# The double rhythm development and characteristics of the *Gasterophilus pecorum* (Diptera: Gasterophilidae) population in desert steppe

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**Abstract**

**Background:** The departure of the mature larvae of the horse stomach bot fly from the host indicates the beginning of a new infection period, and the *Gasterophilus pecorum* becomes the dominant species in the desert steppe, showing its special biological characteristics. The population dynamics of *G. pecorum* were studied to reveal the population development rule of *G. pecorum* in the arid desert steppe.

**Method:** The larvae were collected and recorded in the newly excreted feces by tracking the Przewalski's horses (*Equus przewalskii*), meanwhile, the larval pupation experiments were carried out under natural conditions.

**Results:** (a) There was a positive correlation between the survival rate and the number of larvae (r = 0.630, $p < 0.01$), indicating that the species development had the characteristics of centralized occurrence; (b) The main periods of mature larvae discharge were from early April to early May (peak I) and from mid-August to early September (peak II), and the larval population curve showed a sudden spike in increase and gradual decrease at both peaks; under higher temperature, the number of adults from peak II had higher survival rate, higher pupation rate, higher emergence rate and less eclosion time than that of peak I; (c) Although it has one generation a year, the occurrence peak twice annually displaying a unique “ double rhythm development ” phenomenon, which forms a “ dual rhythm parasitism ” pressure on the local host. This phenomenon is very rare in the study of insect life history, especially in the parasite epidemiology.

**Conclusion:** The natural discharge period of the *G. pecorum* larvae in Kalamaili Nature Reserve (KNR) is longer than 7 months and have the potentially long term infection effect on the host. The above phenomenon is one important reason for the local equine animals to be severely infected with equine myiasis.

**Keywords**: Desert Steppe, *Gasterophilus pecorum*, Mature larvae, Population dynamics, Double rhythm development, Survival rate

## Background

The horse stomach bot flies (*Gasterophilus* spp.) are common obligate parasites in equids [1–2]. *Gasterophilus* larvae parasitize the digestive tract of equine animals and causes inflammation, ulcer, hernia and other phenomena [3–5]. The larvae absorb nutrition from the host and secrete toxins, and may leads to host death when infected severely [6–7]. The genus *Gasterophilus* includes nine species in the world [8–9], and six of them are recorded in China [10]. The infection of bot flies in local equine is 100% in Kalamaili Nature Reserve (KNR), Xinjiang located in the desert grassland [11]. Among the 6 species of horse stomach bot flies, *G. pecorum* infections are extremely high, accounting for 89-98% of all bot flies infections [11–12], which is different from the occurrence of *G. intestinalis* and *G. nasalis* in other regions as the dominant species [13–14].

The horse stomach flies are insect of complete metamorphosis and have four developmental stages, namely, egg, larva, pupa and adult, one generation a year [8]. Them develop in the digestive tract of equines until the mature larva leaves the host, thus starting a new life cycle. The mature larva is the first state of the host’s life in vitro, and its population dynamics determines the subsequent population development process. The most significant feature in the life cycle of the *G. pecorum* is its "unusual" reproductive strategy of laying eggs on grass [8]. Most of the studies on *G. pecorum* originated from the early biological observation by Chereshnev in Kazakhstan [15], the mature larvae mainly appear in early August to early September. However, a study reported that a significant number of *G. pecorum* larvae were collected in KNR to study the development period of pupae, and it was considered that the high incidence period of mature larvae was in spring [16]. Based on the differences between some recent findings and the existing life history records of *G. pecorum*, a systematic study of the population dynamics and developmental process of the local *G. pecorum* in vitro of the host was carried out in order to understand the development characteristics of this species in the desert steppe.

Insect are poikilothermal animals whose metabolism, life cycle and lifespan are subject to the temperature of the environment [17–18].The horse stomach flies have a relatively stable environment in the digestive tract of host, and once discharged from the host, it will be affected by the external temperature [19]. Horse stomach flies are thermophilic insects that tend to live at high temperatures (in warm regions or on a warm-blooded host) [20].The higher temperature is conducive to accelerate the development rate of insects in the suitable temperature range [21–22]. In addition, the survival rate of metamorphosis insects between different states is also affected by temperature [23–25]. The developmental response of insects to temperature is important in understanding the ecology of insect life histories [26]. So, we conducted an experiment to investigate the effect of different temperatures on the developmental rate, survival, pupation, emergence of *G. pecorum* in order to provide parameters for forecasting and management systems in KNR.

