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Assessment of color response and activity rhythms of the invasive black planthopper Ricania speculum (Walker, 1851) using sticky traps

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Abstract:	To be effective, management strategies of invasive alien species, particularly those exerting serious damages to human activities, cannot ignore their spatiotemporal behavior. The black planthopper Ricania speculum is an Asian insect which has been reported as an alien invasive species in Italy, where it threatens local plant diversity, including important crops. In our work, we analyzed the activity rhythms of this species through circular statistics and the efficiency of chromotropic traps to capture adult individuals. Captures were carried out in Central Italy, where the black planthopper is showing a remarkable range expansion, after it first introduction in 2009. We observed that the species was mainly crepuscular, with a high intersexual activity overlap. Activity rhythms changed between July-August and September-October, with changing heliophany, but peaked at sunset and were the lowest in the second half of the night and early morning. The species mostly selected green traps, particularly in September, which is the period of egg-laying over leaves; conversely orange ones were avoided, and yellow ones used proportionally to their local availability. Strategies for controlling this species should consider concentrating trapping effort during the activity peak, using green sticky traps to enhance the capture success of each trap, with the lowest impact over non-target species.

Assessment of color response and activity rhythms of the invasive black planthopper *Ricania speculum* (Walker, 1851) using sticky traps

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Running head: *Ricania speculum* response to chromotropic traps

Abstract

To be effective, management strategies of invasive alien species, particularly those exerting serious damages to human activities, cannot ignore their spatiotemporal behavior. The black planthopper *Ricania speculum* is an Asian insect which has been reported as an alien invasive species in Italy, where it threatens local plant diversity, including important crops. In our work, we analyzed the activity rhythms of this species through circular statistics and the efficiency of chromotropic traps to capture adult individuals. Captures were carried out in Central Italy, where the black planthopper is showing a remarkable range expansion, after it first introduction in 2009. We observed that the species was mainly crepuscular, with a high intersexual activity overlap. Activity rhythms changed between July-August and September-October, with changing heliophany, but peaked at sunset and were the lowest in the second half of the night and early morning. The species mostly selected green traps, particularly in September, which is the period of egg-laying over leaves; conversely orange ones were avoided, and yellow ones used proportionally to their local availability. Strategies for controlling this species should consider concentrating trapping effort during the activity peak, using green sticky traps to enhance the capture success of each trap, with the lowest impact over non-target species.

Key words: chromotropic traps, Fulgoromorpha, Hemiptera, Italy, monitoring, Ricaniidae.

Introduction

Trapping techniques are frequently used to monitor pest species and to provide data on their occurrence, even at low density, as well as reasonably precise estimates of densities and sex ratios. As with most trapping methods, characteristics of the trap may influence its effectiveness and trap colors are a key factor in attracting and trapping most insect species (e.g. Broadbent, 1948; Capinera & Walmsley, 1978; Ali, 1993; Cho *et al*., 2011; Rodriguez-Saona *et al*., 2012; Vrdoljak & Samways, 2012). Traps based on the response of insects to colors have been applied worldwide to sample harmful and beneficial insects, in wild and cultivated plants (Capinera & Walmsley, 1978; Cho *et al*., 2011; Vrdoljak & Samways, 2012). For instance, colored traps are commonly used to attract several insect pests belonging to the orders Hemiptera (Rodriguez-Saona *et al*., 2012), Thysanoptera (Atakan & Bayram, 2011), Diptera (e.g. Haniotakis, 1986), Coleoptera (e.g. Petrice *et al*., 2013; Ávalos & Soto, 2015), as well as for many beneficial flower-visiting insects (anthophiles), e.g. Hymenoptera (Vrdoljak & Samways, 2012). Effectiveness of colored traps is strictly related to the spatiotemporal behavior and reproductive phenology of the target species (see Green & Flint, 1986; Abrahamczyk *et al.*, 2010). Furthermore, colored sticky traps are a simple, rapid and cost-effective method to monitor population dynamics of harmful species and their natural enemies (Atakan & Bayram, 2011) as well as to control insects' numbers in crop protection (Gu *et al*., 2008; Broughton & Harrison, 2012). Invasive insects, particularly those affecting human activities (e.g. agriculture, tourism, ornamental species plantation) and wellness, deserve well-addressed monitoring programs to limit their damages (Francese *et al*., 2005; Hoodle & Hoodle, 2011; Maistrello *et al.*, 2017; Masetti *et al*., 2018).

