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1 **Survival analysis of brown plant hoppers (*Nilaparvata lugens*) in rice using video recordings of**  
2 **predation events**

3

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16

17 **Author contributions:**

18 Planning and design of experiment: IW, WW, LH, FB, YZ, HX

19 Data collection: IW, DF

20 Data analysis and interpretation: LH, IW, WW, FB

21 Writing of publication: FB, LH, WW

22 **Abstract**

23 The brown plant hopper, *Nilaparvata lugens* Stål, is a major rice pest in South-East Asia. While brown  
24 plant hopper (BPH) populations can be regulated by natural enemies, there is limited quantitative  
25 information available about the contribution of different predator species to BPH mortality. Our study  
26 has three aims: (i) assess the relative contribution of different predator species to BPH mortality in rice  
27 fields, (ii) assess diurnal patterns in BPH predation, and (iii) assess the seasonal variation in BPH  
28 predation. We quantified predation of live mobile BPH in three rice fields using video recording and  
29 assessed densities frogs, a major predator group, by direct counts. In 864 hours of video recording, 102  
30 mortality events were observed. Frogs (Ranidae), wolf spiders (Lycosidae) and jumping spiders  
31 (Salticidae) were the main predators, accounting for 76%, 13% and 9% of the BPH predation events,  
32 respectively. There were large differences in frog density across fields, and there was more predation  
33 during the evening (63% predation events) than during the day (37%). Survival analysis indicated that  
34 predation risk quickly decreased with time after the onset of recording sessions and that most  
35 predation happened within the first 10 minutes. The results confirm the often overlooked contribution  
36 of frogs to BPH predation, but also highlight the substantial variation in predator pressure and frog  
37 abundance across farmers' fields. While camera observations provide compelling information on the  
38 identity and relative importance of natural enemies in predation of pests, further development of  
39 methods is needed to minimize possible biases resulting from disturbance when making camera  
40 observations to quantify predation risk.

41

42 **Keywords:** biocontrol, survival analysis, visual observation, pest management

## 43 **Introduction**

44 Rice is a major global staple food and is attacked by several insect pests, of which brown plant hopper  
45 (BPH) *Nilaparvata lugens* Stål (Homoptera: Delphacidae) is considered the most important in South-  
46 East Asia and China (Heong et al., 2015). While rice has a high potential for biocontrol (Dale, 1994;  
47 Settle et al., 1996), this ecological function can easily be disrupted by insecticides (Heong &  
48 Schoenly, 1998). Indeed, BPH typically becomes a pest when the natural enemy complex is decimated  
49 by chemical insecticide applications and pesticide resistant populations of BPH are released from top-  
50 down natural enemy control (Settle et al., 1996; Heong et al., 2015a). The current increase of BPH  
51 infestations in (South-) East Asia hints at disruption of biocontrol by increased reliance on broad-  
52 spectrum insecticides for hopper control in this region (Heong et al., 2015b), and highlights the need  
53 for conservation of natural enemy populations (Bottrell & Schoenly, 2012). Insight in the identity and  
54 predation impact of key natural enemies of rice pests is crucial to set effective conservation targets.

55 Research on biocontrol of BPH in rice has historically been conducted by entomologists, and  
56 concentrated on the contribution of arthropod natural enemies, such as spiders, predatory beetles,  
57 parasitoids and ladybeetles (Chiu 1979; Shepard et al., 1987; Settle et al., 1996; Heong et al., 2015b).  
58 However, recent studies have shown that frogs are important predators of BPH, potentially having a  
59 larger contribution to BPH mortality than arthropod natural enemies (Khatiwada et al., 2016; Zou et  
60 al., 2017). That the role of frogs in biocontrol of BPH has been underappreciated until recently can be  
61 explained by the widespread use of techniques for measuring predation rates that are unsuitable to  
62 quantify predation by frogs, for instance use of immobilized prey (Zou et al., 2017). Furthermore,  
63 previous studies have relied strongly on the use of sampling techniques that are biased towards  
64 arthropod predators, while neglecting other predator groups, such as frogs and birds (Zou et al., 2017).  
65 While the role of non-arthropod predators as biocontrol agents has important implications for  
66 sustainable pest management in China, the evidence base is still small and the contribution of  
67 vertebrate and invertebrate predators to biocontrol under practical farming conditions needs further  
68 study.

69 Here we present a study on the quantification of predation on freely moving BPH populations in rice  
70 in farmer's fields using video cameras. We use video recording as a method because cameras can  
71 record predation events irrespective of whether the predator is an arthropod, frog or bird. Cameras can  
72 document predation events over periods of many hours without an observer being present, thus  
73 reducing disturbance and enabling the collection of data on a greater number of predation events. The  
74 objective of our study was to (i) assess the relative contribution of predators in the suppression of  
75 BPH, (ii) assess the diurnal activity of BPH predators, and (iii) elucidate the dynamics of predation in  
76 farmer field rice systems over the season. Furthermore, with this study we aimed to obtain an estimate  
77 of the predation probability per unit time (i.e. the relative mortality rate), which is a key parameter in  
78 population models that may be used to assess whether predation is sufficient to suppress population  
79 growth of BPH.

80

## 81 **Materials and methods**

### 82 *Field sites and insect culture*

83 The study was conducted in three rice fields at and nearby the Jiangxi Agricultural University in  
84 Nanchang, China, from July to August 2016. All fields were planted with rice variety Y2You1, which  
85 is not resistant to BPH.

86 Field A (400 m<sup>2</sup>) was located in a relatively undisturbed fenced area on the campus of Jiangxi  
87 Agricultural (Longitude, Latitude = E: 115.83815°, N: 28.76623°). Field B (476 m<sup>2</sup>; E: 115.83817°, N:  
88 28.76939°) and Field C (600 m<sup>2</sup>; E: 115.83206°, N: 28.77816°) are farmer's fields where people and  
89 cattle were passing by, but did not enter the fields. No pesticides were used during the crop cycle in  
90 any of the three fields. However, Field C was bordered by a stream which was used for washing  
91 spraying equipment and could have been contaminated. The greatest distance between fields was 1.5  
92 km.

93 BPH for experiments were reared on rice plants in four cages (0.4 m x 0.6 m x 1.5 m) inside a  
94 glasshouse at Jiangxi Agricultural University.

95

#### 96 *Cameras*

97 Three surveillance cameras (DFD<sup>®</sup>, Shenzhen, China), powered by 120V household electricity were  
98 used in Field A (which was fenced and with electricity accessible). Three DV cameras (Pamiel<sup>®</sup>  
99 DIGIPO HDV-S790 CMOS Camcorder, Shenzhen, China), powered by a 12V lead-acid E-bike  
100 battery with 12Ah capacity, were used alternately in Fields B and Field C (farmers' fields, with  
101 electricity unavailable) with a person present near the field to guard the equipment. Both the DV  
102 cameras (with 10x optical zoom) and surveillance cameras (8 mm fixed lens and focus distance of 30  
103 cm) had a resolution of 1280 x 720 pixels per square inch and included infrared (IR) night vision.  
104 Cables and batteries of the DV cameras were placed in plastic containers with lid, and covered with  
105 aluminium foil to protect against rain and heat. The data of the DV cameras were stored on 32G SD  
106 cards, enough for 5 hours of video recording, and were replaced every 4 hours, while the data of the  
107 surveillance cameras were directly stored on a hard drive of a computer that was connected to the  
108 cameras.