## Result

### Population dynamics of mature larvae of *G. pecorum*

A total of 2,021 piles of equine feces were examined, where 443 piles (21.92%) contained *G. pecorum* larvae (Table 1). The proportion of feces containing larvae (PF) in early April and mid-to-late August were 56.03% and 53.23%, respectively. There were two obvious larval peaks with a significant range of changes. In the following May and September, the PF gradually decreased and remained low. Among them, the PF was less than 20% during the three periods of March, mid-to-late May to the end of July, and mid-to-late September.

Table 1 The number of equine feces and *G. pecorum* larvae collected in KNR in 2018

| Date | Feces investigated ($N_{i,}$) | Feces containing larvae ($n_i$) | PF $n_i/N_i$ (%) | Larvae ($m_i$) | NL ($m_i/N_i$) | Proportion of larvae ($m_i/\Sigma m_i$) (%) |
|---|---|---|---|---|---|---|
| 3.15 | 98 | 2 | 2.04 | 2 | 0.02 | 0.28 |
| 3.22 | 94 | 6 | 6.38 | 7 | 0.07 | 0.99 |
| 3.29 | 48 | 3 | 6.25 | 5 | 0.10 | 0.71 |
| 4.05 | 59 | 34 | 57.63 | 103 | 1.75 | 14.63 |
| 4.12 | 82 | 45 | 54.88 | 94 | 1.15 | 13.35 |
| 4.19 | 71 | 37 | 52.11 | 62 | 0.87 | 8.81 |
| 4.26 | 58 | 18 | 31.03 | 40 | 0.69 | 5.68 |
| 5.3 | 59 | 27 | 45.76 | 30 | 0.51 | 4.26 |
| 5.10 | 54 | 13 | 24.07 | 14 | 0.26 | 1.99 |
| 5.17 | 43 | 8 | 18.60 | 8 | 0.19 | 1.14 |
| 5.24 | 55 | 9 | 16.36 | 9 | 0.16 | 1.28 |
| 5.31 | 51 | 4 | 7.84 | 4 | 0.08 | 0.57 |
| 6.7 | 47 | 5 | 10.64 | 5 | 0.11 | 0.71 |
| 6.14 | 57 | 3 | 5.26 | 3 | 0.05 | 0.43 |
| 6.21 | 38 | 4 | 10.53 | 5 | 0.13 | 0.71 |
| 6.28 | 88 | 5 | 5.68 | 5 | 0.06 | 0.71 |
| 7.5 | 75 | 5 | 6.67 | 5 | 0.07 | 0.71 |
| 7.12 | 100 | 3 | 3.00 | 3 | 0.03 | 0.43 |
| 7.19 | 50 | 2 | 4.00 | 2 | 0.04 | 0.28 |
| 7.26 | 100 | 4 | 4.00 | 4 | 0.04 | 0.57 |
| 8.2 | 80 | 1 | 1.25 | 1 | 0.01 | 0.14 |
| 8.9 | 39 | 3 | 7.69 | 3 | 0.08 | 0.43 |
| 8.16 | 54 | 18 | 33.33 | 30 | 0.56 | 4.26 |
| 8.23 | 108 | 61 | 56.48 | 108 | 1.00 | 15.34 |
| 8.30 | 93 | 46 | 49.46 | 60 | 0.65 | 8.52 |
| 9.6 | 79 | 28 | 35.44 | 36 | 0.46 | 5.11 |
| 9.13 | 72 | 22 | 30.56 | 25 | 0.35 | 3.55 |
| 9.20 | 100 | 17 | 17.00 | 20 | 0.20 | 2.84 |
| 9.27 | 69 | 10 | 14.49 | 11 | 0.16 | 1.56 |
| Total | 2021 | 443 | 21.92 | 704 | 0.35 | 100 |

Note: The corresponding information of each date represents the larva data between the time point and the previous time point, for example, 9.27 represents the larva collection during 9.21-9.27.

A total of 704 larvae of *G. pecorum* were collected in this study. The average number of larvae per feces (NL) was 0.35 during the entire investigation period, among which the highest was in early April with an average of 1.40, followed by mid-to-late April and mid-to-late August, with 0.79 and

0.84 respectively. The NL showed the same trend as the PF (Figure 1).