The black planthopper *Ricania speculum* (Walker, 1851) (Hemiptera: Fulgoromorpha: Ricaniidae) is an invasive alien species of Far Eastern origin, reported in Europe since 2009 (Mazza *et al*., 2014). As to Italy, this species has rapidly spread across Liguria (Mazza *et al*., 2014; Rossi & Lucchi, 2015), Tuscany (Silvestri, 2017), Veneto, Piedmont and Latium (Mazza *et al*., 2018). The black planthopper feeds and lays eggs on a wide range of host plants and its large polyphagia has been highlighted since a few years (e.g. Mazza *et al*., 2014; Rossi & Lucchi, 2015; Rossi *et al*., 2015; Lucchi & Rossi, 2016; Laudonia *et al*., 2017; Silvestri, 2017; Mazza *et al*., 2018). The fast range expansion of this species in Italy makes it as a new possible threat to native plants and human activities including olive crops. The species is usually monitored through classical entomological methods and, in Italy, the reports concerning its distribution come mainly from visual inspection (e.g. Rossi *et al*., 2015; Mazza *et al*., 2018). Only, Li *et al*. (2015) used sticky colored traps for capturing this planthopper in mangrove forests in China, i.e. in its native range. Aims of this study were: *i*) to evaluate the use of the sticky color traps to capture the black planthopper and *ii*) to study in depth the phenology (seasonal occurrence) and the activity rhythms of this species, to apply effective monitoring methods and correct pest control measures.

Materials and Methods

Study area

The study was performed in Tuscany (Central Italy), in three rural landscape areas in the municipality of Viareggio (Province of Lucca: 43°50’40’’ N 10°16’15’’ E, 5 m a.s.l.). The three areas are 500 meters far apart each other and covered mostly with human settlements, surrounded by orchards and gardens, with several native and exotic plant species. Sampling areas were selected as hosting high densities of *R. speculum* (Mazza *et al*., 2018).

Traps and captures

Yellow (Y), green (G) and orange (O) sticky traps (FLORTIS®, Settimo Milanese, Italy: http://www.flortis.it/it) were used for this study. The spectral reflectance of the colored traps was analyzed by a spectroradiometer FIELDSPEC 3 HI-RES (ASD inc., Malvern Panalytical, New York, USA), with a spectral resolution of 3 nm and a sampling interval of 1.4 nm. White reference was made by a calibrated panel (SPECTRALON®, ASD inc., Malvern Panalytical, New York, USA), ~100% reflective within the instrument wavelength range of 350-2500 nm. The green trap showed the maximum reflectance between 475 and 525 nm (35-38% of reflectance), the orange one between 600 and 650 nm (~40% of reflectance), the yellow one between 540 and 600 nm (~50% of reflectance) (Fig. 1).

Traps consisted of a 10 × 22 cm rectangular adhesive plastic boards. Boards were adhesive on both sides. In each area, three cross bamboos frames were used as support for the suspension of the chromotropic traps, which were spaced 10 m apart and located at a minimum of 10 m from bushes/trees. One trap per color was attached approximately 180 cm above the ground on the frame and the three traps were spaced 20 cm each other (Fig. 2). All the color combinations (YGO, GOY, OYG) were present in each area, for a total of 27 installed traps.

Traps were positioned on the 1st July, removed on the 31st October 2018 and replaced once a week since the first adult capture (Mazza *et al*., 2014). Traps were visually inspected about once/1h during the collection time (06:00-01:30 hours), following Fanini *et al*. (2014). Despite the interruption in the late night, no individuals were found attached to the trap in early morning, when the inspection started again. During the inspections, the time of arrival of the specimens was reported and adults were gently removed from the traps and sexed by observing the shape of abdomen (Rossi & Lucchi, 2015), through a hand lens 10× magnification. Unsexed individuals referred to adults escaped during the manipulation time or to portions of adults attached to the traps without abdomen (i.e. the body part required for a reliable sex attribution).

Activity rhythms and intersexual temporal overlap

Activity rhythms and patterns of intersexual temporal overlap have been calculated through the R packages *overlap* (Meredith & Ridout, 2014).

