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Water intake by the adult mayfly *Epeorus ikanonis* (Ephemeroptera: Heptageniidae) and its effect on their longevity

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Water drinking habits during flight in adult mayflies of *Epeorus ikanonis* Takahashi were observed and the effect of water intake on their longevity was examined. The study was carried out in a mountain stream in western Japan. Adult males collected a water droplet under the head capsule while alighting on the water surface and consumed it after moving to nearby riparian vegetation. The amount of water intake was experimentally estimated to be 9.7% of the bodyweight for males. Although females did not show the water drinking behavior in the field, they imbibed as much as 5.5% of the bodyweight of water in an experimental situation. Morphological observation of the mouthparts revealed that the water was drawn in through the pits at the base of the labrum. In the field caging experiment, males with a water supply had a substantially longer life span than those without. Increase in adult life span by drinking water was less marked in females. Multiple copulation was observed in both sexes during the experiment. The water drinking behavior of the males may closely relate to mating success through increased longevity. Variation in the longevity of adult mayflies was discussed with regard to the possibility of water intake.

Key words: adult longevity; mayfly; mouthparts; multiple copulation; water intake.

INTRODUCTION

Because the mouthparts of adult mayflies are vestigial (Needham *et al.* 1935; Burks 1953), they are believed to be non-functional (Traver 1925; Illies 1968; Edmunds *et al.* 1976). Nevertheless, the possibility of using the vestigial mouthparts for water intake remains, even if feeding is impossible. Adult mayflies are considered to have a very short life span based on rearing experiments done without a water supply (Clemens 1917; Rawlinson 1939; Lehmkuhl & Anderson 1970, 1971; Allan & Flecker 1989). However, longevity may be seriously affected by water intake, if it occurs.

Longevity in the reproductive stage closely relates to the mating success of males when multiple copulation occurs (Thornhill & Alcock 1983). The possibility of multiple copulation in mayflies has been disputed because of the shortness of their life span (Thornhill & Alcock 1983; Eberhard 1985). If male mayflies can live long enough in a flight period, however, they would be under pressure from sexual selection to attempt multiple copulation.

During observation of swarming males of the mayfly *Epeorus ikanonis* Takahashi at Kibune Stream in 1986, one individual was seen to alight on the water surface and then settle on the bankside vegetation carrying a water drop in the underside of the head capsule. This droplet was then imbibed gradually. In this paper, the water drinking behavior and the morphology of the head capsule and mouthparts of *E. ikanonis* are described and the

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effect of water provision on adult longevity is examined. Variation in the longevity of other species of mayflies is discussed with regard to the possibility of water intake.

METHODS

The heptageniid mayfly *E. ikanonis* inhabits the upper to middle reaches of Japanese mountain streams (Imanishi 1941; Kani 1944; Yamasaki 1987) and has a univoltine life cycle with an emergence period in early spring (Gose 1970). Water drinking behavior of this species was observed at Okunomiya (340 m a.s.l.) and at Yuyagadani-deai (350 m a.s.l.) along Kibune Stream (width 2–5 m), a tributary of the Kamo River in Kyoto City (35°0′N, 130°0′E), on 15 and 16 April 1987, and on 16 and 27 April 1988. Adults of this species swarmed and mated in the daytime, mainly between 10.00 and 17.00 h (Takemon 1990a). Continuous observations of adults in a *ca* 7 m stretch of stream were made during the daytime. When an individual alighted on the water surface, it was traced until it flew up to the riparian vegetation, and was checked to see whether or not a water droplet was held under the head capsule. The duration required for water drinking was also measured.

The amount of water intake was estimated as the difference in bodyweight before and after the water intake. Adult males and females were captured in the field on 29 March 1991 and maintained at 6°C in the laboratory. On the following day a water droplet was supplied to the mouthparts of each individual using a pair of tweezers, and after 2 min the water remaining on the mouthparts was removed with absorbent paper. Since the time required for intake of the whole water droplet was less than 2 min in the field experiment, this time was adopted for the duration of water supply. Bodyweight was measured to the nearest 0.1 mg using a micro-balance (HL-40, Hansen, Kobe, Japan).

