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20 **Spring Temperature Predicts the Long-term Molting Phenology of Two Cicadas,**

21 **_Cryptotympana facialis_ and _Graptopsaltria nigrofuscata_ (Hemiptera: Cicadidae)**

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Abstract

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36   To investigate how seasonal insects respond to changing environments, nymphal skins of the two

37   cicadas *Cryptotympana facialis* (Walker) and *Graptopsaltria nigrofuscata* (Motschulsky)

38   (Hemiptera: Cicadidae) were monitored weekly from late July to August over 12 years in a

39   suburban habitat of central Japan. Based on over 8,000 skins collected from 1995 to 2006, we

40   estimated the period during which temperature or precipitation impacted skin abundance and

41   phenology. Adults of *C. facialis* tended to emerge earlier than those of *G. nigrofuscata*, for which

42   total skin counts fluctuated up to sevenfold among years. The effective accumulated temperature

43   from the previous 3.0–3.5 months to the most recent 1.0–1.5 months at a threshold of

44   approximately >18°C showed the best fit to the cumulative skin counts within a season.

45   Temperature explained 47% and 64% of the total variation in the skin counts for *C. facialis* and *G.*

46   *nigrofuscata*, respectively. The point at which temperature had this effect was consistent between

47   male and female cicadas. Conversely, accumulated precipitation accounted for <16% of the

48   variation in the skin counts for both species. In summary, our long-term study revealed that

49   late-spring temperature plays a key role in predicting the molting phenology of *C. facialis* and *G.*

50   *nigrofuscata* but does not necessarily explain a large amount of the abundance fluctuation.

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52   Keywords: *Cryptotympana facialis*, Effective accumulated temperature, *Graptopsaltria*

53   *nigrofuscata*, Phenology, Population fluctuation

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Introduction

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61   Understanding the responses of organisms to changing environments is important for

62   predicting how climate change threatens biodiversity and the ecosystem services provided by

63   particular species. Evidence has accumulated to suggest that recent climate warming has shifted

64   the phenology (Roy and Sparks 2000, Bale et al. 2002), distribution (Bale et al. 2002, Hagen et al.

65   2007, Harsch et al. 2009), and population dynamics (Jilliard et al. 2004, Kiritani 2006, Chaves et

66   al. 2012) of species based on their physiological constraints. Seasonal outbreaks are known to

67   occur in several insect taxa, such as mayflies (Harper and Peckarsky 2006), bush crickets

68   (Hochkirch and Damerau 2009), and winter moths (Hagen et al. 2007). The phenology of seasonal

69   organisms is considered an indicator of climatic impacts on local biodiversity (Roy and Sparks

70   2000, Gibbs and Breisch 2002, Ogawa-Onishi and Berry 2012). However, both snapshot

71   abundance measurements and long-term presence/absence records are unable to disentangle the

72   seasonality and increase/decrease of population size. Thus, a long-term survey considering both

73   phenology and abundance is necessary to combine the physiological response of a species to the

74   changing environment with its population fluctuation.

75   Cicadas (Hemiptera: Cicadidae) provide an important ecosystem function, as they

76   transfer a significant flux of nutrients from belowground to aboveground (Karban 1982,

77   Menninger et al. 2008). The black cicada, *Cryptotympana facialis* (Walker), and the large brown

78   cicada, *Graptopsaltria nigrofuscata* (Motschulsky), are common species distributed across

79   western Japan. Unlike periodical cicadas, these Japanese cicadas emerge every summer (Dunn

80   2000). *Cryptotympana facialis*, is distributed more southerly and occurs intensively in urbanized

81   habitats. Conversely, *G. nigrofuscata* is usually observed in suburban and rural areas covered by

82   trees. Because of the gradual habitat partitioning between *C. facialis* and *G. nigrofuscata*, these

83   two cicadas are regarded as index species of urbanization (Shiyake 2008). Recently, *C. facialis*

84   has been shown to be increasing as *G. nigrofuscata* decreases across the most urbanized area of

85   western Japan, Osaka City (Kitamoto 1997, Shiyake 2012). Several previous studies have

86   attributed this compositional shift of the cicada community to the heat island phenomenon

87   (Moriyama and Numata 2011) or to habitat fragmentation combined with avian predation

88   (Takakura and Yamazaki 2007).

89   The physiological responses of *C. facialis* and *G. nigrofuscata* have been well-studied in

90   terms of local climate change in urbanized areas. For example, temperature determines the timing

91   and rate of egg hatching, and the eggs of both *C. facialis* and *G. nigrofuscata* exhibit some degree

92   of cold tolerance (Moriyama and Numata 2008, 2009). In the field, adults of *C. facialis* are known

93   to emerge earlier as temperature increases (Shiyake 2012). Humidity is also important for the

94   hatching rate of *C. facialis* and *G. nigrofuscata* (Moriyama and Numata 2010). Thus, in

95   conjunction with the desiccation dependence of egg hatching, warming influences cicada fitness

96   by synchronizing the hatching phenology of *C. facialis* (Moriyama and Numata 2011). However,

97   the extent to which these climate variables explain long-term phenology and population

98   fluctuation remains insufficiently understood.

99   In this study, we employed a 12-year field survey and statistical modeling to estimate

100   how abiotic factors influence the molting phenology and abundance of the two species of

101   Japanese cicada. Specifically, three questions were addressed in this study: Which abiotic factor,

102   temperature or precipitation, influences the molting phenology of *C. facialis* and *G. nigrofuscata*

103   to a greater degree? Do the duration and degree of change for this factor necessary to influence

104   molting phenology differ between *C. facialis* or *G. nigrofuscata*? Finally, to what extent do these

105   environmental factors explain the variation in cicada abundance? Based on the present results and

106   other previously documented evidence, we discuss the factors responsible for long-term variation

107   in the phenology and abundance of cicada populations.

