more than 63 min before or 36 min after sunrise. Though wind-induced fluctuations occurred at Wakulla Beach, the range of capture times at both sites were similar, and peak catch times appear strongly influenced by morning civil twilight. Of the 521 adult male *E. koebelei* caught over the course of the study, only the 391 captured alive at known times are included in the graph.

eclosed *E. koebelei* can be captured at a site. Finally, promising sites may be worth revisitation, because early in a season hosts may be infected without external indication. New locations may even be colonized in particularly productive years.

DIEL ACTIVITY

At the onset of this study, the active period of *E. koebelei* was unknown. We hypothesized that the species is crepuscular, given that:

1. Host activity patterns are important indicators of strepsipteran activity, and delphacid planthoppers, especially the brown planthopper, *Nilaparvata lugens* (Stål 1854) (Hemiptera: Delphacidae), are known to migrate at dusk or dawn (Pender 1994; Qi et al. 2014)
2. Muir (1906) found that all specimens of the closely related species, *E. tenuicornis*, that he reared out in Hawaii eclosed between sunrise and 7 AM. [Time reckoning precedes the introduction of Daylight Saving Time.]

Table 2. Catch statistics and variation in *Elenchus koebelei* sampling duration. On average, all Strepsiptera were caught within a 20 minute period each day at Guana Tolomato Matanzas National Estuarine Research Reserve (GTM), and in just over a half hour at Wakulla Beach (WB). Due to logistics and inclement weather, the 2014 field season began late and was curtailed (no specimens were collected from WB, and only a few from GTM). Both locations are coastal sites in Florida, GTM on the Atlantic, and WB on the Gulf.

Year	Site	Average start of sampling before sunrise (minutes ± SD)	Average minutes sampling	Average minutes until first catch	Absolute max and (average max minutes) between catches	Average minutes between first and last catch	Average minutes sampling after last catch
2013	GTM	42 ± 17	81	21	32 (14)	19	33
2013	WB	59 ± 26	85	27	19 (13)	32	29
2014	GTM	48 ± 7	66	36	20 (14)	7	19
2014	WB	51 ± 14	49	—	—	—	—
2015	GTM	49 ± 8	55	24	32 (12)	19	11

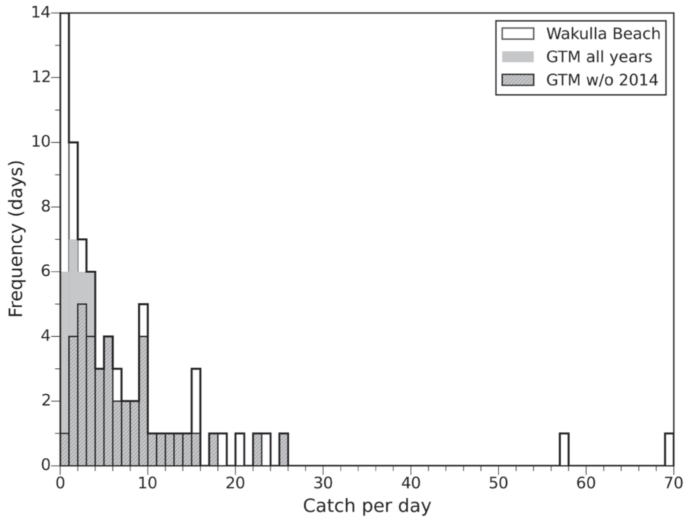

Fig. 6. Stacked frequency distributions of captured *Elenchus koebelei* males. The white area under the outline illustrates the number of days with a given catch at Wakulla Beach, the gray area depicts that for all years at Guana Tolomato Matanzas National Estuarine Research Reserve (GTM), and the hashed gray area represents the portion of the catch at GTM without 2014. Lastly, the broad outline represents the combined catch frequencies from both sites for all 3 years, 2013–2015. More than half the days with no catches occurred in 2014, when sampling began in mid-Oct.