Record of each individual was categorized as: daytime (between 30 minutes after sunrise and 30 minutes before sunset), nighttime (between 30 minutes after sunset and 1 hour before sunrise), dawn (from 30 minutes before sunrise to 30 minutes after sunrise) and dusk (from 30 minutes before sunset to 30 minutes after sunset) (see Carnevali *et al*., 2016). Sunrise and sunset times were calculated through the R package *NightDay* for every trapping day (Hughes-Brandl, 2011). The Rayleigh test was computed through the R package *circular* (Lund *et al*., 2017) to evaluate the distribution uniformity of the daily activity data, to assess whether the black planthopper showed a random activity pattern over the 24-hours cycle (Lioy *et al*., 2016). Intersexual comparisons were carried out to estimate the coefficient of overlapping (Δ) between temporal activity patterns of both sexes. A total activity rhythm was then estimated to account also for unsexed individuals. The coefficient of overlapping ranges between 0 (no overlap) and 1 (total overlap: Linkie & Ridout, 2011; Meredith & Ridout, 2014). We calculated the Δ_4 estimator as the smallest sample of each pairwise comparison was always > 0.75 (Linkie & Ridout, 2011; Meredith & Ridout, 2014). An overlap coefficient included between 0.50 and 0.75 was considered as "intermediate" (Monterroso *et al*., 2014). Temporal overlap was considered as "high" if $\Delta_4 > 0.75$ (Monterroso *et al*., 2014) and as "very high" when $\Delta_4 > 0.90$.

We divided our four-month study period into 2 two-month periods according to heliophany, i.e. July-August (mean heliophany > 9.5 hours) and September-October (mean heliophany < 8 hours), which is suggested to affect the activity rhythms of *Ricania speculum*. Analyses were carried out at both total and two-month scales. The Watson's Two-Sample Test of Homogeneity (U^2) was used to compare intersexual overlap (Torretta *et al*., 2016), using circular statistics with the R package *circular* (Lund *et al*., 2017).

Trap-color selection

Trap-color selection was assessed using collected individuals and computed through the Ivlev's Electivity Index (hereafter, E-index: Ivlev, 1961), which compares the availability of a trap with its use by the species, following the formula:

$$E = \sum_{i=1}^{n} \frac{(Ui - Ai)}{(Ui + Ai)}$$

where U_i is the proportion of use of traps of the "i" color and A_i is its availability (i.e. 33.3% for each of the three color-traps). The E-index values vary between −1 (total avoidance) and + 1 (total selection): thus, any positive value indicates that selection occurs, whereas negative ones show avoidance. Following previous works (e.g. Lazzaro, 1987; Lückstädt & Reiti, 2002; Laurenzi *et al*., 2016; Mori *et al*., 2018) we assessed a strong selection/avoidance respectively at values > + 0.3 or < −0.3. E-indices were calculated for both sexes and total (to account for unsexed individuals), at both total and two-month scales.

Results

General results

In 123 days of captures, we collected a total of 827 specimens of *Ricania speculum* (421 males, 154 females, 252 unsexed). The captures were concentrated in 78 days: the first adults were captured on 18th July 2018, whereas since the 4th October 2018 no individual was any more attracted to the traps. The species in Tuscany (Central Italy) is univoltine, as illustrated in Fig. 3, with a peak of adults captured in September.

Activity rhythms and intersexual temporal overlap

Ricania speculum is mostly a crepuscular species, which activity peaks at sunset (Fig. 4). When heliophany was the highest (i.e. July-August), *R. speculum* adults were active for a high number of hours, i.e. between 6:00 to midnight, with a significant peak at 20:43 (Rayleigh test: Z = 56.42, r = 0.71, P < 0.001), i.e. at sunset. When heliophany was the lowest (i.e. September-October), activity of *R. speculum* was concentrated between late morning to midnight, peaking at 17:17 (Rayleigh test: Z = 93.18, r = 0.77, P < 0.001), i.e. at sunset time (Fig. 5). Different heliophany may explain the intermediate coefficient of overlap (Δ_4 = 0.605).

Intersexual overlap did not change between the 2 two-month periods (Watson's Two-Sample Test of Homogeneity U^2= 0.18, P = 0.08: Fig. 6) and it was high or very high.

Trap-color selection

Females were more selective than males and tend to avoid orange traps in both periods, whereas selecting green ones in the September-October. From a general point of view (i.e. including unsexed individuals), orange traps were avoided, yellow ones used in proportion to their availability and green traps positively selected (Table 1).

Discussion

The black planthopper is an alien invasive species which is becoming abundant in Central Italy, as confirmed by the high number of captured individuals in a short period. This result is noteworthy since this species seems to be spreading in Italy and it can feed and lay eggs in a long list of plants ranging from angiosperms to gymnosperms (Mazza *et al*., 2018).

Our study confirmed that this species is univoltine in the introduction area and that it overwinters as eggs (cf. Rossi *et al*., 2015, for Liguria). Conversely, in the native range, the black planthopper may shows two generations per year, overwintering as juveniles (Yu, 2007, for China).