109

#### 110 *Observations*

111 Video recordings were conducted one day per week during day time (8:00 – 16:00 h) and one time  
112 during the evening (17:00 – 01:00 h). The recordings were made from July 1 until August 24, for a  
113 total of nine weeks. Recordings were made in two fields (each with three cameras) at the same time,  
114 resulting in a total of six recordings during day time and six in the evening on each day of weekly  
115 observations (Table 1). No recordings were made on sunny days because the high temperatures (>40  
116 °C maximum temperature) could damage the equipment, or during heavy rain and storms because  
117 BPH area easily dislodged from plants under such weather conditions making observations  
118 impracticable.

119 BPH adults and late-instar nymphs were collected from the insect rearing and transferred to a 30 cm  
120 plastic tube containing three rice stems. BPHs were allowed to settle for 30 - 40 minutes and then the  
121 rice stems with BPHs were inserted in the ground next to a randomly chosen rice plant in the field.  
122 The initial number of BPHs per observation was  $21 \pm 8$  (mean  $\pm$  SD). Cameras were located  
123 approximately 30 cm from the BPH infested rice stems and rice leaves blocking the camera view were  
124 removed. For each recording session new random locations were selected, and locations were always  
125 at least 3 m apart.

126 On the day after the recordings, frog densities were determined around each field using transect counts  
127 (Fujita et al., 2015). To do so, an observer walked slowly over the bund around the rice field recording  
128 all frogs spotted within half a meter to the left and the right of the walking path. Counts were  
129 converted to frog densities by dividing the count by the surveyed area of a 1 m wide strip around the  
130 field (82, 88 and 121 m<sup>2</sup> for Fields A, B and C, respectively).

131

### 132 *Analysis*

133 The video recordings were reviewed and all BPH removal events were denoted and predators were  
134 identified from the images. We report summary statistics for the full nine weeks of observation, and  
135 analysed a subset of six weeks using survival analysis. For the survival analysis, the data of week two  
136 and seven were not analysed because heavy rain and wind caused severe disturbance of the  
137 measurements. Data of week three were excluded because no mortality was observed and survival  
138 analysis requires at least one mortality event.

139 Survival analysis focusses on the time at which a certain event occurs (Kleinbaum and Klein, 2006).  
140 Here, the event of interest is predation of BPH. In the terminology of survival analysis, a predation  
141 event is a “failure”. The zero point (time  $t = 0$ ) to calculate a failure time was set at the start of the  
142 video observation and the end point was the time  $T$  at which the BPH was predated. The time until the  
143 predation event is called the survival time  $T$  (Lee and Wang, 2003). Individuals can also get lost from  
144 an experiment for other reasons than the event of interest (predation). For these individuals the exact

145 survival time  $T$  is unknown, but we know when the individual was last seen alive and this time is  
 146 referred to as a censored observation; it is the time a hopper “left” the study while still being alive  
 147 (Kleinbaum and Klein, 2006). In our study we have right censored data, which occurs when an  
 148 individual is not killed in the eight hour observation period or when a hopper walks out of sight of the  
 149 camera before the end of the eight hour observation period. Despite being censored, such observations  
 150 still contain the information that the individual survived up to the censoring time. The data from  
 151 censored and uncensored observations are combined to produce the so-called Kaplan-Meier estimator,  
 152 which visualizes the data obtained, without fitting a model (Kalbfleisch and Prentice, 2002).

153 Thereafter, we used the Cox proportional hazards model to account for the effect of the three  
 154 covariates (Field (A, B, C), Week (1, 4, 5, 6, 8, 9) and Time of Day (day time, evening)) on mortality  
 155 risk. The Cox proportional hazards model has the advantage that the effect of the covariates ( $\sum\beta$ ) can  
 156 be estimated without a parametric baseline hazard (Sun, 2007).

157 Finally, we used a parametric survival analysis and tested both an exponential distribution and a  
 158 Weibull distribution to describe the distribution of survival times. The hazard function (i.e., the  
 159 instantaneous death rate at time  $t$ , given that the BPH was still alive before time  $t$ ) for the exponential  
 160 distribution is given by  $h(t) = \frac{1}{\lambda}$  where  $\lambda$  is a time scale equal to the average survival time. The  
 161 survival function  $S(t)$  gives the proportion of individuals from the initial population that is still alive at  
 162 time  $t$ , or more formally, the probability that the survival time of individuals is larger than  $t$ , so in  
 163 general  $S(t) = P(T>t)$  (Kleinbaum and Klein, 2006). The survival function of the exponential  
 164 distribution is  $S(t) = \exp\left(-\left(\frac{t}{\lambda}\right)\right)$ . For the Weibull distribution, the hazard rate and survival function are  
 165 given by:

$$166 \quad h(t) = \frac{k}{\lambda} \left(\frac{t}{\lambda}\right)^{k-1} \quad \text{and} \quad S(t) = \exp\left(-\left(\frac{t}{\lambda}\right)^k\right) \quad (1)$$

167 where  $k$  is a shape parameter which defines whether the instantaneous death rate (hazard function)  
 168 increases with time ( $k>1$ ), stays constant over time ( $k=1$ ) or decreases with time ( $0<k<1$ ). Note that the

169 exponential distribution is a special case of the Weibull distribution for  $k=1$ . The average survival  
 170 time for the exponential distribution is  $\lambda$  and for the Weibull distribution  $\lambda \Gamma(1+(1/k))$ . The survival  
 171 function is estimated using both the uncensored observations (i.e., survival times) and the censored  
 172 observations (when an individual was lost or is still alive at the end of the observation), thus the  
 173 estimated survival time differs from the average survival time of the uncensored observations.  
 174 The effect of covariates was then incorporated in the parametric survival models. The extended hazard  
 175 function for the Weibull distribution is:

$$176 \quad h(t) = \frac{k}{\lambda} \left( \frac{t}{\lambda} \right)^{k-1} \exp \left( k \sum_{i=1}^p \beta_i x_i \right) \quad (2)$$

177 The cumulative hazard is obtained by integrating the hazard function (2) from 0 to  $t$ . The  
 178 corresponding survival function can be found by taking All possible models were fitted to the data:  
 179 with main effects of week, time of day and field (1 model), with two out of the three factors (3  
 180 models), with one out of the three factors (3 models) and the null model (no covariate effects). The  
 181 best model was chosen using Akaike's information criterion (AIC; Akaike, 1974). A difference in AIC  
 182 ( $= \Delta AIC$ ) between two models larger than 2 was used as criterion to conclude that the model with  
 183 minimum AIC received substantially more support from the data than the alternative model (Bolker,  
 184 2008). Significant effects of explanatory factors were determined by inspecting P-values ( $<0.05$ ).

185 The survival function is again the e-power of minus this cumulative hazard:

$$186 \quad S(t) = \exp \left( - \int_0^t \frac{k}{\lambda} \left( \frac{s}{\lambda} \right)^{k-1} \exp \left( k \sum_{i=1}^p \beta_i x_i \right) ds \right) = \exp \left( - \exp \left( k \sum_{i=1}^p \beta_i x_i \right) \left( \frac{t}{\lambda} \right)^k \right) \quad (3)$$

187 We use this formula to estimate the time until a certain fraction of survival is reached and compare  
 188 these times between fields, weeks and times of day (4).

$$189 \quad t_{fraction=f} = \lambda \left( \exp \left( -k \sum_{i=1}^p \beta_i x_i \right) \ln \left( \frac{1}{f} \right) \right)^{\frac{1}{k}} \quad (4)$$

190 The relationship between the observed BPH kills by frogs and the frog densities in the field were  
191 analysed using simple linear regression. All analyses were performed with R version 3.1.0 (R core  
192 team, 2016), and the survival analysis was conducted using the package “survival” (Therneau, 2015)  
193 while simple linear regression was done using the base function `lm()`.