The morphology of the head capsule and mouthparts was observed using a binocular microscope (× 40) and a stereoscopic scanning electron microscope (× 150–500) (JSM-5400LV, Jeol, Tokyo, Japan). Mouth structures were identified with reference to those of *Stenonema* in Needham *et al.* (1935) and *Ephemera* in Shiraki (1972).

Longevity in the adult stage of both sexes was measured by rearing adults from subimagines to death at Okunomiya from 29 March to 28 April 1988. Subimagines emerging from the water surface were captured by nets and kept in cages (30 cm × 30 cm × 40 cm) covered by a board roof near the stream. All adults were marked individually on a wing using lacquer dots, on the day of moulting into the adult stage. Those that failed to moult were excluded from analysis. The adults (70 males and 27 females) were randomly separated into two groups, one with a water supply (2.1 μL on average to each individual) every day and the other without. The air temperature and humidity were recorded throughout the study period using a thermo-hygrograph adjacent to the cages (Fig. 1).

Copulation behavior in the cage, between nine males and six females, was observed between 14.58 and 16.25 h on 14 April 1988. These adults were obtained by rearing subimagines captured at their emergence on 6 April. They moulted into adults on 13 or 14 April. Each adult was identified by paint marking on the forewing.

RESULTS

Water drinking behaviour

A total of seven males was observed to alight on the water surface of the stream. Five of them were hovering above the stream at a height of 1.0–3.5 m and two were sitting on the shore before alighting on the stream surface. They alighted either in rapids or in slow-flowing parts of the stream. All the males took off from the water surface immediately after alighting. Only four of the seven males could be observed closely at their perching sites on the bankside vegetation after leaving the water surface. All of them held a water droplet under the head capsule. The time required for disappearance of the droplet was 48, 105, 112 and 119 s from the time they alighted on the stream surface. After drinking the water, two males remained at the perching sites and two flew up to higher tree canopies. Alighting on the stream surface was observed only in the afternoon between 12.20 and 17.00 h; that is, 12.22 and 15.48 h on 15 April 1987, 15.31 h on 16 April 1987, 14.00, 16.04, and 16.33 h on 16 April 1988, and 16.56 h on 27 April 1988.