According to our study, the species showed a flight period of few months in summer, with a peak in September, when over 200 individuals were captured in one week. Activity of both sexes significantly peaked at dusk, making it a mainly crepuscular insect. Also, *Ricania japonica* Melichar, 1898, now *Orosanga japonica* (Melichar, 1898) seems a crepuscular/nocturnal insect since it was collected in Bulgaria using light traps during the night (Gjonov, 2011). This crepuscular behavior probably represents the best trade-off between temperature, predator avoidance and host plant searching. Foraging at low light intensities is particularly common for insects in tropical/subtropical areas (e.g. the area of origin of the black planthopper) where severe food interspecific competition and a high risk of predation occur (Greiner, 2006). Moreover, the laying behavior is a ritualized and time-consuming process (Rossi *et al*., 2015), which may expose planthoppers to predation: thus, eggs are probably mostly laid at low light intensity.

We showed that the colored sticky traps are effective tools to monitor the black planthopper; thus, they may be used for early detection in new areas, as well as for mass trapping programs. More in-depth studies are however required to optimize trap height and number as in Wang *et al*. (2015) for *Aleurocanthus spiniferus* (Quaintance, 1903) (Hemiptera: Aleyrodidae).

Captures provided us with an accurate idea on the phenology patterns, but a wrong idea of the sex ratio. Despite the high percentage of unsexed individuals, the sticky traps captured more males than females and similar male-biased trapping has been reported for some species of leafhoppers captured with sticky traps (e.g. Meyer & Colvin, 1985; Kersting & Baspinar, 1995). This bias has also been reported for the Diptera Agromyzidae (Scheirs *et al*., 1997): male-biased catches in colored traps can be explained by sex dependent feeding and/or mating strategies (Scheirs *et al*., 1997).

Concerning the color selection, only Li *et al*. (2015) investigated the sticky colored traps in mangrove forest in Shenzen (China) for capturing this species: ten colors (but not the orange) were tested, with the yellow traps showing the highest attractive effect. This study, however, does not represent a valid comparison with our one, as it has been carried out in a completely different study site (i.e. a tropical mangrove forest).

From a general perspective, yellow is the color preferred by most insects and the attraction to yellow may constitute a stimulus eliciting food and/or host plant search (Prokopy, 1972). Visual cues are important to locate and select the host plants and several leafhoppers such as *Empoasca* spp. are attracted to colors commonly associated with living foliage (Rodriguez-Saona *et al*., 2012). The attraction to a color may also change following physiological stages (Francese *et al*., 2010). Accordingly, we showed that yellow traps were used in proportion to their availability, orange ones were avoided, and green ones positively selected. In our study, females were more selective than males and tended to avoid orange traps in both periods, whereas being particularly attracted by the green traps in September-October. Attraction to green may be host-related since this color is typical of foliage and this color preference may be related also to the peak of egg-laying. Eggs in *R. speculum* are usually laid in clusters within the tissues of young twigs or in the leaf midrib (Rossi *et al*., 2015). Given that the species overwinters in the egg stage in the invasive range (Rossi *et al*., 2015), early Autumn (September-October) may also represent the peak of egg-laying.

To conclude, green sticky traps seem to be the most suitable ones to sample *Ricania speculum* adults in the invasive range and they might be used both for the early detection and for mass trapping. The use of green traps may also reduce the impact on non-target species, such as the beneficial ones (e.g. Hymenoptera parasitoids, bees, coccinellids), since these species are mainly attracted by yellow traps (Wallis & Shaw, 2008). Our captures (Fig. 7) seem to confirm this trend, but further studies are necessary to confirm it.

Concentrating the trapping effort during the activity peak with green sticky traps may *i*) enhance the capture success of each trap, *ii*) reduce the impact on non-target species and *iii*) increase the number of captures, to reduce the population densities and, in turn, possible damages to cultivated areas.

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Table 1. E-indices for trap-color selection. Asterisks highlight significant selection (positive values) and avoidance (negative values).

	Orange	Yellow	Green
Males (July-August)	-0.13	0.13	-0.01
Females (July-August)	-0.30*	0.14	0.07
Males (September-October)	-0.21	-0.05	0.20
Females (September-October)	-0.31*	0.05	0.37*
Total	**-0.31***	**0.06**	**0.34***

Figure legends

Fig. 1. Reflectance spectra for the yellow (Y), green (G) and orange (O) sticky traps used to capture of *Ricania speculum* adults between July and October 2018.

Fig. 2. Orange, yellow and green sticky traps used for the capture of *Ricania speculum* adults in July-October 2018.

Fig. 3. Number of *Ricania speculum* adults captured per week from July to October 2018 using chromotropic traps.