194

## 195 **Results**

196 In 864 hours of video material collected over nine weeks, 2194 BPHs were observed and a total of 102  
197 mortality events were recorded. Frogs (Ranidae), wolf spiders (Lycosidae) and jumping spiders  
198 (Salticidae) were the main predators, accounting for 78, 13 and 9 kills, respectively. A ground beetle  
199 (Carabidae) and a water strider (*Aquarius elongatus*) killed both one BPH. Mortality was most  
200 frequently observed in Field A (88), followed by Field C (9) and Field B (5), and there was substantial  
201 variation in predation over time (Figure 1). While frogs were responsible for 74 and 4 BPH mortality  
202 events in Field A and B, no BPH predation by frogs was observed in Field C. In Field C jumping  
203 spiders were responsible for 7 of the 9 kills. Thirty-eight mortality events were recorded during the  
204 day and 64 during the evening. The transect counts indicated that frog densities were highest in Field  
205 A, lower in Field B and the lowest in Field C (Figure 2). There was a weak and marginally significant  
206 association between observed BPH kills by frogs and the frog densities in the field ( $R^2 = 0.12$ ,  $P =$   
207  $0.063$ ; Figure 3).

208 The six-week dataset used for the survival analysis (weeks 1, 4, 5, 6, 8 and 9) comprised 576  
209 observation hours and 93 mortality events. Kaplan-Meier curves indicated that Field and Time of Day  
210 had multiplicative effects on the hazard rate, i.e. survival curves in different fields or at a different  
211 time of day had the same overall shape, but differed by a constant (“proportional”) factor affecting the  
212 time scale of the curve (multiplication or contraction along the time axis). In contrast, the curves for  
213 the different weeks crossed, i.e. the time courses were dissimilar between different weeks not only in  
214 time scale (related to  $\lambda$  in the Weibull model), but also in shape (related to  $k$  in the Weibull model;  
215 Figure 4). When fitting the exponential and Weibull distribution with covariates to the stratified data

216 for each week separately, assuming that the effect of the covariates of Field and Time of day was  
217 constant over the weeks, the Weibull model received substantial more support from the data than the  
218 exponential model ( $\Delta AIC = 65.2$ ), indicating that mortality risk was not constant over time. Indeed,  
219 the estimates for the shape parameter  $k$  of the Weibull model were less than 1 for all weeks, indicating  
220 that the risk of predation was highest at the start of the measurements and decreased over time (Table  
221 2). A common scale parameter  $\lambda$  of approximately 99 hours was fitted for the whole data set, meaning  
222 that if hoppers were not killed at the start, it would take a very long time ( $\sim 4$  days) before a predation  
223 event would happen. The killing rate for a BPH was therefore highest within approximately the first  
224 10 minutes of the experiment, when the BPH density was still relatively high. Thereafter, the killing  
225 rate quickly diminished to almost zero. The Weibull model further indicated that mortality risk in  
226 Fields B and C was significantly lower than in Field A, and that mortality risk was significantly higher  
227 in the evening than during the day ( $P < 0.05$ ; Table 2; Appendix 1). In Field A, the estimated time of  
228 80% survival was 9.5 hours during day time and after 4.4 hours in the evening (Table 3). In fields B  
229 and C, survival was still above 95% after 3 hours (Figure 4B).

230

## 231 **Discussion**

232 Our study has three key findings. First, we show that predator communities predated upon BPH differ  
233 per rice field, resulting in different BPH mortality rates. Second, predation pressure is higher in the  
234 evening than during day time, and third, mortality rate was highest at the start of the experiment when  
235 BPH densities were still relatively high, but quickly decreased after that. Our study confirms the  
236 findings of Zou et al. (2017) and Khatiwada et al. (2016) that frogs are the main predators of BPH. In  
237 the study area *Rana limnocharis* is the most common species of frog (Xiao, unpublished data). The  
238 contribution of frogs to BPH predation was 77% in our current study which is similar to the 75%  
239 reported by Zou et al. (2017).

240 There were clear differences in predation rate between the three fields (Figure 1). The differences in  
241 frog predation between the three fields are likely to be related to the differences in frog densities,

242 which was highest in Field A ( $0.173 \pm 0.028$  frogs/m<sup>2</sup>; mean  $\pm$  SEM), followed by Field B ( $0.083 \pm$   
243  $0.010$ ) and Field C ( $0.017 \pm 0.004$ ), respectively (Figure 2). However, predator density is not the only  
244 factor affecting predation risk; this risk is expected to depend also on prey density. With higher prey  
245 density in a field, the risk of predation per individual prey is diminished as a result of lower  
246 predator/prey ratio. In Field A, BPH density ranged between 0.45 and 2.57 BPH per plant  
247 (Supplementary information Table S1), which can be considered as low and well below economic  
248 damage thresholds (Sogawa & Cheng, 1979). While no formal BPH density assessments were made in  
249 fields B and C, BPH were much more commonly observed in these fields than in field A, indicating  
250 that densities of hoppers were substantially higher in fields B and C than in field A. These two factors,  
251 the difference in frog density between the fields, and the difference in BPH density, combine to have a  
252 high predator: prey ratio in Field A, an intermediate ratio in Field B, and a low ratio in Field C. The  
253 difference in predator: prey ratio between the fields provides a plausible explanation for the large  
254 differences in predation observed between Field A versus Fields B and C. While different camera  
255 systems were used in Field A (surveillance cameras), and Field B and C (DV cameras), we do not  
256 consider the difference in equipment a plausible explanation for the differences in predation observed  
257 between the fields. Both video recording systems provided clear images of predation, and we cannot  
258 think of plausible reasons (e.g. colour, size, sound, or odour) why one type of camera should attract or  
259 repel more frogs than another type of camera.

260 Many different factors may be responsible for the differences in frog abundance between the fields.  
261 First, Field C and the surrounding area consisted of grassland and a tree nursery for more than five  
262 years before the land was converted into cropland in early 2016. Grassland and nurseries are less  
263 suitable habitat for frogs and therefore the initial frog density in 2016 may have been low. Second,  
264 even though no pesticide was used in field C, it was bordered by a stream in which farmers emptied  
265 and cleaned their spraying devices. Possibly, pesticides of the stream ended up in Field C impacting  
266 frog populations. Indeed, frogs can be directly affected by pesticides (Hayes et al., 2006), or indirectly  
267 via cascading effects in the food chain (Relyea & Diecks, 2008). Thus, even though frogs may be  
268 effective predators of BPH in relatively undisturbed systems, frogs may be sensitive to human-induced

269 disturbances. As such, on-farm conservation biocontrol measures and/or agri-environment schemes  
270 that provide undisturbed aquatic habitat and refuge for frogs may contribute to frog-mediated  
271 biocontrol (Holland et al., 2016).

272 The correlation between estimated frog densities and frog-mediated kills was weak, and for specific  
273 combinations of field and time of day, negative correlations were found between estimated frog  
274 densities and frog-mediated kills (Fig. 3). This indicates that visual frog counts cannot easily be used  
275 as predictors of frog-mediated BPH predation. Possibly, predator/prey ratios would have provided a  
276 better predictor of predation risk. In future studies, it may be considered to not only assess predator  
277 densities to explain predation risk but also the background density of BPH and other prey.