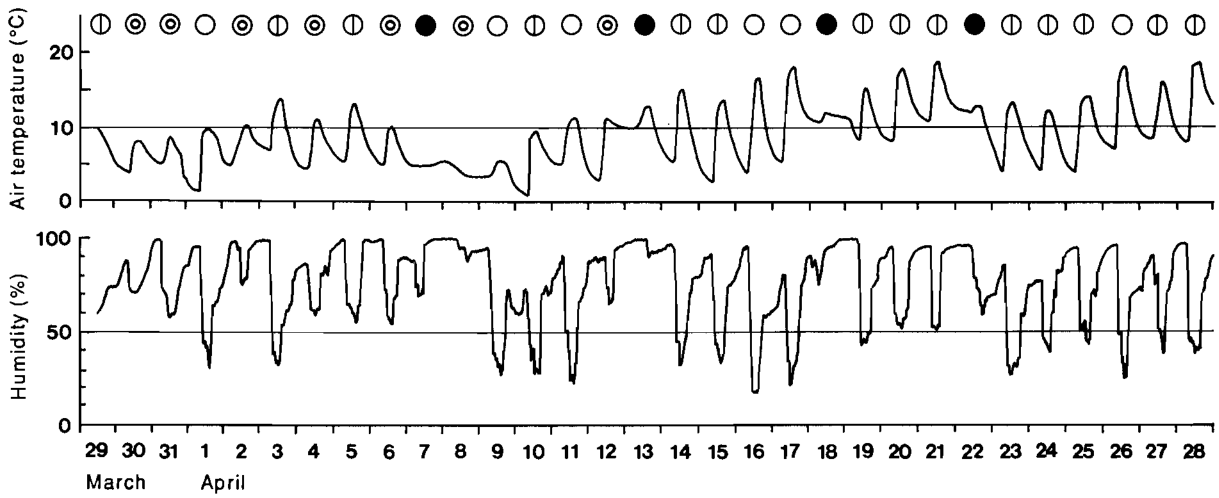

Fig. 1. Diel and seasonal changes of air temperature and humidity at Okunomiya in the study period from 29 March to 28 April 1988. Symbols represent the weather conditions: (○) clear fine; (◐) fine; (◎) cloudy; and (●) rainy.

Although more than 30 females were observed to fly across the field or to lay eggs on the stream shore, they did not alight on the stream surface to drink water.

Morphology of the head capsule and mouthparts

Figure 2 shows the morphology of the head capsule of *E. ikanonis*. The frontal margin of the head capsule extended forward and downward forming a concave fringe surrounding the mouthparts (see arrows in Fig. 2) in both sexes. Sexual dimorphism was noticeable in the relative size of compound eyes but the frontal margin of the head capsule was morphologically similar in both sexes.

Figure 3 shows electron-microscopic photographs of the mouthparts of the mayfly. The mouthparts of both sexes were highly degenerate and may be immovable. The labrum was only a protuberance without segmentation. The main pit and side pits opened at the posterior edge and at both sides of the labrum, respectively. The water seemed to be taken in through these pits. The pharynx and outer sides of the side pits had thick bristles. The mandibles were degenerated into two protuberant tips and the canines and lacinia were not differentiated. The maxillae showed a segmented structure with two segmented palps. The hypopharynx was rather indistinct in shape. The labium was the largest part, composed of a mentum, glossa and labial palps. The ventral side of the labial palps was also bristled.

Amount of water intake

The amount of water taken at one time is shown in Table 1. Although males were relatively smaller than females in bodyweight, the amount of water intake was not different between males and ovigerous females. Consequently, the ratio of water intake to bodyweight was larger in males. Spent females took a significantly smaller amount of water than ovigerous females.

Effect of water intake on adult life span

Survival curves of individuals with and without a water supply are shown in Fig. 4. Males and females with a water supply lived up to 16 and 10 days, but those without water supply only 6 and 7 days, respectively. The average longevity differed significantly between the two groups in each sex, but the difference was more marked in males (Table 2). The bodies of individuals not given water were apparently wrinkled on death due to desiccation, whereas those supplied with water were soft even after death.

Multiple copulation in both sexes

Mating pairs in the cages were found frequently, on 14, 15, 16, 17, 20, and 21 April 1988, when it was fine and the air temperature exceeded 10°C (Fig. 1). A total of 11 copulations occurred between