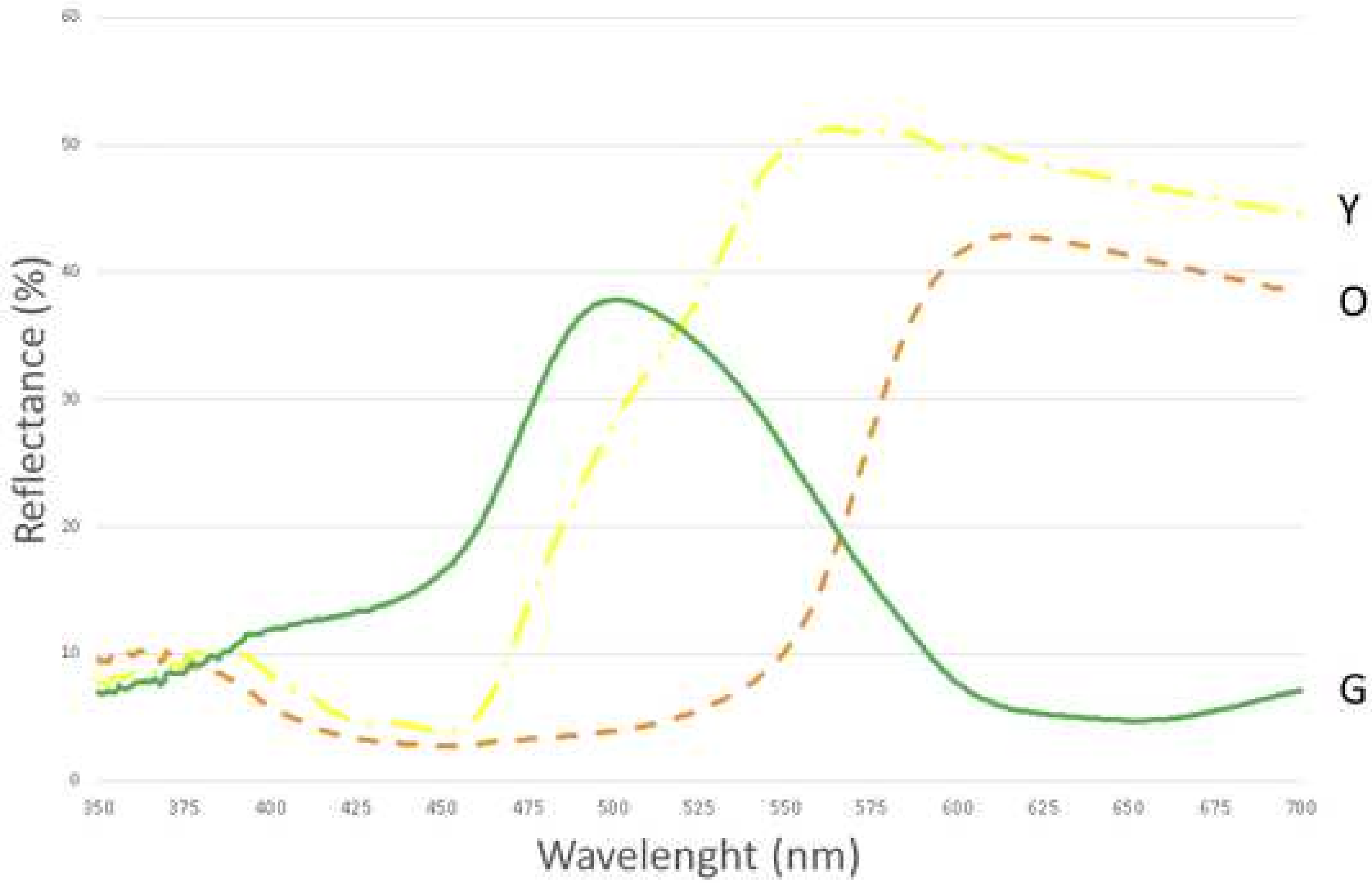
Fig. 4. Activity rhythms of *Ricania speculum* during the four-month period.

Fig. 5. Activity rhythms of *Ricania speculum* during two-month periods at different heliophany, and interperiod overlap. Red arrows show sunset time.

Fig. 6. Overlap of activity rhythms of the *Ricania speculum* males and females in both two-month periods and total.

Fig. 7. Impact of the green, yellow and orange sticky traps used for the capture of black planthoppers on non-target species.

