UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE ECOLOGIA

Ecología

(PROYECTO)

La interacción depredador-presa entre la luciérnaga carnívora *Photuris lugubris* y la luciérnaga sincrónica *Photinus palaciosi* (Coleoptera: Lampyridae).

TESIS

(**POR ARTÍCULO CIENTÍFICO**)

***Photuris lugubris* Female Fireflies Hunt Males of the Synchronous Firefly *Photinus palaciosi* (Coleoptera: Lampyridae).**

QUE PARA OPTAR POR EL GRADO DE:

MAESTRA EN CIENCIAS BIOLÓGICAS

PRESENTA:

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P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **27 de noviembre de 2023**, se aprobó el siguiente jurado para el examen de grado de **MAESTRA EN CIENCIAS BIOLÓGICAS** en el campo de conocimiento de **Ecología** de la alumna **MAQUITICO ROCHA YARA** con número de cuenta: **522003996,** por la modalidad de graduación de **tesis por artículo científico** titulado: **"Photuris lugubris Female Fireflies Hunt Males of the Synchronous Firefly Photinus palaciosi (Coleoptera: Lampyridae)"** que es producto del proyecto realizado en la maestría que lleva por título**: "LA INTERACCIÓN DEPREDADOR-PRESA ENTRE LA LUCIÉRNAGA CARNÍVORA *Photuris lugubris* Y LA LUCIÉRNAGA SINCRÓNICA *Photinus palaciosi* (COLEOPTERA: LAMPYRIDA)"** ambos realizados bajo la dirección del **DR. CARLOS RAFAEL CORDERO MACEDO,** quedando integrado de la siguiente manera:

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"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 26 de enero de 2024

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Índice

Resumen

El término *femme fatale* se asocia de manera general a todas las hembras cazadoras de especies pertenecientes a la subfamilia Photurinae, de manera implícita se les adjudica la cualidad de practicar el mimetismo agresivo para cazar, haciéndose pasar por hembras receptivas. Si bien las *femme fatales* son un ejemplo clásico de mimetismo agresivo, han sido documentados otros métodos de caza para diferentes especies del género *Photuris*. A través de la observación en campo del comportamiento sexual y depredador de *Photuris lugubris* a lo largo de tres temporadas de cortejo-caza, describimos la interacción de depredación entre las hembras *P. lugubris* y los machos *Photinus palaciosi*, una luciérnaga sincrónica, endémica de México. Además, calculamos tasas de atracción y captura de las hembras *P. lugubris* sobre los machos *P. palaciosi*, como una aproximación acerca de la eficacia en la captura de las presas por parte de las hembras. Nuestras observaciones demuestran que las hembras *P. lugubris* atraen machos *P. palaciosi* respondiendo a sus señales bioluminiscentes, además demostramos experimentalmente que los machos *P. lugubris* no depredan machos *P. palaciosi*. También presentamos evidencia experimental acerca de que las hembras cazadoras se aparean con múltiples machos. Finalmente mostramos evidencia acerca de que el éxito de caza de las hembras *P. lugubris* es alto en cautiverio, pero bajo en la naturaleza, ya que, aunque atraen a una cantidad considerable de machos, la mayoría de ellos logra evitar el ataque. Describimos los comportamientos de los machos de *P. palaciosi* que reducen el éxito de captura de las hembras de *P. lugubris*, así como el comportamiento que les ayuda a estas hembras a capturar machos presa. Este patrón de adaptaciones y contra adaptaciones es consistente con un proceso coevolutivo depredador-presa.

Abstract

The *femme fatale* term is generally associated with female fireflies of the subfamily Photurinae that hunt males of other firefly species. This term implicitly assigns the practice of aggressive mimicry to these females via the mimicking of the response pattern of the females of their male prey to attract and capture the males. While *femme fatales* are a classic example of aggressive mimicry, other hunting methods have been documented for different species within the *Photuris* genus. Through field observation of the sexual and predatory behavior of *Photuris lugubris* over three courtship and hunting seasons, we describe the predator-prey interaction between *Photuris lugubris* females and *Photinus palaciosi* males, a synchronous firefly, endemic of Mexico, whose courtship display is exploited as a tourist attraction in several states of our country. In addition, we calculated attraction and capture rates of *P. lugubris* females on *P. Palaciosi* males, as an approximation of the effectiveness of females in capturing prey. Our observations demonstrate that *P. lugubris* females attract *P. palaciosi* males by responding to their bioluminescent signals, and we also experimentally demonstrate that *P. lugubris* males do not prey on *P. Palaciosi* males. Furthermore, we present experimental evidence that these *femmes fatales* mate with multiple males. Finally, we show evidence that the hunting success of *P. lugubris* females is high in captivity, but low in nature, since, although they attract a considerable number of males, most of them manage to avoid the attack. We describe behaviors of *P. palaciosi* males that reduce the capture success of female *P. lugubris*, as well as behavior of these females that help them capture prey males. This pattern of adaptations and counter-adaptations is consistent with a predator-prey coevolutionary process.

Introducción

El eminente biólogo James Lloyd utilizó por primera vez el término *femme fatale* para referirse a las hembras cazadoras de algunas especies dentro de la familia Lampyridae, quienes atraían machos de otras especies de luciérnagas sirviéndose del mimetismo agresivo, con el único fin de devorarlos (Lloyd, 1965). Las *femme fatales* responden a los destellos de los machos presa imitando la respuesta bioluminiscente de las hembras de su especie, haciéndose pasar por parejas potenciales y manteniendo comunicación con ellos hasta que éstos se acercan los suficiente para someterlos y alimentarse de ellos.

Según Eisner y colaboradores (1997), las hembras cazadoras obtienen de sus presas, lucibufaginas, compuestos que son incapaces de producir por sí mismas y cuyo secuestro supone una disminución potencial de sus riesgos de depredación, así como los de sus huevos. Estos esteroides defensivos tienen una función emética y otorgan un sabor desagradable a quien los posee (Gonzàlez *et al*., 1999). No hay registros en la literatura sobre machos de especies con *femmes fatales* que también cacen y se alimenten de otras luciérnagas. Sin embargo, los registros en hemolinfa de pequeñas cantidades de lucibufaginas llevaron a suponer a Eisner *et al*. (1997) que ellos también podrían cazar.

Este comportamiento depredador se ha reportado sólo dentro de la subfamilia Photurinae y en el continente Americano. En Norteamérica se han reportado hembras cazadoras del género *Bicellonycha*, *Crematogaster* y varias especies del género *Photuris* pertenecientes al grupo llamado Division II por Lloyd (2018). En Estados Unidos el fenómeno ha sido ampliamente estudiado para especies del género *Photuri*s, cuyas presas suelen pertenecer a los géneros *Photinus* y *Pyractomena*.

En general, el comportamiento reportado para las hembras cazadoras es similar. Emergen al principio de la temporada de cortejo para aparearse con sus machos, posteriormente su comportamiento cambia modificando postura y agresividad, transformándose en *femmes fatales* y dispersándose hacia los sitios de cortejo de sus machos presas con el fin de atraerlos utilizando el engaño con señales bioluminiscentes. Nelson *et al*., (1975) reportan que las hembras vírgenes de *Photuris versicolor* responden casi exclusivamente a los destellos artificiales que imitan los de sus machos coespecíficos e ignoran aquellos flashes artificiales que imitan los de sus presas, machos de *Photinus macdermotti*. Sin embargo, una vez que se han apareado, la situación se invierte y las hembras de *P. versicolor* responden casi exclusivamente a los flashes artificiales que imitan a los de sus machos presa ignorando los de sus machos coespecíficos. Se desconoce si otras especies de *femmes fatales* necesiten solo una cópula para cambiar al modo cazador, considerando que dentro de otros géneros de la familia Lampyridae es común la poliandria y que Eisner *et al*., (1997) experimentaron ofreciendo machos *Photinus* a hembras vírgenes de *P. versicolor*, quienes rápidamente los atacaron y comieron.

Según Lloyd (2018), el mimetismo agresivo es común en las *femmes fatales*. No obstante, es posible que las hembras cazadoras atraigan machos presa simplemente respondiendo a los destellos de éstos, utilizando el mismo patrón bioluminiscente que utilizan para responder a los machos de su especie. Jamie (2017) explica que en el ejercicio del mimetismo agresivo, la imitación de la señal puede requerir ser precisa solo en aquellos aspectos a través de los cuales el receptor de la señal la reconoce como similar a la señal real, abriendo la puerta a señales imperfectas, que funcionan para atraer machos presa.

Considerando lo anterior, la evolución del mimetismo agresivo dependería de la tendencia a aproximarse hacia una señal por parte de los machos presa (selectividad) y de la estructura de la señal en sí: tiempo de respuesta y número de señales que emiten las hembras. De acuerdo con Lloyd (1966), estos parámetros son esenciales para el reconocimiento intraespecífico y sexual de las especies de lampíridos que utilizan la bioluminiscencia para comunicarse. La precisión con la que se imitan los parámetros del patrón bioluminiscente de

las hembras de la especie presa por parte de las *femmes fatales*, podría evolucionar en función de la selectividad de los machos presa sin que necesariamente las señales lleguen a ser idénticas.

Las *femmes fatales* son un ejemplo clásico de mimetismo agresivo. Según Lloyd (1984), algunas de estas hembras son capaces de imitar con "cierto grado de refinamiento" la respuesta de las hembras de sus machos presa y en casos aún más extremos, son capaces de imitar el patrón especifico de las hembras de varias especies presa a medida que se les presenta la oportunidad (Lloyd, 1965, 1975, 2018). Los machos presa por su parte, podrían desarrollar contra-estrategias para evitar ser atraídos por señales falsas y depredados, tal como lo ha documentado Lloyd (1966, 1983, 1984). Lo anterior llevó al profesor Lloyd (1984) a considerar que el mimetismo agresivo de *Photuris* es una de las presiones de selección más importantes que afectan el comportamiento de señalización de las luciérnagas en el continente Americano.

