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Flight energetics in relation to sexual differences in the mating behaviour of a mayfly, *Siphlonurus aestivalis*

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Summary. Mayflies (Ephemeroptera) are known to have short adult life-spans. Adults are unable to feed, and they utilize reserves stored during their aquatic larval stage. Energy reserves (fat, glycogen, and free sugars) of mature larvae, subimagoes and imagoes of both sexes of *Siphlonurus aestivalis* Eaton were compared. All the stages of both sexes had low glycogen and free sugar contents, and the only significant change occurred during the transformation of the mature larva to subimago when almost all the reserves of free sugars were used up. Glycogen and free sugars may serve as energy sources permitting individuals to swim and fly out of the water during emergence. Fat made up most of the energy reserves of mature larvae and was the main source of energy used during the final development of both sexes. Young adult males had high fat reserves which they used as a source of energy for their swarming flights. In contrast, females did not seem to use a significant amount of fat for flight. This difference is probably related to the different mating strategies of the sexes in this species. Males perform long flights waiting for females, whereas females perform only brief flights to mate and reproduce.

Key words: Flight energetics – Lipids – Carbohydrates – Sex differences – Mating behaviour

Flight is the most energy-demanding activity performed by insects (Candy 1989; Casey 1989), and either fat or carbohydrates (glycogen and free sugars) can be used as the main source of energy (Beenakkers 1969; Downer and Matthews 1976; Kammer and Heinrich 1978; Beenakkers et al. 1985).

Mayflies (Ephemeroptera) are known to have short adult life spans during which they do not feed. Thus their imagoes utilize reserves stored during the aquatic larval stage. Mayflies are also unique among insects since they moult after becoming fully winged, i.e. they have a winged preadult life stage, called the subimago (see Edmunds and McCafferty 1988 for review).

Little attention has been paid to the physiology of these insects, especially the type of and timing of accumulation of energy reserves for their adult life. Only a few studies (Sweeney 1978; Meyer and Walther 1988; Meyer 1990) have been devoted exclusively to the energetic compounds of the larvae. The intentions of this work were (i) to identify the source of energy used by individuals for flying, and (ii) to investigate whether typical sexual differences in mating behaviour, where males form dense swarms and females perform much shorter flights toward the swarm before mating and ovipositing (Allan and Flecker 1989), may be associated with physiological differences. In order to study these questions, we used *Siphlonurus aestivalis* Eaton (Siphlonuridae). This is a relatively large mayfly species which has the advantage of being easily collected in the field, and it is easy to rear in the laboratory. For both sexes the amount of fat, glycogen, and free sugar reserves were quantified from the mature larvae stage (at which time mayflies have terminated feeding) until the imagoes reproduce.

Material and methods

S. aestivalis larvae inhabit slow-running or standing waters (Brittain 1978; Sartori 1987). Mature larvae (ML), as well as reproductive male and female imagoes (RI) were collected at the river Le Veyron and in the nearby pond, Les Monneaux (canton de Vaud, Switzerland), between 20 May and 14 June 1989. After collecting, individuals were immediately frozen. To obtain subimagoes and imagoes of known age, nymphs were reared in the laboratory. Young subimagoes (YS) were collected just after they had left the water. Older subimagoes (OS) were frozen 35 h after emergence, i.e. after roughly half of their subimaginal life at a constant temperature of 16.5° C (Sartori and Sartori-Fausel unpublished). Young imagoes (YI) were collected just after their moult when they were less than one hour old. These specimens were also frozen immediately after collecting. A total of 245 specimens were used for the analyses.

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Quantification of lipid and carbohydrate content was performed separately for both sexes in these five stages. Two different sets of individuals were used for lipid and carbohydrate analysis.

Lipid analysis

The procedure followed that of Peakin (1972) (see Keller and Passera 1989). Each specimen was first weighed (fresh weight) and then dried for 24 h at 70° C for dry weight measurment. Fat was extracted with petroleum ether (boiling point 40–60° C) for 24 h, and then the specimens were dried again for 24 h and weighed to measure the weight loss (= weight of fat extracted) of each specimen.