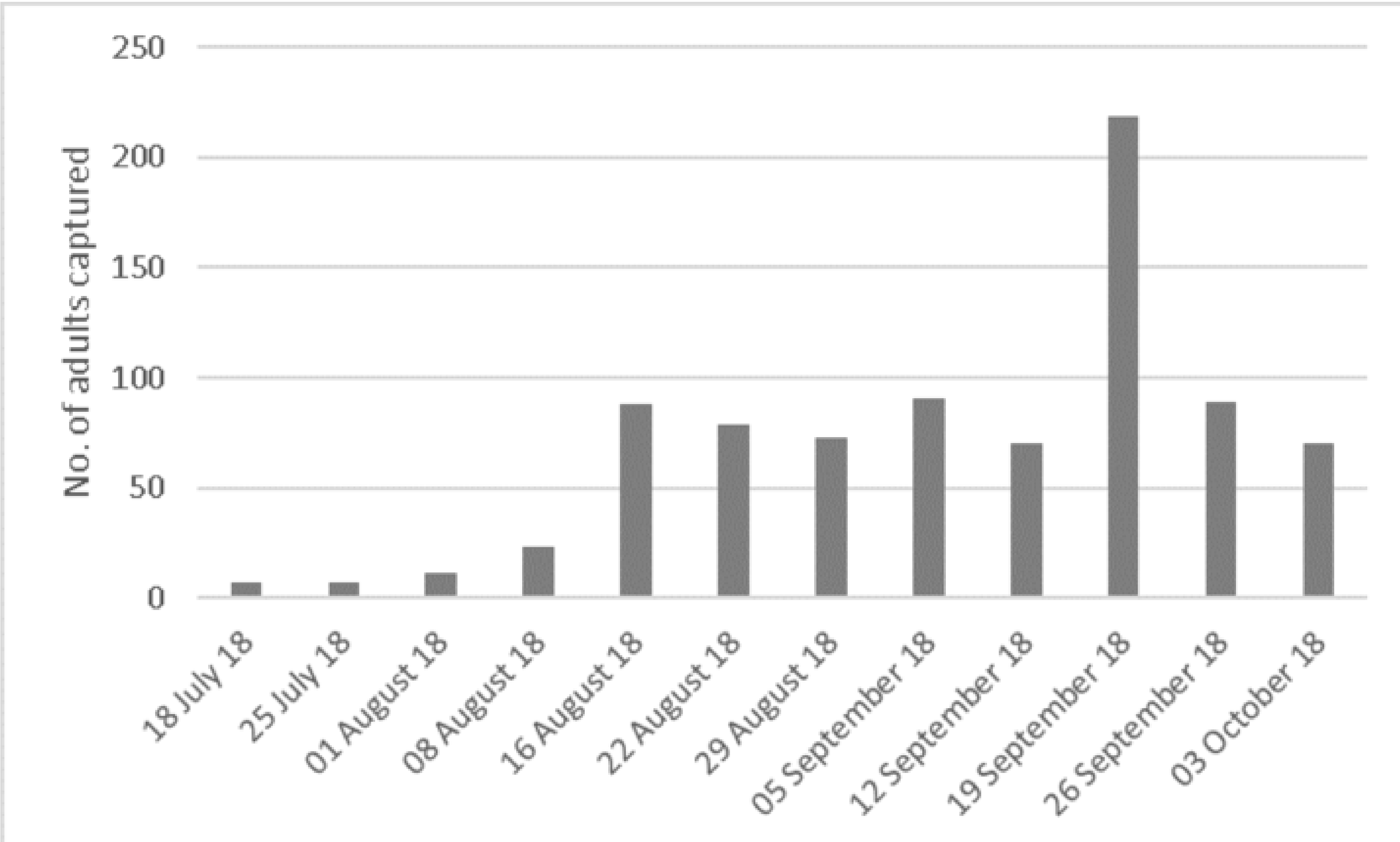
Figure 1



60
50
40
30
20
10
0
350
375
400
425
450
475
500
525
550
575
600
625
650
675
700
Reflectance (%)
Wavelenght (nm)
Y
O
G