3. Eclosed *E. koebelei* are not encountered in diurnal sweep netting, even when net mesh sizes are suitably small and the percentage of stylopized hosts may approach 40% (Stiling et al. 1991b).

It is possible *E. koebelei* is diurnal. However, that is unlikely because on several mornings the final catch of the day occurred before sunrise (Fig. 4). In fact, more than 50 live *E. koebelei* were caught before daybreak on 2 separate mornings (Fig. 4; Supplemental data). Although the detectability of a light trap is reduced with increasing ambient light (particularly at distance), at Wakulla Beach we observed 2 additional peaks in captures that occurred from 10 to 15 min after sunrise, and from 20 to 25 min after sunrise, indicating that the trap was sufficiently conspicuous to attract *E. koebelei* well after sunrise. (This also indicates that the Strepsiptera did not have far to fly: at even moderate distances after sunrise, artificial light should fade into the background.) It is also possible *E. koebelei* is nocturnal. However, we conclude that is highly unlikely, given the brief lifespan of eclosed adult male Strepsiptera and that some specimens were captured over 30 min after sunrise.

Because *E. koebelei* are eclosed at dawn, they are visually capable of flying at dusk as well. However, on the windless evening of 27 Sep 2013, no *E. koebelei* were captured or sighted in sampling at Wakulla Beach, where the insects were known to be present. Sampling began 13 min before sunset and ended 70 min thereafter. On that evening, dragonflies were flying in great numbers, some as long as 28 min after sunset. Although their hunting success would decline sharply with increasing darkness, dragonflies likely pose a threat to Strepsiptera at dusk. It is unknown how the eyeless calling females would be aware of dragonflies at whatever density, or even the sighted males, before having cast off their pupal caps and therefore committing to eclosure. Judging from their treatment of other variable parameters such as moon illumination and tide height (see below), the strepsipteran approach appears to be to ignore them in favor of consistent factors. It is therefore most likely that adult *E. koebelei* are active only around dawn (i.e., strictly matinal), when most bats are finished feeding, and larger predatory insects are not yet active.

OTHER CONDITIONS INFLUENCING CATCH

Factors such as temperature, timing, wind speed, and the absence of precipitation, significantly influence *E. koebelei* catch. In this study, most data were collected and disseminated by area weather stations. Such records are not as accurate as local measurements, but are more readily available, are still very useful in determining sampling success, and provide data *before* arriving at a site, which can save a great deal of turmoil. Over the course of the study, a local thermometer was nearly always on hand. After 4 Aug 2015, a portable weather station also was available to record local wind speed, temperature, and humidity.

TEMPERATURE

The most productive temperature range for catching *E. koebelei* was found to be from 21.7 to 25.6 °C (71–78 °F), within which 93% of all *E. koebelei* were caught. This range also contained the most favorable ratios of catch to days at temperature. No *E. koebelei* were captured at temperatures below 17.2 °C (63 °F). *Elenchus koebelei* eclosed at the highest temperature encountered (27.8 °C (82 °F)); however, sampling efficiency declined sharply for temperatures above 25.6 °C (78 °F) (see Fig. 7.) Avoiding eclosing when it is warmer may help protect *E. koebelei* from attack by aerial predatory insects, which generally have higher take-off temperatures.

TIMING

We found it best to have the trap completely operational from 35 min before sunrise to about 15 min after sunrise at Guana Tolomato Matanzas Research Reserve, where strong breezes are rare. However, at Wakulla Beach, a windier site, sampling from 45 min before until 25 min after sunrise produced better results. These findings should generalize to other locations.