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109   **Materials & Methods**

110   **Study site.** We conducted the study at a suburban site located in Hokusei-cho, Mie

111   Prefecture, central Japan (34°09′N, 136°31′E, approximately 100 m in altitude). The study site

112   consisted of 208 m^2 of shady area near a small park surrounded by residential houses. The ground

113   was covered with gravel in which a few weeds grew. Twenty-one trees of ten species were planted

114   sparsely within the study area. These trees consisted of the evergreen magnolia *Magnolia*

115   *grandiflora* L. ($n = 1$, diameter at breast height = 18 cm), the empress tree *Paulownia tomentosa*

116   (Thunb.) Steud. ($n = 1$, DBH = 32 cm), the chinaberry *Melia azedarach* L. ($n = 1$, DBH = 51 cm),

117   *Acer palmatum* Thunb. ($n = 1$, DBH = 51 cm), the hemp palm *Trachycarpus fortunei* (Hook.)

118   H.Wendl. ($n = 1$, DBH = 11 cm), the Japanese cypress *Chamaecyparis obtusa* (Siebold et Zucc.)

119   Endl. ($n = 1$, DBH = 46 cm), the Japanese persimmon tree *Diospyros kaki* Thunb. ($n = 1$, DBH =

120   51 cm), the wild cherry tree *Cerasus jamasakura* (Siebold ex Koidz.) H. Ohba ($n = 2$, DBH = 50,

121   50 m), *Camellia* sp. ($n = 2$, DBH = 13, 22 cm), and *Podocarpus macrophyllus* (Thunb.) Sweet (n

122   = 10, DBH = 10 cm for all individuals). The Japanese persimmon tree died in 1999, and the

123   magnolia and empress tree were cut in 2006 due to the development of the nearby area.

124   **Collection of cicada skins.** *Cryptotympana facialis* and *Graptopsaltria nigrofuscata*

125   individuals spend a nymphal period of several years on tree roots belowground. When the final

126   instar is ready to molt, it emerges from the ground and sheds its skin on a nearby tree or artificial

127   structure. The adult cicada flies away after molting, while its exuvia remains for several weeks.

128   The two species in this study can be distinguished by these skins based on the width, darkness,

129   and color pattern of the abdomen. Thus, skin number is an excellent predictor of cicada

130   abundance within a site.

131   We visited the site and collected all cicada skins at least weekly from late July to the

132   end of August for 12 years, from 1995 to 2006. The cicada skins were collected by hand from the

133   ground, tree surfaces, and artificial structures (such as fences and poles) within the site. We also

134   used an insect net (4 m in length) to collect skins attached to elevated places (up to approximately

135 6 m in height). The skins were taken to the laboratory and counted. From 2001 to 2006, sexes

136 were also identified from the cicada skins based on the presence/absence of an ovipositor on the

137 tip of the abdomen. From 1997 to 2000, the number of holes on the ground was also counted; the

138 holes were buried after each survey to avoid counting them repeatedly. The surveys were

139 conducted on sunny or cloudy days. Skins were collected during the morning, because the

140 nymphal cicadas emerged during the evening and molted during the night. Detailed survey dates

141 and skin counts are available in the supplementary material (Supp. Table S1).

142   **Weather data.** Data were downloaded from the website of the Japan Meteorological

143 Agency (http://www.data.jma.go.jp/gmd/risk/obsdl/index.php). The weather stations nearest to the

144 study site were selected for temperature (Kuwana, Mie Prefecture: 35°03′N, 136°41′E, 3 m in

145 altitude) and precipitation data (Hokusei-cho, Mie Prefecture: 35°08′N, 136°32′E, 125 m in

146 altitude). Daily mean and maximum temperatures, as well as daily total precipitation, were

147 compiled from 1994 to 2006. Several time points were not available (one point for temperature

148 and three points for precipitation) from the database; these values were estimated using the

149 average of the neighboring two days. To improve the presentation of data in the figure, the daily

150 temperature was smoothed using a spline method. We used the smooth.spline function

151 implemented in R version 3.0.1 (R Core Team 2013).

152   **Data analysis.** We estimated the period during which the temperature or precipitation

153 impacted the skin counts. The response variable was the cumulative number of skins during one

154 season (which was $\ln(y+1)$ transformed to improve normality). The explanatory variable was the

155 effective accumulated temperature or precipitation, which was calculated using daily mean

156 temperature (°C) or daily total precipitation (mm). The effective accumulated temperature or

157 precipitation was modeled as follows: $E = \Sigma[t = t_0 \to t_1] (x_t - th)$ {if $(x_t - th) > 0$, otherwise 0}. The

158 effective accumulated temperature or precipitation E represents the sum of the precipitation or

159 temperature x at day t, higher than the threshold value th, from the past t_0 to the most recent t_1

160 days. The effect of E was analyzed using the linear regression $\ln(y+1) = a \times \ln(E) + b$. We then

161 determined the unknown parameters a, b, t_0, t_1, and th that minimized the ordinary least-squares of

162 the cumulative skin counts. The coefficient a and slope b were estimated on an exponential scale

163 to obtain positive values (i.e., $a = \exp(a)$, $b = \exp(b)$). For the least-square optimization, we used

164 the simulated-annealing method (Belisle 1992) and the Nelder-Mead method (Nelder and Mead

165 1965) because of their applicability to nondifferentiable functions (implemented in the optim

166 function in the R software). To avoid local optima, we first applied the simulated-annealing

167 method (which is robust for the initial parameters but requires a long calculation time) with the

168 following initial parameters: $t_0 = 90$; $t_1 = 30$; $th = 15$ (for temperature) and 5 (for precipitation); $a =$