278 Frog-BPH interactions are complex and are not yet well understood. There was a striking pattern of  
279 high frog densities in week 1 and 2, and then a sudden drop in week 3, after which the frog densities  
280 increased again (Fig 1). This drop in observed density is unlikely to be related to population dynamic  
281 phenomena since *R. limnocharis* may live for several years (Liao et al., 2011). Potentially, the drop in  
282 frog counts in transect walks at the edge of the fields in week 3 may be explained by a reduction in  
283 activity of frogs or relocation to other areas, rather than a transition from one frog generation to  
284 another. The field specific density of frogs suggest that (i) these may be influenced by field-specific  
285 management practices, including pollution with agrochemicals, and that (ii) frog-prey interactions  
286 mainly operate at the field scale rather than at the landscape scale, because the three study sites were  
287 essentially in one and the same landscape, but showed diverging results regarding the importance of  
288 frogs in predation. The scale at which landscape factors affect frog populations is unknown and needs  
289 to be studied further.

290 Our finding that predation rate of BPH was higher in the evening than during the day time points  
291 towards diurnal patterns in predation. Diurnal predation patterns have also been reported previously in  
292 tropical rice systems. For example, de Kraker et al. (2000) found that predation rates of the crickets  
293 *Metioche vittaticollis* and *Anaxipha longipennis* on eggs of leaf folder *Cnaphalocrocis medinalis* in  
294 the Philippines were the lowest around noon and highest in the early morning and late evening.

295 Kiritani et al. (1972) found that *Enoplognatha*, *Tetragnatha* and *Lycosa* spiders were most active  
296 during the evening and night, while *Oedothorax* spiders were most active during the day. In addition,  
297 many amphibians, including frogs, are nocturnal and are relatively inactive during the day (Wells,  
298 2007). In our study which was conducted during the height of summer with very hot day temperatures,  
299 frogs may have been more active in the evening when the risk of predation may be lower and  
300 temperatures are less extreme than during day time.

301 Survival analysis indicated that mortality risk was highest at the start of the experiment. Similar  
302 dynamics were observed in Field A in 2015 (Zou, pers. obs.). This phenomenon may be explained by  
303 the high BPH densities at the start of the experiment (approximately 21 BPH on experimental rice  
304 stems versus 0.45-2.57 BPH per rice plant in Field A), and introduced BPH were more mobile and  
305 therefore easier to spot by predators. Density-dependent predator-prey interactions may involve a  
306 numerical response (predators aggregating in locations with high prey density) and/or a functional  
307 response (predators attain higher *per capita* predation rate in locations with high prey density).

308 Kenmore et al. (1984) reported density-dependent regulation of BPH populations in rice, which was  
309 associated with a positive numerical response of spiders to plant hopper density. While we are not  
310 aware of studies that investigated density-dependent responses of frogs to prey density, we consider  
311 density-dependent responses of frogs to BPH densities plausible since this is a common ecological  
312 phenomenon (Holling, 1959; Hassell & May, 1974).

313 We cannot rule out the possibility that predators exhibited learning behaviour, aggregating to the sites  
314 where the cameras were set up and where soon prey would be introduced, even though we tried to  
315 account for such effects by changing the locations in the field of the cameras in each recording  
316 session. If this would be the case, our estimation of predation risk of BPH by frogs would be an  
317 overestimate. Surprisingly, the window of high predation risk was only short, in the order of ten  
318 minutes, after which the risk of predation quickly declined to virtually zero. This finding indicates that  
319 predation by frogs may be enhanced by aggregation of hoppers, by movement of the hoppers, and  
320 possibly by learning behaviour of frogs.

321 The short period of intensive predation explains the low overall predation pressure in the nine-week  
322 observation period (102 kills in 864 observation hours, which is approximately 0.12 predation events  
323 per hour). This low predation pressure seems to be atypical because strong top down control on BPH  
324 populations in rice has been reported in several studies (Kenmore et al., 1984; Settle et al., 1996;  
325 Claridge et al., 2002), including predator exclusion studies that were conducted in the same study  
326 region (Zou et al., unpublished data). This paradox merits further investigation. Nevertheless, camera  
327 observations provide important information on biocontrol of agricultural pests by providing contextual  
328 and direct information about the identity and relative contribution of natural enemies (Frank et al.,  
329 2007; Grieshop et al., 2012). Disturbances associated with the establishment of the equipment and  
330 introduction of experimental insects (BPH prey) may be an important factor in behavioural studies  
331 with frogs, and further development of experimental methods is needed to minimize biases in data  
332 collection. Furthermore, the very large field-to-field variation in frog abundance and predation rates  
333 points to the importance of crop management, and the potential role of pesticides, land use history or  
334 other anthropogenic effects as factors affecting biological control. Before a general conclusion on the  
335 importance of frogs as a natural enemy of BPH can be drawn, further studies are needed on the  
336 importance of frogs, birds and arthropod natural enemies of BPH in rice growing regions at other  
337 locations. Such studies would provide important input for crop and habitat management measures that  
338 may improve BPH control in rice.

339

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426 Zou, Y., de Kraker, J., Bianchi, F.J.J.A., van Telgen, M.D., Xiao, H., van der Werf, W., 2017. Video  
427 monitoring of brown plant hopper predation in rice shows flaws of sentinel methods. Scientific  
428 Reports 7: 42210 DOI: 10.1038/srep42210

429

430 Table 1. Overview of the number of predation events of brown plant hoppers in three fields (A, B, and  
 431 C) during nine weeks (1: July 1, 2: July 6, 3: July 13 or 16, 4: July 20, 5: July 27, 6: August 2, 7:  
 432 August 11, 8: August, 17, and 9: August 24). Recordings were made each week in Field A, and in  
 433 alternating weeks in fields B and C. Three cameras were used in each field during day time (8:00-  
 434 16:00) and evening (17:00 – 01:00 h).

Field	Time	Week									Total
		1	2	3	4	5	6	7	8	9	
A	Day	6	0	0	3	0	6	10	3	3	31
	Evening	21	4	0	2	5	4	3	10	8	57
B	Day	1	- <sup>1</sup>	0	-	0	-	1	-	0	2
	Evening	1	-	0	-	2	-	0	-	0	3
C	Day	-	0	-	1	-	3	-	1	-	5
	Evening	-	0	-	0	-	3	-	1	-	4

435 <sup>1</sup>A dash indicates that no observations were made during that week in that field.

436

437 Table 2. Estimates of the stratified Weibull model that best fitted the data. The geometric mean of all  $k$   
 438 parameters is 0.64, indicating a concentration of predation events near the start of the measurements.  
 439 Field A during the day was used as a baseline. Regression parameter values ( $\beta$ ) represent departures  
 440 from the reference, where a  $\beta$ -value equal to 0 means no difference from the baseline.

Effects of covariates ( $\exp(\beta)$ )				
Factor	Effect size ( $\exp(\beta)$ )	$\beta$ (s.e.)	z-score	$P$
Field B	0.08	-2.4940 (0.875)	-2.849	0.0044
Field C	0.15	-1.9082 (0.670)	-2.848	0.0044
Evening	2.17	0.7716 (0.386)	-1.997	0.0458
Weibull model parameters				
parameter	estimate	Overall/ week number		
$\lambda$	99.41 h	Overall		
$k$	0.59	Week 1		
	0.96	Week 4		
	0.76	Week 5		
	0.55	Week 6		
	0.53	Week 8		
	0.55	Week 9		