WIND

Elenchus koebelei are quite capable fliers, but they are minute and lightweight, so substantial local winds could blow them well off course, and also obscure the location of calling females. Furthermore, it is likely 'strong' wind is the predominant factor in patchy strepsipteran distribution. In the absence of a local wind meter, the Beaufort scale or the wind speed from an area weather report can be used. Wind speeds from about 1.6 to 9.7 km/h (1–6 mph) appear to be best (Fig. 8). Still air conditions were found to be good, and wind speeds from 9.7 to 12.9 km/h (6–8 mph) were suitable. *Elenchus koebelei* rarely flew at wind speeds above 16 km/h (10 mph). No Strepsiptera ever were captured when the trap's collection sheet billowed enough to destabilize it. However, when stronger winds died down several min before dawn, males still eclosed and were caught. At Guana Tolomato Matanzas Research Reserve, wind speed was greatly reduced and regularized by nearby trees, and eclosed *E. koebelei* exhibited a smooth single-peaked distribution of catch times relative to sunrise. At Wakulla Beach, the frequency of windy conditions between sampling days greatly increased catch variance, while within sampling days, the prevalence of wind gusts likely prevented a unimodal distribution (Fig. 5).

For consistency, all analyses were done on wind data from area weather stations. However, those may overstate the wind speed in protected areas, such that still wind conditions could in fact yield the best results. Unfortunately, on-site wind speed data were available only for the last 7 d of trapping in 2015. Over that time period, they averaged 5.6 km/h (3.5 mph) less than area wind measurements, with 5 d registering 0 km/h (Supplemental data). To obtain a more in-depth treatment, wind speed should be monitored continuously and matched against individualized catch times.