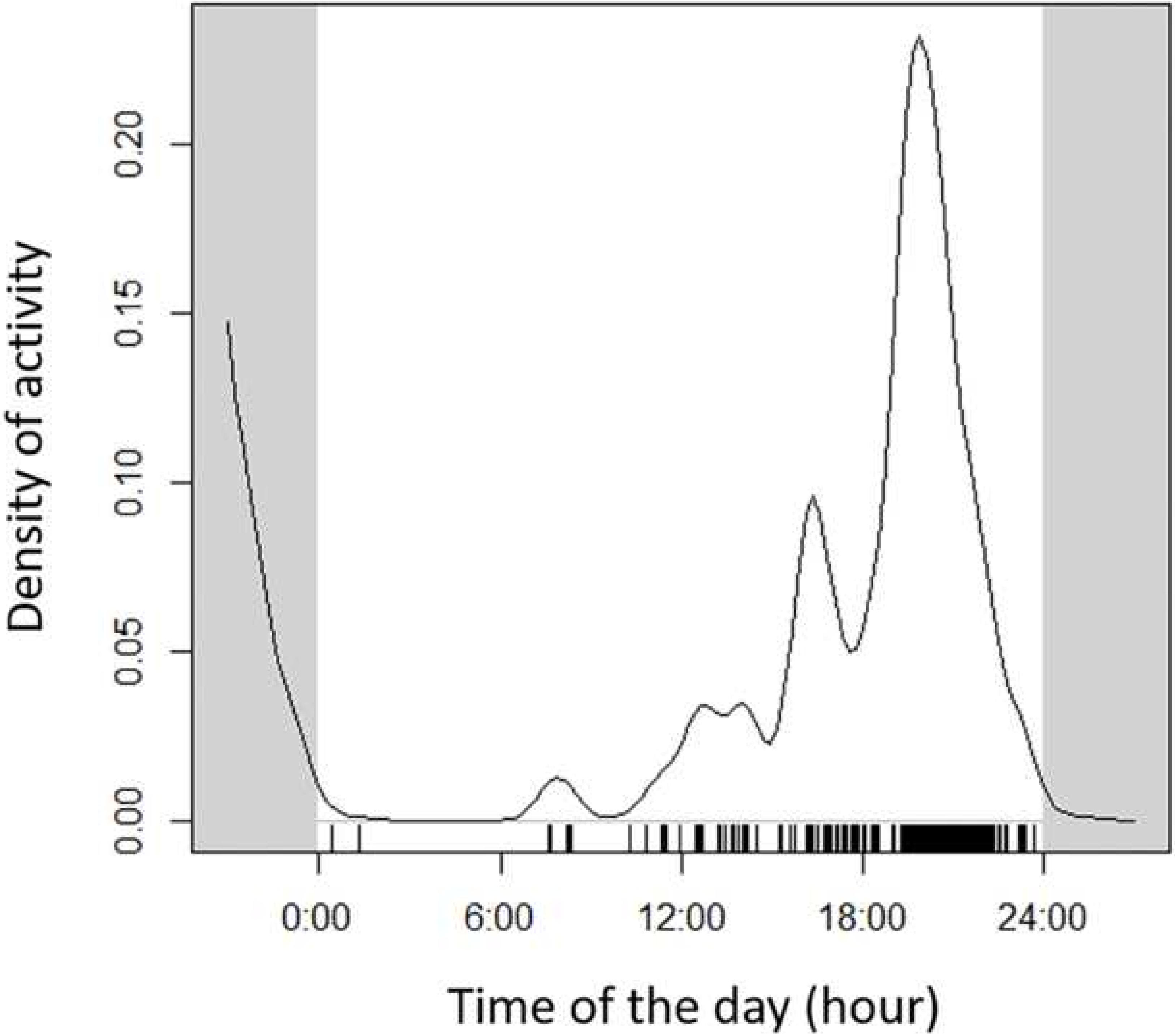
Figure 2

Figure 3



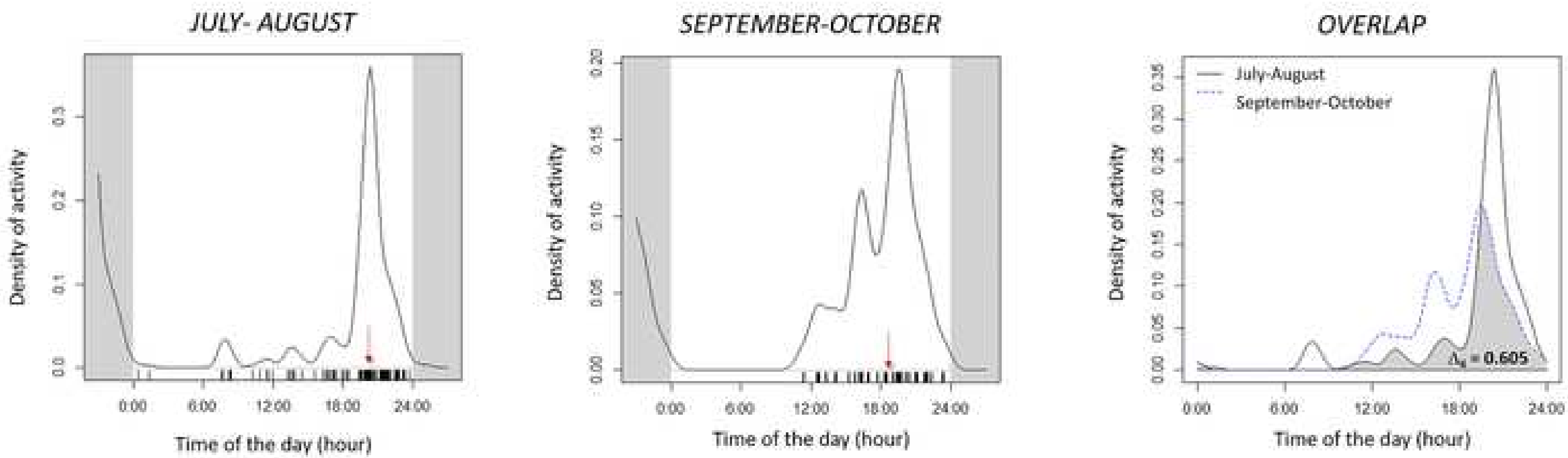
250
200
150
100
50
0
No. of adults captured
18 July 18
25 July 18
01 August 18
08 August 18
16 August 18
22 August 18
29 August 18
05 September 18
12 September 18
19 September 18
26 September 18
03 October 18