Este proceso evolutivo podría ser el responsable de la complejidad y plasticidad frecuentemente observadas en los sistemas de comunicación de las especies de la familia Lampyridae (Lewis & Cratsley, 2008). Evaluar la estructura de la señal real y de la señal "imitada" podría ofrecer a través del grado de similitud una medida del grado de engaño, sin embargo, en escenarios evolutivos intrincados como el aquí descrito, una mejor manera de medir la eficacia de las señales engañosas de las hembras de *Photuris* es midiendo el éxito de atracción y captura de presas, considerando que se relaciona mejor con las presiones de selección y las adaptaciones desarrolladas por presa y depredador.

Casi la totalidad de las investigaciones que involucran depredación por alguna especie miembro de la subfamilia Photurinae, se han realizado al norte del continente Americano, principalmente en Estados Unidos, aun cuando la distribución del grupo y de las especies presa abarca norte, centro y sur de América (Lloyd, 2018). En nuestro país existe un amplio conocimiento acerca de la taxonomía y distribución de las especies pertenecientes a la familia Lampyridae (Zaragoza *et al*., 2020), pero se conoce poco sobre las interacciones ecológicas entre los miembros de la propia familia, puntualmente sobre las repercusiones demográficas y selectivas de la depredación por *femmes fatales* en las poblaciones de las especies presa. Por ejemplo, el efecto de esta interacción en las características de la población, su dimensión, la proporción de sexos e incluso la distribución espacial de machos y hembras de la especie presa. Evolutivamente hablando, una presión de selección como esta podría favorecer el cambio de las frecuencias fenotípicas en el comportamiento de los machos presa en función de disminuir los riesgos de ser depredados por *femme fatales*, afectando indirectamente el comportamiento de cortejo de la especie presa.

El **objetivo** de esta tesis fue describir la interacción de depredación entre *Photuris lugubris* y *Photinus palaciosi*, a través de observaciones de campo y datos experimentales recabados durante la temporada de cortejo de los años 2021 y 2022 y posteriormente evaluar el éxito de atracción y captura de las hembras de *P. lugubris* a través de observaciones de campo realizadas durante la temporada de cortejo del año 2023. Hasta donde sabemos, existen pocos estudios en México sobre las interacciones de depredación a través de mimetismo agresivo dentro de la familia Lampyridae (Lloyd & Ballantyne, 2003). La interacción de depredación por *femme fatale*, aquí descrita, toma una relevancia mayor al involucrar como presa a los machos de una especie sincrónica explotada como atractivo turístico en México.

insects

MDPI

Communication

Photuris lugubris Female Fireflies Hunt Males of the Synchronous Firefly *Photinus palaciosi* (Coleoptera: Lampyridae)

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Simple Summary: Adults of most fireflies do not feed, except for the females of several species of *Photuris*, which eat males of other firefly species. In most cases, female *Photuris* attract their prey by responding with glows or flashes to the male's bioluminescent signals, as if they were potential mates. For this reason, female *Photuris* are also called *femmes fatales*. In this paper, we provide evidence that the females of *Photuris lugubris* are *femmes fatales* of the males of the firefly *Photinus palaciosi*. We present different types of field observations suggesting that the females of *P. lugubris* attract males of *P. palaciosi* by responding to their bioluminescent signals and that, as a consequence, sometimes these are captured and eaten. We demonstrate experimentally that male *P. lugubris* are not predators of *P. palaciosi*. We also present experimental evidence that females of *P. lugubris* mate with multiple males and discuss the potential implications of this observation for the switch between mating and hunting behaviour. *P. palaciosi* is a firefly that reaches very high densities of flashing males during the reproductive season and that, intermittently, they synchronize their flashes providing a magnificent show that in the last few years has been the focus of tourist activities. Thus, our study also adds to the knowledge on the natural history of a species of economic interest.

Abstract: *Femmes fatales* (*Ff*) are female fireflies that hunt and feed on the males of other firefly species that they attract by responding with glows or flashes to their bioluminescent signals. Here, we present field observations demonstrating that *Photuris lugubris* females are *Ff* of male *Photinus palaciosi*, a synchronous firefly exploited as a tourist attraction in the mountains of central Mexico. We show that the hunting success of the *Ff* is low, as observed in previous studies, suggesting that the impact of predation on the prey population is low. We present experimental data showing sex-specific hunting behaviour, since only female *P. lugubris* fed on *P. palaciosi.* We also present experimental data showing that at least some female *P. lugubris* mate multiple times; we discuss the implications of this discovery for the switch between the mating and hunting modes of *Ff*. We discuss open questions, as well as the possible impact of *Ff* on tourism focused on synchronous fireflies.

Keywords: bioluminescence; deceptive behaviour; predation; behavioural dimorphism; polyandry; Mexico

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1. Introduction

Deception has evolved multiple times in the context of antagonistic interactions between species [1–4]. Members of the "deceiving species" transmit misleading information

("fake signals") to members of the "deceived species" which respond in a way that is detrimental for them, but beneficial for the deceiving species. The costs paid by the deceived species generate selection pressures that could start antagonistic coevolutionary races [5]. For example, many palatable insect species have evolved morphologies, odours and colour patterns that make them similar to toxic or dangerous species (Batesian mimicry) and help them deceive potential predators [1–4].

Femmes fatales (*Ff* hereafter) are female fireflies that hunt and feed on males of other firefly species [6]; the males of species with *Ff* have not been observed hunting or feeding on fireflies. Firefly *Ff* are a textbook example of deceptive behaviour [2,4,7,8]. *Ff* attract their male prey by responding with glows or flashes to their bioluminescent signals, as if they were potential mates. *Ff* behaviour has been observed exclusively in the subfamily Photurinae [9]. In North America, *Ff* have been observed in *Bicellonycha* [10] and in several (but not all) species of *Photuris* belonging to the group called Division II by Lloyd [9]. According to Lloyd [9], this behaviour is also present in other Neotropical genera of Photurinae. In the North American species studied, *Photuris* females mimic "with 'some degree' of refinement" [11] (p. 370) the bioluminescent responses of the females of their prey species (this is called aggressive mimicry), mainly members of *Pyractomena* and *Photinus* [9,11–13]. Although males of prey species can escape before or during the attack, the predatory females eat many of them [12] and acquire, besides nutrients, defensive steroids called lucibufagins that *Photuris* are unable to produce [14]. Lucibufagins protect adult *Photuris* from predatory spiders [13] and protect their eggs from insect predators [14].

Photuris females enter the *Ff* predatory mode after mating [15], frequently changing location from their own mating site to the mating site of their prey species during the daily mating period [9,10]. Nelson et al. [15] reported that *Photuris versicolor* virgin females respond almost exclusively to artificial flashes mimicking the bioluminescent signals produced by conspecific males, and ignoring artificial flashes mimicking those of their male prey (*Photinus macdermotti*). In contrast, once mated, *P. versicolor* females respond exclusively to artificial heterospecific male flashes, ignoring flashes mimicking those of males of their own species [15]. The posture of virgin and mated female *P. versicolor* during the daily mating period also differs, with mated females being apparently more alert and ready to attack: "erect posture, extended mandibles and elevated antennae" [15] (p. 629); a photograph of this posture is also on page 629 of this paper. However, it is not known if other species with *Ff* need only one mating to switch to the *Ff* mode, since fireflies of other genera (e. g. *Photinus*) mate multiple times with different males [16,17]. On the other hand, Eisner et al. [13] described experiments in which they offered *Photinus* males to virgin *P. versicolor* females, which quickly attacked and consumed the males.

According to Lloyd [9,11], aggressive mimicry seems to be very common in *Photuris Ff*. However, at least in theory, it is possible that *Ff* attract prey fireflies by simply answering male prey with the same bioluminescent response they use to answer their own courting males, even if this response is different to that of females of the prey species. The evolution of aggressive mimicry in *Ff* depends on the shape of the preference function of males, defined as the relation between the tendency to approach a signal and the "structure" (the intensity, duration and time intervals between flashes) of the signal. If males are very selective (i.e., if they only approach females emitting a narrowly species-specific bioluminescent signal), aggressive mimicry will be selected in *Ff*. On the contrary, if males of the prey firefly respond (approach) to a broad range of female signals, selection for aggressive mimicry will be null or very weak. Intermediate situations are possible, and factors such as the abundance of *Ff* (and, thus, the strength of the selective pressure exerted on prey fireflies) and the presence and abundance of other firefly species whose signals interfere with intraspecific communication in the prey species, will determine the position along this gradient of a particular species.

In this paper, we present field observations and experimental data showing that *Photuris lugubris* females are *Ff* of male *Photinus palaciosi*, a synchronous firefly exploited as a tourist attraction in Mexico [18]. We also present data showing that at least some female

P. lugubris mate multiple times before feeding on *P. palaciosi*. *Photinus palaciosi* is endemic to the mountains of Central Mexico [19], and its use in tourism is relatively recent [18]. To the best of our knowledge, this is the first report of an interaction between *P. palaciosi* and a species with *Ff*, and until now, it has been observed only in our study site (Rancho del Valle) near the town of Amecameca, Estado de México, México. For simplicity, we will refer to *P. lugubris* as "*Photuris*" and to *P. palaciosi* as "*Photinus*".