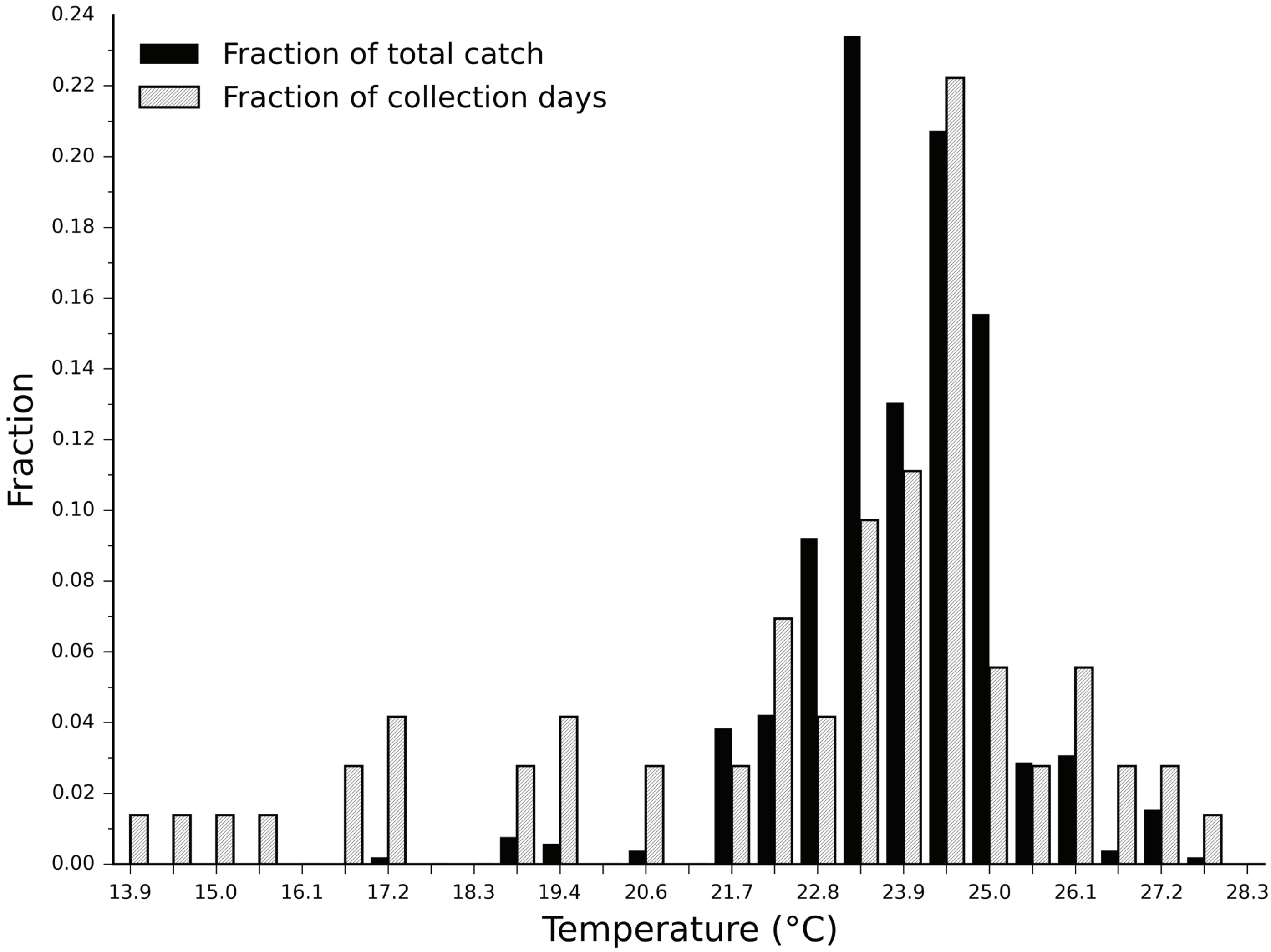

Fig. 7. Influence of initial temperature on total catch. Black boxes indicate the fraction of total Strepsiptera captured at the given temperatures; adjacent hashed boxes represent the fraction of mornings at each temperature. The most productive days were those with dawn temperatures ranging from 22.8 to 25 °C (73–77 °F). *Elenchus koebelei* was not found to fly on mornings when the temperature was below 17.2 °C (63 °F). The ratio of *E. koebelei* caught to collection days drops off dramatically for temperatures above 25.5 °C (78 °F).

RAIN

Though very light drizzle was tolerated, *E. koebelei* were found to prefer mornings free of precipitation. Males appear to be highly sensitive to barometric pressure, such that they did not fly on mornings that seemed very promising, but suddenly degenerated into substantial rainfall. However, when rain was light enough to be of no concern for shorting out exposed electrical connections, it was also suitable for collecting *E. koebelei*.

TIDE

Tide height was found to be of little significance, particularly at Guana Tolomato Matanzas Research Reserve. It may increase the difficulty of collection, but does not appear to actually influence strepsipteran eclosion. There is some indication that the host insect, *P. marginata*, may avoid immersion, ostensibly to escape consumption by predatory fish. At Guana Tolomato Matanzas Research Reserve, the Pearson correlation coefficient between tide height and the number of *E. koebelei* caught was 0.12, with an estimated 62% chance of having arisen randomly. At Wakulla Beach, the correlation coefficient was –0.29 with an estimated 21% chance of having arisen randomly. However, when corrected for the range of water heights that occurred during collection episodes, the correlation coefficient fell to –0.02 (95%) and –0.21 (37%), respectively. The persistent slight negative correlation found at Wakulla Beach was due to the catch characteristics of the 2 extremely high yield days, and not due to consistent differential tide-based eclosing of male *E. koebelei*.

MOONLIGHT

Only moon rise, moon set, and lunar visibility as a function of phase were recorded consistently. No data on cloud cover were noted regularly, and a light meter was not available to record relative brightness. However, within these confines, it was repeatedly found that *E. koebelei* did not eclose differently despite the greatly increased availability of light during full, and nearly full, moons. This lack of response suggests strongly that in addition to being completely blind, adult female *E. koebelei* also do not adjust their calling times on the basis of lunar light intensity.