169 $\exp(0) = 1$; and $b = \exp(0) = 1$. We then utilized the Nelder-Mead method (which requires less

170 calculation time than the simulated-annealing method) after the parameters obtained from the

171 previous calculation were set as the initial parameters. This Nelder-Mead optimization was

172 repeated until the algorithm converged. Model fitting was evaluated by the proportion of the

173 explained sum of squares (representing the coefficient of determination, R^2: Kvålseth 1985), and

174 statistical significances were examined by F-tests for the mean squares of the explained and

175 residual sums of squares ($df1$ = no. of parameters – 1, and $df2$ = sample size – $df1$). These

176 analyses were performed for the two cicada species and two climate variables (temperature and

177 precipitation). Temperature and precipitation were separately fitted to avoid multicollinearity. The

178 best-fitted temperature and precipitation were further analyzed using multiple regressions to test

179 their combined effects on the skin counts. Two additional analyses were also conducted to confirm

180 the robustness of the parameter estimation. First, we repeated the temperature analysis using the

181 daily maxima instead of the means. Second, for the data from 2001 to 2006, we separately

182 analyzed male and female skins for *C. facialis* and *G. nigrofuscata*. The R code and input data

183 files are provided as supplementary materials (Supp. Appendix 1 and 2 for the code and input data

184 files, respectively).

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Results

        Patterns of molting phenology. Skins of *C. facialis* were observed earlier than those of *G. nigrofuscata* (Fig. 1). Total skin counts fluctuated up to sevenfold among the study years. The yearly total counts fluctuated at a similar level between *C. facialis* and *G. nigrofuscata* (coefficient of variation = SD/Mean = 0.55 and 0.60, respectively). The total number of ground holes roughly corresponded with the total skin count for each year (1349, 960, 683, and 550 holes for 1997, 1998, 1999, and 2000, respectively). From 2001 to 2006, male cicadas emerged earlier than females for both *C. facialis* and *G. nigrofuscata* (Fig. 1). Two other cicada species, *Platypleura kaempferi* (Fabricius) and *Meimuna opalifera* (Walker), were also observed at the site, but their abundances were much lower than those of *C. facialis* and *G. nigrofuscata* (at most, eight skins per day; see Supp. Table S1). In total, 8,216 skins of the four species were collected across 12 years. During the study years, daily mean temperature ranged from approximately 0°C to 30°C near the study site (Fig. 2). Daily precipitation tended to increase with temperature (Fig. 2).

        Climatic effects on phenology. The accumulated temperatures >17.9°C and >18.9°C over the previous 101 and 109 days best fit the observed patterns for the cumulative skin counts of *C. facialis* and *G. nigrofuscata* within a season, respectively (parameters t_0 and *th*, respectively: Table 1). The points until which temperature was accumulated, t_1, were approximately 38 and 44 days in the two respective species (Table 1). The accumulated temperature explained 47% and 64% of the total variation in the cumulative skin counts of *C. facialis* and *G. nigrofuscata*, respectively (Table 1; Fig. 3). Temperature was also analyzed using daily maxima, but similar results were obtained (t_0 = 102 and 107, t_1 = 37 and 29, *th* = 23 and 23, R^2 = 0.40 and 0.51 for *C. facialis* and *G. nigrofuscata*, respectively). Regarding the sexes of the two cicada species, t_0 was estimated at 88 and 92 days, while the threshold temperatures were 17.4°C and 21.3°C, respectively (Table 1). The parameter t_1 ranged from 28 to 37 days among the sexes and species.

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210   R^2 varied from 30% to 74% (Table 1). In contrast, the effective accumulated precipitation

211   explained at most 16% of the variation in the cumulative skin counts of *C. facialis* and *G.*

212   *nigrofuscata* (Table 2). The variation explained by precipitation was at most 8% for male and

213   female cicada skin counts (Table 2). We also performed multiple regressions using the best-fitted

214   temperature and precipitation. However, the models including the temperature, precipitation, and

215   interaction term did not considerably improve the variation explained (*C. facialis*, $R^2 = 0.48$; *G.*

216   *nigrofuscata*, $R^2 = 0.69$), indicating weak combined effects of the two climatic factors.

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219   **Discussion**

220   Our long-term survey and quantitative analysis uncovered several characteristics of the

221   molting phenology of *C. facialis* and *G. nigrofuscata*. First, the effective accumulated temperature

222   explained significantly more of the variation in the cumulative skin counts than did precipitation.

223   Second, the time over which past temperature had an effect was consistent from the past 3.0–3.5

224   months to the most recent 1.0–1.5 months. Conversely, the threshold temperature ranged from

225   18°C to 23°C depending on the species, temperature criterion (daily mean or maximum), and sex.

226   These results indicate that the accumulated temperature during late spring is a key predictor for

227   the molting phenology of both cicada species, whereas the current year's precipitation is likely a

228   less influential factor. Third, temperature accounted for 17% more of the variation of the

229   cumulative skin counts in *G. nigrofuscata* compared to *C. facialis*. Physiological and other

230   possible factors are therefore discussed in to the following section regarding the temporal

231   fluctuation of skin counts within and among years.

