International Journal of Odonatology, 2016
Vol. 19, No. 3, 95–105, http://dx.doi.org/10.1080/13887890.2015.1129990

The factors influencing the length of the terrestrial period in the final instar larvae of *Epiophlebia superstes* (Selys, 1889) (Anisozygoptera: Epiophlebiidae)

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(*Received 10 October 2015; final version received 7 December 2015*)

The terrestrial period before adult eclosion of the final instar larvae of *Epiophlebia superstes* was studied during spring 2008 to 2012, at a mountain stream in Nurukawa, Aomori prefecture, northern Japan. The average terrestrial period of larvae that left the water during early, middle and late April, estimated by mark-release-recapture and caging methods, was 45.3 days ($\pm$ 1.3 days, $n = 8$), 35.8 days ($\pm$ 1.8 days, $n = 9$) and 29.0 days ($\pm$ 1.4 days, $n = 2$), respectively. The date on which a larva left the water was negatively correlated with the terrestrial period ($r^2 = 0.80$, df $= 17$, $p < 0.01$). Also, indoors, the terrestrial period was negatively correlated to air temperature ($r^2 = 0.76$, df $= 7$, $p < 0.01$).

Keywords: Odonata; Epiophlebiidae; *Epiophlebia superstes;* imaginal ecdysis; larva; terrestrial period

Introduction

Final instar (F-0) larvae of almost all odonate species crawl out of the water before casting off their larval cuticle to emerge as adults (Corbet, 1999). In this paper I will refer to the period between the day of "landing" (i.e. emergence from the water) and the day of adult eclosion as the terrestrial period.

In most odonate species, the length of the terrestrial period falls between a few hours to a few days (Fukushima, 1968), while in some other species it is much longer and the landed larvae spend time hiding themselves under stones, etc., near their eclosion spots (Corbet, 1999). For example, the terrestrial period is six days in *Mnais pruinosa* Selys, 1953 (Fukushima, 1968), 10 days in *Boyeria maclachlani* (Selys, 1883) (Aida, 1974; Yamaguchi, 1961), 3–25 days in *Anotogaster sieboldii* (Selys, 1854) (Yamaguchi, 1961) and 20–30 days in *Epiophlebia superstes* (Eda, 1964, 1966). The terrestrial period of *E. superstes* is considerably longer than that of other odonate species and this fact stimulates researchers to study the actual length of the period, what influences the length, and why *E. superstes* has such a long terrestrial period.

Eda (1964, 1966) reported that F-0 larvae of *E. superstes* spent this period hiding themselves under stones or leaf litter near their eclosion sites at Mt Takao (35°48′ N, 139°05′ E), Tokyo, Japan. This species occurs also in Nurukawa village (40°30′ N, 140°48′ E), Aomori Prefecture, where the climate is colder than in Mt Takao and the snow cover remains until early May.

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Therefore the length of the terrestrial period of *E. superstes* is expected to be longer in Nurukawa than in Mt Takao. In my previous study (Naraoka & Takahashi, 2007), it was estimated to be 25–35 days. But the method used for this estimation involved a defect: the terrestrial period was estimated from the date of eclosion in larvae kept outdoors in the lowlands, although the larvae had landed and then been captured on the shore of a mountain stream.

To estimate more precisely the length of the terrestrial period of *E. superstes* and to clarify the influence of ambient temperature and the date of landing on the length of the terrestrial period, I have improved the method. The methods adopted for the present study were: field cage observations, a mark-release-recapture method and laboratory rearing experiments.