Carbohydrate analysis

The specimens were dried at 90° C for 45 min in order to inactivate the enzymes, and then stored at room temperature until they were subjected to a procedure adapted from van Handel (1985) (see Passera et al. 1990 and Passera and Keller 1990) employing anthrone as the colour reagent. This procedure permitted the determination of the amount of glycogen and free sugars from the same individual.

Statistical analysis

Means were compared by ANOVA. Multiple comparisons among pairs of means (different stages) of the same sex were carried out using Scheffe F-test. Means are listed plus or minus standard deviation and are referred to as not significantly different when $P > 0.05$.

Results

Females of all stages were significantly heavier than their male counterparts ($F_{1,243} = 515.74$, $P < 0.001$) (Fig. 1). In both sexes there was a significant decrease in weight between the mature larval and reproductive imaginal stages ($F_{4,117} = 38.41$, $P < 0.001$ and $F_{4,118} = 7.88$, $P < 0.001$ for males and females, respectively). For both males and females most of the weight loss occurred between the mature larval and subimaginal stages (33% for males: Scheffe F-test = 10.61, $P < 0.001$ and 26% for females: Scheffe F-test = 4.81, $P < 0.001$). Later in their development the weight remained stable in females, whereas male imagoes lost more weight during the swarm flight, and the reproductive male imagoes' weight was about 20% less than that of young imagoes (Scheffe F-test = 5.29, $P < 0.001$). This weight decrease in males resulted primarily from fat use during the swarm flight (see below).

The relative importance of fat and carbohydrates as a source of energy was assessed by studying the decline of these substrates from full grown larvae until swarming imagoes. Fat provided most of the energy used by all stages. Overall, males used 2.05 mg fat, 0.14 mg glycogen, and 0.13 mg free sugar (Figs. 2–5). Using caloric values of 9.45 kcal . g^{-1} and 4.10 kcal . g^{-1} for lipids and carbohydrates respectively (Winberg 1971), it appears that fat provided more than 95% of the total amount of energy used by males between the mature larval and reproductive imago stages. Similarly, the amount of fat used by females (3.52 mg) was much higher than the amounts of glycogen (0.17 mg) and free sugar (0.13 mg) used. Overall, fat provided 97% of the total energy used by females from full grown larvae until swarming imagoes. Most of the carbohydrate reserves were used before the imago stage in both sexes (Figs. 4 and 5, see below).

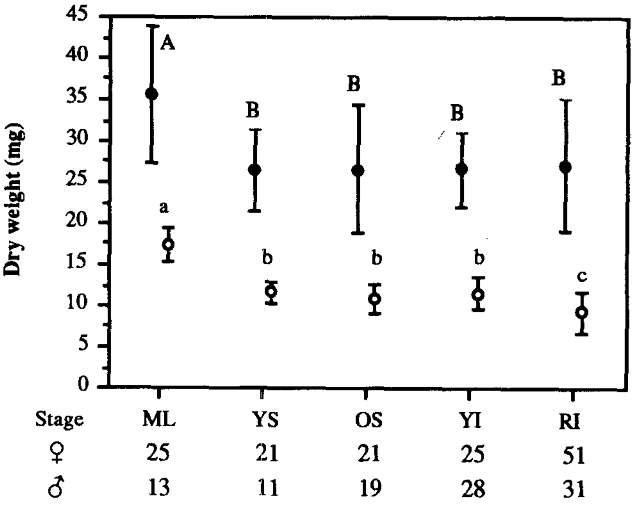

Fig. 1. Mean dry weight (± SD) of males (*open circles*) and females (*filled circles*) of mature larva (*ML*), young subimago (*YS*), old subimago (*OS*), young imago (*YI*), and reproductive imago (*RI*). Number of specimens studied are given for both sexes for each stage. Means with different *letters* (*lower case*: males; *upper case*: females) are significantly different (Scheffe F-tests, $P < 0.01$)