232   Several previous studies have suggested that warming can shift the first singing day or

233   date of peak abundance in Japanese cicadas (Shiyake 2008, Kamitani 2010, Ogawa-Onishi and

234   Berry 2012). Our quantitative results support the potential impact of temperature on molting

235 phenology. In addition, the physiological evidence reported to date has shown that temperatures

236 ranging from 10°C to 30°C strongly affect the timing and rate of egg hatching (Moriyama and

237 Numata 2008), whereas low temperature has a relatively weak impact on the egg mortality of *C.*

238 *facialis* and *G. nigrofuscata* (Moriyama and Numata 2009). Thus, the nymphs of the two cicadas

239 are likely more sensitive to moderate temperatures during late spring than to winter coldness. In

240 contrast, we found limited evidence regarding the impact of precipitation on the molting

241 phenology of *C. facialis* and *G. nigrofuscata*, although rainfall is known to be critical for egg

242 hatching in Japanese cicadas (Hirose 1977; Moriyama and Numata 2010, 2011). As the rainy

243 season often ends in mid-July (known as the "Baiu" period in Japan), the limited effect of

244 precipitation may be due to the emergence of final-instar nymphs of *C. facialis* and *G.*

245 *nigrofuscata* in late July or early August, allowing them to avoid the direct impacts of the rainy

246 period. Additionally, we sampled the nymphal skins on days with no rain, which may have

247 prevented us from detecting the influence of precipitation on skin counts. Further investigations

248 are required to evaluate the relative importance of temperature and precipitation throughout the

249 cicada life cycle, including egg hatching and adult emergence.

250   Our results should be interpreted carefully regarding the confounding effects of the

251 distribution of peak abundance within the study season. The proportion of explained variation

252 tended to increase as the time point of peak abundance became later in the season (i.e., descending

253 order of male *C. facialis*, female *C. facialis*, male *G. nigrofuscata*, and female *G. nigrofuscata*:

254 Table 1; Fig. 1). In addition to these sexual differences, the start dates of our survey were too late

255 to follow the phenology of *C. facialis*, particularly when abundance peaked earlier than usual (e.g.,

256 1998 and 2004: Fig. 1). Therefore, the differences in predictability between the species and sexes

257 might reflect the differences of the observed abundance peaks. To deal with these potential

258 limitations, multiple populations should be compared in the future.

259   In Japanese cicadas, an abundance fluctuation of approximately threefold is considered

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260   moderate, as documented for *Platypleura kaempferi* (Hirose 1977). Despite the significant

261   predictability of temperature for the phenological pattern, the actual variations in yearly

262   abundance (up to sevenfold) were often larger or smaller than those predicted by the temperature.

263   Thus, we believe that temperature is unlikely a regulator of cicada abundance among years.

264   Regarding factors other than climate that regulate cicada populations, predation and intraspecific

265   competition can be hypothesized as ecological mechanisms. While resource competition has

266   rarely been reported for cicadas, several studies have suggested the importance of predation in

267   their population fluctuations (e.g., Karban 1982, Williams and Simon 1995, Koenig and Liebhold

268   2005). For example, Takakura and Yamazaki (2007) revealed that avian predation on *G.*

269   *nigrofuscata* was moderate in a dense plantation because adult cicadas were able to hide from

270   predators. As the suburban habitat in the present study was covered with secondary forest, the

271   higher predictability of *G. nigrofuscata* abundance could be explained by this relatively moderate

272   avian predation. Furthermore, the total number of ground holes was similar to the yearly skin

273   count, suggesting that predation (such as ant predation on aboveground nymphs) was unlikely

274   pivotal during molting. If predation were involved, we hypothesize that the population fluctuation

275   of skin abundance may be attributed to avian predation or other belowground agents.

276   In summary, our study revealed that late spring temperature is a key predictor for the

277   summer outbreaks of two Japanese cicadas. We also found that the duration over which

278   temperature was influential was consistent between the species and their sexes. These quantitative

279   results provide critical insight into how seasonal insects respond to changing enviroments.

280   Furthermore, the long-term abundance data posed a further question regarding the population

281   stability of seasonal insects, i.e., what factors regulate yearly abundance? Another factor, such as

282   predation, must be evaluated carefully to obtain a comprehensive understanding of the fluctuation

283   of *C. facialis* and *G. nigrofuscata* populations.

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376 **Table 1** Effects of daily mean temperature (°C) on cumulative skin counts of *Cryptotympana*

377 *facialis* and *Graptopsaltria nigrofuscata* within a season.

Species	Parameters estimated					SS	R^2
	a	b	t_0	t_1	th		
C. facialis	1.26	0.14	101	38	17.9	97.9	**0.47**
G. nigrofuscata	1.29	0.01	109	44	18.9	112.0	**0.64**
C. facialis M	1.09	0.12	92	28	17.4	54.1	**0.30**
C. facialis F	0.98	1.24	88	35	21.3	57.6	**0.53**
G. nigrofuscata M	0.98	0.91	91	37	21.2	28.7	**0.68**
G. nigrofuscata F	1.16	0.00	91	35	21.3	39.6	**0.74**

378 Shown are the estimated parameters for the time over which daily mean temperature had an effect

379 (from the past t_0 days to the most recent t_1 days), the threshold (th) of effective accumulated

380 temperature, and the slope coefficient (a) and intercept (b) of the linear regression for temperature.

381 Sum of squares (SS) and coefficient of determination (R^2) values are listed to the right of the

382 parameters. The estimated results for male (M) and female (F) skins of the two species (from

383 2001 to 2006) are also listed below the table. Bold values of R^2 indicate <5% significance

384 according to the *F*-test.

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394   **Table 2** Effects of daily precipitation (mm) on cumulative skin counts of *Cryptotympana facialis*

395   and *Graptopsaltria nigrofuscata* within a season.