Methods

1. Study site

The investigation was conducted at a small branch stream of the Aseishigawa River at Nurukawa (40°30′ N, 140°48′ E, 520–550 m asl), Hirakawa City, Aomori Prefecture for five years from 2008 to 2012 (Figure 1). The stream is 2–3 m wide and flows northward, with the slope of the hill on the east side, while on the west bank was 4–5 m of flat land, beyond which a steep slope

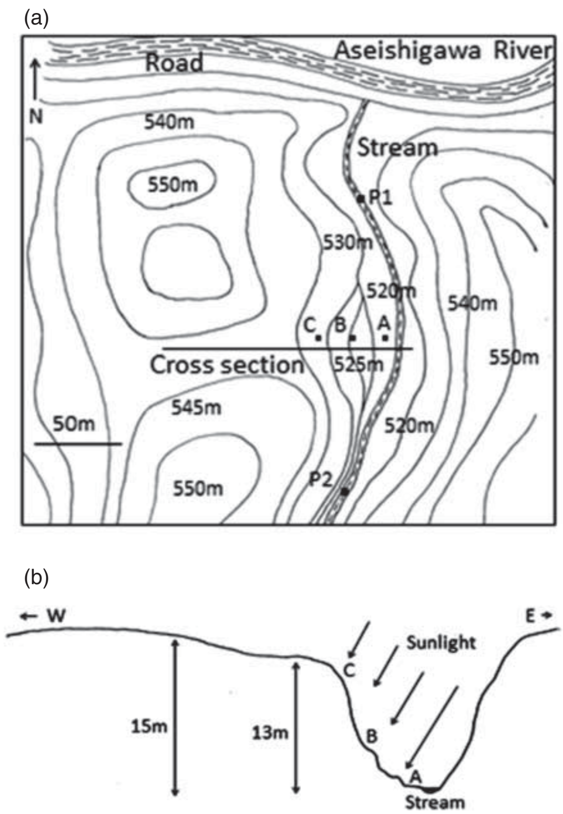

Figure 1. (a) Map showing the study area at Nurukawa, Hirakawa City, Aomori Prefecture, Japan. A, B and C: measuring points of air temperature in 2008. (b) A cross section shows the typical topography of the study area. Sunlight direction during 7:00–8:00 h of late May is shown.

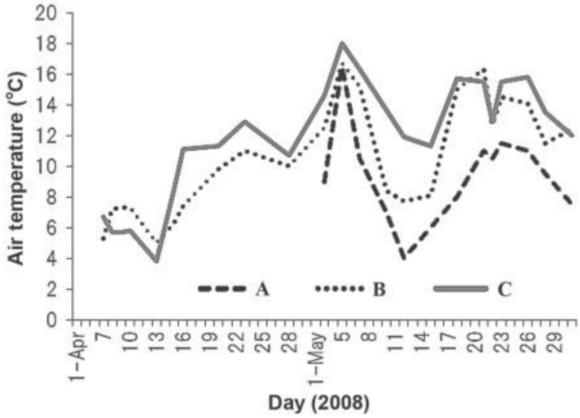

Figure 2. Seasonal changes of air temperature at the study site in 2008. Points A, B and C are stream side, sunny slope and sunny ridge, respectively, as shown in Figure 1.

(60–90° gradient) of a small hill reaching 13–15 m above the bank is situated. The slope was covered with low bushes and grasses after thawing.

The larval census area was 200 m along the stream (from P1 to P2, Figure 1), covering 40 m of the east side and 100 m of the west side of the stream. The adult census was conducted around every two days. When I found an adult eclosed, I recorded its eclosion hour and sex. I also recorded the position of the site, i.e. horizontal distance from and vertical height above the stream.

I measured the air temperature at three points in the census area (A, B and C, Figure 1) using a temperature recorder (TG846, Empex Corporation, Tokyo) in 2008 (Figure 2). Measuring points A, B and C are located at 0.1 m (stream side), 6 m (sunny slope) and 13 m (sunny ridges) above the stream surface, respectively.

For the other survey years, however, I used data of a meteorological observatory at Ikarigaseki, Hirakawa city that is 11 km west from the survey area, as Ikarigaseki and the survey area are located relatively nearby and are topographically closely similar.

In late March about 50% of the water surface of the investigated stream appeared from under the snow and in the middle of April it expanded to 60–70% by thawing. By late May, the west slope of the stream was exposed to the sun (Figure 1): the sunny period began at *c.*6:00 h at the ridge (near point C), 6:00–7:00 h at the middle of the slope (near point B) and at *c.*8:00 h at the bottom (near point A). The west slope was shaded in the afternoon, however, while the east slope of the stream was exposed to the sun between *c.*10:00 h and 17:00 h. For more details about the study site, consult Naraoka and Takahashi (2007).