2. Materials and Methods

2.1. Field Study

The study area is a pine-oak forest within a private property named "Rancho del Valle", located in the Santiago Cuahutenco village, municipality of Amecameca, Estado de Mexico, Mexico. The main business of "Rancho del Valle" is tourism, including firefly sightings. The area of "Rancho del Valle" is 21 square kilometres, and although most of its area is occupied by forest, it has cabins, a restaurant, and other facilities. The fireflies were identified with the keys provided by Zaragoza-Caballero et al. [20] for the fireflies of Central Mexico.

Our observations and experiments were made during the reproductive seasons of two consecutive years: June 14th to July 21st in 2021, and May 18th to July 21st in 2022. In 2021 the reproductive season was already in progress when we started our observations, while in 2022 we started the study from the beginning of the season. Two to four observers per night walked along dirt paths between 20:00 and 24:00 h, a time interval that completely covers the nightly display period of *Photinus* and *Photuris* (~20:30–22:00 h). During the walks, we looked for female *Photuris* and once we located one, we recorded its activity. In some cases, we made focal observations of females, making voice or video recordings of their behaviour and interactions with *Photinus* or male *Photuris*. Most audio and video recordings were made with cell phones, although we also used a GoPro® Hero 9 camera. Most of our observations were made in the dark, and are based on the luminescent signals emitted by the fireflies. Occasionally, we briefly used the light of a lantern or of a cell phone screen to check species or behaviours. Our observations were made mostly at a distance of at least one meter. The fireflies, perched either alone or interacting with other individuals never stopped their activities or moved away from the site when we observed them. For the experiments, we collected female *Photuris* and male *Photinus* (experiment 1) or *Photuris* couples and males (experiment 2), by hand or by using an entomological net. We took the collected specimens to the lab (less than 15 min away) in 125 mL plastic containers, one firefly per container.

2.2. Experiment 1: Do Male and Female Photuris Feed on Photinus Males?

In 2021, between June 18 and July 13, when female *Photuris* were frequently found in *Photinus* display areas, we captured *Photuris* females (N = 16) and males (N = 10) and kept them individually in ~1 litre empty plastic containers. The same night they were captured, we introduced one field-captured *Photinus* male to each of the containers. We maintained the containers in the dark and inspected them briefly and intermittently under a dim light throughout the night or until the *Photinus* male was eaten. We exposed all experimental *Photuris* just one night to a *Photinus* male and returned the live specimens to the field site after the experiment.

2.3. Experiment 2: Do Female Photuris Mate Multiple Times?

In 2022, we captured fourteen *Photuris* females at the beginning of their mating season (between May 28th and June 8th), twelve in copula and two when courting with a male (these couples mated in captivity the night they were captured); some of these females could have mated before. We kept the females individually in 1.1 litre plastic containers with a layer of soil—collected from the study site—in the bottom and $\frac{1}{4}$ of a small apple as a source of liquids. On consecutive nights, we exposed the females individually to a new male *Photuris*, also captured in the field. We observed the couples briefly and intermittently,

under a dim light, until 1–1:30 am—three or four hours after the natural mating period—or before if they mated.

3. Results

3.1. Photuris lugubris Females Are Femmes Fatales of Photinus palaciosi Males

We made several field observations supporting the hypothesis that *Photuris* females are *Ff* of *Photinus* males. First, having studied *Photinus* during several years in other localities, mainly in the state of Tlaxcala, in our first year of study (2021) in Rancho del Valle, we did not expect to find *Photuris* in our study area. In fact, a recent article synthesizing information about the firefly species known from México and their distribution within the country did not report *Photuris lugubris* from the Estado de México [19], the state where our study site is located. However, in the first year of study (2021), we observed male and female *Photuris* in their mating display area, and later in the season, mainly females in or near *Photinus* display areas (see Section 3.2). Before knowing that it was a *Photuris* species, on at least 11 occasions we confused a *Photuris* female for a *Photinus* female in display areas of the last species.

Secondly, we observed several *Photuris* females perching on plants at different heights (from low grasses to leaves at 2 m in height) attracting and interacting at close range with *Photinus* males. We recorded this on 20 occasions (8 females in 2021 and 12 in 2022). In some of these cases, we observed *Photuris* females emitting glows (faint flashes that extinguished gradually) or flashes in response to (i.e., in the direction of) the male *Photinus* passing flashing, sometimes at distances as far as 2 m; when emitting flashes, females sometimes put their lanterns close to the leaf they were perching on, thus reducing the amount of light beholders could perceive. Some male *Photinus* responded by flying in the direction of the female. Some of these males landed on plants near the female ($\leq$50 cm) and started flashing interactions with her, while other males stayed flying around the female, sometimes as close as 30 cm. In other cases, we observed *Photuris* females when they were already interacting with one or more *Photinus* males that were perched on nearby plants or flying around the female. During these interactions males emit flashes that the female responds with glows and flashes, however, not all *Photinus* flashes are responded to. In some interactions, the males came gradually closer to the female, usually walking or "jumping" on plants. We also observed females "jumping" on males when they were very close. Interestingly, males frequently "jumped down" when the female approached, or jumped in their direction, when they were a few cm from the female. The interactions between a female *Photuris* and an individual male *Photinus* can last just a few instants, but sometimes they extend for several minutes (our longest observation was up to 40 min). According to our observations, many (most?) of these interactions were unsuccessful hunting events. We have two clear examples of this in two females that interacted with several males in a relatively short period of time (our observations suggest that the rate of interaction varies broadly): (a) During 21 min of continuous observation, one female *Photuris* interacted with six *Photinus* males without success, even though she approached two of the males with her lantern off when they were close to her (these males jumped down from their perches before being captured); (b) During 36 min of continuous observation, one female *Photuris* interacted with five *Photinus* males unsuccessfully, although one of them barely escaped.

Thirdly, we observed five *Photuris* females (two in 2021 and three in 2022) that had already captured and were feeding on a male *Photinus*. Female *Photuris* frequently emit light when feeding on *Photinus*; we never observed *Photinus* flashing when attacked by *Photuris* (in contrast, males flash continuously when wrapped in spiderwebs). Finally, we observed four predation events from the moment the male approached the female until he was captured and eaten (two in 2021 and two in 2022), and one event (in 2021) in which the female captured the male and then lost him. As mentioned above, the female approached the male with her lantern off and captured the males, sometimes jumping on them.

3.2. Hunting Photuris lugubris Females Move to the Display Areas of Photinus palaciosi

The annual mating season of *Photuris* starts and finishes earlier than that of *Photinus*, although there is some overlap (personal observation). Our observations indicate that *Photuris* males emerge a few days before females (i.e., they are protandrous). In 2022, the first males were observed on May 12th, while the first females were observed on May 28th. At the beginning of the mating season, *Photuris* courts (i.e., perform bioluminescent displays and male–female interactions) exclusively on a slightly sloped area of a little less than 1 ha, where pine trees were planted more than 20 years ago; despite their age, these pines are small (<2m tall). When the mating season of *Photinus* begins, *Photuris* females start appearing in or near the display areas of *Photinus* (the first hunting female was observed on 9 June), sending bioluminescent responses to the displaying heterospecific males. *Photinus* also appears to be protandrous (personal observation). At the beginning of its mating season, *Photinus* mainly courts in a few, localized display areas (that do not coincide with the pine plantation display area of *Photuris*), and then later in the season, the display area expands to different parts of the forest. Accordingly, *Photuris* females are also present in these display zones at these times.

3.3. Only Female Photuris lugubris Eat Photinus palaciosi Males

None of the *Photuris* males confined with a *Photinus* male for one night (N = 10) killed or ate the *Photinus*, and although we only inspected them intermittently, they were never observed attempting to attack (Figure 1). In contrast, 15 out of 16 *Photuris* females (~94%) attacked the *Photinus* male and 13 of them ate the male (Figures 1 and 2); one female attacked and then left the male alive—possibly because he faked death—and another female captured and then lost the male due to human disturbance. Although we only intermittently inspected the female containers, in several cases we observed *Photinus* males forcefully trying to escape, sometimes succeeding. However, eventually most were eaten, probably because they were confined with the female for several hours.

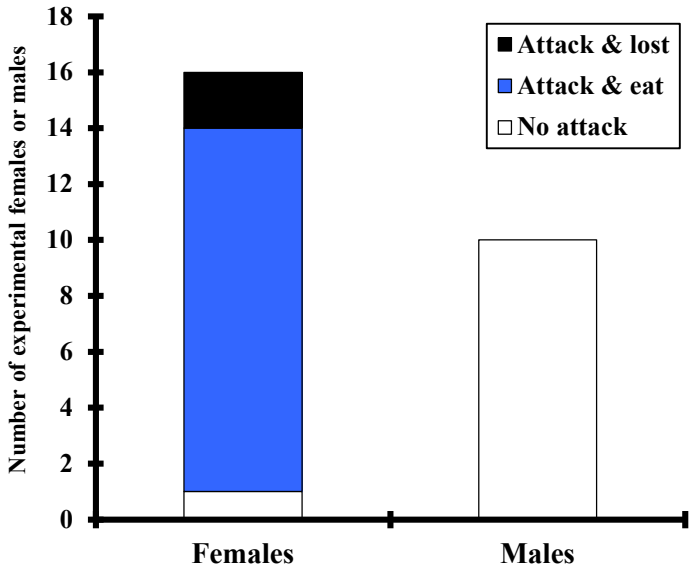

Figure 1. Number of experimental, field-collected *Photuris lugubris* females and males that did not attack, attack-and-ate or attack-and-lost the male *Photinus palaciosi* introduced to their containers.