Figure 4



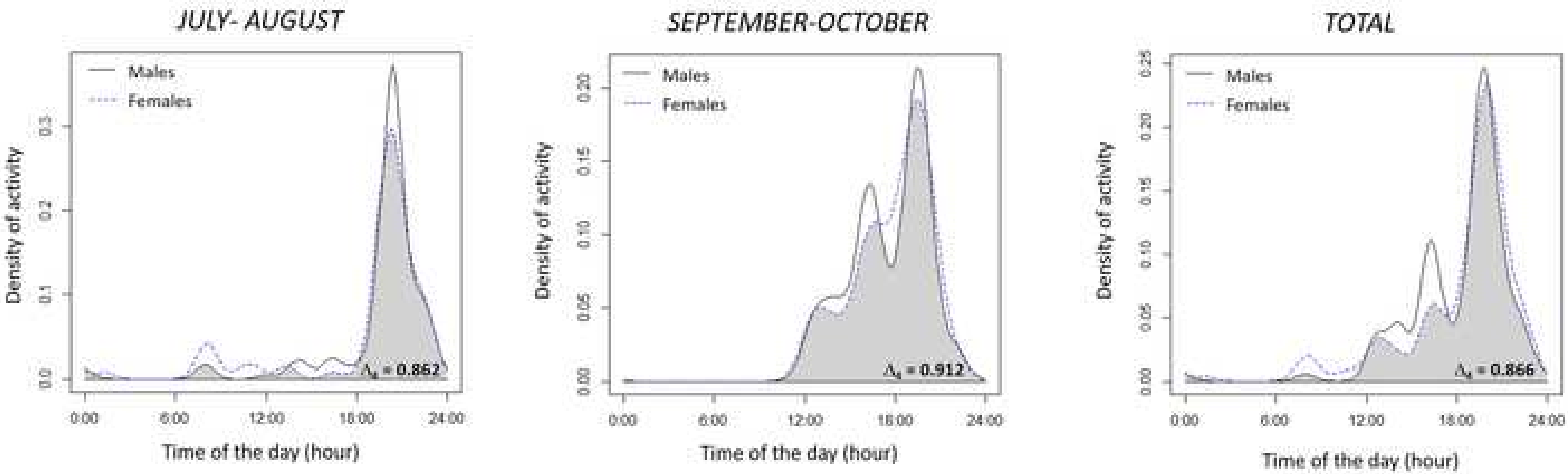
Density of activity
0.00
0.05
0.10
0.15
0.20
0:00
6:00
12:00
18:00
24:00
Time of the day (hour)

Figure 5



JULY- AUGUST
Density of activity
0.0
0.1
0.2
0.3
0:00
6:00
12:00
18:00
24:00
Time of the day (hour)
SEPTEMBER-OCTOBER
Density of activity
0.00
0.05
0.10
0.15
0.20
0:00
6:00
12:00
18:00
24:00
Time of the day (hour)
OVERLAP
July-August
September-October
Density of activity
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
Δ_4 = 0.605
0:00
6:00
12:00
18:00
24:00
Time of the day (hour)

Figure 6



JULY- AUGUST
SEPTEMBER-OCTOBER
TOTAL
Males
Females
Density of activity
Time of the day (hour)
0:00
6:00
12:00
18:00
24:00
Δ_4 = 0.862
Δ_4 = 0.912
Δ_4 = 0.866

Figure 7