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Source: Zoological Science, 20(3) : 377-382

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.20.377
Mating and Reproduction of Predaceous Diving Beetles, *Dytiscus sharpi*, Observed Under Artificial Breeding Conditions

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ABSTRACT—Mating season and embryonic development of the predaceous diving beetles, *Dytiscus sharpi* (Coleoptera; Dytiscidae) were observed under artificial breeding conditions. Female and male adult insects started mating from November to March and gave first instar larvae mainly in April. When the mating was artificially delayed until February, first instar larvae appeared from the end of March to the middle of May. I also investigated the effects of temperature on larval development. Apparent hatchability of eggs was not affected by high temperature, however, their normal development after hatching was significantly interfered. Most of the first instar larvae kept at 20–25°C from before hatching died within one day after hatching. By contrast, juveniles kept outdoors (7.0–20.9°C) could develop at least until second instar larvae. Temperature >23°C after hatching had no effects on larval development. From these observations, it was concluded that the reproduction strategy of *Dytiscus sharpi*, i.e. mating in late autumn and hatching in early spring would be the reasonable results of adaptation to the warm habitats where they are collected.

Key words: *Dytiscus sharpi*, reproduction, diving beetles, artificial breeding, mating season

INTRODUCTION

In Japan, three *Dytiscus* species (*D. marginalis czerskii*, *D. dauricus* and *D. sharpi*) and one subspecies (*D. sharpi validus*) have been described. *D. marginalis czerskii* and *D. dauricus* inhabit the northern areas of Japan, e.g. Hokkaido or a part of Tohoku districts. The distribution of these species indicates that they can breed even in cold regions as well as other species of the same genus reported so far (Roughley, 1990).

The distribution of *D. sharpi*, however, is known to be restricted in southern areas. Wehncke (1875) published the first description of *D. sharpi* in Japan. Collection at Ueno in Tokyo was also reported (Sharp, 1884). Since there were no records of collection except for in Kanto area (Chiba, Tokyo and Kanagawa) of Japan and in a part of China (Wu, 1937), *D. sharpi* would have southern restricted habitat in comparison with many other *Dytiscus* species. Therefore, one of the most interesting open questions is what physiological mechanism or ecological strategy of this species determines the habitat.

Reports on the mating behavior and life cycle of the genus are rather complicated. Yamaguchi (1992) observed that *D. sharpi validus* mated from December to April and eggs were laid from April to May. Embryos hatched from the middle of April to the middle of May. Hilsenhoff (1993) also described field or aquarium observations of eight *Dytiscus* species (*D. alaskanus*, *D. carolinus*, *D. cordieri*, *D. dauricus*, *D. fasciventris*, *D. harrisii*, *D. hybridus* and *D. verticalis*) in Wisconsin, U.S.A. His records of collection indicated that all species had a univoltine life cycle, and adults mated early in spring. He also reported that they oviposited when their breeding habitat became free of ice, which may be from late March to early May, depending on habitat and latitude. Aiken and Wilkinson (1985) reported that *D. alaskanus* collected from Alberta Lake in Canada had two mating seasons: one is in spring (April to June) and the other is from late summer to autumn (from late August to early October). They also reported that females after wintering mated in April and oviposited as soon as the lake was free of ice. First instar larvae appeared from mid to late in May, and the third instar larvae were most abundant throughout July. Blunck (1912) also found that *D. marginalis* in Europe showed a similar annual cycle of life. These studies suggest that different species of *Dytiscus* have their own mating seasons and different life cycles having adapted themselves to their own specific habitat conditions.

The present circumstances for wild *D. sharpi* are getting worse. Due to rapid destruction of environment by land development, the habitat for insects became narrower and...
the population size is decreasing. On the other hand, few studies have been reported on the biological and ecological features of this species. Understanding the life history should help us to conserve the insects as well as its related subspecies, *D. sharpi validus*, which are both included in the list of critically endangered species by Ministry of the Environment, Government of Japan (2000).

In the present study, I investigated the life history of *D. sharpi* to clarify the mechanism how they adapt themselves to a warm restricted area of Japan. Using an artificial breeding system based on our previous study (Inoda and Kamimura, 2003), I observed the mating and breeding behavior of insects. Detailed relationship between the mating season and hatching of eggs was clarified. The hatchability and survival rate of first instar larvae was also examined under various temperature conditions. How the prolonged seasons of mating and the temperature sensitivity during development would be related to the ecological strategy of *D. sharpi* will be discussed in detail.