The percentage of larvae in April and August were 42.47% and 28.55%, respectively. Among them, 27.98% and 14.79% were in early and mid-to-late April, and 23.86% in mid-to-late August, respectively, which was significantly higher than the other periods ($p < 0.05$). The number of larvae collected from early April to early May and from mid-August to early September accounted for 48.72% and 32.52% of the total number of larvae, respectively, and the cumulative proportion of the two stages accounted for 81.24%. Therefore, the main periods of mature larvae discharge in KNR were from early April to early May (peak I) and from mid-August to early September (peak II) (Figure 2) while maintaining continuous larvae production throughout March to September.

The NL was derived from obtaining an average of 10 piles of feces per adult Przewalski's horse per day, as observed from 10 wild horses in temporary captivity. These horses defecated an average of 10.1 piles of feces daily. It is estimated that 749 larvae of *G. pecorum* were discharged from each horse, which was close to the annual average infection amount of *G. pecorum* released locally [11].

The results showed that with time, the cumulative number of larvae collected during the survey period showed a "double S" growth curve (Figure 3). The two spikes were concentrated in April and August, the slope of the curve showed that the first growth of the cumulative growth curve of total larvae (Figure 3, A) and survival larvae (Figure 3, B) were increased fastest on April 5, and the second growth curve (Figure 3, C) and (Figure 3, D) were both on August 23.

**Population development analysis**

The results showed that the survival rates of the two peaks were 69.57% (peak I) and 73.27% (peak II) ($p$ = 0.183, t = -1.727), with higher pupation rate from peak II (89.19%) than that of peak I (66.83%) ($p$ = 0.002 < 0.05, t = -10.547). The eclosion rate of peak II (87.88%) was also higher than that of peak I (63.31%) ($p$ = 0.002, t = -9.525) (Figure 4, B).

The average pupal period of the two peak stages lasted for 34.05 days and 20.2 days respectively ($p$ < 0.001, t = 15.513) (Figure 4, A). The longest and shortest development period of peak I was 39 days and 26 days, and the longest and shortest development time of peak II was 24 days and 18 days.

Spearman correlation analysis showed that there was a positive correlation between the survival rate and the number of larvae (r = 0.630, $p$ < 0.01). That is, the survival rate of *G. pecorum* larvae was higher during the two peak periods.

Population decreases in three stages throughout the development from larvae (N) to adults (N'): survival rate (α), pupation rate (β) and eclosion rate (γ), yielding:

$$N' = \alpha\beta\gamma N \tag{6}$$

The number of adults developed from larvae in peak I and peak II were N1' and N2', respectively, thus:

$$N_1' = \alpha 1\beta 1\gamma 1\ N1 = 69.57\% \text{ x } 66.83\% \text{ x } 63.31\% \text{ x } (51.70\% \text{ x } N) = 15.22\%N$$

$$N_2' = \alpha 2\beta 2\gamma 2\ N2 = 73.27\% \text{ x } 89.19\% \text{ x } 87.88\% \text{ x } (41.76\% \text{ x } N) = 23.98\%N$$

The results showed that the larvae that emerged into adults from peak I accounted for 15.22% of the total larvae, and peak II was 23.98%. The number of larvae in peak I was 1.48 times as much as that in peak II, but the higher survival rate, pupation rate and emergence rate in peak II resulted in 1.32 times more of adults than that of peak I.

Table 2 The sex ratio of *G. pecorum* adults in the two peak periods

| Period | Proportion of females (%) | Proportion of males (%) | Total (%) | Male to female ratio |
|---|---|---|---|---|
| Peak I | 57.95 | 42.05 | 100 | 0.73 |
| Peak II | 43.10 | 56.90 | 100 | 1.32 |

The number of female adults in peak I and peak II were $N_{FI}$ and $N_{FII}$, respectively, therefore:

$$N_{FI} = 15.22\%N \times 57.95\% = 0.09\%N$$

$$N_{FII} = 23.98\%N \times 43.10\% = 0.10\%N$$

In addition, there were differences in the sex ratio between the two peak periods. The ratio of male to female in peak I was 0.73, which was lower than that in peak II (0.76) (Table 2). When comparing the proportion of female adults in the total larvae in the two peak periods, it was found that peak II (0.10%) was slightly higher than peak I (0.09%), signifying that peak II had greater infective potential to equine animals in KNR.