2. Field cage observations

The terrestrial period of F-0 larvae was studied in the field using mesh-bags (cages), into which marked larvae were introduced. In April 2008 and 2009, I collected F-0 larvae found walking on the snow within 5 m of the stream. I assumed that these larvae had landed from the stream on the same survey day, because F-0 larvae that landed on previous days were never found walking close to the stream; the latter were hidden under fallen leaves or were walking more than 10 m from the stream. They were individually marked with combinations of body parts and color: I used three different body parts (dorsal and both lateral surfaces of abdomen) and three different marker pens (white, blue and red). For each individually marked larva, the landing date and sex was recorded.

One to three marked larvae were put in a nylon fine-mesh cage (25 × 30 × 60 cm), lined with leaf litter and broken twigs. I prepared 15 cages (nine in 2008, six in 2009). All cages were brought to the west slope where many eclosions of *E. superstes* were observed. The cages were immobilized using strings so as to keep the bottom in close contact to the ground. When I found an adult newly eclosed, I recorded the eclosion time, sex and individual marks left on its exuviae.

3. Mark-release-recapture method

In addition to the field cage method mentioned above, I used a mark-release-recapture technique in 2010 and 2012. I collected F-0 larvae found walking on the snow within 5 m of the stream. They were marked individually and released on the slopes of the same study area. The handling and marking procedures apparently did not cause ill effects on the behavior of larvae.

As in the field cage observations, I recorded the eclosion time, sex and marks of individuals, as well as the position of the eclosion site.

4. Laboratory observations

I collected newly landed F-0 larvae at occasional opportunities during the whole study period between 2009 and 2012 (eight males and one female). They were brought to the laboratory to examine the effect of temperature on the terrestrial period. Larvae were introduced to a clear acrylic tank (35 × 20 × 27 cm high) with moist dead leaves and twigs. The tank, covered by a net, was placed beside the window without temperature control, and the date of the eclosion of each larvae was recorded.

Air temperature within the acrylic tank was measured every hour using a thermo-recorder (TR-72Ui, T & D Corporation, Nagano, Japan).

Results

1. Seasonal change of the physical environment within the habitat

Figure 2 shows the change in air temperature during the survey period in the study site in 2008. Air temperature increased as the season progressed. Site C was regularly warmer than Site A or B during May.

2. Eclosion in the natural habitats

Figure 3 shows the seasonal change in the number of adult eclosions. Because sample size is small each year and there was no apparent annual variability, all eclosion data from 2008 to 2012 were combined. The total number of eclosions for all study years was 46 (30 males and 16 females). Adult eclosion was observed between 9 May and 1 June, and peaked around 23 May. No significant difference in sex ratio was detected ($F = 1.09$, $df = 45$, $p > 0.25$).

Figure 4 shows the influence of weather conditions on the number of adult eclosions. In total, 42 adult eclosions were observed on 35 sunny census days, while only four eclosions were seen on two of 21 cloudy days, when mean air temperature was 17.7°C. Eclosion was not observed when mean air temperature was below 17°C, irrespective of the weather. No eclosion was observed during eight rainy days.