3.4. At Least Some Photuris lugubris Females Mate Multiple Times

We exposed fourteen mated females to between three and six males in captivity. Six of these females (42.9%) mated a second time and two of these re-mated females mated multiple times (three and six times in total, respectively) (Figure 3). Besides the fact that some of the females could have mated before we captured them for the experiment, a significant, positive correlation between the number of nights a female was exposed to

a male and the number of copulations achieved (Spearman rank correlation: $r_s = 0.65$, $p = 0.011$, $n = 14$; Figure 3) suggests that we underestimated both the proportion of re-mated females and the number of matings per female.

Figure 2. Experimental female *Photuris lugubris* (right) feeding on a male *Photinus palaciosi* (left). Notice that the prey is almost decapitated.

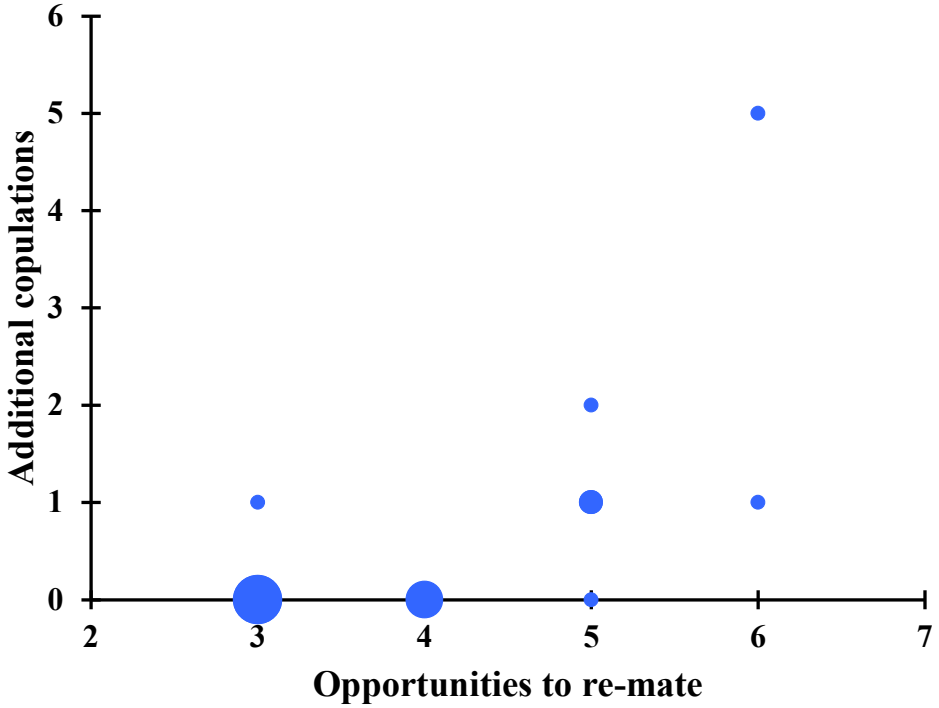

Figure 3. Number of copulations performed in captivity by females collected in copula, as a function of the number of opportunities to re-mate given in captivity. The positive correlation is statistically significant (Spearman rank correlation: $r_s = 0.65$, $p = 0.011$, $n = 14$). The size of the blue dots indicates the number of females (smallest dot: one female; largest dot: four females).

4. Discussion

Our observations show that female *Photuris lugubris* prey on *Photinus palaciosi* males in our study site. Female *Photuris* attract males by responding with glows and flashes to the bioluminescent signals of male *Photinus*, and then try to capture them once they are close. So, the behaviour of female *Photuris* fits the definition of a *Ff* [6,21]. Published discussions of *Ff* biology, e.g., [6,11,12,21], appear to suggest that species with *Ff* exhibit aggressive mimicry, however, as discussed in the introduction, this is not a necessary condition. We have not made a quantitative comparison of the bioluminescent signals displayed during courtship by *Photinus* females with those produced by *Photuris* females when courting and hunting, and thus we still do not know if the *Ff* of our study exhibit aggressive mimicry.

Although the hunting success of *Photuris* females in captivity was high—probably because they were confined in a relatively small space—our preliminary field observations suggest that the hunting success of *Photuris* females is relatively low, as we attested more failed than successful interactions. In most cases in which *Photinus* males interacted with a *Photuris* female while in flight or perched on a plant, either they never approached close enough to be attacked or they escaped by dropping from their perch when the females approached them. Our observations qualitatively agree with previous studies, estimating that between 10% and 15% of hunting attempts result in prey capture [12,22].

Our experimental results show that only female *Photuris* prey on *Photinus* males, as neither experimental (Figure 1) nor field-observed males attacked or fed on male *Photinus*. Although Eisner and collaborators have shown that by feeding on *Photinus Ff* obtain a type of defensive steroid (lucibufagins) that protects the female and their eggs from several predators [13,14], our observations indicate that *Photuris* consumes most of the soft parts of the body of their prey and thereby should also obtain energy and other nutrients from *Photinus* males. There are few studies on the number of prey eaten by *Ff* in the wild (according to Lewis [21], females of some species are capable of eating several males per night, and Lewis et al. [23] report that one female *Photuris* ate eight out of 11 *Photinus* males offered in captivity over a period of seven days), and we have not found any publication on the effect of the variation in the number of prey eaten on different components of their reproductive success. Why males do not feed on *Photinus* males is also an intriguing question that remains to be answered.

A previous study suggests that females are either in a sexually receptive state or in hunting mode [15]. However, if females mate multiple times and capture multiple prey, it is possible that they experience an intermediate phase in which they are still sexually receptive but are already trying to feed on *Photinus* males. We do not know if in our study site female *Photuris* attempted to eat several *Photinus* males, but we know that at least some females mated multiple times (Figure 3). We also observed one female *Photuris* courting with a male that on several occasions responded to the signals emitted by *Photinus* males flying nearby; the signals employed by the female while courting and when responding to *Photinus* males were clearly different.

Finally, measuring the impact of *Photuris* predation on *Photinus* demography is an interesting question for evolutionary and applied reasons. Lloyd [11] (p. 370) considers that predation by *Photuris* females is "probably one of the most important selection pressures affecting firefly signalling behaviour in the Western Hemisphere". On the other hand, *Photinus palaciosi* is a synchronous firefly that is the focus of growing tourism activities in Central Mexico [18,24]. Since the success of firefly-watching tourism depends strongly on the large numbers of flashing fireflies, typically of synchronous fireflies, one could ask about the risks of severe population declines resulting from *Photuris* predation, both in our study area (the only site known to date in which *Ff* attacking *P. palaciosi* has been observed) or in other *P. palaciosi* populations where *Photuris* could exist or invade. The large numbers of *Photinus* males relative to the number of *Photuris* females observed in our study site, together with the apparently low hunting success, suggest that under the present conditions, the effect on *Photinus* mortality is small. However, the quantitative investigation

of the demographic effects of *Ff* on their prey and modelling studies of different scenarios (for example, a population explosion of *Photuris*) seem worth pursuing.

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Article

Deceptive Seduction by *Femme Fatale* Fireflies and Its Avoidance by Males of a Synchronous Firefly Species (Coleoptera: Lampyridae)

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Simple Summary: Predatory female fireflies of several species in the genus *Photuris* attract males from other firefly species by responding to their flash signals, and then, the females try to capture and feed on the attracted males. Predatory female *Photuris*, called "femmes fatales", are considered a serious threat to males of other firefly species; however, there are very few quantitative data on the strength of this menace. We measured the attraction of *Photinus palaciosi* males to predatory females of *Photuris lugubris* in the field, as well as the number of prey captured. We observed that females, in general, attract several males of the prey species but capture relatively few; only nine out of 92 (9.8%) observations of predatory females resulted in successful capture. Our observations show that the low hunting success of *Photuris* females could be explained in part by male behaviors. First, the males attracted behave in a way that we call "cautious" or "hesitant", which could prevent attacks, and second, these males also behave in ways that probably reduce the success of attacks at close distance, such as approaching in flight and "dropping" in the face of an imminent attack. Female *Photuris* also behave in ways that probably improve their success in attracting and capturing prey, such as perching in or near display areas of their prey species, and partially concealing their own lantern on the substrate to avoid revealing their much larger size and/or to better mimic the responses of *Photinus* females. Our observations are thus consistent with the idea that predators and prey are engaged in a coevolutionary race of adaptation and counter-adaptation.

Abstract: *Photuris* female fireflies attract males of different firefly species by responding to their flashing signals; then, they try to capture and feed on them. This aggressive mimicry is considered a major selective pressure on the communication systems of the fireflies of the American continent. The intensity of this selective pressure is a function of its efficiency in prey capture. In this study, the rates of attraction and capture of males of the synchronous firefly *Photinus palaciosi* by the predatory females of *Photuris lugubris* are reported. Although the females attract numerous males, their hunting success is low. This result is consistent with the few previous measurements published. In agreement with the predicted coevolutionary race between predator and prey, behaviors consistent with predation avoidance in *P. palaciosi* and increasing prey encounters and prey deception by *P. lugubris* were observed.