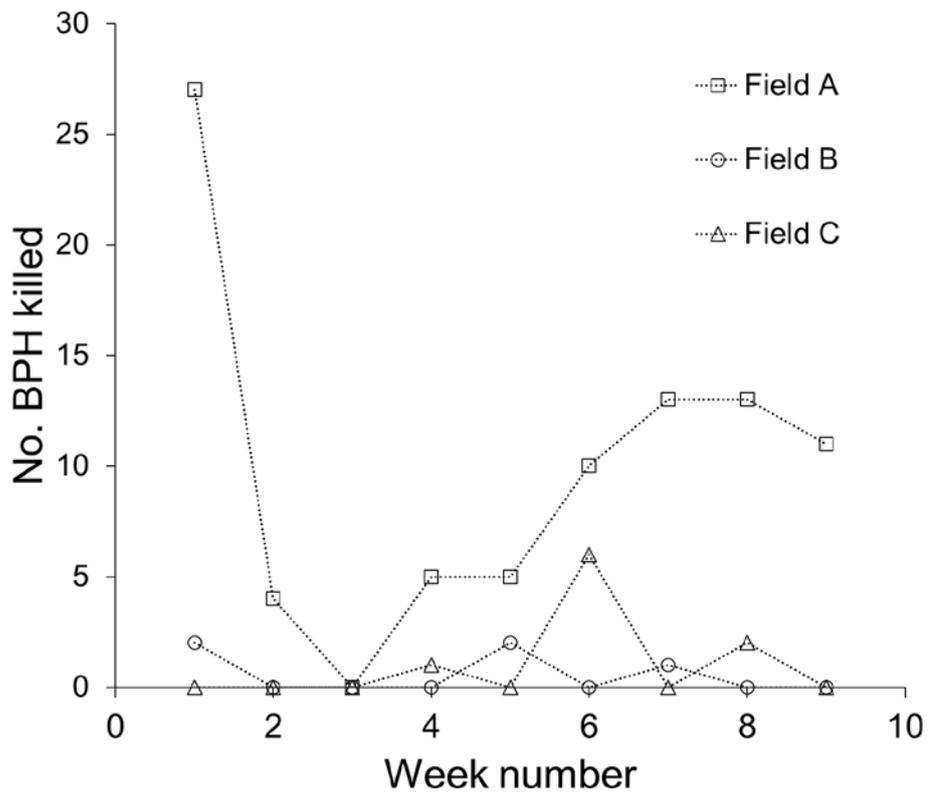
441

442

443 Table 3. Estimated times (in hours) at which 95, 90, 85 or 80% of the initial numbers of BPH survived  
444 in Field A based on the model presented in Table 2.

	95%	90%	85%	80%
Field A, day time	0.96	2.95	5.81	9.54
Field A, evening	0.44	1.37	2.69	4.41

445

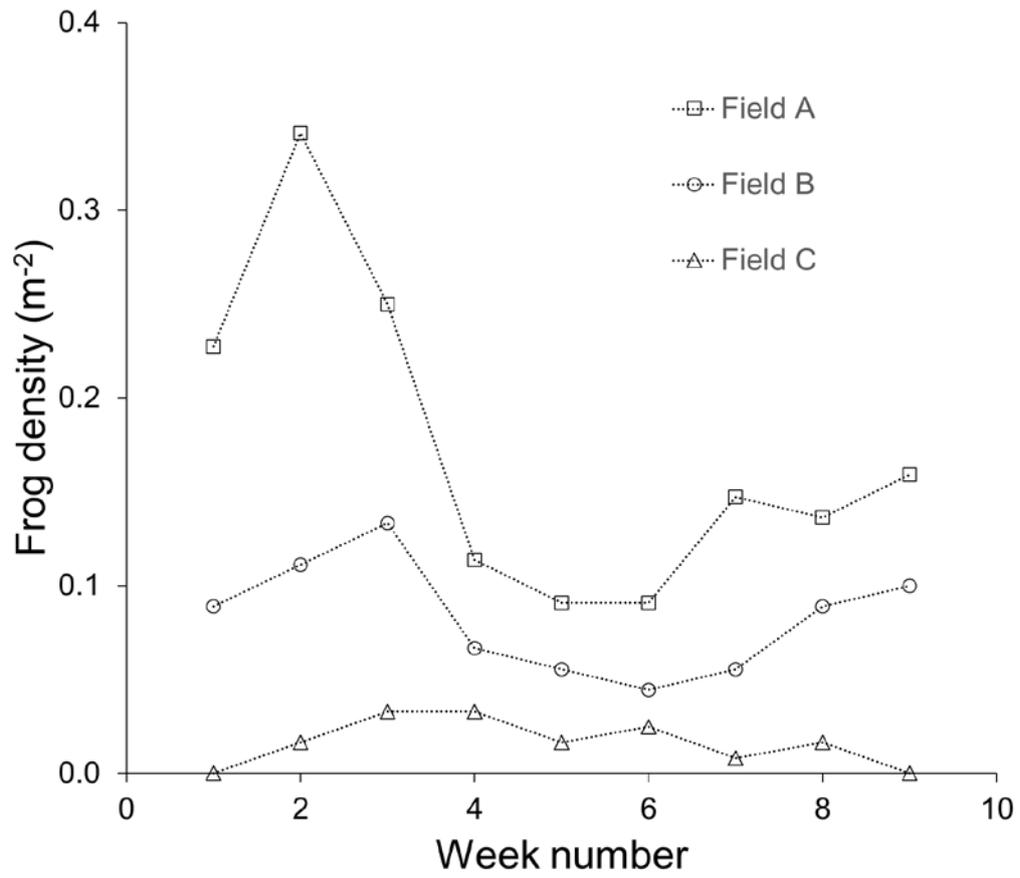


446

447 Fig. 1. Number of brown plant hopper predation events during nine weeks in rice fields A, B and C.

448 Week 1 started on the 1<sup>st</sup> of July and week 9 ended on the 30<sup>th</sup> of August 2016. For this figure,

449 observations of day and night observations were combined.



450 Fig. 2. Weekly estimates of adult frog density (m<sup>-2</sup>) in three rice fields A , B and C. The first count was  
 451 made on 28 June 2016.