Moonlight intensity does appear to influence catch, albeit negatively. That is, as found for other nonaquatic insects, the number of Strepsiptera eclosing ostensibly remains constant with respect to

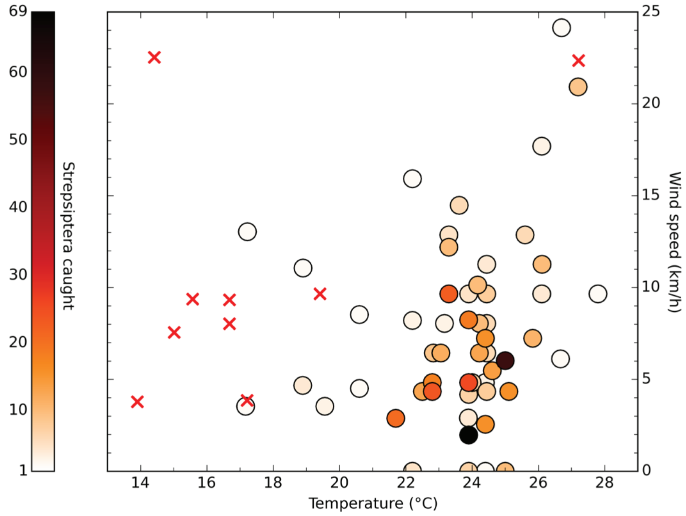

Fig. 8. Daily Strepsiptera catch versus temperature and wind speed. Ninety-three percent of the *Elenchus koebelei* were caught at temperatures between 21.7 to 25.6 °C (71–78 °F) inclusive. Strepsiptera catch suffered markedly when it was too cold. Similarly, most *E. koebelei* were captured when the wind was blowing slightly, perhaps owing to the role pheromones play. The ×'s indicate conditions in which no Strepsiptera were caught but sampling was attempted. Graphed wind speeds were measured at area weather stations rather than locally.

moonlight, but on brightly moonlit nights the light trap has to contend with increased ambient light, and is therefore less efficient (Williams et al. 1956). Because *E. koebelei* is crepuscular, the effect is less pronounced than what could be expected of nocturnal insects. However, it is worth noting that both cases of catches during astronomical twilight occurred on moonless nights.

LIGHT SENSITIVITY

Eclosed *E. koebelei* were most often captured during twilight, the period between night and day when no sunlight reaches an observer directly, but sunlight redirected by the atmosphere still does (unlike at night). Twilight is divided into 3 formalized stages, based on the sensitivity of the human eye (timeanddate.com n.d.; US Naval Observatory 2011). However, despite this origin, the subdivisions also have relevance to *E. koebelei*, as can be seen in Figures 4 and 5. In the morning, twilight begins at dawn and ends at sunrise. Astronomical twilight is the first stage of morning twilight; it occurs when the geometric center of the sun is between 18° (inclusive) and 12° (exclusive) below the horizon. At the beginning of astronomical twilight, the intensity of scattered sunlight is less than that from weak stars, and remains barely perceptible for a considerable interval thereafter (US Naval Observatory 2011). Throughout this stage of twilight the horizon is indistinct to a human observer. Nautical twilight occurs when the sun is between 12° and 6° below the horizon. It is named for nautical navigation by star and horizon sighting because in nautical twilight, the horizon remains discernible even on moonless nights, but most stars visible to the naked eye can be seen. In the absence of moonlight, during nautical twilight artificial illumination is required for most outdoor activities. Civil twilight occurs from when the sun is 6° below the horizon until sunrise. Under good atmospheric conditions in civil twilight, artificial illumination is not necessary to clearly distinguish terrestrial objects, and only the brightest celestial bodies can be seen by the naked human eye. Sunrise begins the moment any portion of the solar disk breaches the horizon.