Species	Parameters estimated					SS	R^2
	a	b	t_0	t_1	th		
C. facialis	0.88	0.34	71	33	0.0	163.7	**0.12**
G. nigrofuscata	0.85	0.00	70	34	9.4	258.5	**0.16**
C. facialis M	0.10	4.37	82	27	20.0	75.8	0.02
C. facialis F	0.64	0.43	88	49	0.0	117.0	0.05
G. nigrofuscata M	0.34	2.42	74	49	36.3	83.1	0.08
G. nigrofuscata F	0.00	3.13	98	27	3.0	151.5	0.00

396   Shown are the estimated parameters for the time over which daily mean temperature had an effect

397   (from the past t_0 days to the most recent t_1 days), the threshold (th) of effective accumulated

398   precipitation, and the slope coefficient (a) and intercept (b) of the linear regression. Bold values of

399   R^2 indicate <5% significance according to the F-test. The interpretation follows that of Table 1.

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412   **Figure legends**

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414   **Figure 1** Molting phenology of *Cryptotympana facialis* (black line) and *Graptopsaltria*

415   *nigrofuscata* (grey line) over 12 years at the study site. The upper figure shows the skin counts of

416   the two species at each survey date over the entire study period, while the lower two figures

417   present the number of male (solid line) and female (dashed line) skins collected from 2001 to

418   2006 for the two species.

419

420   **Figure 2** Temporal variation in daily mean temperature (°C: solid line) and daily precipitation

421   (mm: vertical bars) from 1994 to 2006 around the study site (northern Mie Prefecture, central

422   Japan). Daily mean temperature was smoothed using a spline method for legibility.

423

424   **Figure 3** Observed (circles) and predicted (vertical bars) values of the cumulative skin counts of

425   *Cryptotympana facialis* (closed: upper figure) and *Graptopsaltria nigrofuscata* (open: lower

426   figure). Predictions were made based on the effective accumulated temperature according to the

427   estimated parameters in Table 1. Dashed lines indicate intervals between the study years. The skin

428   counts are shown on a ln-scale.
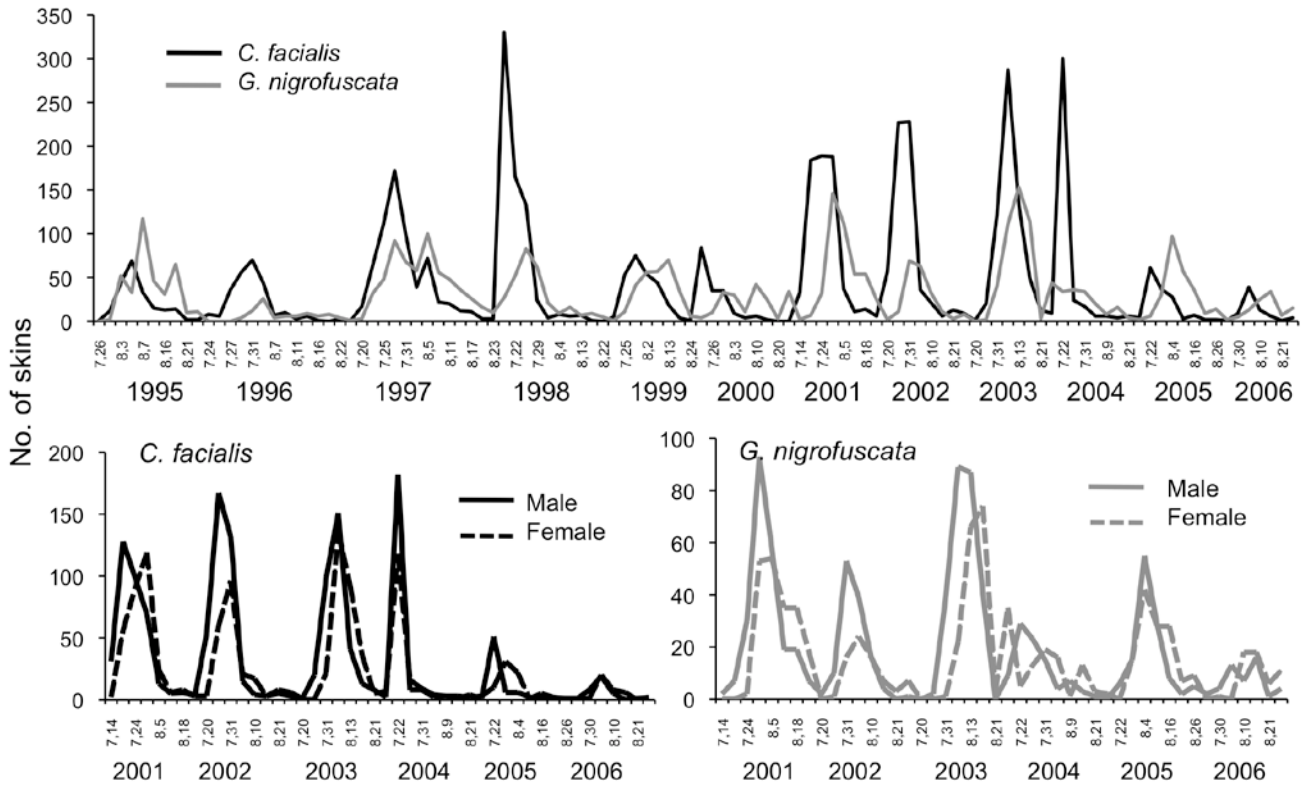
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437   Figure 1

