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Daily egg production in *Pantala flavescens* in relation to food intake (Odonata: Libellulidae)

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Abstract. The migratory dragonfly, *Pantala flavescens* (Fabricius, 1798), arrives in Japan from tropical regions every spring. Although the population increases as autumn nears, it dies in the winter cold. The adults often form foraging swarms above open grasslands when feeding on small insects, while oviposition occurs at diverse open water bodies throughout the day. Although oogenesis requires a daily intake of nutrition from prey, there has been little consideration of the relationship between food intake and the number of eggs produced. In the early morning, females of reproductive age were captured from foraging swarms in grasslands. Immediately after capture, an artificial oviposition technique was applied to each female to release all mature eggs loaded. Then, the females were kept until death, up to 5 days, in envelopes in the laboratory. They were starved but hydrated daily, and the dry weight of faeces excreted during 24 h after capture was measured. Females excreted 8.4 mg of faeces within 24 h after capture. Then, they released about 840 mature eggs at 24 h after capture, suggesting that when females take in a sufficient amount of daily food, they can oviposit a large number of eggs every day. The rapid egg production might enable the population of *P. flavescens* to grow. A positive correlation was found between the food intake on the previous day and the number of eggs produced within a 24 h of capture. The act of ingesting fresh nutrients derived from the prey might promote rapid release of reserves in the female fat body, resulting in the oogenesis. Females able to encounter available foraging sites might produce a large number of mature eggs in the subsequent day to be laid.

Further key words. artificial oviposition technique, faeces, fertility, inter-clutch interval, mature egg.

Introduction

Since odonate adults have little fat body immediately after their emergence, throughout their lives they must receive ongoing nutrition for somatic maintenance, and in females, also for oogenesis. Well nourished insects show increased longevity as well as a high reproductive output (Plaistow

& Siva-Jothy 1999). The intake of nutrition during the pre-reproductive stage of adults helps to develop their flight muscles, to harden the exoskeleton, and to develop eggs in the ovaries (Marden 1989). In male *Erythemis simplicicollis* (Say, 1839), individuals that consumed more food in their pre-reproductive stages attained sexual maturity more rapidly (McVey 1985). Koch & Suhling (2005) reported that additional food intake provided by hand feeding significantly increased the survival rate during the pre-reproductive stage of *Orthetrum coerulescens* (Fabricius, 1798) adults in the laboratory. After sexual maturation, foraging activity continues nourishing the fat body, but this nutrition is mainly allocated to reproduction (Baird & May 1997). Anholt et al. (1991) pointed out that food intake during the reproductive stage was related to success in territorial conflict among males and egg production in females.

Corbet (1999: 37) stated that females of most dragonfly species lay almost all of the mature eggs held in the ovaries in single oviposition bouts, so that they must return to foraging sites in order to produce more eggs. Banks & Thompson (1987) noted that the inter-clutch interval is one of the important factors determining the number of eggs laid throughout the dragonfly life-span. About 2.2 days and 3–4 days were needed to produce one clutch of eggs in *Plathemis lydia* (Drury, 1773) (Koening & Albono 1987) and in *Sympetrum danae* (Sulzer, 1776) (Michiels & Dhondt 1989), respectively. Food intake during the inter-clutch periods for females has been quantified in *S. frequens* (Selys, 1883) (Higashi 1973) and *Pachydiplax longipennis* (Burmeister, 1839) (Baird & May 1997). Watanabe et al. (2011) measured the relationships between food intake and egg production in *Sympetrum infuscatum* (Selys, 1883) females using hand-feeding and artificial oviposition techniques. All species previously studied have been "perchers" (Corbet 1999: 373). In perchers for each adult a long observation period in the field is possible, so the foraging success in each foraging flight can be observed. In addition, it is relatively easy to rear adults in the laboratory in percher species. On the other hand, little information about the egg production of "flier" species in relation to food intake has been reported, mainly due to the difficulty of making observations.

Pantala flavescens is a well known flier dwelling in tropical and subtropical regions. It migrates to Japan every spring, and the population rapidly in-

creases, with frequent foraging swarms of adults from summer into autumn (Arai 2007: 90). Hirake (2012) pointed out that *P. flavescens* in the foraging swarms often attacked swarming insects such as Ephemeroptera over open fields in late summer. Ichikawa & Watanabe (2015) estimated that *P. flavescens* females consumed about 185 small insects daily.