**MATERIALS AND METHODS**

**Collecting and keeping of insects**

Adult diving beetles (*Dytiscus sharpi*) were collected in Chiba prefecture in the middle of October and kept in outdoor aquariums (14 females and 8 males). Female *Dytiscus* usually have a white mating plug covering the posterior end of abdomen only when they mated (Blunck, 1912). In the present study, females collected in the field had no mating plugs. In November, males and females were randomly chosen and paired as described below. Each pair was kept in a plastic chamber (74×39×40 cm²) containing water of 10 cm depth. The insects were fed with small dried sardines once a week. Fresh water was supplied every 6 hr by overflowing the chamber with de-chlorinated tap water. For oviposition, live Japanese parsley (*Oenanthe javanica*) was placed in the same chamber.

Usually, oviposition was observed from March to May under the present breeding conditions. After hatching, first instar larvae were collected and transferred individually into cylindrical plastic containers (diameter 4 cm, height 7 cm, water depth 5 cm) equipped with plastic mesh for footholds. They were fed with five *Asellus hilgendorfi* everyday. The de-chlorinated tap water was supplied everyday.

**Eggs**

To investigate temperature effects on the embryonic development, eggs were collected in 12 hr after oviposition (all the spawned eggs were collected every 12 hr) from the stems of Japanese parsley and transferred into a cylindrical plastic container (diameter 4 cm, height 7 cm, water depth 5 cm). Five eggs were kept outdoors (averaged temperature at 11.3 °C ranging from 0.6 to 20.6 °C) and the others were at first kept at approximately 13 °C for half a day and then transferred into chambers under temperature-controlled conditions (incubators at 15, 20 and 25 °C). The hatched larvae were used for the experiments to investigate the effects of temperature on survival rate of first instar larvae and on the periods until hatching. The water was not changed before hatching. Hatchability and development of the larvae were investigated.

**Larvae**

Larvae hatched outdoors were collected in 6 hr after hatching (all the larvae were collected every 6 hr) and transferred into a container and kept individually (see above) at various temperatures, *i.e.* outdoors (7.0–20.9 °C, mean water temperature: 15.1 °C) or at 23 and 25 °C until second instar larvae. Seven to twenty first instar larvae were used for the experiments to investigate the effects of temperature on the survival rate (Table 2). Duration of larval stage was also investigated.

### Table 1. Effects of temperature on hatchability, survival rate of first instar larvae and period of eggs.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th><em>Outdoors</em></th>
<th>15</th>
<th>20</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eggs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival rate [%]</td>
<td>100 (5)</td>
<td>80 (5)</td>
<td>75 (4)</td>
<td>75 (4)</td>
</tr>
<tr>
<td>First instar larvae&lt;sup&gt;a)&lt;/sup&gt;</td>
<td>100 (5)</td>
<td>100 (4)</td>
<td>33.3 (3)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>Period of eggs [days]</td>
<td>27.2±3.0 (5)</td>
<td>11.3±2.1&lt;sup&gt;b)&lt;/sup&gt; (4)</td>
<td>6.3±0.6&lt;sup&gt;b)&lt;/sup&gt; (3)</td>
<td>5.7±0.6&lt;sup&gt;c)&lt;/sup&gt; (3)</td>
</tr>
</tbody>
</table>

<sup>a)</sup> 0.6–20.6 °C, average: 11.3 °C. Parentheses indicate the number of specimens.  
<sup>b)</sup> There are significant differences among these experimental conditions (*p*<0.01).  
<sup>c)</sup> Significant difference comparing with the outdoor condition (*p*<0.001).

### Table 2. Effects of temperature on the survival rate and the stage periods of first instar larvae.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th><em>Outdoors</em></th>
<th>23</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival rate [%]</td>
<td>90.0 (20)</td>
<td>88.9 (9)</td>
<td>58.3 (12)</td>
</tr>
<tr>
<td>Period of first instar larvae [days]</td>
<td>8.5±1.9&lt;sup&gt;a)&lt;/sup&gt; (11)</td>
<td>4.0±0.6&lt;sup&gt;b)&lt;/sup&gt; (7)</td>
<td>4.0±0.5&lt;sup&gt;a)&lt;/sup&gt; (8)</td>
</tr>
</tbody>
</table>