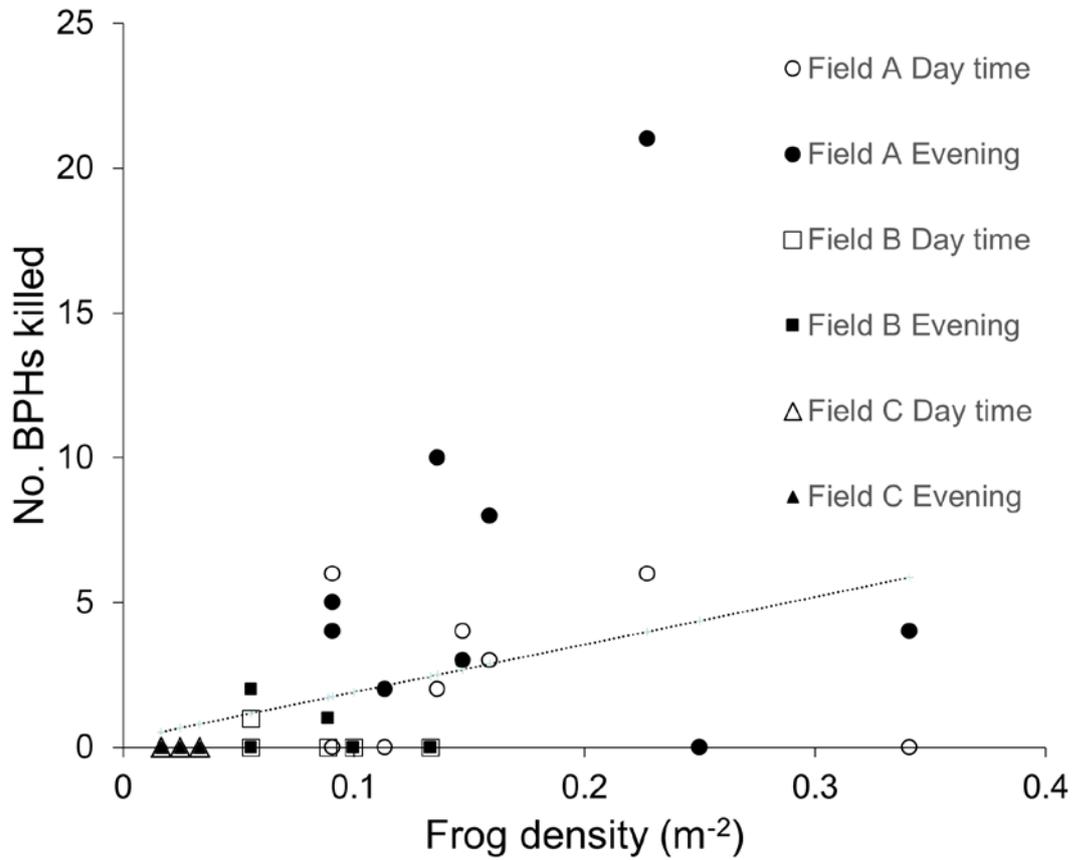
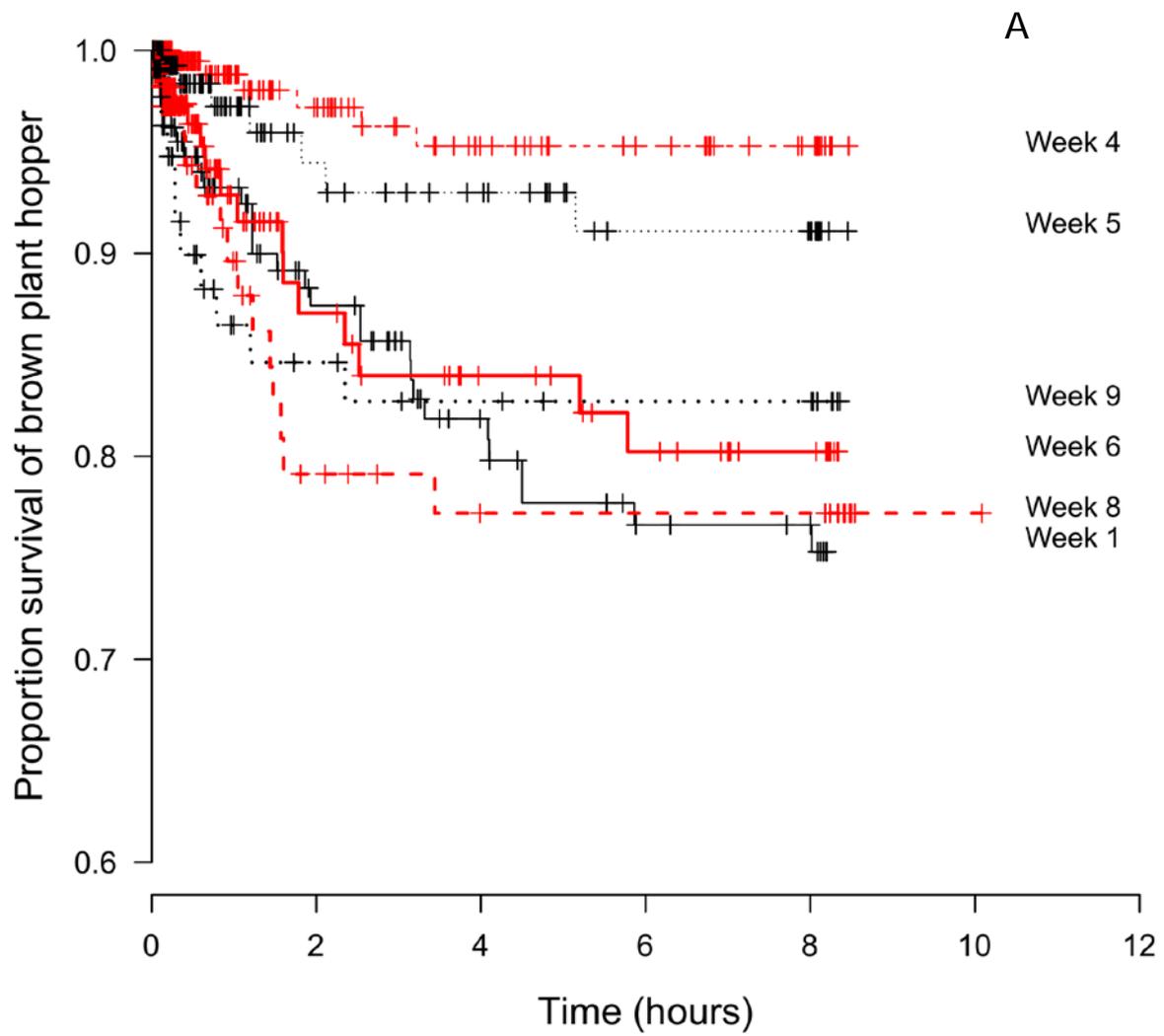
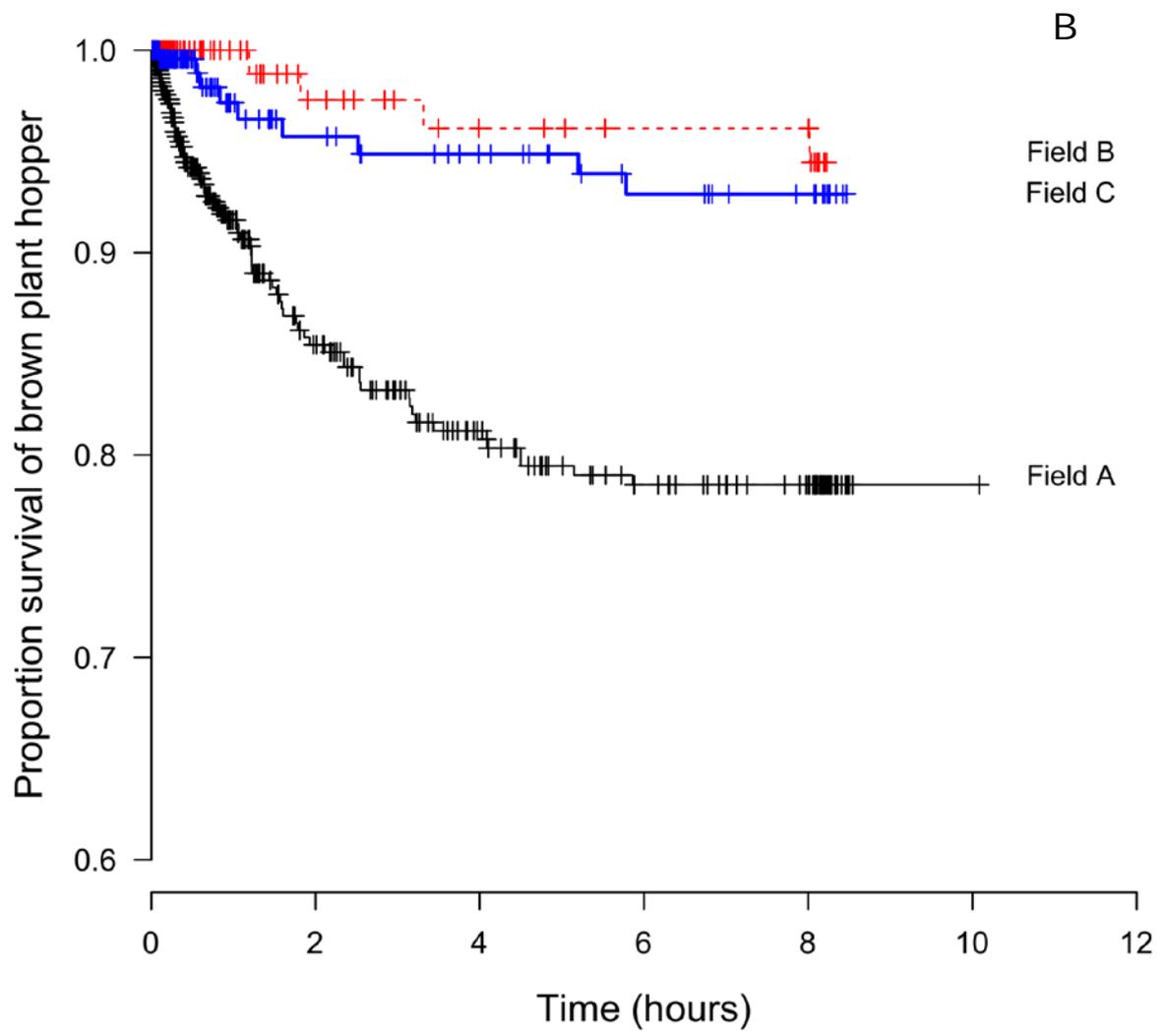