**Temperature characteristics during larvae discharge period**

The daily temperature of KNR depicted that the temperature rose rapidly from March to April, and slowed down from April to July, and began to decline after reaching the highest temperature of 38 °C (July 20) (Figure 5, A). The maximum daily temperature difference is 20 °C, the minimum and the average daily temperature differences are 3 °C and 12.78 °C respectively. After fitting the trend of temperature change, it was found that the curve showed a parabola characteristic of "rising first and then decreasing" ($y = 0.8570 + 3.7449x + 0.1031x^2 - 0.0240x^3$, $R^2$=91.18%) (Figure 5, B). The maximum value of the fitting curve was found to be July 19.

## Discussion

Insect population dynamics is an important part of insect ecology research [30–31]. In the reports on the fauna of horse stomach bot fly, the prevalence of *G. pecorum* was low in most countries and regions [2, 13, 32], but it is more common in the digestive tract of equines in Mongolia-Xinjiang and Qinghai-Tibet regions of China [12, 33], central and northern Kazakhstan [14], the Republic of Mongolia [34] and the Yakut Republic of Russia [35]. This high incidence of this species showed a certain regionality. In terms of basic research on stomach bot fly, a study was carried out on the pupal development period of *G. pecorum* and presented a prediction model for adult occurrence period [16]. The study on the egg development period and the survival period of the first instar larvae of *G. pecorum* has also been completed (in submission). The results will further improve the understanding of this species and contribute to the understanding of its epidemiology.

The parasites are also adaptable to the environment and have different performance characteristics in different environments [36–38]. The KNR is a desert grassland with high temperature and drought in summer, severe cold and long winter, and little annual precipitation. The unique environmental conditions have formed a specific occurrence of *G. pecorum*. This special situation is different from that of the early discovery in Kazakhstan that the larvae of *G. pecorum* were excreted only in August [15]. It is also different from the report that the larvae of *G. pecorum* were found in January, March and July in southern Italy [32]. And different from that reported in South Africa that the adult of *G. pecorum* only appeared in February to May and August [8].

Phenology affects the development dynamics of insects [39–40]. The development rhythm of some insects of one generation a year is closely related to phenology [41–42]. With the change of phenology, the population dynamics showed a peak period of correlation [43]. In this study, the larval population of *G. pecorum* indicated two peaks annually, which were closely related to the special arid climate of the desert steppe. In the desert steppe, water is the most crucial factor for life [44–45], therefore, many plants derived characteristics of rapid development of ephemeral plants. Due to the characteristics of local precipitation [46], some *Stipa* sp. showed a special secondary growth phenomenon after adapting to the environment [47]. Through field investigation, we found that the local dominant plant Stipa began to resume growth at the end of March and early April, head sprouting in May, and began to regenerate tender leaves in late August, which confirmed the phenomenon of secondary growth. Some studies have shown that once water conditions are suitable, many desert plants formed the characteristics of rapid development and life cycle [48–50], and the effects are also reflected in the ecosystem based on this: the phenomenon of simultaneous occurrence of *G. pecorum* and vector plants. Different from the other five species which lay eggs directly on the horse, the female of *G. pecorum* lay eggs on Stipa [51]. Under the influence of phenology, the secondary growth of Stipa was observed, and parasite population dynamics matched perfectly with the growth of vector plants and phenological changes, which also led to the local double rhythm development phenomenon of *G. pecorum*. This phenomenon indicates that there are two centralized infections per year for the host, which is different from the annual infection of parasitic linear animals [52–53], also differs from the special infection types associated with phenology, such as most arthropod infections [54–56].

In the appropriate temperature range, the higher the temperature, the more beneficial the development of insects [57]. The average temperature of peak I (11.3 ± 5.3 °C) was significantly lower than peak II (24.4 ± 2.7 °C) ($t = -11.083$, $p < 0.001$), which affected the survival rate of mature larvae. In the subsequent pupal development stage, the environmental temperature in peak II is more conducive to the life process of this stage. This resulted in mature larvae of peak II with higher survival rate (SR, PR and ER) and shorter pupal stage, thus eventually exceeding the number of the adults in peak I.

## Conclusion

The natural discharge period of the *G. pecorum* larvae in Kalamaili Nature Reserve is longer than 7 months, and it's the first time that the unique phenomenon of "double rhythm development" has been found in stomach bot flies. The close relationship between the double rhythm development of *G. pecorum* and the secondary growth of Stipa in the desert steppe is an important reason for the high infection rate, high infection amount of *G. pecorum* and the absolute dominance of *Gasterophilus* spp. in local equine animals. The population in peak II had higher survival rate than peak I because of the suitable development conditions. This study demonstrated the highly co-evolutionary phenomenon of the desert steppe ecosystem and revealed the tenacious adaptability of life under adverse conditions.