<sup>a)</sup> 7.0–20.9 °C, average: 15.1 °C. Parentheses indicate the number of specimens.  
<sup>b)</sup> There are significant differences comparing with the outdoor condition (*p*<0.001). Period of first instar larvae indicate mean±SD.
Fig. 1. Paring experiments and the time courses of hatching under various breeding conditions in 1998–1999 (Experiments 1–4) and 1999–2000 (Experiments 5–9). Open arrows indicate the time when wild insects were collected. Solid black bars indicate the periods when females and males were kept in pairs for mating. White bars indicate the periods when each female was kept isolated. Solid arrows are the date when mating plug was found.
Mating experiments
In order to determine when insects started mating, nine independent pairing conditions were chosen as follows. Since the available number of materials was restricted due to their limited distribution and the species has been long listed in endangered species, I carried out the following experiments with 22 adults (14 females and 8 males).

As for the first series of experiments (1998–1999), male and female pairs of insects were kept in the same chamber for various periods as shown in Fig. 1. Females were then kept isolated from males and hatched larvae found in the tanks of females were counted.

Experiment 1: Control experiments to keep 6 females isolated from males.
Experiment 2: A female being paired with a male from November in 1998 to May in 1999 (for seven months).
Experiment 3: Paired from November to January in 1999 (for the first three months).
Experiment 4: Paired from March to May in 1999 (for the latter three months).

To determine more precisely the mating season required for normal oviposition, a second series of experiments (1999–2000) were carried out in which insects were paired in the same chamber only for a month as shown in Fig. 1. The number of hatched larvae and the date of hatching were determined as described above. I also recorded the dates when mating plugs in females were found.

Experiment 5: A female being paired with a male from November in 1999 to May in 2000 (for seven months) as in the Experiment 2.

Experiment 6–9: Paired only for a month (November, December, January, or February in 1999-2000). After paring, each female was kept isolated until May in 2000.

Statistical analysis
For the statistical analysis of the survival rate of eggs and first instar larvae under various temperature conditions (Tables 1, 2), the Fisher’s exact test was executed using the software ‘R’ (Ihaka and Gentleman, 1996) as well as a software provided by Aoki (2002). Differences in mean period of eggs and first instar larvae (Tables 1, 2) were analyzed by the Tukey multiple comparison. p values less than 0.05 were considered to be statistically significant on both sides.

RESULTS
Pairing for mating and oviposition
When 6 adult females were kept without mating from November 1st to May 31st (Experiment 1), any larvae did not appear throughout a year (Fig. 1, Exp. 1).

When a female and a male were kept together in the same chamber from November 1st to May 31st (Experiment 2) as a control experiment, first instar larvae appeared from the end of March to April (Fig. 1, Exp.2). Total number of the obtained first instar larvae was 67.

Almost the same result was obtained in the Experiment 3, where a female and male pair was kept from November 1st to January 1st (for three months) and 77 first instar larvae were found from the end of March to April.

When they were paired from March 1st to May 31st (Experiment 4), first instar larvae were obtained exclusively at the end of April (Fig. 1, Exp.4). The total number of the first instar larvae was 6. No dead eggs were found in the tank. To determine the mating season more in detail, the second series of experiments, Experiments 5–9, were carried out under the outdoor breeding conditions in 1999–2000.

When a female and a male were paired from November 1st to May 31st (for seven months, Experiment 5) as a control experiment, hatched juveniles were found from the end of March to the middle of May in 2000. 9.2, 60.5 and 30.3% of first instar larvae appeared in March, April and May, respectively. The total number of the obtained first instar larvae was 76.

The following detailed observations revealed that the mating season of insects could be artificially restricted. Hatched juveniles were found from the beginning of April to the middle of May in 2000 even if a female was paired with a male only for one month in November of 1999 (Fig. 1, Exp.6). In this case, the total number of the first instar larvae was 26. Mating plug of the female was found in November 5th. Paring in December yielded 113 first instar larvae (Fig. 1, Exp.7). 9.7, 84.1 and 6.2% of the first instar larvae appeared in March, April and May, respectively. Mating plug was found in December 4th. Similarly paring in January (Experiment 8, the formation of mating plug was on January 2nd) and in February (Experiment 9, the formation of mating plug was on February 2nd) resulted in hatching of 39 and 47 first instar larvae, respectively. 0, 89.7 and 10.3% (in Experiment 8) and 0, 87.2 and 12.8% (in Experiment 9) of the first instar larvae appeared in March, April and May, respectively.