At Guana Tolomato Matanzas Research Reserve, the largest number of Strepsiptera were caught from 25 to 15 min before sunrise, the beginning of which roughly coincides with the onset of civil twilight. At Wakulla Beach, the majority of eclosed *E. koebelei* were caught in 2 waves, the first from 30 to 25 min before sunrise, just prior to the start of civil twilight, and the second from 5 minutes before sunrise until sunrise itself, which marks the end of civil twilight (Fig. 5). Although the boundaries of twilight correspond to human visual sensitivity, it appears that civil twilight had strong bearing on the mating dynamics of both strepsipteran populations nonetheless, though at Wakulla Beach the preferred flight time may have been split to better coincide with troughs in wind activity. Unlike at Guana Tolomato Matanzas Research Reserve, the site at Wakulla Beach was not partially enclosed by trees, thus allowing for stronger winds and also greater ambient light intensity, either or both of which might have encouraged *E. koebelei* to eclose at altered times. Accordingly, Strepsiptera at Wakulla Beach were more apt to fly during nautical twilight, and only *E. koebelei* at Wakulla Beach were found to fly during astronomical twilight (Table 3). Likewise, more *E. koebelei* were collected after sunrise at Wakulla Beach than at Guana Tolomato Matanzas Research Reserve. This may be due to a post-dawn reduction in wind at the site.

The visual sensitivity of *E. koebelei* has not been determined experimentally, but given these findings, we hypothesize that *E. koebelei* see at roughly 1 twilight stage brighter than do humans, such that our astronomical twilight corresponds to their nautical twilight, human nautical twilight is the civil twilight of *E. koebelei*, human civil twilight is their dawn, and full dawn and beyond may require significant internal shielding in *E. koebelei*, which otherwise could adversely affect their visual acuity.

Table 3. Adult male *Elenchus koebelei* captured during different phases of morning. *Elenchus koebelei* had a propensity to approach the light trap during civil twilight at both sites. However, because there are no nearby bordering trees, it is significantly windier and also brighter at Wakulla Beach (WB). These conditions appear to have extended acceptable flight times even into astronomical twilight at the site.

Site	Male *Elenchus Koebelei* captured at known times	Number	Proportion
GTM	During astronomical twilight	0	0.00
	During nautical twilight	42	0.21
	During civil twilight	123	0.62
	After sunrise	34	0.17
	Total	199	1.00
WB	During astronomical twilight	7	0.04
	During nautical twilight	66	0.34
	During civil twilight	77	0.40
	After sunrise	42	0.22
	Total	192	1.00

WING EXPANSION

It typically took several minutes for any *E. koebelei* to visit the light trap regardless of when sampling began (Fig. 4). Furthermore, their wings are not furled as one would expect had they already been expanded within the pupal case, as is typical of male Strepsiptera. As a result, *E. koebelei* walk with wings held aloft. Because of these attributes, we hypothesize that *E. koebelei* do not fly immediately upon eclosing—unusual for Strepsiptera—but must first expand their wings in the manner of other flying insects, as Hassan (1939) reported of *E. tenuicornis*. It may be that during this time, *E. koebelei* can become fixated on a present light trap. If so, this would help explain the effectiveness of deploying the trap several minutes before the first Strepsiptera were expected (Fig. 4), and perhaps the unit's strong appeal so long after sunrise, as well. One also wonders if this approach improves immediate flight performance, and what effect such a 'trial period' might have on vision.

On several occasions we noted that moving the trap a few meters revived flagging catch totals. This indicated that many *E. koebelei* did not have far to fly to arrive at the trap, yet despite their considerable flight velocity, it still took several minutes for the first of them to do so. Although the unaccounted-for time could have been occupied by mating, many captured *E. koebelei* had very noticeably distended abdomens. Because Strepsiptera do not feed as adults and may spend substantial time in a pharate state awaiting appropriate conditions, the distension was probably due to sperm reserves, indicating that the males had not yet mated. Additionally, the act of strepsipteran mating is unlikely to last long (Muir 1906) or to be repeated (Hughes-Schrader 1924). Furthermore, in at least 2 strepsipteran species it has been noted that males die just a few minutes after mating (Kathirithamby 1989; Beani et al. 2005), but our (presumably unmated) specimens normally survived for hours when allowed to do so.