Keywords: aggressive mimicry; deception; hunting success; predation avoidance; sexual signaling

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1. Introduction

Deception is a ubiquitous feature of life [1,2]; evolutionarily speaking, deception has been selected to improve escape from natural enemies [1,3] and increase reproduction (as in pollination by deceit [1]) or food acquisition [1]. A fascinating example of interspecific deception is aggressive mimicry, which occurs when "the mimic signals a fitness benefit to the receiver and the mimic's signal is deceptive" ([4], p. 4). Aggressive mimicry as an aid to capture prey has been described in a wide range of animals, including spiders [5], aphids [6], katydids [7], fish [8,9], and *Homo sapiens* [10]. In North America, predatory firefly females from species belonging to three genera of the subfamily Photurinae (*Bicellonycha*, *Crematogaster*, and *Photuris*) emit bioluminescent responses to the flashing signals of males of other firefly species (several species of *Photinus* are common prey, although males from other genera, including other *Photuris* species, are also hunted); in this way these "femmes fatales" attract the males and then try to capture them and feed on them [11,12]. By feeding on the males of other species, predatory females not only obtain nutrients and energy but also defensive steroid pyrones called lucibufagins (molecules that *Photuris* cannot produce) that protect the female [13] and her eggs [14] from predators. Thus, firefly femmes fatales [11,12,15] are classical and extreme examples of aggressive mimicry: classical because the femme fatale sends fake signals indicating the presence of a sexually receptive female and extreme because it can result in the deceived individual being killed.

In many cases, *Photuris* females mimic the specific response signals of the females of the prey species [12,15], and individual females of some species have been shown to mimic the specific response of females from several species depending on the identity of the potential prey [12,15,16]. These studies led the most important researcher of Photurinae and femme fatale behavior, the late Prof. James Lloyd, to think that femmes fatales are the source of selective pressures that have been crucial for the evolutionary divergence of bioluminescent signals in prey firefly species of the American continent ([15]; see also [12,15,17,18]). Females of predatory *Photuris* have morphological adaptations apparently designed to subdue and consume their prey [19], and recent molecular studies have identified genes involved in resistance to lucibufagins [20] and several candidate genes that could represent diverse physiological adaptations for the consumption of potentially toxic prey [21]. Thus, it is expected that *Photuris* predation is the source of selective pressures promoting the evolution of countermeasures in their prey fireflies [15,18], which resulted in a coevolutionary process that could be responsible for the complexity and plasticity frequently observed in the communication systems of fireflies [12,16,18,22–26]. Since, in many places, several potential prey firefly species coexist, sometimes with more than one species of femmes fatales, the coevolutionary process, rather than a product of pairwise interactions, is more likely to be of the type known as "diffuse coevolution" [27,28]. In this context, some prey counter-adaptations could select for an increased degree of deceptiveness in the signals produced by *Photuris* females. This has led to the study of the match between the response signals produced by females of prey species and the deceptive responses emitted by femmes fatales, implicitly considering the degree of match as a measure of the degree of deceptiveness (see review in [12]). However, a perfect copy of the signal is not necessary for successful mimicry; instead, only the components used by the receiver to assess the signal need to be mimicked [4].

On the other hand, since aggressive mimicry selects for male prey counterstrategies to avoid being attracted and captured, a more direct way of measuring the efficiency of the deceptive signals of *Photuris* females is measuring their success in attracting, capturing, and consuming prey. These measurements are more directly related to the selective pressures exerted and the adaptations developed by both predator and prey. When the courting areas of femmes fatales and prey fireflies do not overlap, it is reasonable to expect that hunting females move to places where the probability of finding prey is higher and then start to attract and attempt to capture prey. Previous studies (summarized in [12]) and a recent study of *Photuris lugubris* [29] indicate that mated females move from their

own courtship display areas to, or close to, the courtship display areas of their prey fireflies. However, the consequences of this movement for prey encounters, although apparently obvious, have not been quantified. Similarly, rates of prey attraction and capture by firefly femmes fatales are rarely reported. Lloyd [16] studied *Photuris versicolor* hunting two species of *Photinus* and two species of *Photuris* in the field and observed wide interspecific variation in the rates of capture of the different prey species (4%, 9%, 20%, and 54%). However, Lloyd did not report or discuss the causes of this variation. In a laboratory experiment, Lewis et al. [30] observed large differences in the consumption rates of females of several *Photuris* species confined with males from eight species of Lampyridae, including five species of *Photinus* (one of them, *P. carolinus*, a synchronous firefly). The causes of this variation were not determined, but the fact that the species with low consumption rates showed evidence of having been attacked suggests between-prey differences in defensive chemistry, nutritional value, or ability to escape once captured. Furthermore, since only 11 females from at least three *Photuris* species were studied, interspecific differences in hunting ability cannot be ruled out. Finally, Lloyd [12] also reported an observation of one female *Photuris carrorum* that answered the signals of twenty-five male *Photinus macdermotti* before capturing one (a success rate of 3.8%), although he did not mention if the twenty-five males that were not captured approached the female or if they were unsuccessfully attacked.

In this paper, we report field measurements of rates of attraction and capture for male *Photinus palaciosi*, a synchronous firefly from the mountains of Central Mexico [31], by *Photuris lugubris* femmes fatales [29]. This study is part of a broader project aimed at understanding the evolution of the femme fatale behavior of Photurinae fireflies.

2. Materials and Methods

2.1. Firefly Natural History in "Rancho del Valle"

This study was conducted in the pine–oak forest of "Rancho del Valle", a private ecotourism ranch located in the village of Santiago Cuauhtenco, Municipality of Amecameca, Estado de México, Mexico. This section is based on observations made during three mating seasons (2021 to 2023). The mating season of *P. lugubris* (*Photuris* hereafter) starts about the middle of May, when males begin flying and signaling (flashing); one or two weeks after, the first females are observed perching in vegetation at heights ≤2 m exchanging signals with conspecific males (i.e., the species is protandrous). During this period, the fireflies occupy a relatively small section of the ranch (<1 ha) dominated by grasses and planted pine trees of heights ≤2 m; larger trees are virtually absent in this area [29]. Both males and females can fly, but females remain perched during the nightly courtship period. Most of the courtship and mating activity occurs between 19:30 and 22:00 h (although a few individuals have been observed as early as 19:00 h and later than 22:00 h). To the human eye, males and females are very similar and can be distinguished only by their behavior and the type of bioluminescent signals they produce: males produce intermittent flashes while flying, and the perching females respond with glows produced at much lower rates. When a male succeeds in approaching a female, he briefly touches her with his front legs and antennae and quickly tries to mount her and copulate. The mating season of this species ends about the second week of June; after this time, females move to areas where male *Photinus* are common and start hunting them using aggressive mimicry [29], while male *Photuris* are only occasionally observed courting hunting females. However, in 2023, the rainy season started late in Mexico, adult emergence dates were somewhat delayed, and more males were observed courting hunting females. The first author had the impression that these males were more "cautious" when courting hunting females, and the signaling interactions lasted much longer. Previous studies led us to think that the hunting females were already mated [32,33]. However, females mate multiple times [29], and thus, it is possible that some hunting females are still sexually receptive. In support of this hypothesis, five males were observed mating with hunting females, and

in one case, the female discarded the partially eaten prey to mate (this was the first female observed capturing a male in 2023). In contrast to the mating period, during the hunting period, female *Photuris* are observed until 23:00 h. The last *Photuris* females can be observed between the second and third week of July.

P. palaciosi (*Photinus* hereafter), a synchronous firefly, is a protandrous species (females appear around a week after the first males) whose females cannot fly because they have reduced wings (i.e., they are brachypterous) [31]. The mating season of this species starts between the first and second week of June, so males appear approximately when *Photuris* females start hunting. Courtship and mating occur more commonly in areas covered by the foliage of trees, all over the forest. Males usually fly at heights ≤2 m, flashing intermittently in search of females perching in low vegetation (<0.5 m). Females respond with glows produced at much lower rates. When a male succeeds in exchanging signals with a female, he flies toward the female, lands close to her, and finally approaches walking. Once he contacts the female, he starts tapping the dorsum of her body with his legs and antennae in a way suggesting contact courtship; this interaction can extend for several minutes until copulation ensues or until the male departs, possibly because the female rejects copulation (females must cooperate by opening her valves to allow intromission of the aedeagus). Although a few males can be observed flying as early as 19:00 h, most of the courtship activity occurs between 19:30 and 22:00 h, with a few signaling individuals observed later. The mating season of *Photinus* finishes about the first week of August.

The two focal species of this study coexist with a third firefly, *Photinus extensus*. This species is less abundant than the others, it is possibly protandrous, and its females are also brachypterous. Males of this species begin to be observed by the middle of June, and its mating season finishes by the end of July. The nightly courtship period is shorter than that of the other two sympatric species, between 19:00 and 21:00 h. The flying males emit intermittent flashes that are responded to with glows by the females perched on herbs at heights ≤ 0.5 m. The courtship and mating of this species were observed all over the forest. In three consecutive mating seasons (2021–2023), *Photuris* females have never been observed attacking or feeding on male *P. extensus*; in fact, no male *P. extensus* has been observed flying toward or inspecting a female *Photuris*. P. *extensus* males are larger than *Photuris*, which, in turn, is larger than *P. palaciosi*. The first author once exposed a male *P. extensus* to a female *Photuris* (in a container similar to those previously used to test that *Photuris* females feed on *Photinus* males [29]) and observed that the female approached the male only once without attacking, and the male was alive and intact the next morning (in contrast, 15 out of 16 *Photuris* females attacked the *P. palaciosi* males they were confined with).