Temperature effects on the hatchability and survival rate of first instar larvae
As shown in Table 1, high hatchability of embryos (>75%) was observed under the present experimental conditions regardless of the incubation temperature (There was no significant difference among data of these conditions).

However, the survival rate of hatched larvae showed high sensitivity to the incubation temperature before or during hatching. The larvae kept outdoors from eggs (averaged temperature at 11.3°C ranging from 0.6 to 20.6°C) survived more than one day. On the contrary, 66.7% of the first instar larvae died within one day in the case they had been exposed at 20°C from eggs. All the juveniles died within one day after hatching when the embryos had been kept at 25°C. There was clear significant difference among these breeding conditions (p<0.01). It was also clarified that the rates of development was higher at higher temperatures, i.e. first instar larvae appeared in 27.2, 11.3, 6.3 and 5.7 days in average when they were kept outdoors, at 15, 20 and 25°C, respectively (Table 1).

Temperature effects after hatching was also investigated. As shown in Table 2, the survival rates of the first instar larvae were 90, 89 and 58% when they were kept outdoors, at 23°C and at 25°C, respectively. In all the cases, they developed into normal second instar larvae. At 25°C, 42% of the larvae could not survived, however, there were no statistical difference comparing with the survival rate under other temperature conditions. Period of the stage of first instar larvae was 8.5, 4.0 and 4.0 days when they were
kept at outdoor, 23 and 25°C, respectively (Table 2). There were significant differences in the period of first instars stage between outdoor and 23°C (p<0.001), and outdoor and 25°C (p<0.001).

DISCUSSION

Relationship between mating and hatching

Depending on species of Dytiscus, various mating seasons from autumn to spring have been reported (Blunck, 1912; Hilsenhoff, 1993). Aiken and Wilkinson (1985) also showed that the adults of D. alaskanus mated in spring or autumn after wintering and then laid eggs as soon as the lake became free of ice. The eggs then hatched from the middle to end of May. Yamaguchi (1992) reported another case of mating before wintering. He observed that copulation of D. sharpi validus occurred from December to April and first instar larvae appeared from the middle of April to May. However, the exact time of mating could not be determined from these observations since they described only the formation of mating plugs or the mating behavior (male mounting on females) of wild insects. Therefore, observation under artificial breeding conditions has been required to clarify the details.

In this report, it was clearly shown that copulation started late in autumn, more precisely from November to March for the most effective mating (Fig.1). I could also show new evidence that hatching was mainly in April even though the mating season was artificially delayed or restricted (Fig.1).

Prolonged season of mating may help males to ensure paternity as described in D. alaskanus (1992) and D. marginalis (1912). This could be also the case for D. sharpi (Fig.1). However, since most of wild and bred females with mating plugs were found from November to December (data not shown), actual copulation would be finished in relatively early seasons.

Other species of the same genus living in boreal areas has to spend a long season of cold weather when the habitats were covered with deep snow and ice (Aiken, 1986). The habitats of D. sharpi, however, do not become frozen completely since the mean lowest temperature in a year is usually not less than 1–2°C (Japan Meteorological Agency, 1991). Therefore, they would be able to continue mating even during winter although the activity becomes lowered. Biological importance of wintering was still unknown in the Dytiscidae. Further investigations are needed to solve the problem.

As Aiken (1992) reported that there could be 9-month delay between mating and oviposition for females in the case of D. alaskanus, quite a long span of time for sustained sperm fertility is expected. In the case of present study, D. sharpi, spermatozoa could keep their ability to fertilize eggs at least for four months in spermatophores in female bodies. Further detailed investigation should be awaited to clarify what mechanism enables such longevity of sperm fertility.

Temperature effects on eggs and first instar larvae

In the present study, it was clearly demonstrated that periods between the embryos and the first instar larvae within one day (<12hr) after hatching are selectively sensitive to higher temperatures (Table 1). Additionally, my observation showed the first instar larvae can survive at 25°C (Table 2). As my preliminary observation indicate that adult insects could be reared at 30°C, D. sharpi may have temperature sensitivity only in a short period of the life cycle.