RESISTANCE TO DROWNING

Unlike gnats, alate ants, and other flying insects attracted to the light trap, *E. koebelei* did not drown when water affixed their bodies to the trap's support beams. This was discovered accidentally after a rainstorm, when the erected trap was carried through wet grass, and exploited thereafter. In stunted, less dense *Spartina* such as that at Wakulla Beach, attaching shorter legs to the trap to adjust for the reduced canopy height and wetting the trap base, together act as another means of ensnarement. Capturing Strepsiptera in this manner tended to ruin their remarkable wings, but did assist in obtaining larger numbers of live Strepsiptera at Wakulla Beach, particularly on the 2 mornings with the greatest live catch counts: 63 on 17 Sep 2013, and 57 on 22 Sep 2013 (57 and 51 caught live at known times, respectively). Strepsiptera can survive at least 29 min in such conditions.

This ability to avoid immersion asphyxiation may relate to the poorly understood strepsipteran "balloon gut" (Pohl & Beutel 2005; Beutel & Pohl 2006), or to the thinness of the strepsipteran cuticle, such that they may be able to breathe through it, as they ostensibly do as fully embedded larvae. The loss of all but 1 spiracle that has occurred in Elenchidae and many other strepsipteran clades also is worth noting (Pohl & Beutel 2005).

VISION

There is discrepancy over whether Strepsiptera see well with so few facets, which some expect to effectively act as pixels (Pix et al. 2000), while others hold that each 'ommatidia' should behave as a separate image-forming *eyelet* (Buschbeck et al. 1999; Maksimovic et al. 2007). The capture of large numbers of eclosed males below the grass canopy at Wakulla Beach suggests that *E. koebelei* can fly between grass blades before sunrise, and therefore must see well enough to do so. In addition to helping protect against larger insect predators, flying through *Spartina* should provide some buffer against wind, and also is where one might expect planthoppers—and calling females—to be located.

CAVEAT AND COMMENDATION

The data leading to this report were amassed as by-products of collection forays for detailed eye studies. Because of this, there were problems with its procurement, such as not having an anemometer collecting continuous local wind measurements, not beginning collection at the same time relative to sunrise each morning, and, due to processing limitations, not taking the maximal number of Strepsiptera available for collection each day in 2015. Despite these deficiencies, to the authors' knowledge, the Supplementary Data of this report comprise the most complete set of capture characteristics from any Strepsiptera collection effort so far conducted.

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References Cited

Beani L, Giusti F, Mercati D, Lupetti P, Paccagnini E, Turillazzi S, Dallai R. 2005. Mating of *Xenos vesparum* (Rossi) (Strepsiptera, Insecta) revisited. Journal of Morphology 265: 291–303.

Beutel RG, Pohl H. 2006. Head structures of males of Strepsiptera (Hexapoda) with emphasis on basal splitting events within the order. Journal of Morphology 267: 536–554.

Buschbeck E, Ehmer B, Hoy R. 1999. Chunk versus point sampling: visual imaging in a small insect. Science 286: 1178–1180.

Cook JL. 2014. Review of the biology of parasitic insects in the order Strepsiptera. Comparative Parasitology 81: 134–151.

Cvačka J, Jiroš P, Kalinová B, Straka J, Černá K, Šebesta P, Tomčala A, Vašíčková S, Jahn U, Šobotník J. 2012. Stylopsal: the first identified female-produced sex pheromone of Strepsiptera. Journal of Chemical Ecology 38: 1483–1491.

Denno RF. 1994. Life history variation in planthoppers, pp. 163–215 *In* Denno RF, Perfect TJ [eds.], Planthoppers: Their Ecology and Management, Chapman & Hall, New York, USA.

General Electric. 2017. GE Lighting. http://consumer.gelighting.com/catalog/ (last accessed 20 Nov 2017).

Green EE. 1902. A stylopid attracted by light. Entomologist's Monthly Magazine 38: 219.

Griffith GE, Omernik JM, Pierson SM. 2001. Level III and IV Ecoregions of Florida (map). Scale: 1:940,000. Revised 1999–2001 from 1991–1993 original.

Hassan AI. 1939. The biology of some British Delphacidae (Homopt.) and their parasites with special reference to the Strepsiptera. Transactions of the Royal Entomological Society of London 89: 345–384.