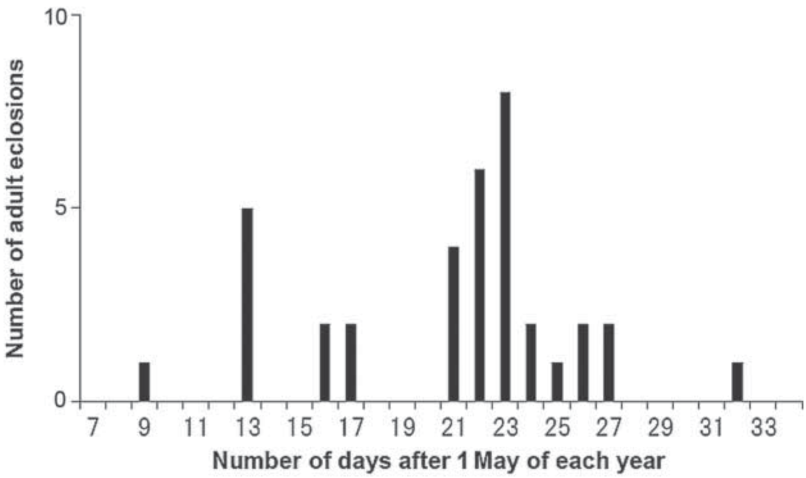

Figure 3. Seasonal changes of the number of adult eclosions in the study area. Records are from 2008 to 2012.

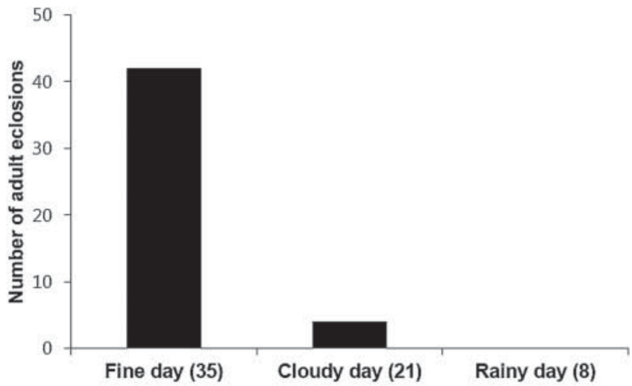

Figure 4. The number of adult eclosions found on fine, cloudy and rainy days during five years from 2008 to 2012. Numbers in parentheses show the number of census days for each weather condition.

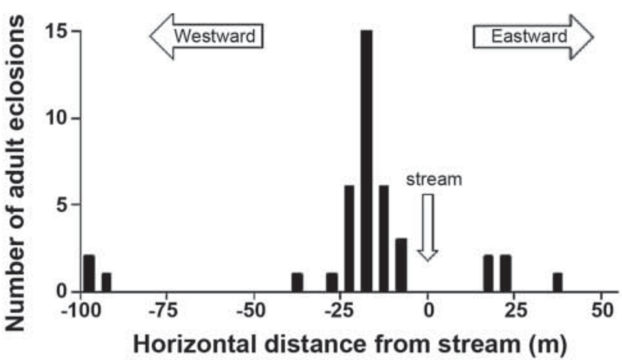

Figure 5. Horizontal distance of eclosion site from the stream. Direction toward the west and the east are shown as negative and positive figures respectively. Records are from 2008 to 2012.

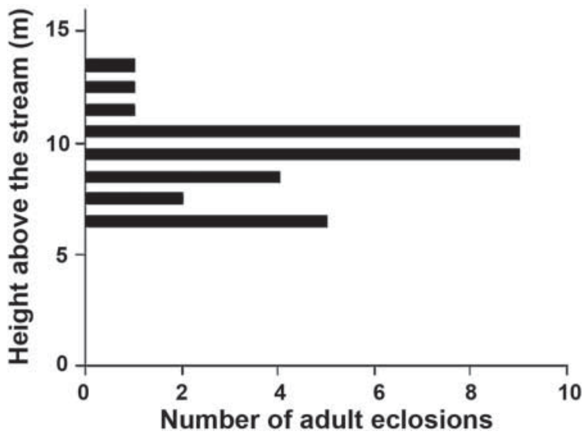

Figure 6. Height of eclosion site above the stream surface. Records are from 2008 to 2012.

Most adult eclosions were observed on the west side of the stream (47/52, 90%, Figure 5), with only five on the east side. The westward horizontal distance between the adult eclosion site and the stream was bimodal: mostly between 10 and 25 m, but with some around 100 m. This gap is simply due to lingering snow accumulated on the relatively flat surface at 40–90 m (Figure 1). The average distance westward from the stream was 26.5 ± 23.9 m ($n = 38$) while eastward it was 26.0 ± 8.2 m ($n = 5$).