Although the frequency of *P. flavescens* visits to water is unknown, oviposition behaviour in Japan has been observed on various types of open water, such as small ponds, rice paddy fields, swimming pools, and puddles (Arai 2007: 110). Ichikawa & Watanabe (2014) reported that *P. flavescens* females of stage M, just after sexual maturation, had about 1 100 ovarioles, each containing about four submature eggs, i.e., oval eggs without chorion, suggesting that they might have the ability to develop a large number of mature eggs in a short time.

In the present study, we captured reproductively mature *P. flavescens* females (stage M) from the foraging swarms and estimated the amount of food intake in relation to the potential ability to produce eggs.

Material and methods

Swarms of *Pantala flavescens* foraging low above grasslands are usually found on sunny and windless days from August to mid-September in Tsukuba City, Ibaraki Prefecture, central Japan. Females in the swarms were opportunistically sampled within limits determined by the length of the net handle (about 2 m) before the onset of foraging (07:00–09:00 h JST [UTC+9], 07:20–09:20 h solar time) for seven days in 2014. Adults flying less than 3 m above the ground could be captured. The stage of each female sampled was identified by using the criteria established by Ichikawa & Watanabe (2014). We then used females of reproductive stage M, just after sexual maturation, which showed partly brownish wings without visible damage. Females identified in the pre-reproductive stages and in older reproductive stages were not used and were therefore released immediately following capture. The hind wing length of each female was measured with electronic callipers (accuracy, 0.01 mm) to examine the body size variation.

Immediately after capture, in the field, oviposition was artificially induced in each female using the technique of Watanabe & Higashi

(1993). Females were gently held by the wings, and the tip of the abdomen was repeatedly dipped vertically into a vial of water once per second in order to allow the female to release mature eggs, continuing until she stopped releasing eggs. The water temperature in the vial was equal to the air temperature (around 30°C). Even if a female released no eggs, the dipping procedure was conducted for 1 min, after which the female was considered to be unable to oviposit. *Pantala flavescens* females released almost all of their mature eggs, irrespective of the number of mature eggs present in their ovaries (Ichikawa & Watanabe 2014). Thus, females that released no eggs by this technique must be non-gravid. The eggs released were counted under a binocular microscope in the laboratory. Because the surface colour of fertilized eggs darkens one day after oviposition in many libellulid species (Corbet 1999: 50), the colour change of each egg released was examined a couple of days after the artificial oviposition in order to assess fertility.

After artificial oviposition, each female was kept individually in an envelope under laboratory conditions of 26°C and a 14L:10D light regime. As described in detail by Ichikawa & Watanabe (2015), we used envelopes instead of plastic cups for holding adults because mortality from injury can occur with activity even within a small cup. Every day, each dragonfly was fed by syringe a single drop of water (about 50 µl) in the morning, at noon, and in the afternoon. The faeces excreted by females in the envelope consisted of rather dry granular pellets, which were easily collected from the envelope using a brush. During the first 24 hours after capture, the faeces excreted by females were collected and then dried in an oven at 80°C for 8 hours prior to weighing.

During the first 5 days after capture, the females were starved but hydrated. The eggs released by artificial oviposition were counted at 24 hour intervals (24, 48, 72, 96, and 120 h after capture). No eggs were released in the envelope throughout the experiment. The fertility of the eggs released was also examined.

All statistical analysis was carried out using R 3.1.1. Pearson's correlation test was used to examine the relationship between the number of eggs released when females were captured and the number of eggs released at 24 h after capture. The relationship between food intake and egg production was analysed.

Results

Each swarm contained both sexes in various stages of sexual maturity. Although there were some differences in the extent of fluttering among the stages, most of them showed little active fluttering, but rather, glided, before 09:00 h. Some females occasionally landed on the grass. Eventually, 13 females of reproductive stage M were captured. The hind wing length of females captured was 40.1 ± 0.8 mm (mean ± SD, n = 13).