2.2. Field Study

From June 30 to July 22, 2023, between 19:00 and 23:00 h (an interval that covers the nightly display period of *P. palaciosi* and *P. lugubris*), two to four trained observers walked along paths in the forest looking for *Photuris* females. The paths were those where fireflies have been observed in previous years [29] and are usually located in or near *Photinus* courtship sites (personal observation). Once a female *Photuris* was found, usually by detecting one of her glows, one observer continuously monitored the behavior and interactions of the female (thus, we used the focal sampling method [34]) from a distance between 50 cm and 1 m until the female either flew away from the site or stopped performing relevant activity (i.e., responding to male flashes), which usually occurred after the nightly courtship display period of *Photinus*. Since the population of *Photuris* is small (personal observation), and it is the only one that has been found so far coexisting with *Photinus palaciosi*, we decided not to mark *Photuris* females to prevent potential negative effects on the fireflies. Furthermore, hunting females are easily disturbed and fly away, drop to the ground and hide, or stop producing signals when touched. The observations were recorded with the audio-recorder app of the observer's cell phone; occasional photographs and short videos were also taken.

The number of *Photinus* males that flew within 3 m around the female was recorded. These males were usually detected via their flashes; this distance was used because Lloyd ([11], p. 654) mentions that female fireflies rarely answer to flashing males at distances greater than 3 m. These numbers allowed us to measure the effect of local prey density on the attraction and capture of prey and, thus, indirectly, the effect of female location on hunting success. The following behaviors were recorded: (1) *Communication*: An event of communication was considered to occur when individual A (*Photinus* flying male or *Photuris* perched female) responded with a flash or glow to a signal emitted by individual B (*Photuris* perched female or *Photinus* flying male), with B then responding with a glow or flash to the signal emitted by A; the pair could continue exchanging signals as a part of the same communication event. A female *Photuris* was considered to answer a specific *Photinus* male when she responded with a glow to one of his flashes and moved her lantern in his direction (this behavior is like that observed during courtship, both in *Photuris* and *Photinus*). If the female had more than one male *Photinus* flashing around her, the female was considered to answer if she produced glows in response to some of the flashes; in these cases, she usually directs her lantern to a (preferred?) male. A male *Photinus* was considered to answer a female *Photuris* when he produced flashes after the female glowed and remained close to her (<1 m). (2) *Approach*: A male was considered to approach a female when he changed the trajectory of his flight in the direction of the signaling female during or after a communication event; the male could approach the female flying or, at some point, alight on the ground or on a plant and approach walking. It was recorded if the male approached the *Photuris* female at 5 cm or less because these are the more frequent distances at which we have observed females initiating an attack. (3) *Attack*: The female suddenly approaches a nearby male (usually when the distance between them is ≤ 5 cm), either walking or jumping, and tries to capture him. Although the attacked males usually exchanged signals with the female before the attack, the female turned off her lantern sometime before the attack. It was recorded if the male escaped the attack or if he was captured and eaten by the female. Female *Photuris* eat most of the tissues of their prey, discarding only the exoskeleton; eating one prey takes several hours.

2.3. Statistical Analyses

The data were analyzed considering that hunting is a sequential process in which females first locate themselves in areas of higher probability of finding prey and then attract males and finally attempt to capture them. The potential relationships between the following variables were calculated with Pearson correlations: (1) the number of males passing at a distance ≤3 m from a *Photuris* female and the number of males that approached that female to a distance ≤5 cm; (2) the number of communication events involving a female and the number of males that approached that female to a distance ≤5 cm. These correlations measured the potential effect of local prey density (and thus, indirectly, female location) and the aggressive mimicry of signals on the attraction of prey within attack distance. Fisher's exact tests were used to investigate the potential relationship between (3) the number of males that approached a female at a distance ≤5 cm and the probability of a successful attack and between (4) the number of attacks performed by a female and the probability of a successful attack. All tests were performed using the open-access statistical software available on the website www.socscistatistics.com, accessed on 2 October 2023.

3. Results

A total of 92 observations of *Photuris* females were made. Since the females were not marked, it is not known how many different females were studied. Some females were observed in, or very close to, places where females were observed on previous nights, and for this reason, we suspect that at least some females were observed on more than one night. The total time looking for females was 153.2 h, and the amount of time observing *Photuris* females was 56.8 h, for a total of 210 h of fieldwork.

Prey density, measured as the number of male *Photinus* observed flying at 3 m or less from the female *Photuris*, had a positive effect on the number of males that approached the female close enough to risk being attacked (≤5 cm) (Figure 1; $r = 0.46$, $p < 0.00005$, $n = 71$; sample size is less than 92 because one of the observers did not record the number of males passing at a distance ≤3 m). The number of communication events, a direct measure of aggressive mimicry, also had a positive effect on the number of males that approached a female *Photuris* at attack distance (Figure 2; $r = 0.65$, $p < 0.00001$, $n = 71$). Although in some cases males approached females without a previous communication event (for example, sometimes they appeared to be attracted by the flashes of other males close to the female), in 83 of the 92 observations (90.2%), there was at least one communication event between a female *Photuris* and a male *Photinus*.

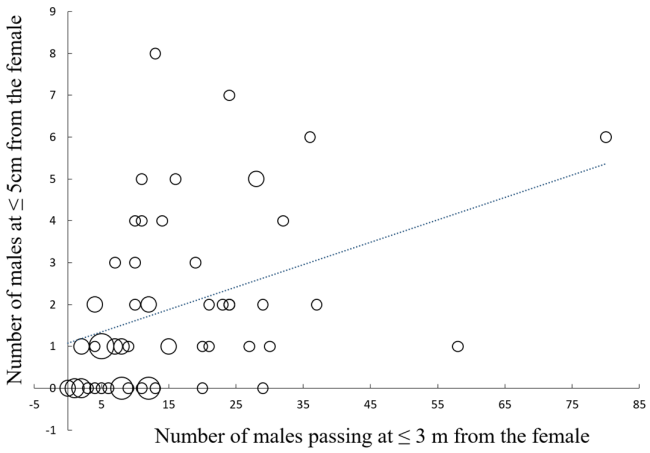

Figure 1. Relationship between the total number of *Photinus palaciosi* males observed flying within 3 m or less of a predatory female *Photuris lugubris* during a nightly courtship period and the number of males approaching that female at 5 cm or less. The size of the circles is proportional to the number of females represented by the circle (1, 2, 3, 4, or 5). The correlation was positive and statistically significant ($r = 0.46$, $p < 0.00005$, $n = 71$).

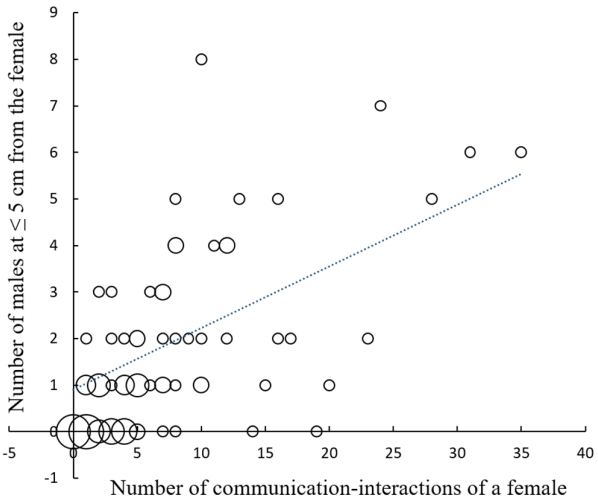

Figure 2. Relationship between the number of "communication" events performed by a predatory female *Photuris lugubris* during a nightly courtship period and the number of *Photinus palaciosi* males approaching that female at 5 cm or less. The size of the circles is proportional to the number of females represented by the circle (1, 2, 3, 4, 5, or 9). The correlation was positive and statistically significant ($r = 0.65$, $p < 0.00001$, $n = 92$).

Only 9 of the 92 observations (9.8%) ended in successful hunting. The number of successful attacks was independent of the number of *Photinus* males that approached a female *Photuris* at an attack distance (≤5 cm) (Figure 3; Fisher's exact probability test, $p = 1$). Although females were observed attacking males up to six times in a night, all nine successful hunts occurred in the first attack of the night (Figure 4; Fisher's exact probability test, $p = 0.041$).

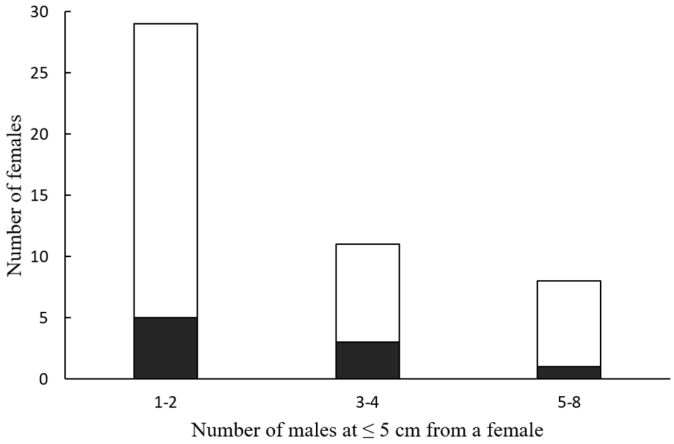

Figure 3. Number of *Photuris lugubris* females that attracted a given number of *Photinus palaciosi* males at 5 cm or less and number of these females that captured one male (shaded area). Fisher's exact probability test was not significant ($p = 1$).

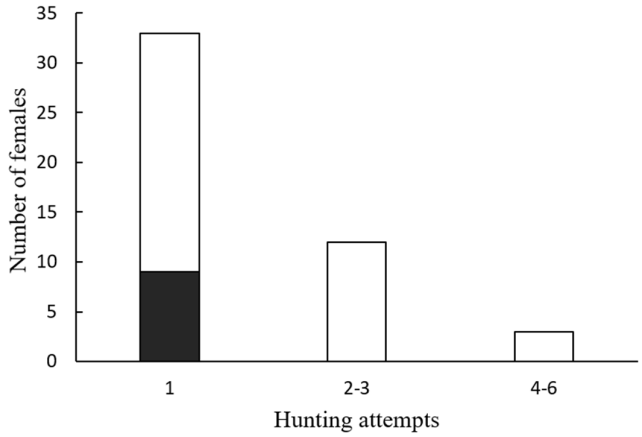

Figure 4. Number of *Photuris lugubris* females that attacked a given number of *Photinus palaciosi* males and number of these females that captured one male (shaded area). Fisher's exact probability test was significant ($p = 0.041$).