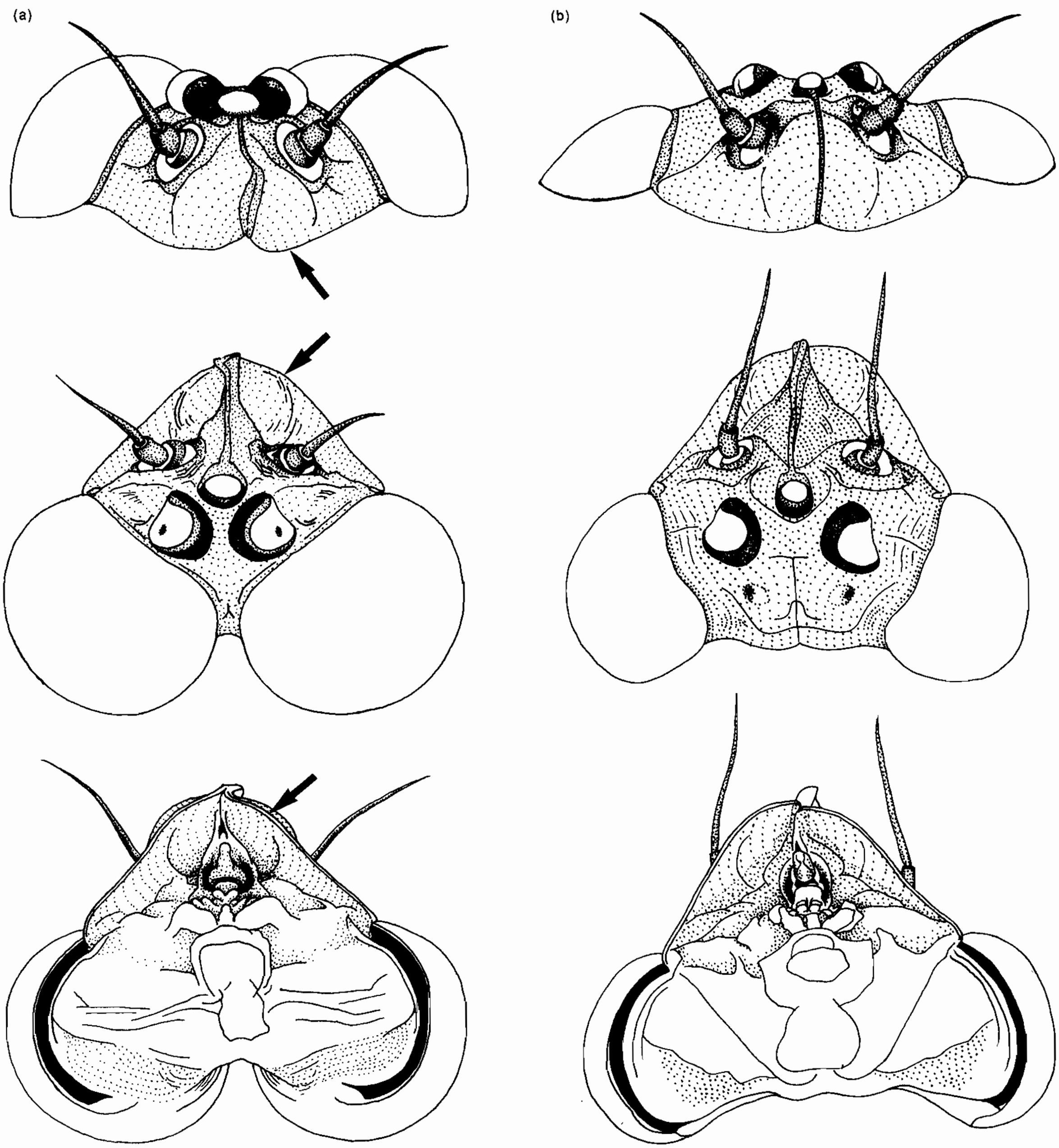

Fig. 2. Head capsule morphology of the adult mayfly *Epeorus ikanonis*. (a) Male and (b) female. Top: front view; middle: dorsal view; and bottom: ventral view. Arrows indicate the frontal margin of the head capsule forming a concave fringe.

seven males and five females of the nine males and six females kept in the cage during the 87 min observation on 14 April 1988. Four females and three males performed multiple copulation; two females and one male copulated three times, and two females and two males copulated twice successively with different mates. Mating was always initiated by the male, which approached the female walking on the floor of the cage. With his abdomen held upward, the male followed the female quickly, crept under her from behind, seized her body with the fore legs and then copulated with her. The copulation lasted an average of 7.8 min (n = 11, range = 2.2–16.3 min, SD = 4.9 min). These mating behaviors were almost identical to those observed among individuals in the field aggregating