The number of eggs released by artificial oviposition immediately after capture was 446.1 ± 176.6 (n = 13), though two females released no eggs. The average time during which eggs were released was 1.2 ± 0.9 min (n = 11). The maximum number of eggs released was 1 527 within 2.3 min. About 97.6 % (n = 11) of the eggs released were fertile.

Faeces excreted within 24 h of capture were dark brown oval pellets. They contained many cuticle fragments, probably remnants of the prey insects consumed on the previous day, as described by Ichikawa & Watanabe (2015). The dry weight of faeces excreted during the 24 h after capture was 8.43 ± 0.84 mg (n = 13). Because most females that were captured from 07:00 to 09:00 h had not yet fed that day, the weight of faeces excreted during the 24 h after capture must reflect the quantity of food intake from the previous day.

The number of eggs released at 24 h after capture in the laboratory was 835.2 ± 79.2 (n = 13). There was no relationship in the number of eggs released between immediately after capture and at 24 h after capture ($r = -0.10$, $p = 0.754$). The fertility rate was 98.1 % (n = 13).

When females continued to be kept in envelopes and received only water, most females released few eggs (15.3 ± 13.6 eggs, n = 12) at 48 h after capture. Then, the numbers of eggs released at 72 h, 96 h, and 120 h after capture were 0.3 ± 0.2 (n = 8), 0 (n = 6), and 0 (n = 3), respectively. Females that released no eggs by the artificial oviposition technique developed no more mature eggs in their ovaries, confirmed by the dissection. All of the eggs released were fertilized.

As shown in Figure 1, there is a positive correlation between the dry weight of faeces excreted during 24 h after capture and the number of eggs released at 24 h after capture. Table 1 represents the results of the multiple line regression analyse on the egg production of females for the food intake

Table 1. Results of multiple regression analysis on the number of eggs released by females of *Pantala flavescens* at 24 hours after capture. n = 13, *F* = 14.62, *d.f.* = 10.00, *p* = 0.0011.

	Partical regression confficient	t	*p*
Length of hind wing	57.21	0.83	0.43
Dry weight of faeces excreted within 24 hr after capture	71.85	3.95	0.0027

and body size, though little variation in the hind wing length of females was found. The result shows that the number of eggs released at 24 h after capture depended on the dry weight of faeces excreted within 24 h after capture ($p = 0.0027$), not on the length of hind wing ($p = 0.43$).

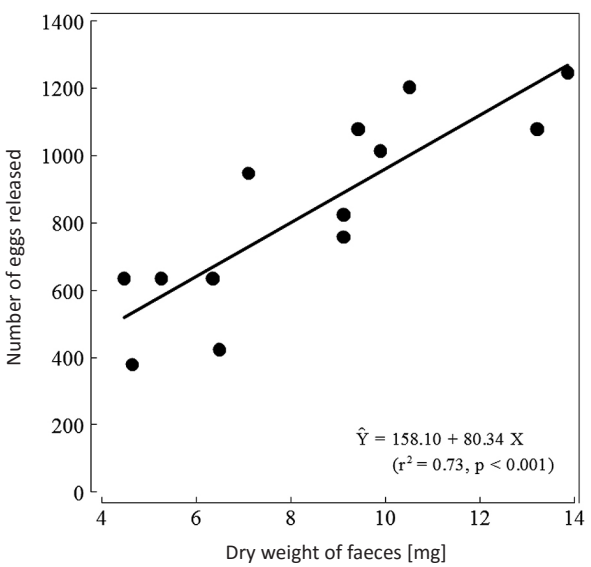

Figure 1. Relationship between the dry weight of faeces excreted by *Pantala flavescens* females during 24 h after capture and the number of eggs released 24 h after capture during artificial oviposition.

Discussion

In the present study, there was little variation in the hind wing length of *Pantala flavescens* females. This in agreement with the results of Samways & Osborn (1998) who reported a low coefficient of variation of the hind wing length of *P. flavescens* females captured in South Africa and on Easter Island between December and February. Little seasonal change in the hind wing length of females from July to October in Japan was also found by Ishizawa (2007). Therefore, little variation in body size of *P. flavescens* females might be appeared, irrespective of sampling sites and seasons.