Hrabar M, Danci A, McCann S, Schaefer PW, Gries G. 2014. New findings on life history traits of *Xenos peckii* (Strepsiptera: Xenidae). The Canadian Entomologist 146: 514–527.

Hubbard HG. 1892. The life history of *Xenos*. The Canadian Entomologist 24: 257–262.

Hughes-Schrader S. 1924. Reproduction in *Acroschismus wheeleri* Pierce. Journal of Morphology 39: 157–205.

Hunter JD. 2007. Matplotlib: a 2D graphics environment. Computing In Science & Engineering 9: 90–95.

James M, Nandamuri SP, Stahl A, Buschbeck EK. 2016. The unusual eyes of *Xenos peckii* (Strepsiptera: Xenidae) have green– and UV–sensitive photoreceptors. Journal of Experimental Biology 219: 3866–3874.

Kathirithamby J. 1989. Review of the order Strepsiptera. Systematic Entomology 14: 41–92.

Khalaf KT. 1968. The seasonal incidence of free Strepsiptera (Insecta) males in southern Louisiana. American Midland Naturalist 80: 565–568.

Maksimovic S, Layne JE, Buschbeck EK. 2007. Behavioral evidence for within-eyelet resolution in twisted-winged insects (Strepsiptera). Journal of Experimental Biology 210: 2819–2828.

Meadows K. 1967. Distribution of two Strepsiptera in Florida: 1960-1962. Florida Entomologist 50: 137–138.

Muir F. 1906. Notes on Some Fijian Insects. Hawaiian Sugar Planters' Association, Honolulu, (H. T.), Hawaii.

Pender J. 1994. Migration of the brown planthopper, *Nilaparvata lugens* (Stål) with special reference to synoptic meteorology. Grana 33: 112–115.

Pix W, Zanker J, Zeil J. 2000. The optomotor response and spatial resolution of the visual system in male *Xenos vesparum* (Strepsiptera). Journal of Experimental Biology 203: 3397–3409.

Pohl H, Beutel RG. 2005. The phylogeny of Strepsiptera (Hexapoda). Cladistics 21: 328–374.

Qi H, Jiang C, Zhang Y, Yang X, Cheng D. 2014. Radar observations of the seasonal migration of brown planthopper (*Nilaparvata lugens* Stål) in Southern China. Bulletin of Entomological Research 104: 731–741.

Shepard WD. 1979. Occurrence of *Triozocera mexicana* Pierce (Strepsiptera: Corioxenidae) in Oklahoma, with a brief review of this genus and species. The Coleopterists Bulletin 33: 217–222.

Stiling P, Throckmorton A, Silvanima J, Strong DR. 1991a. Biology of and rates of parasitism by nymphal and adult parasites of the salt-marsh-inhabiting planthoppers *Prokelisia marginata* and *P. dolus*. Florida Entomologist 74: 81–87.

Stiling P, Throckmorton A, Silvanima J, Strong DR. 1991b. Does spatial scale affect the incidence of density dependence? A field test with insect parasitoids. Ecology 72: 2143–2154.

Time and Date AS. Twilight, dawn, and dusk. timeanddate.com. http://www.timeanddate.com/astronomy/different-types-twilight.html (last accessed 20 Nov 2017).

Tolasch T, Kehl S, Dötterl S. 2012. First sex pheromone of the order Strepsiptera: (3R,5R,9R)-3,5,9-trimethyldodecanal in *Stylops melittae* Kirby, 1802. Journal of Chemical Ecology 38: 1493–1503.

U.S. Naval Observatory. 2011. Rise, set, and twilight definitions. Astronomical Applications Department. http://aa.usno.navy.mil/faq/docs/RST_defs.php (last accessed 20 Nov 2017).

Williams CB, Singh BP, Ziady SE. 1956. An investigation into the possible effects of moonlight on the activity of insects in the field. Proceedings of the Royal Entomological Society of London. Series A, General Entomology 31: 135–144.