450 Figure 2
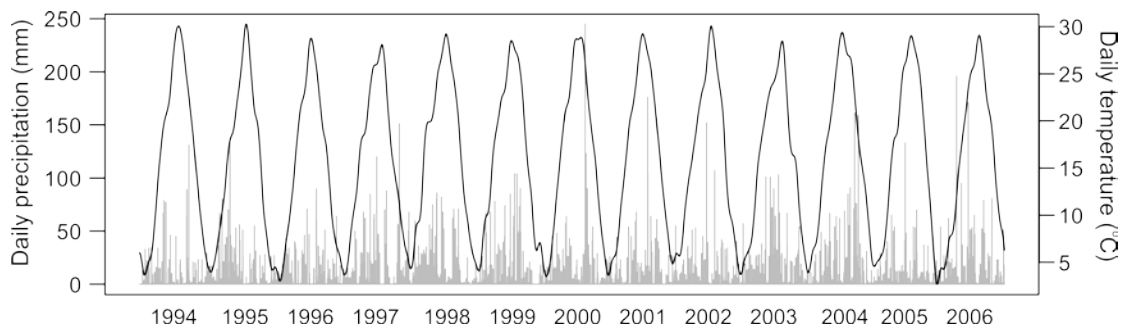
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469   Figure 3
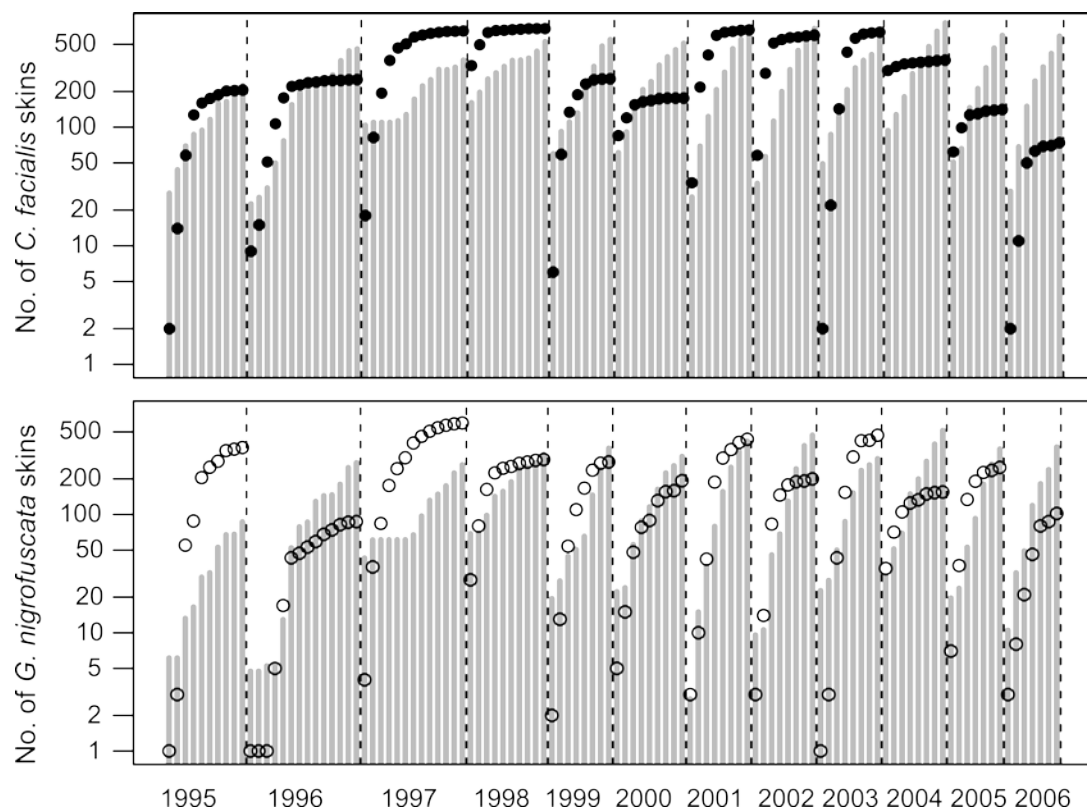
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482   **Supplemental Table S1.** Detailed survey dates and numbers of skins collected for four cicada

483   species. Cf, *Cryptotympana facialis*; Gn, *Graptopsaltria nigrofuscata*; Pk, *Platypleura kaempferi*;

484   Mo, *Meimuna opalifera*. Sex information (M, Male; F, Female) are not available from 1995 to

485   2000 (indicated by "NA").

Year	Month	Date	Cf	Cf_M	Cf_F	Gn	Gn_M	Gn_F	Pk	Pk_M	Pk_F	Mo	Mo_M	Mo_F
1995	7	26	1	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA
1995	7	29	12	NA	NA	2	NA	NA	1	NA	NA	0	NA	NA
1995	8	3	44	NA	NA	52	NA	NA	0	NA	NA	0	NA	NA
1995	8	5	69	NA	NA	33	NA	NA	0	NA	NA	1	NA	NA
1995	8	7	33	NA	NA	117	NA	NA	0	NA	NA	0	NA	NA
1995	8	10	15	NA	NA	46	NA	NA	0	NA	NA	0	NA	NA
1995	8	16	13	NA	NA	31	NA	NA	0	NA	NA	2	NA	NA
1995	8	18	14	NA	NA	65	NA	NA	0	NA	NA	3	NA	NA
1995	8	21	2	NA	NA	10	NA	NA	0	NA	NA	3	NA	NA
1995	8	23	2	NA	NA	11	NA	NA	0	NA	NA	0	NA	NA
1996	7	24	8	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA
1996	7	26	6	NA	NA	0	NA	NA	1	NA	NA	0	NA	NA
1996	7	27	36	NA	NA	0	NA	NA	2	NA	NA	0	NA	NA
1996	7	29	56	NA	NA	4	NA	NA	0	NA	NA	1	NA	NA
1996	7	31	70	NA	NA	12	NA	NA	0	NA	NA	0	NA	NA
1996	8	5	44	NA	NA	26	NA	NA	0	NA	NA	1	NA	NA
1996	8	7	7	NA	NA	4	NA	NA	0	NA	NA	2	NA	NA
1996	8	8	10	NA	NA	6	NA	NA	0	NA	NA	1	NA	NA
1996	8	11	3	NA	NA	6	NA	NA	0	NA	NA	0	NA	NA
1996	8	13	6	NA	NA	9	NA	NA	0	NA	NA	2	NA	NA
1996	8	16	1	NA	NA	6	NA	NA	0	NA	NA	0	NA	NA
1996	8	19	0	NA	NA	8	NA	NA	0	NA	NA	0	NA	NA
1996	8	22	3	NA	NA	4	NA	NA	0	NA	NA	3	NA	NA
1996	8	23	1	NA	NA	1	NA	NA	0	NA	NA	2	NA	NA
1997	7	20	17	NA	NA	3	NA	NA	0	NA	NA	0	NA	NA
1997	7	23	64	NA	NA	32	NA	NA	0	NA	NA	0	NA	NA