The behavior frequently observed during *Photuris–Photinus* interactions helps us understand the low rates of hunting success. *Photinus* males attracted by *Photuris* females behave as if they are "hesitant" (or very "cautious") to approach *Photuris* females because they frequently remain in flight exchanging signals for several minutes at a relatively close range (<1 m) but without further approaching. This behavior is different from the direct male approaches observed when *Photinus* males court females of their own species (personal observations). Also, males frequently, upon alighting, approach the females slowly, in contrast to the faster approximations observed in sexual interactions between male and female *Photinus* (personal observations). Generally, when a female *Photuris* approaches a *Photinus* male that is flying or perching close to her, he suddenly "drops" several centimeters, possibly to avoid

contact; in these cases, the female does not follow the male. On the other hand, hunting females appear to partially conceal their lanterns by pushing them against the surfaces of their perches (usually leaves), which makes sense considering that female *Photuris* are much larger than *Photinus* females, although this behavior could also help them to mimic the response pattern. Additionally, sometime before an attack, females usually turn off their lanterns. This behavior led one of the reviewers of this paper to the reasonable suggestion that the hunting behavior of *Photuris* is best described as a combination of aggressive mimicry and, in the instants before the attack, stalking.

Besides the nine females observed hunting and feeding on their prey, four other *Photuris* females were found already feeding on a male *Photinus*. Most of the observations of successful hunting occurred during the first 10 days of observation (10/13; Figure 5), approximately corresponding to the first half of the sampling period. Since there were fewer observers per night in the second half of the study season (Figure 5), this number could be an underestimate, although the observers had the impression that the number of predatory females was decreasing during the second half of the study period. Most of the successful attacks (8/9) were observed before 21:00 h (Figure 5), which is consistent with the observation that successful attacks were the first attacks of the night (Figure 4).

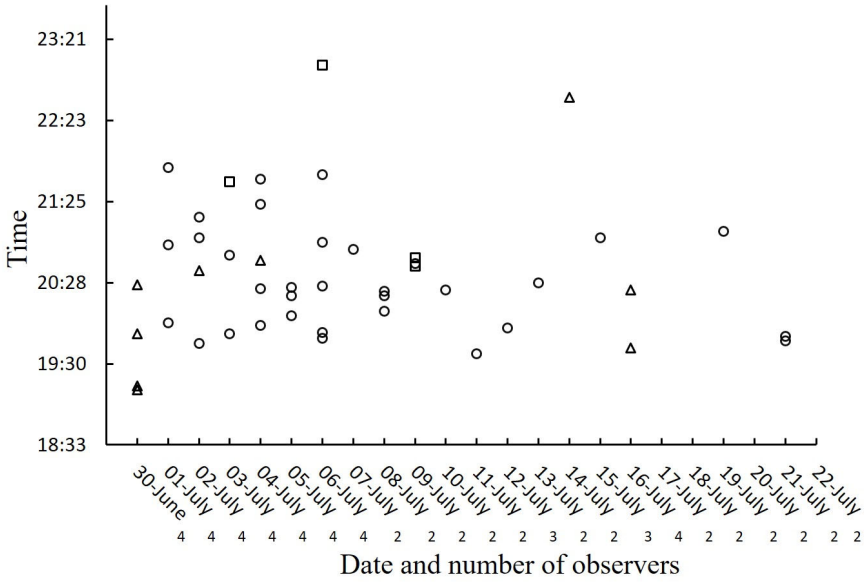

Figure 5. Timing of predator–prey interactions between *Photuris lugubris* females and *Photinus palaciosi* males. Distribution of unsuccessful (the male escaped; circles) and successful (the male was captured; triangles) attacks in relation to the time of the night and date; squares correspond to females that had already captured a male when first observed. The number of researchers in the field is given for each night below the date.

4. Discussion

Since the courting and mating area of *Photuris* in the study site is very small and barely overlaps with the courting area of *Photinus*, hunting females move to, or close to, the courting areas of their prey species [29]. The observations reported here indicate that the relocation of *Photuris* females during the hunting period is important to attracting more potential prey (Figure 1). Previous studies (summarized in [12]) indicate that mated females of other predatory firefly species also move from their own courtship display areas to, or close to, the courtship display areas of their prey fireflies; however, the quantitative data (see Supplementary Materials) presented here are the first documenting a possible advantage in female relocation. The location of females within, beside, or between

the congregation sites of *Photinus* males—as well as variations in the number of males in these congregations and in the number of responding *Photinus* females and other "competing" *Photuris* females—could result in variations in the number of males interacting with females. The results also provide one of the few published quantitative measures of the importance of firefly aggressive mimicry for attracting prey since *Photuris* females attract *Photinus* males mainly by responding to their flashing signals (Figure 2). However, males apparently are also attracted to other males flashing at *Photuris* females in close range (see [35] for a similar observation). We observed that the glowing responses of *Photuris* females to males of their own species are different from the signals used to attract *Photinus* males (personal observations). It is also interesting that *Photuris* females apparently partially conceal their lanterns by pushing them against the surfaces of their perches as if trying to look smaller, like *Photinus* females. It will be interesting to test this hypothesis. These observations are consistent with the existence of aggressive mimicry in this species. Detailed studies comparing the courtship signals of both species and the hunting signals of *Photuris* are currently underway.

Although *Photuris* femmes fatales attract a considerable number of potential prey at attack distance (Figure 2), their hunting success is low, as only 10% of the females were observed capturing and feeding on a male *Photinus* (Figure 3). These observations are consistent with low success rates documented in most of the few previous studies on attraction and hunting success in *Photuris*, as noted in the Introduction section. Some species of *Photuris* femmes fatales have been observed employing alternative hunting tactics [12,17,30,36,37], and thus, if *P. lugubris* females also exhibit some of these behaviors, it is possible that the low success rate documented in this study could be an underestimation of the actual rates of capture. Two of these strategies involve eavesdropping on the bioluminescent signals produced by their prey: "stalking" and "aerial hawking" [12,17,30,36]. As mentioned in the Results section, in the moments before attacking a deceptively attracted *Photinus*, female *Photuris* turn off their lanterns and attack, a behavior that led one of the reviewers of this paper to suggest that the hunting tactic of *P. lugubris* is a combination of aggressive mimicry and stalking. However, we have never observed a female *Photuris* stalking a prey firefly that she did not attract after it responded to her signals. It can be argued that documenting this behavior requires the continuous observation of perched signaling *Photinus*. During the mating season of 2021, we made observations of female *Photinus* to describe their courtship behavior and never observed a case of stalking by *Photuris*; similar observations of perched males flashing were not made, although this male behavior is relatively rare. With respect to "aerial hawking", in three consecutive mating seasons (2021–2023), we never observed flying *Photuris* females pursuing and capturing *Photinus* males in flight. However, in 2023, two local tour guides described one observation each of behavior suggesting that this "aerial hawking" strategy might be employed by some females, although probably sporadically. It must be mentioned that the tour guides did not confirm that these observations represented examples of aerial hunting. A third "hunting" strategy reported in *Photuris* fireflies is the stealing of fireflies trapped in spiderwebs (kleptoparasitism) [37]. Although it is common to observe *Photinus* trapped in spiderwebs (but never *Photuris*), frequently with their lanterns on, a case of kleptoparasitism, or even a *Photuris* female close to a spiderweb with *Photinus* trapped, has never been observed. However, with the information available, it is not possible to discard this hunting strategy or consider it rare. Thus, further studies are necessary to determine if female *Photuris* employ other hunting strategies in "Rancho del Valle" and, in case they do, how much prey they obtain in these other ways. What is clear is that many *Photuris* females behave as femmes fatales in our study area and that this strategy has a high rate of failure.

Another intriguing observation of the present study is the fact that all successful hunts occurred during the first attack of the femme fatale, while other females failed after several attacks. This could be the result of individual variation in the ability to deceive and capture male prey by female *Photuris*. Unfortunately, given that female *Photuris* were not

individually identified, it is not possible to assess this idea. Studying the possible existence of this variation and its correlates (genetic, developmental, phenotypic, environmental) seems a promising line of research.

One reviewer of this paper pointed out two potential biases in the prey capture estimates. First, the observation that most successful attacks occurred early in the mating period could bias down the prey capture estimate because, later in the night, successful femmes fatales would be hard to detect because they would be feeding instead of interacting with prey. However, we think this possible bias is not very important because *Photuris* females always produce glows, albeit at low rates, when feeding. In fact, thanks to these glows, females already feeding were detected in this study and in a previous one [29]. Second, if there is individual variation in hunting ability (a reasonable expectation), hunting females observed late in the night would tend to be unsuccessful females that would bias down the overall capture rate estimate. This is true; some successful femmes fatales were probably missed early in the night given the limited number of field researchers.