Most of the eclosion sites were located on slopes higher than 5 m above the stream (Figure 6), where they were exposed to sunlight in the morning (Figure 1).

3. Terrestrial period of F-0 larvae

The results of the field cage studies are shown in Table 1. Twenty-nine F-0 larvae were used in this method (2008: 11 males and eight females; 2009: five males and five females). Among them 13 adults (45%) eclosed (2008: six males and four females; 2009: two males and one female). I examined the cages in the middle of June and found dead bodies of eight male and three female larvae; the other five larvae were missing.

Table 1. Terrestrial periods of final instar larvae of *E. superstes* based on the field cage method. Larvae were collected immediately after landing in Nurukawa field in 2008 and 2009. (Mean air temperature during the terrestrial period is estimated using data at Ikarigaseki meteorological observatory).

Individual No.	Sex	Landing	Eclosion	Terrestrial period (days)	Mean air temperature (°C)
1	Male	6 April 2008	21 May 2008	45	10.8
2	Male	6 April 2008	22 May 2008	46	10.9
3	Female	6 April 2008	19 May 2008	43	10.5
4	Female	6 April 2008	22 May 2008	46	10.8
5	Male	7 April 2008	22 May 2008	45	10.9
6	Female	7 April 2008	24 May 2008	47	11.1
7	Male	13 April 2008	17 May 2008	34	10.9
8	Male	15April 2008	19 May 2008	34	11.3
9	Male	15April 2008	22 May 2008	37	11.7
10	Female	15 April 2008	19 May 2008	34	11.3
11	Male	17 April 2009	25 May 2009	38	11.3
12	Male	17 April 2009	21 May 2009	34	10.9
13	Female	17 April 2009	25 May 2009	38	11.3

The terrestrial period, estimated as the duration between the day of landing and eclosion, ranged from 34 to 47 days ($n = 13$), for the two years and sexes combined. Average terrestrial period was 40.1 ± 5.3 (SD) days.

The results of the mark-release-recapture study are shown in Table 2. In total 103 marked F-0 larvae (48 males and 55 females) were released (2010: 17 males and 15 females; 2011: 17 males and 26 females; 2012: 14 males and 14 females). Among them, six exuviae were recovered in 2010 (three males) and 2011 (one male and two females). No marked exuviae were found in 2012. The rediscovery rate was 5.8%. The estimation of terrestrial period in this method ranged from 28 to 46 days and the average including the two study years and both sexes was 36.8 ± 7.2 (SD) days, which was similar to results of the field cage method ($F = 1.22$, $df = 18$, $p > 0.25$).

The average terrestrial period of the F-0 larvae landed in early, middle and late April, combining the two methods was 45.3 days (±1.3 days, $n = 8$), 35.8 days (±1.8 days, $n = 9$) and 29.0 days (±1.4 days, $n = 2$), respectively. Details of the behavior of four larvae during the terrestrial period are given in the Appendix.

Figure 7 shows the relationship between the landing day and the adult eclosion day after 1 April based on the data obtained from either method described above. The straight line is the line of x–y equivalence. Thus the vertical distance between each point of adult eclosion day and the straight line indicates the terrestrial period of this individual. Records are from 2008–2012.

Table 2. Terrestrial periods of final instar larvae of *E. superstes* based on the mark-recapture-release method. Larvae were collected immediately after landing in Nurukawa field in 2010 and 2011. (Estimation method of mean air temperature is the same as Table 1).

Individual No.	Sex	Landing	Eclosion	Terestrial period (days)	Mean air temperature (°C)
1	Male	9 Apr. 2010	23 May 2010	44	9.4
2	Male	22 Apr. 2010	22 May 2010	30	10.8
3	Male	24 Apr. 2010	22 May 2010	28	11.3
4	Female	10 Apr. 2011	26 May 2010	46	9.8
5	Male	20 Apt. 2011	26 May 2010	36	10.7
6	Female	20 Apt. 2011	27 May 2010	37	10.8