It has been previously reported that the rate of development was affected by temperature in the case of D. alaskanus (Aiken, 1986) and D. marginalis (Blunck, 1916) as well as other insects (Campbell et al., 1974). Aiken (1986) also described that the development time from eggs to larvae for D. alaskanus and D. marginalis were 130 and 190 degree-days, respectively. From the previous data by Aiken (1986) and Blunck (1916), I tentatively calculated the threshold temperature required for egg development according to the method by Campbell et al. (1974). It was approximately 4.8 and 3.2°C for D. alaskanus and D. marginalis, respectively. In the case of D. sharpi, the development time and the threshold temperature of eggs were estimated to be 120 degree-days and 3.8°C, respectively. A preliminary analysis of covariance indicated that the hatch rate (inverse of mean hatch time) of the three Dytiscus species (D. sharpi, D. alaskanus and D. marginalis) were not significantly different (F=3.914, p>0.05) to each other. Messenger (1959, 1970) has shown that threshold and thermal constant of development may be useful indicators to show the potentials of insect distribution and abundance. However, there was no obvious relationship between the climates of their habitats and the development time of eggs. It suggests that some regulatory mechanism of developmental time in a temperature-dependent manner might not be helping Dytiscus species to adapt the circumstances. On the contrary, mating in early seasons and oviposition in spring before getting warm seem to be indispensable for the adaptation of D. sharpi to southern climates.

Since the periods from eggs to early first instar stage (within one day after hatching) of D. sharpi were sensitive to temperature (Table 1), hatching before June (temperature <15°C by Japan Meteorological Agency, 1991) of wild insects should be one of the most crucial strategies to survive. At the same time, it should be noted that they have chosen risky southern habitats rather than cooler northern areas in Japan, although the ecological meaning was not clarified yet. Interestingly, many other abundant species of Dytiscus have the similar seasonal transients of larval stages (Larson, 1985). Those who can develop juveniles at lower temperature seem to be adapted much better to the area of colder climates and could be successfully distributed in broad arctic areas in contrast with D. sharpi.

Adaptation to the environment

Ecological fitness is best measured by the reproductive success or the survival rate of juveniles to stages of repro-
ductive maturation (Williams, 1966). Such maturation and reproduction of insects is usually triggered by environmental cues, mostly photoperiod and temperature, being mediated with neuroendocrine system (Tauber et al., 1986). In the case of black fly species, for example, the optimal growth of larvae and reproduction can be accomplished in a distinct and narrow range of temperature (from late winter to early spring). It results in the growth of larvae of big size and high fecundity (Merritt et al., 1982). Polyphagous gypsy moths, Lymantria dispar, prefer soft young foliage during the initial stage of larvae but are susceptible to hot weather. The lar-vae hatch in spring, and growth completed before the peak of summer is crucial for their adaptation (Goldschmidt, 1929, 1933, 1934, 1938). D. alaskanus in Canada, also need to lay eggs and grow in a short time because they have limited ice-free periods when they can complete the important stages of their life cycle (Aiken, 1986). According to the investigations by Larson (1985), dytiscids are shown to have relatively shorter span of larval stages (than adults) comparing with most of other aquatic insects. The instability of habitats as well as predation and food competition would be the factors that reduce the duration of larval stages. In this manner, every species has different strategy for ecological adaptation by adjusting their life cycles to each environmental condition.

In the present study, it was shown that D. sharpi has a vulnerable stage (eggs to early first instar larvae) that has high sensitivity to higher temperatures (Table 1). The properties would be one of the major limiting factors for the effective adaptation since they need to oviposit in early (cold) seasons to reduce the risk of temperatures. Subsequently, copulation needs to be started earlier before wintering to accomplish enough fecundity. It may be concluded that D. sharpi have obtained ecological strategy that the mating started from early season before wintering. It results in oviposition and hatching in early spring and can avoid embryonic development during warm seasons.

In the present study, I revealed a part of bionomics in D. sharpi that had been obscured because of critically endangered species for a long time. I hope the present study would be contributing to the preservation plan of this species.

ACKNOWLEDGMENTS

I thank Shinji Kamimura for the critical reading of the manuscript. I appreciate the supports of statistical analysis by Sachiko Koyama and Kazuo Shigemasu. I also thank Yuichi Tsuzuki and Akinori Taniwaki for their help in the fieldwork. I am deeply grateful to my wife, son and daughter who were kind enough to support and allow me to accomplish the study.

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(Received June 6, 2002 / Accepted December 18, 2002)