As mentioned in the Introduction, the aggressive mimicry of female *Photuris* is considered a major selective pressure on the communication systems of fireflies in the American continent, and thus, counter-adaptations in prey species are expected to evolve [18]. The low hunting success of *Photuris* femmes fatales found in this and previous studies could be explained by countermeasures that coevolved in the prey [15,18]. Although the existence of a very efficient predatory *Photuris* species cannot be discarded, the general pattern suggests that an asymmetry in the strength of the selective pressures experienced by predators and prey could explain the low hunting success of *Photuris* females and the relatively good ability to escape of the prey fireflies. This asymmetry is known as the "life–dinner principle" [38], which proposes that selective pressures are stronger in the prey species than in the predator because an unsuccessful predator loses a dinner but unsuccessful prey loses its life. In the present study, although *Photinus* males probably paid time, energy (especially when they spent long periods in flight communicating with the predatory females [36]), and opportunity costs by being attracted by *Photuris* females, they were reasonably good at avoiding predation. On the other hand, exploring the possibility of individual variation in *Photinus* males in their ability to avoid being deceived and captured by femmes fatales seems an interesting and complementary line of research for the future.

Although a detailed study of the behavioral strategies used by *P. palaciosi* males to reduce predation by *P. lugubris* females remains to be performed, the "hesitant" and "dropping-when-approached" behaviors observed in males and the "concealing strategies" of females are consistent with the idea of the late Prof. J. Lloyd [18,25] that prey fireflies should evolve a variety of strategies aimed at uncovering the identity of the female they are interacting with. In this context, the absence of "reflex bleeding", a defense strategy present in several fireflies [39], is intriguing. In three consecutive mating seasons (2021–2023), reflex bleeding was never observed when *Photuris* females attacked males, either in the field or in an experimental setting [29] or when researchers manipulated individuals (both males and females) by hand. In previous studies involving the manipulation of *Photinus* males and females by hand in localities where *Photuris* is absent [31,40] but other predators are present, in the states of Tlaxcala, Puebla, and Estado de México, reflex bleeding was also never observed.

The aggressive mimicry of sexual signals seems to be rare across the tree of life, but the range of organisms exhibiting this behavior is broad. An incomplete list includes female bolas spiders (*Mastophora* sp.) mimicking the sexual pheromones of its moth prey *Spodoptera frugiperda* [5] and males and females of the katydid orthopteran *Chlorobalius leucoviridis* attracting and capturing males of several species of cicadas by responding with sound signals like those of its prey's females [7]. Pollination via sexual deceit in orchids and other flowering plants is similar because of the imitation of sexual signals; however, successful deception does not mean the death of the cheated receiver [41].

5. Conclusions

We measured the attraction of *Photinus palaciosi* males to predatory females of *Photuris lugubris* in the field, as well as the number of prey captured. We observed that females, in general, attract several males of the prey species but capture relatively few (9.8%). Our observations suggest that the low hunting success of *Photuris* femmes fatales could be explained by male behaviors. The attracted males behave in a way that we call "cautious" or "hesitant" that could prevent attacks, and they also behave in ways that probably reduce the success of attacks at a close distance, such as approaching in flight and "dropping" in the face of attack. Females also behave in ways that probably improve their success in attracting and capturing prey, such as relocating in or near the display areas of the prey species, partially concealing the lantern on the substrate, possibly to avoid showing their much larger size and/or to better mimic the responses of *Photinus* females, or turning off their lanterns sometime before attacking. Our observations are consistent with the idea that predators and prey are engaged in a coevolutionary race of adaptation and counter-adaptation. The investigation of possible alternative hunting strategies in *Photuris* and the investigation of individual variation both in the ability to deceive and capture prey by *Photuris* femme fatales and in the ability to detect and escape by *Photinus* males are promising lines for future research.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/insects15010078/s1, Excel file with the quantitative raw data used in the analyses presented in this paper.

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Conclusiones

Nuestras observaciones en campo muestran que las hembras de *Photuris lugubris* depredan a los machos de *Photinus palaciosi* en nuestro sitio de estudio. Su comportamiento concuerda con la descripción de la conducta de *femme fatale* discutida en la literatura (Lloyd, 1965; Lewis, 2016). Posterior al apareamiento, las hembras de *P. lugubris* se desplazan hacia los sitios de cortejo de *P. palaciosi*, ahí atraen a los machos de *P. palaciosi* contestando a sus destellos y una vez que están lo suficientemente cerca los someten y devoran. El número de interacciones entre una hembra cazadora y sus machos presa potenciales, puede variar dependiendo de si la hembra se ubica dentro, al lado o entre sitios de congregación de machos *P. palaciosi* y de que tan abundantes en machos sean estos sitios. La presencia cercana de hembras de *P. palaciosi* y de otras hembras cazadoras activas, también podría interferir en el número de machos presa que se comunican y o se aproximan a una hembra *P. lugubris*.

Aunque nuestros datos no permiten determinar si ejercen o no mimetismo agresivo a través de las señales bioluminiscentes, hemos observado que la repuesta bioluminiscente que las hembras de *P. lugubris* emiten para contestar a sus machos coespecíficos es distinta a la que utilizan cuando se comunican con sus machos presa. Nuestras observaciones también nos han llevado a considerar que la estrategia de caza de las hembras de *P. lugubris* no se limita a la imitación de las señales bioluminiscentes de las hembras de sus machos presa, sino a un conjunto de estrategias de comportamiento que les permiten camuflar su identidad e intenciones. Por ejemplo, pegando su linterna al sustrato en el que están posadas durante la comunicación con sus machos presa o el cese de la emisión de señales bioluminiscentes segundos antes de brincar sobre el macho presa para someterlo.

El éxito de caza de las hembras *P. lugubris* es alto en cautiverio, debido probablemente al confinamiento en un espacio reducido, pero en la naturaleza es bajo, 10%, lo cual concuerda con lo reportado por Lloyd (2018), 10-15%. De acuerdo con nuestros datos, las hembras atraen hasta una distancia de caza a una gran cantidad de machos, sin embargo, los machos presa parecen ser hábiles al identificar a las hembras cazadoras ya que en la mayoría de los casos logran huir evitando el ataque. Adicionalmente calculamos que más del 70% de los ataques exitosos ocurrieron dentro de la primer mitad de la temporada de caza.

Nuestros resultados muestran que solo las hembras de *P. lugubris* depredan machos de *P. palaciosi*. Ni nuestros datos experimentales ni nuestras observaciones en campo muestran a ningún macho de *P. lugubris* atrayendo o atacando machos de *P. palaciosi*. Las hembras de *P. lugubris* consumen todas las partes blandas del cuerpo de sus presas, ricas en lucibufaginas y otros nutrientes, lo que sugiere que las hembras de *P. lugubris* no solo se benefician de los compuestos esteroideos de sus presas. Un estudio previo sugiere que posterior a la cópula, las hembras *Photuris* se transforman en *femme fatales* (Nelson *et al*., 1975), aunque, si las hembras se aparean varias veces y capturan varias presas a través de toda la temporada, podría ser posible que experimenten una fase intermedia en la que son receptivas sexualmente pero también están listas para cazar. En nuestras observaciones varias hembras de *P. lugubris* se aparearon múltiples veces, por desgracia, carecemos de información acerca del número de veces que cazan durante toda la temporada. Sin embargo, observamos hembras cazadoras que respondían a machos de su propia especie utilizando respuestas distintas a las que dirigían hacia sus presas. En un caso extremo se observó a una hembra que fue cortejada mientras se alimentaba de un macho de *P. palaciosi* que abandonó a su presa y aceptó copular.

Medir el impacto de la depredación de *P. lugubris* en la demografía de *P. palaciosi* es una tarea interesante por cuestiones evolutivas y aplicadas. El éxito de caza de las hembras de *P. lugubris* podría no limitarse a ser el resultado de la exactitud con la que se imita el patrón de las hembras de la especie presa. La fluctuación de esta medida a través de la temporada podría responder a la interacción de una variedad de factores demográficos y conductuales

entre especies. A inicios de la temporada de caza, cuando las hembra cazadoras son abundantes y la densidad de las hembras de la especie presa es baja. La inexperiencia de los machos de *P. palaciosi* interactuando con hembras impostoras podría favorecer que el número de ataque exitosos de estas hembras sea mayor. Bajo esta hipótesis el éxito de las hembras cazadoras disminuiría a medida que los machos presa sean capaces de evaluar los patrones bioluminiscentes y discriminar entre las hembras de su especie y las hembras impostoras. Empero, cuando la temporada avanza y la densidad poblacional de las hembras de ambas especies se invierte, el éxito de caza de las hembras de *P. lugubris* podría estar sujeto a selección dependiente de frecuencia negativa. Considerado que interactuar con una hembra cazadora es poco probable y que evaluar por demasiado tiempo a una hembra de su especie durante el cortejo podría disminuir sus probabilidades de aparearse, mantener una estrategia de comportamiento "cauteloso", fruto de experiencias de cortejo previas, podría no ser la estrategia más adecuada para los machos de *P. palaciosi* a mediados o finales de su temporada de apareamiento, beneficiando indirectamente a las hembras cazadoras. De esta manera la dinámica poblacional de ambas especies podría influir en el éxito de caza de las hembras de *P. lugubris*.

Lo anterior es especialmente relevante considerando que la especie presa es una especie endémica de México y que es utilizada como atracción turística en varios estados de nuestro país. En este contexto, los grandes números de machos de *P. palaciosi* necesarios en los avistamientos sincrónicos podrían verse afectados ante un desajuste ecológico y a su vez la vulnerabilidad que le otorga a la especie la explotación turística podría poner en riesgo no solo a las poblaciones de *P. palaciosi*, sino también a las de su depredador *P. lugubris*. Ante ambos escenarios la investigación cuantitativa acerca de los efectos demográficos de la depredación por *femme fatales* parece ser oportuna y necesaria.

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