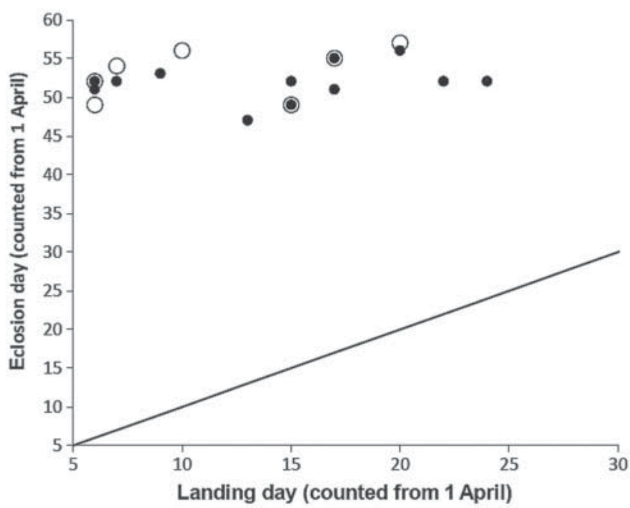

Figure 7. The relationship between the landing day and the adult eclosion day counted from 1 April. The straight line is the x–y equivalence. Records are from 2008 to 2012. Male and female are shown by black and open circles, respectively.

Table 3. Terrestrial periods of final instar larvae of *E. superstes* under uncontrolled temperature conditions (range 3.1–29.1°C) in the laboratory. Larvae were collected immediately after emergence in Nurukawa field between 2009 and 2012. (Mean air temperature is the average of temperatures measured at 1 h intervals during the terrestrial period.)

Indiv. No.	Sex	Emergence	Eclosion	Terrestrial period (days)	Mean air Temperature (°C)
1	Male	6 Apr. 2009	22 Apr. 2009	16	15.7
2	Male	6 Apr. 2009	22 Apr. 2009	16	15.7
3	Female	5 Apr. 2010	2 May 2010	27	9.6
4	Male	16 Apr. 2010	6 May 2010	20	12.0
5	Male	6 Apr. 2011	30 Apr. 2011	24	11.7
6	Male	6 Apr. 2011	1 May 2011	25	11.8
7	Male	25 Apr. 2011	9 May 2011	14	14.3
8	Male	9 Apr. 2012	27 Apr. 2012	18	13.6
9	Male	19 Apr. 2012	5 May 2012	17	16.6

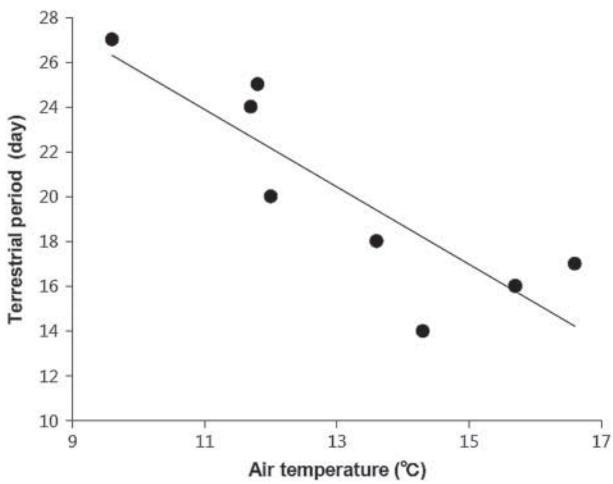

Figure 8. The duration of the larval terrestrial period in relation to the mean air temperature during the terrestrial period of each larva, under laboratory conditions. The line indicates the linear regression.

It is apparent that the terrestrial period was longer for larvae that landed from the water earlier than those that landed later. The terrestrial period was negatively correlated with the landing day ($r^2 = 0.80$, df = 16, $p < 0.01$). Most eclosions occurred during middle and late May irrespective of the landing day (Figure 7). No significant difference between sexes in the eclosion day was detected ($F = 2.65$, df = 20, $p > 0.25$).

4. Effect of air temperature on the terrestrial period

The terrestrial period of F-0 larvae measured in the laboratory is shown in Table 3. All captive larvae eclosed between 22 April and 9 May, earlier than the eclosion under natural conditions (Table 3). Larvae usually clung to an eclosion site (twig) during 5:30–7:00 h and completed eclosion by 8:00 h.