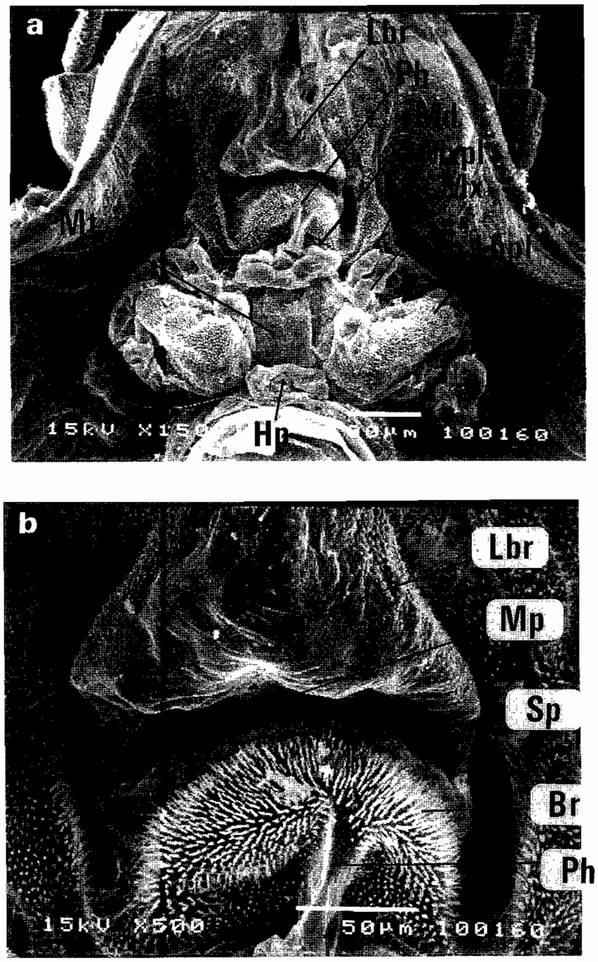

Fig. 3. Electron micrographs of the mouthparts of the adult male *Epeorus ikanonis*. (a) A whole picture of the mouthparts (× 150); and (b) a magnified picture of the main pit and side pits behind the labrum (× 500). Abbreviations: Br = bristles, Hp = hypopharynx, Lbpl = labial palp, Lbr = labrum, Md = mandible, Mp = main pit, Mt = mentum, Mx = maxilla, Mxpl = maxillary palp, Ph = pharynx and Sp = side pit.

around the oviposition sites on the stream shore (Takemon 1990a).

DISCUSSION

Function of head capsule and mouthpart morphology

The mouthparts of adult mayflies have been believed to be non-functional (Traver 1925; Illies 1968; Edmunds *et al.* 1976) or only capable of taking air for flight (Shiraki 1972) because of their degenerative state. In the present study it has been demonstrated for the first time that the mouthparts of *E. ikanonis* can be used to take water, and this ability seems extremely important in lengthening the adult life span. How males catch water during a brief contact with the water surface using such vestigial and probably immovable mouthparts remains to be investigated. It is possible that the concave edge of the frontal margin of the head capsule assists in adhering a water droplet to the mouthparts by surface tension. An ephermerid mayfly adult has a narrow pharynx with a complex muscle which has been believed to regulate the amount of air intake into the gut (Shiraki 1972). This muscle system may also be used for water intake.

Relations between water intake and evaporation

Mayflies lose about 22% of their bodyweight when they cast the skin of subimagines (Lameere 1917). The weight lost through evaporation constitutes more than 90% of the total loss, while the weight of the subimaginal skin amounts to only about 1.5% of the bodyweight (Lameere 1917). Adults may thus need to compensate for the water lost during and after moulting by drinking water. Evaporation from the body is influenced by atmospheric humidity and therefore the water requirement will increase when adults are exposed to dry air.

The results described here showed the diel timing of water drinking to occur in the afternoon, while mating and oviposition activities also occur in the morning. This seems to relate to lower humidity in the daytime (Fig. 1). Moreover, males stay in the sunny place along the stream through the daytime, whereas females visit the stream shore only for a short time during copulation and oviposition and spend most time resting under the leaves of tree canopies (Takemon 1990a). The long time spent in the sunny places by males will lead to greater evaporation and an increased requirement for water. This may be one of the reasons why only males show water drinking behaviour.