The number of eggs released by artificial oviposition immediately after capture and the period of egg release showed large variation in the present study. Because females captured from foraging swarms may have had various oviposition experiences on previous days, the clutch size should theoretically vary from zero to a very high value. Ichikawa & Watanabe (2014) applied the artificial oviposition technique for the reproductive stage of *P. flavescens* females captured from foraging swarms at various time of day and reported that the average number of eggs released was 640, though some females released more than 2 000 eggs. On the other hand, Koch & Suhling (2005) used an artificial oviposition technique with *P. flavescens* females soon after copulation near artificial ponds and concluded that the number of eggs released per female in an oviposition bout was about 982.

Corbet (1999: 62) pointed out that the fertility of odonate eggs released, including *P. flavescens*, was typically 100 % or nearly so, except where very low temperatures affected the efficiency of sperm transfer or where sperm was depleted due to mating having taken place too long previously. These results included artificially induced oviposition (Corbet 1999: 62). In the present study, the fertility of eggs released immediately after capture was also nearly 100 %. Furthermore, the fertility of eggs released by *P. flavescens* females reared with water in the laboratory was 100 %, too.

Because of the starvation regime in the laboratory, the nutrients for egg production of *P. flavescens* must depend on the prey insects eaten the previous day as well as fat body reserves in the present study. Watanabe et al. (2011) reported that *S. infuscatum* females consumed about 17.7 mg of food per day and developed about 72 eggs per day. Higashi & Watanabe (1993) reported that *Orthetrum japonicum* (Uhler, 1858) released about 300 eggs

every day. Therefore, daily egg production by *P. flavescens* females must be high at least among some libellulid species, though in the present study females were kept in envelopes and thus unable to expend energy by flying. Also it must be noted that because of different relative sizes of eggs such cross-species comparisons must be approached with caution.

After releasing eggs at 24 h after capture in the laboratory, starved *P. flavescens* females did not produce additional mature eggs. This indicated that most nutrients taken in on the previous day were apparently exhausted by oogenesis during 24 h after capture. Thus, the daily foraging activity must be essential for *P. flavescens* females to increase their lifetime egg production. Palacino et al. (2012) stated that rains might interfere with dragonflies' ability to fly and to forage on small flying insects. Banks & Thompson (1987) examined the reproductive success of *Coenagrion puella* (Linnaeus, 1758) females and pointed out that the number of sunny days during their reproductive periods affected the lifetime egg production. Therefore, good weather condition for daily foraging activity might increase the lifetime egg production of *P. flavescens* females, though they flew actively in the rain on Easter Island (Moore 1993).

Richardson & Baker (1997) pointed out that food intake is an important determinant of production by the damselfly, *Ischnura verticalis* (Say, 1839), and that body size is less important. Watanabe et al. (2011) also reported a positive correlation between the amount of food intake and the egg production of *S. infuscatum* females. In *P. flavescens* females, food intake on the previous day also had a great influence on their egg production. This indicated that the act of ingesting fresh nutrients derived from the prey might tend to allow rapid releases of reserves in the female body, resulting in oogenesis. However, Cordero (1991) found a partial correlation between the body length of *Ischnura graellsii* (Rambur, 1842) and its clutch size, without any information on food intake. Nevertheless, in *P. flavescens*, females that foraged successfully the previous day appear to develop a large number of mature eggs.

Corbet (1999: 10) pointed out that *P. flavescens* might be an example of an *r*-strategist among odonate species. In the present study, *P. flavescens* females in stage M laid more than 800 mature eggs every day. Ichikawa & Watanabe (2014) reported that the volume of eggs released by *P. flaves-*

cens females was about 0.038 mm³. Among libellulid species, Inoue & Tani (2010: 92 f.; 134) suggested a relatively small egg size in *P. flavescens*. Furthermore, the eggs and larvae of *P. flavescens* develop very rapidly in high water temperatures, completing development within 50 days (Suhling et al. 2004; Iwata et al. 2009). These traits undoubtedly enable *P. flavescens* to increase its population quickly in the summer in Japan and elsewhere. Detailed studies on lifetime egg production in *P. flavescens* to clarify its life history strategies, including immigrant population growth, are needed.

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