The average terrestrial period was 19.7 ± 4.6 (SD) days (Table 3). In contrast to Table 1, the terrestrial period was not correlated with the landing day ($r^2 = 0.32$, df = 7, $p > 0.05$), but it was negatively correlated with air temperature ($y = -1.73x + 42.87$, $n = 9$, $r^2 = 0.76$, df = 7, $p < 0.01$, Figure 8).

Discussion

The majority of *E. superstes* eclosed on the west slope, on days with the sun shining early in the morning. The landing of F-0 larvae from the water also was greater on sunny days than on cloudy days (Naraoka & Takahashi, 2007). Light intensity therefore apparently influenced the eclosion decisions as well as landing decisions by F-0 larvae of *E. superstes*. It is also clear that temperature influences eclosion, because eclosion occurs when air temperature is higher than 17°C even on cloudy days.

Typically, eclosion occurred on slopes 5 m higher than the stream side. This is probably because the larvae select places with higher air temperature and more exposed to the sun than places adjacent to the stream (Figure 2). Under higher air temperature, F-0 larvae can eclose earlier in the morning and the eclosed adult can then make their maiden flight into the forest earlier in the day. Then, the risks during eclosion (the failure to molt or expand and hardening of the wings, and predation by birds) would be decreased.

The terrestrial period estimated from the field cage method and the mark-release-recapture method varied from 28 to 47 days. Although Naraoka and Takahashi (2007) estimated it as 25–35 days for the terrestrial period in Aomori Prefecture, they noted that actual length of the terrestrial periods should be longer than that estimated because of the defect of the method employed in that study.

Why do *E. superstes* F-0 larvae land from streams so early in spring, and why is the terrestrial period until eclosion so long? F-0 larvae are thought to land in response to the stimulus of the rising of water temperature and the sunlight streaming through the open surface of the stream during April (Naraoka & Takahashi, 2007). The landing during April may allow for escape from streams before their predators such as fish, birds and other creatures become active with increasing water and air temperatures. After landing, the larvae may wait a long time for good weather with a high enough temperature for eclosion while basking on the slopes of hillsides. Throughout this period, their imaginal metamorphosis may be completed more quickly with progressive seasonal increase in air temperature than in places adjacent to the stream.

The progressive seasonal increase in air temperature is thought to shorten the terrestrial period, as larvae that landed later than others had a shorter terrestrial period. The effect of temperature is clear also from the fact that indoors the terrestrial period was shorter and negatively correlated with air temperature. It has also been shown in outdoor experiments in the lowlands (Naraoka & Takahashi, 2007) that as the landing day was delayed, the terrestrial period became shorter. Thus, the date of eclosion was concentrated in the period from mid to late May irrespective of the landing day.

The brief terrestrial period of 10 days in the case of the larvae that were recorded by Fukushima (1968) might have been due to high air temperature of the place in which the larvae were kept. The terrestrial period (20–30 days) in Kanto Region, central Japan, estimated by Eda (1964) is not inconsistent with the present result, because air temperature in the Kanto Region is *c*.6°C higher than in Aomori Prefecture.

The ambiguity of the correlation between air temperature and terrestrial period in the natural habitat might be due to a lack of precise measuring of air temperature. The absence of a correlation between landing day and terrestrial period in the indoor experiment may be due to the small fluctuation of air temperature in the shelter of the laboratory. But it is unclear whether the new laboratory environment had a physiological influence on the examined F-0 larvae.

Further studies are required to clarify what decides the date of landing and also the date of adult eclosion.

Acknowledgments

I am grateful to Dr. Yoshitaka Tsubaki and Dr. Michael L. May for their useful comments and the critical reading of the manuscript. Thanks to Mr Naoya Ishizawa for his useful comments and Mr Katsunari Takahashi who offered me his data of eclosion of *E. superstes*.