## Methods

### Study area

Kalamaili Nature Reserve (KNR) is located in the desert subregion of Northwest China (44°36′~46°00′N, 88°30′~90°03′E), with an area of 18,000 km2, an altitude of 600-1,464 m, an average annual temperature of 2.4 °C, an average annual precipitation of 159 mm and an annual evaporation of 2,090 mm. It has a typical temperate continental climate [27–28]. The protected animals are the reintroduced species *Equus Przewalskii* (EN), the endangered species *Equus hemionus* (NT), as well as *Gazella subgutturosa* (VU) and *Ovis ammon* (NT), There are also domestic livestock such as horses there seasonally [29].

### Material

From March to September 2018, the feces of the Przewalski's horses were inspected, with 50-80 piles per week. The larvae of *G. pecorum* in the feces were collected, and the number of stomach bot flies was recorded.

$$\text{Proportion of feces containing larvae (PF, \%)} = \frac{\text{Number of feces with larvae}}{\text{Number of feces investigated}} \times 100\% \quad (1)$$

$$\text{Number of larvae per fece (NL)} = \frac{\text{Number of larvae in feces}}{\text{Number of feces investigated}} \quad (2)$$

Transparent plastic cups (8 cm in diameter, 6 cm in height) were used as the pupation container of *G. pecorum* larvae, five larvae were placed in each cup, the cup mouth was sealed with gauze and cultured in outdoor shade, the pupation and eclosion behavior of the larvae were observed simultaneously. The number of each insect state was recorded, the survival rate, pupation rate and eclosion rate were counted. The daily temperature was also recorded.

**Data analysis**

To evaluate the development status of stomach bot flies, the following formulas were utilized:

$$\text{Survival rate (SR, \%)} = \frac{\text{Number of surviving larvae in feces}}{\text{Total number of larvae in feces}} \times 100\% \quad (3)$$

$$\text{Pupation rate (PR, \%)} = \frac{\text{Number of pupae}}{\text{Number of surviving larvae}} \times 100\% \quad (4)$$

$$\text{Eclosion rate (ER, \%)} = \frac{\text{Number of adults}}{\text{Number of pupae}} \times 100\% \quad (5)$$

Spearman correlation analysis was used to analyze the relationship between the number of mature larvae and its survival rate, and the significant level was set as $\alpha = 0.05$. Data analysis was performed in SPSS 20.0, and the graph was drawn by Sigmaplot 12.0 and Graphpad prism 7.

## List of Abbreviations

KNR - Kalamaili Nature Reserve

PF - Proportion of feces containing larvae

NL - Number of larvae per fece

SR - Survival rate

PR - Pupation rate

ER - Eclosion rate

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### Availability of Data and Material

All data generated or analysed during this study are included in this article.

### Author contributions

K.L., K.Z. and H.Q.H. conceived the study, K.Z. drafted the manuscript, K.Z., H.Q.H. and R.Z. conducted the experiment, K.Z., B.R.Z., C.W. and M.E. carried out the statistics, B.L.L., D.Z. and K.L. revised the manuscript. All authors reviewed the manuscript and approved the final version.

## Ethics Statement

The study was performed in accordance with the relevant guidelines and regulations regarding animal welfare. All experimental protocols were approved by Wildlife Conservation Office of Altay Prefecture and Beijing Forestry University.

## Consent for publication

Not applicable.

## Competing interests

The authors declare no competing interests.

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Figure legends

Figure 1 The population dynamics of *G. pecorum* in 2018 (mean/week).

Figure 2 Proportion of larvae of *G. pecorum* collected weekly (mean/week).

Figure 3 The accumulative collection of *G. pecorum* larvae in 2018.

Figure 4 Comparison of development in vitro of *G. pecorum* between two peak periods. (A) The pupal days of the two peak periods; (B) The survival rate (SR), pupation rate (PR) and eclosion rate (ER) of the two peak periods.

Figure 5 The temperature changes in KNR in 2018. (A) The average daily temperature, the highest temperature, the lowest temperature, and the temperature difference in KNR; (B) The temperature fitting curve.