1997	7	25	112	NA	NA	48	NA	NA	0	NA	NA	1	NA	NA
1997	7	28	172	NA	NA	92	NA	NA	0	NA	NA	0	NA	NA
1997	7	31	100	NA	NA	68	NA	NA	2	NA	NA	0	NA	NA
1997	8	2	39	NA	NA	58	NA	NA	0	NA	NA	0	NA	NA
1997	8	5	72	NA	NA	100	NA	NA	4	NA	NA	0	NA	NA
1997	8	8	22	NA	NA	56	NA	NA	4	NA	NA	0	NA	NA
1997	8	11	20	NA	NA	48	NA	NA	3	NA	NA	0	NA	NA
1997	8	15	12	NA	NA	36	NA	NA	6	NA	NA	0	NA	NA
1997	8	17	11	NA	NA	26	NA	NA	6	NA	NA	0	NA	NA
1997	8	20	3	NA	NA	16	NA	NA	8	NA	NA	0	NA	NA
1997	8	23	3	NA	NA	10	NA	NA	6	NA	NA	0	NA	NA
1998	7	19	330	NA	NA	27	NA	NA	0	NA	NA	0	NA	NA
1998	7	22	165	NA	NA	52	NA	NA	0	NA	NA	0	NA	NA
1998	7	26	133	NA	NA	83	NA	NA	0	NA	NA	1	NA	NA
1998	7	29	24	NA	NA	62	NA	NA	0	NA	NA	0	NA	NA
1998	7	31	4	NA	NA	21	NA	NA	0	NA	NA	0	NA	NA
1998	8	4	8	NA	NA	9	NA	NA	0	NA	NA	0	NA	NA
1998	8	8	6	NA	NA	16	NA	NA	0	NA	NA	0	NA	NA
1998	8	13	7	NA	NA	7	NA	NA	0	NA	NA	0	NA	NA
1998	8	16	1	NA	NA	9	NA	NA	0	NA	NA	0	NA	NA
1998	8	22	0	NA	NA	5	NA	NA	0	NA	NA	0	NA	NA
1999	7	20	5	NA	NA	1	NA	NA	0	NA	NA	0	NA	NA
1999	7	25	53	NA	NA	11	NA	NA	0	NA	NA	2	NA	NA
1999	7	29	75	NA	NA	41	NA	NA	0	NA	NA	2	NA	NA
1999	8	2	54	NA	NA	56	NA	NA	0	NA	NA	1	NA	NA
1999	8	7	44	NA	NA	57	NA	NA	0	NA	NA	0	NA	NA
1999	8	13	19	NA	NA	70	NA	NA	3	NA	NA	0	NA	NA
1999	8	19	4	NA	NA	35	NA	NA	5	NA	NA	0	NA	NA
1999	8	24	1	NA	NA	6	NA	NA	3	NA	NA	0	NA	NA
2000	7	22	84	NA	NA	4	NA	NA	0	NA	NA	3	NA	NA
2000	7	26	35	NA	NA	10	NA	NA	0	NA	NA	1	NA	NA
2000	7	30	35	NA	NA	33	NA	NA	0	NA	NA	1	NA	NA

2000	8	3	9	NA	NA	30	NA	NA	0	NA	NA	0	NA	NA
2000	8	5	4	NA	NA	11	NA	NA	0	NA	NA	1	NA	NA
2000	8	10	6	NA	NA	42	NA	NA	1	NA	NA	0	NA	NA
2000	8	14	2	NA	NA	25	NA	NA	3	NA	NA	0	NA	NA
2000	8	20	0	NA	NA	3	NA	NA	1	NA	NA	0	NA	NA
2000	8	23	0	NA	NA	34	NA	NA	0	NA	NA	0	NA	NA
2001	7	14	33	31	2	2	2	0	0	0	0	0	0	0
2001	7	20	184	128	56	7	7	0	0	0	0	1	1	0
2001	7	24	189	99	90	32	30	2	0	0	0	0	0	0
2001	7	30	188	69	119	146	93	53	0	0	0	2	2	0
2001	8	5	37	13	24	112	58	54	0	0	0	0	0	0
2001	8	13	11	5	6	54	19	35	0	0	0	0	0	0
2001	8	18	14	6	8	54	19	35	0	0	0	0	0	0
2001	8	23	6	3	3	25	7	18	4	3	1	0	0	0
2002	7	20	57	54	3	2	2	0	0	0	0	0	0	0
2002	7	25	227	167	60	11	10	1	1	1	0	0	0	0
2002	7	31	228	132	96	69	53	16	1	1	0	0	0	0
2002	8	6	36	15	21	63	39	24	4	3	1	0	0	0
2002	8	10	21	4	17	32	16	16	1	1	0	0	0	0
2002	8	15	6	3	3	11	4	7	1	0	1	0	0	0
2002	8	21	13	8	5	3	0	3	1	1	0	0	0	0
2002	8	25	9	6	3	8	1	7	0	0	0	0	0	0
2003	7	20	1	1	0	0	0	0	0	0	0	1	1	0
2003	7	26	20	20	0	2	2	0	0	0	0	0	0	0
2003	7	31	121	99	22	40	39	1	0	0	0	0	0	0
2003	8	6	287	151	136	111	89	22	0	0	0	0	0	0
2003	8	13	133	41	92	153	87	66	0	0	0	0	0	0
2003	8	19	49	13	36	114	40	74	3	1	2	0	0	0
2003	8	21	13	8	5	3	0	3	1	1	0	0	0	0
2003	8	25	9	2	7	44	9	35	1	1	0	0	0	0
2004	7	22	300	182	118	34	29	5	0	0	0	1	1	0
2004	7	27	24	8	16	36	23	13	0	0	0	0	0	0