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Appendix: Terrestrial behavior of selected undisturbed F-0 larvae

Larva A

15 April 2007, 11:42 (cloudy occasional rain, 2.5–6.4°C): one male larva was climbing up on the vertical snow wall on the west side at a height of 2.5 m from the surface of the stream. At 13:00 the larva was walking westward on the flat top of a hill at a distance of 30 m from the stream. Thereafter, it got dark and the larva circulated the same place. It eventually reached a hole at the root of a Japanese beech (diameter of the tree, 35 cm). At 14:50 it descended to the hole, then again went up, and finally entered a hole in the snow.

16 April (fine, later cloudy, 1–7°C), 08:43: the larva stayed in the hole, then at 10:16 it exited the hole and at 10:17 it walked westward 30 cm from the hole. At 12:26 it walked 20 m and went down into a hole in a Japanese beech (diameter, 30 cm); however, it walked sideways on the wall and got out of the hole again. It walked westward, and at 13:14 descended to a hole of another Japanese beech (diameter, 30 cm). At 13:32 it climbed up on the opposite wall and went out westward. After walking *c*.30 m, it climbed the slope 1 m up, then at 16:03 hid under the dead twigs of trees in a hollow.

17 April (fine, later cloudy, 1–5°C), 07:06: the larva stayed still at the same hollow as the previous day. At 10:57 it came out on the edge of the hollow and held the same position. However, at 12:47 it was not seen, and I could not find it. It may have entered a hole of a nearby Japanese beech (diameter, 45 cm). The larva had reached a point *c*.93 m from the stream bank.

Larva B

16 April 2007 (fine, later cloudy, 1–7°C), 13:34: one male larva was walking on the flat top of the hill 30 m from the stream. At 15:45 it entered a small hollow in the snow.

17 April (fine, later cloudy, 1–5°C): the larva stayed still in the hollow all day long.

18 April (fine, 2–14°C), 07:00: it stayed in the hollow as it had on the previous day. At 07:09 a frozen male larva was found near the hollow on the snow. It was covered with frost. At 10:26 the larva in the hollow was still there. At 13:30 it could not be seen; it may have moved deep into the hollow.

19 April (fine, maximum temperature: *c*.16°C): no observations.

20 April (fair, 7–14°C), 09:54: the inside of the hollow appeared due to thawing of the ceiling of the hollow, and the larva was dead on its back. The distance from the stream was *c*.40 m.

Larva C

20 April 2012 (cloudy, 7–10°C), 09:49: one male larva was walking on the flat space on the west bank of the stream. At 10:53 it climbed up on the west slope. At 11:10 it went northwards with its back to the sun. At 11:53 it changed its course westwards. At 12:51 it went south, facing the sun. At 12:54 it turned to the west. At 13:50 it turned to the north.

At 14:25 it walked to the hollow of a Japanese beech (diameter, 26 cm). At 14:34 it entered the crack between the snow wall and the trunk of the beech. At 15:02 it disappeared into the hollow. Five hours and 13 minutes had passed from the start of my observation and the walking distance was *c*.30 m.

Larva D

21 April 2012 (fine, 8–12°C), 10:45: one female larva was climbing up the snow wall of the west side of the stream at a height of 2 m from the water surface. At 11:08 it walked westward on the slope. At 12:38 it walked toward a hollow of a Japanese beech (diameter, 80 cm) on the flat top of the hill. At 12:50 it passed by the hollow. At 13:33 it walked southwest facing the sun. At 13:49 it turned to the west. At 14:05 it descended the hill toward the west stream. At 14:50 it climbed up 2.5 m on the wall of the west slope at an inclination of 80°. At 15:17 it went northward. At 16:00 it walked to the hollow of a beech tree (diameter, 16 cm) and at 16:16 it fell into a small hollow in the snow. Then it climbed up from the hollow and walked north. At 16:18 it reached the beech hollow and went into it. At 16:20 it fell 1 cm, then got up and again it went down. At 16:24 it fell 15 cm, then got up and again went down to the bottom of the hollow, and disappeared at 16:27. This took five hours and 40 minutes from the appearance of the larva and the walking distance was *c*.100 m.