Fig 3. Scatterplot of the relationship between frog density and the number of brown plant hoppers killed by frogs (linear regression equation for all data:  $y = 16.465x + 0.2634$ ;  $R^2 = 0.12$ ;  $P = 0.063$ ).





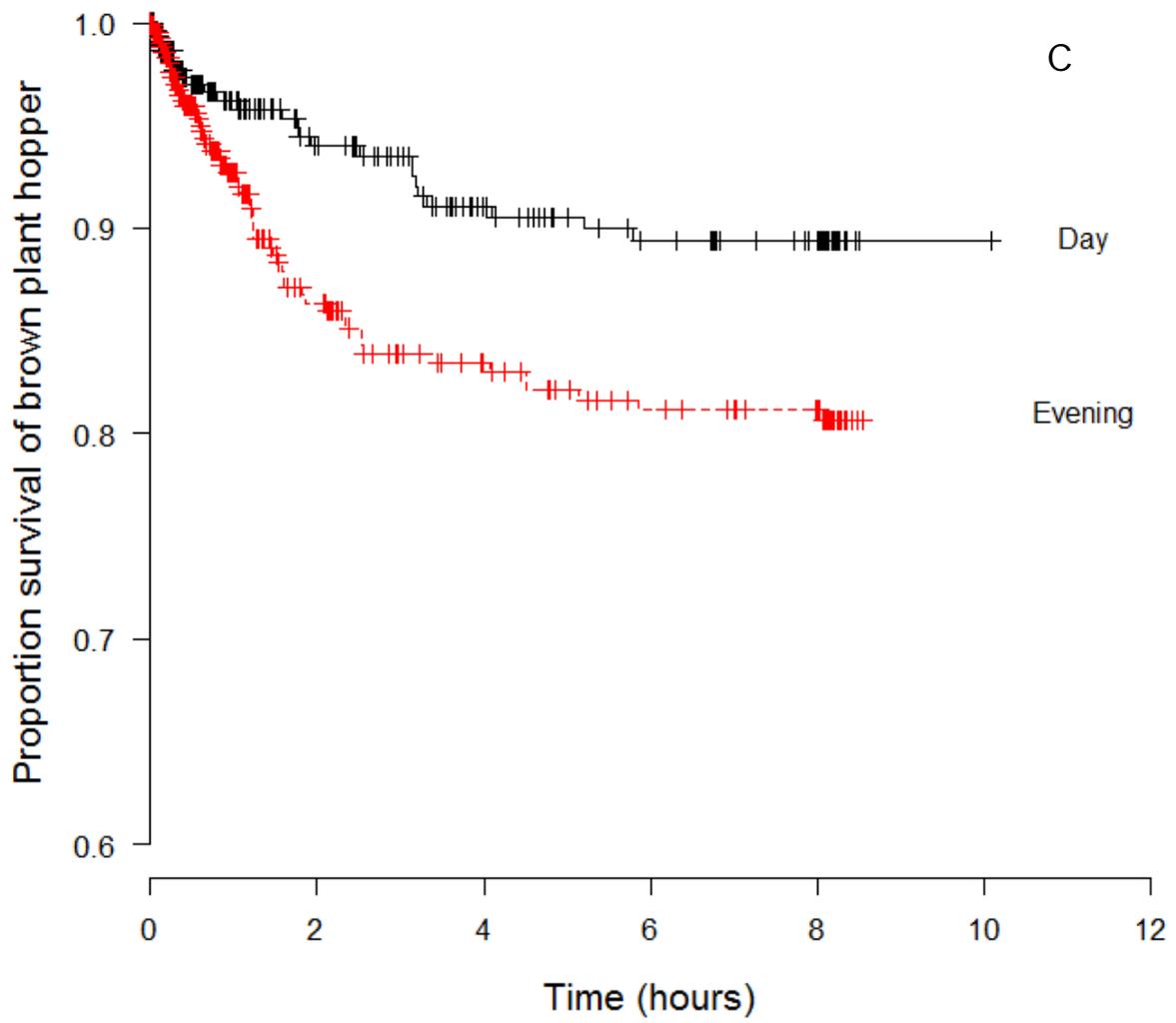


Fig 4. Kaplan-Meier survival curves for the observations in different Weeks (A), Fields (B) and Time of day (C). Censored data are indicated with “+”.

453 **Electronic supplementary material 1**

454 Table S1. Brown plant hopper (BPH) densities per plant (mean  $\pm$  SEM) on 20 plants in Field A from  
455 July to August 2016. BPH densities were assessed by inserting a plate (39 cm  $\times$  29.5 cm  $\times$  2 cm) at the  
456 base of a rice plant, shaking the plant, and counting the number of dropped BPH on the plate (Hu et  
457 al., 2011).