2004	7	31	17	8	9	34	15	19	0	0	0	0	0	0
2004	8	5	6	2	4	20	4	16	0	0	0	0	0	0
2004	8	9	6	3	3	8	7	1	0	0	0	0	0	0
2004	8	15	4	1	3	16	3	13	0	0	0	0	0	0
2004	8	21	6	4	2	4	1	3	1	0	1	0	0	0
2004	8	25	4	1	3	2	0	2	0	0	0	0	0	0
2005	7	22	61	51	10	6	6	0	0	0	0	0	0	0
2005	7	26	37	6	31	30	15	15	0	0	0	0	0	0
2005	8	4	28	6	22	97	55	42	0	0	0	0	0	0
2005	8	10	3	2	1	57	29	28	0	0	0	0	0	0
2005	8	16	7	2	5	36	8	28	1	1	0	0	0	0
2005	8	22	2	0	2	9	2	7	0	0	0	0	0	0
2005	8	26	2	1	1	14	5	9	2	1	1	0	0	0
2006	7	23	1	1	0	2	2	0	0	0	0	1	0	1
2006	7	30	9	8	1	5	4	1	0	0	0	0	0	0
2006	8	4	39	19	20	13	13	0	0	0	0	1	0	1
2006	8	10	13	5	8	25	7	18	0	0	0	0	0	0
2006	8	16	6	0	6	34	16	18	0	0	0	0	0	0
2006	8	21	1	1	0	7	1	6	0	0	0	0	0	0
2006	8	26	4	2	2	15	4	11	3	1	2	0	0	0

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495   **Appendix 1** R code for the parameter estimation

496   ####################################

497   #Sato&Sato: Cicada Phenology, R code##

498   ####################################

499

500   #Note: This is an example for the temperature analysis of C. facialis skins

501   #For the other response variables, please change "Cf.Cum" into "Gn.Cum", "Cf.CumM", "Cf.CumF",

502   "Gn.CumM", or "Gn.CumF".

503

504   #Load data

505   #CicadaMie = read.csv("cicadaSkinMF.csv", header=T) # for Male-Female data

506   CicadaMie = read.csv("cicadaSkin.csv", header=T)

507   ClimateData = read.csv("MieClimate.csv", header=T)

508

509   #ClimateData[,4]:Daily Mean Temp; [,5]: Daily Max Temp; [,6]: Precip

510   calc.CumTemp = function(t0, t1, th) {

511   CumTemp = c()

512   for (k in 1:nrow(CicadaMie)) {

513   t.point = subset(ClimateData, Year==CicadaMie[k,1]&DaysFromNewYear==CicadaMie[k,4])$rowID

514   ThH = (ClimateData[,4][(t.point-t0):(t.point-t0+t1)]>th) #Change [,4] or [,5] or [,6]

515   CumTemp = c(CumTemp, sum(((ClimateData[,4][(t.point-t0):(t.point-t0+t1)]-th)*ThH))) #Note: ThH

516   is a TRUE or FALSE vector

517   }

518   return(CumTemp+1) #+1 to prevent log(0)

519   }

520

521   #Define a target function

522   SS = function(x) {

523   a = exp(x[1]);b = exp(x[2]);t0 = x[3];t1 = x[4];th = x[5] #a and b are exponential to range >0

524   Temp = calc.CumTemp(t0, t1, th)

525   SS = sum((log(CicadaMie$CfCum+1)-(a*log(Temp)+b))^2)

526   return(SS)

```
527    }

528

529    #Parameter optimization

530    par = c(0,0,90,30,15) #Initial values

531

532    #First step: Simulated-annealing

533    res = optim(par,SS, method="SANN")

534

535    #Second step: Nelder-Mead method, repeat until convergence as "res3 = optim(res2$par[1:5], SS...)"

536    res2 = optim(res$par[1:5],SS, method="Nelder-Mead")

537

538    #Calclating R2 value

539    SSnull = sum((log(CicadaMie$CfCum+1)-mean(log(CicadaMie$CfCum+1)))^2)

540    print(1-(res2$value/SSnull))

541

542    #F-test

543    df.param = length(res2$par)-1; MSg = ((SSnull-res2$value)/df.param)

544    df.resi = length(CicadaMie$CfCum)-df.param; MSr = (SSnull/df.resi)

545    Fval = MSg/MSr; pf(Fval,df.param,df.resi,lower.tail=F)

546

547    #Observed vs. Prediction plot

548    plot(exp(exp(res2$par[1])*log(calc.CumTemp(res2$par[3],res2$par[4],res2$par[5]))+exp(res2$par[2])),ty

549    pe="h",lwd=3,col="grey",las=1,ylab="Cumulative shell no.",log="y")

550    points(CicadaMie$CfCum+1,pch=1)

551    abline(v=cumsum(c(10,14,13,10,8,9,8,8,8,8,7,7))+0.5,lty=2,col="grey")

552

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555

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```

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557   **Appendix 2** Input data files for R analyses (SatoSato_Appendix2_inputFiles.xls)

558

559   Download URL

560   →(https://drive.google.com/file/d/0B8lD2dRmBfQdb3RmWGxXMzhhUkU/view?usp=sharing)

561