Adult longevity in relation to mating and oviposition habits

The male-restricted water drinking may be related also to the fact that the males can substantially

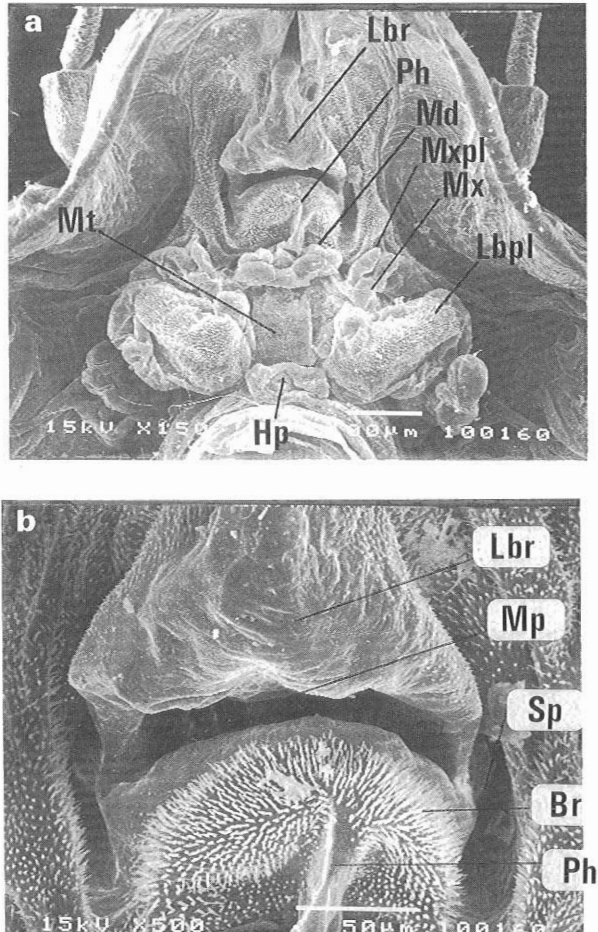

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Table 1 Bodyweight and the amount of water intake in *Epeorus ikanonis*. Bodyweight represents that before water intake

	Bodyweight (mg)	Water intake (mg)	Water intake/Bodyweight (%)	*n*
Male	$17.73^a \pm 2.99$	$1.72^a \pm 0.52$	$9.68^a \pm 2.81$	41
Female (o)	$31.55^b \pm 3.60$	$1.77^a \pm 0.55$	$5.53^b \pm 1.26$	11
Female (s)	$18.41^a \pm 3.33$	$0.48^b \pm 0.31$	$2.53^c \pm 1.36$	10

Values are mean ± SD; female (o): ovigerous females; female (s): spent females. Values with the different letters are significantly different ($P < 0.01$) by Mann-Whitney *U*-test.

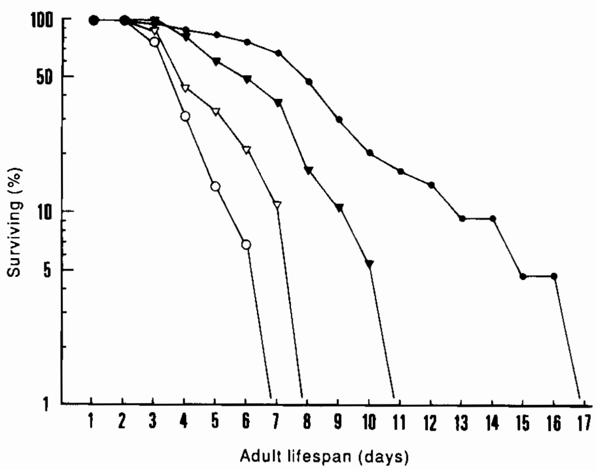

Fig. 4. Survivorship curves of *Epeorus ikanonis* in the adult stage. (●) water supplied males ($n = 41$); (▼) water supplied females ($n = 18$); (○) unsupplied males ($n = 29$); and (▽) unsupplied females ($n = 9$). Day 1 is the day of moulting into adults from subimagines.