458

459

---

460 **Date**                      **BPH/plant**

461

462 7-Jul                      0.45  $\pm$  0.25

463 12-Jul                     1.45  $\pm$  0.25

464 18-Jul                     2.38  $\pm$  0.75

465 3-Aug                      1.64  $\pm$  0.47

466 7-Aug                      2.57  $\pm$  0.61

467 14-Aug                     1.75  $\pm$  0.86

468 22-Aug                     2.13  $\pm$  0.61

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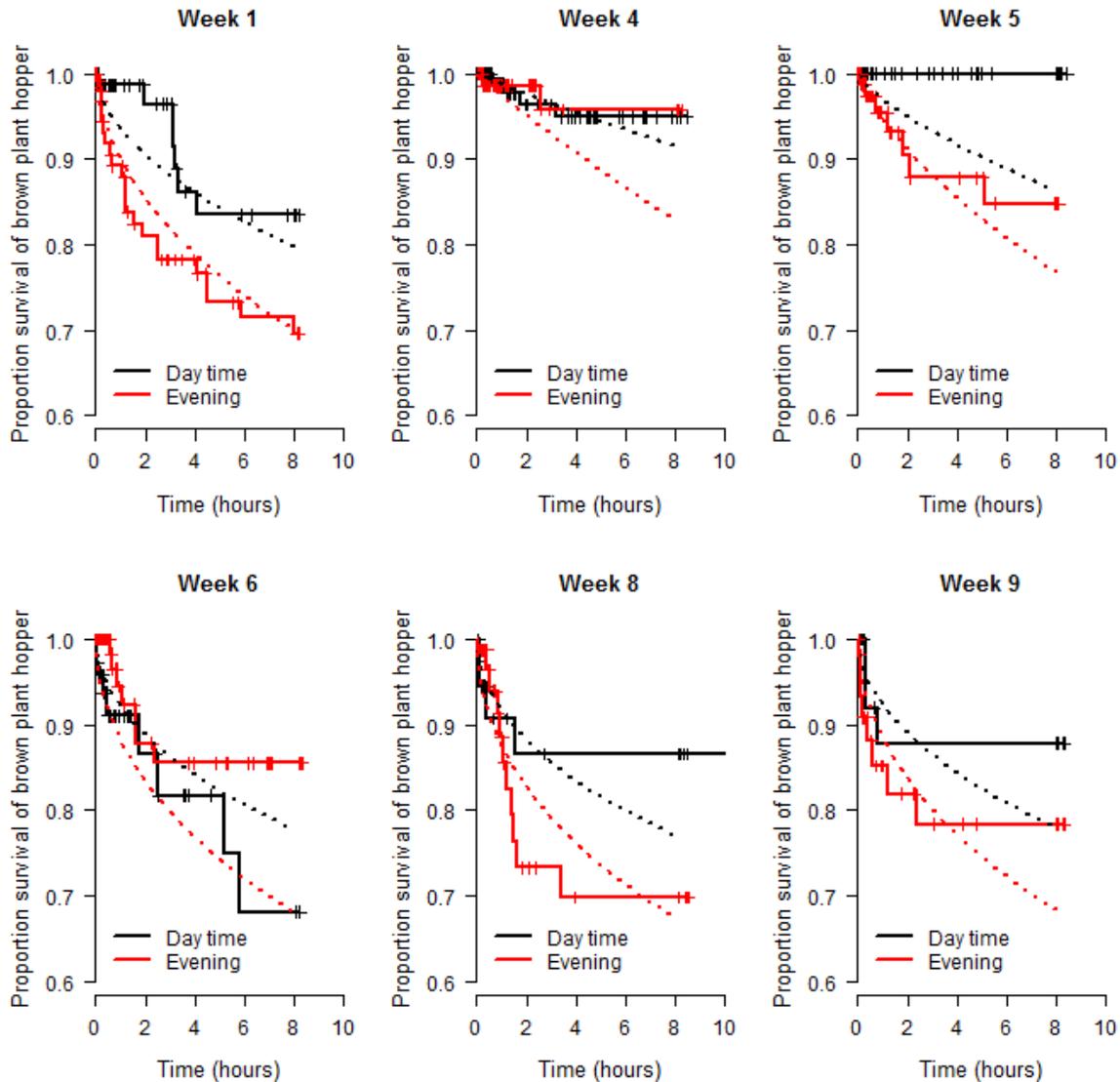
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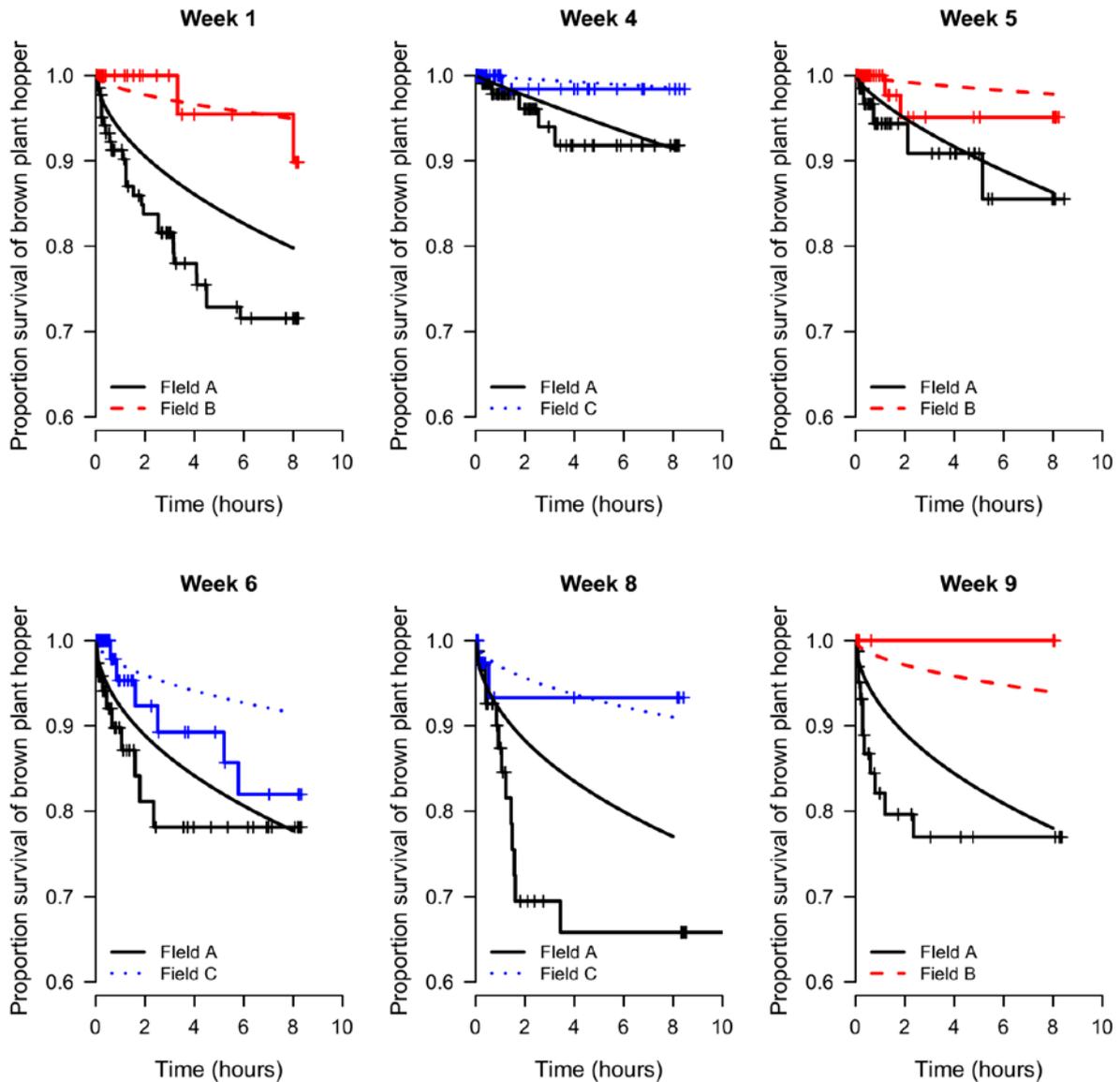
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474

475 The predicted survivor curves in Figure S1 and S2 are based on the analysis of the full six-week  
 476 dataset. Due to the low number of predation events it was not possible to conduct analyses on subsets  
 477 for separate weeks, fields or times of day.



478  
 479 Fig S1. Kaplan-Meier survival curves and best fitting parametric (Weibull) models for observations on  
 480 BPH predation during six weeks during day (black) or evening (red). The Kaplan Meier estimator for  
 481 each week and time of day is shown as a step curve (solid lines) while the best fitting (Weibull) model  
 482 is shown by dotted lines. This best model has a constant proportional effect of time of day and does  
 483 not account for interactions between week and time of day. The scale parameter  $\lambda$  is week-specific.  
 484 Censored data are indicated with “+”.



485  
 486 Fig S2. Kaplan-Meier survival curves and best fitting parametric (Weibull) models for observations on  
 487 BPH predation during six weeks in three fields. Kaplan-Meier survivors are plotted in solid black  
 488 (Field A), red (Field B) and blue (Field C) while the Weibull curves are plotted as dashed lines in the  
 489 same colours. The best fitting Weibull model has a constant proportional effect of field and does not  
 490 account for interactions between week and time of day. Censored data are indicated with “+”.