Table 2 Comparison of longevity in days (mean ± SD) in males and females of *Epeorus ikanonis*, with and without water supply

	Male	Female
With water	$7.8^a \pm 3.3$	$5.7^b \pm 2.1$
Without water	$3.3^c \pm 1.1$	$4.0^c \pm 1.7$

Values with different letters are significantly different: $P < 0.05$ between a and b or b and c; $P < 0.01$ between a and c by Mann-Whitney *U*-test.

increase their life span by drinking, while in females such an increase is limited in extent. The selection pressure for longer life span will work when reproductive success is positively correlated with longevity. Males of this species have an ability to copulate multiply and this probably also occurs in the field, considering a large variation in the amount of sperm remaining in the sperm vesicle of field-caught males (Takemon 1990b). Moreover, sperm mixing in the female vestibule may occur when a female copulates successively with different males (Takemon 1990b). Thus, male reproductive success may be increased by additional matings. In such a case, it is advantageous for males to increase their life span to gain more opportunities for mating. Another aspect of sexual selection for higher mating success concerns protandrous emergence habits: that is, the mean emergence date of males was 4.8 and 4.4 days earlier than that of females in this species in 1982 and 1985, respectively (Takemon 1990a). Protandry has been reported for many species of aquatic insects including mayflies (e.g. Thew 1958; Watanabe *et al.* 1989; Takemon 1990c) and is probably linked to the longer life span of males. In this respect, the habit of drinking water seems to be of particular importance in males.

The lack of water drinking behavior and the shorter life span in females, on the other hand, may relate to their oviposition habit. Females of this species lay all their eggs on a single occasion (Takemon 1990a) and mayfly females cannot produce additional eggs since their ovaries degenerate in the adult stage (Needham *et al.* 1935). Thus, the selection for survival after oviposition should not be intense. As to the stage before oviposition, 80% of field females start oviposition 20 s after copulation, on average ($n = 43$, Takemon 1990a). This habit indicates that females do not need a long lifespan after copulation. Multiple copulation of females is also observed in field individuals but it occurs successively and they oviposit within a day (Takemon 1990a). Therefore the possible advantages of multiple copulation, such as an increase in genetic variation, nutrient and fertilization success, may not work intensively as a selection pressure for longer life span in females, even if any of those advantages exist.

Variation in adult longevity among mayflies

Adult mayflies generally have been considered to be short-lived since they do not feed (Needham *et al.* 1935; Burks, 1953; Illies 1968; Edmunds *et al.* 1976; Thornhill & Alcock 1983; Eberhard 1985; Brittain 1990). Indeed, species such as *Ephoron album* (Crass 1947; Thew 1958; Britt 1962), *Ephoron ladogensis* (Tiensuu 1935) and *Ephoron shigae* (Shioyama 1978; Watanabe *et al.* 1989) are known to die within a few hours of emergence. On the other hand, long-lived ovoviviparous females are also known; for example, *Cloeon* sp. can live for 54 days (Crass 1947) and *Cloeon dipterum* for 28 days (Degrange 1960). Experimental maintenance of adults in cages resulted in relatively short longevity; for example, 4.5 days in *Isonychia bicolor* (Clemens 1917) and 3.5 days on average with the maximum of 9 days in *Epeorus longimanus* (Allan & Flecker 1989). These durations, however, refer to situations without a water supply. Apart from *E. ikanonis* in the present study, water drinking behaviour in males of three other species, *Ephemera strigata*, *Epeorus napaeus*, and *Ecdyonurus tobiironis*, has been noted (Takemon 1990a). Considering the results described here, adult life span of these and other mayfly species may be longer and encompass a wider range of variation than has been accepted generally.

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