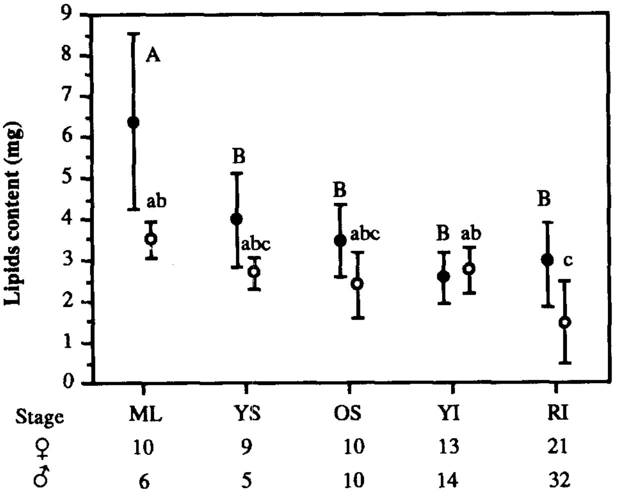

Fig. 2. Mean lipid content of males (*open circles*) and females (*filled circles*) in the five stages examined. For explanations see Fig. 1

On average, females had higher fat reserves than males during the mature larval stage (3.52 ± 0.43 mg and 6.36 ± 2.15 mg, for males and females, respectively; $F_{1,15} = 9.99$, $P < 0.001$). In both sexes we found a significant decrease of fat reserves during the ontogeny ($F_{4,62} = 12.33$, $P < 0.001$; $F_{4,58} = 17.59$, $P < 0.001$ for males and females, respectively) (Fig. 2). However, the time when the fat reserves were metabolised differed between the sexes. Females used fat between the mature larval and young imago stages. In males the fat decline during this period was much lower, and both sexes had about the same

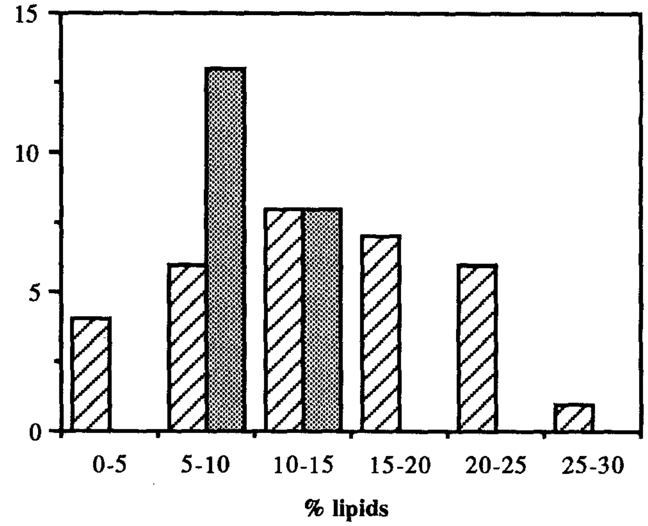

Fig. 3. Distribution of lipid contents in the reproductive male and female imagoes during their swarming flight. *Hatched bars*: males, $n=32$; *filled bars*: females, $n=21$

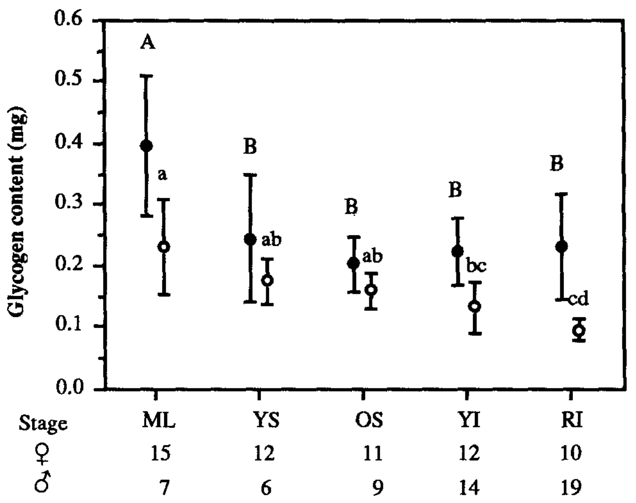

Fig. 4. Mean glycogen content of males (*open circles*) and females (*filled circles*) in the five stages examined. For explanations see Fig. 1

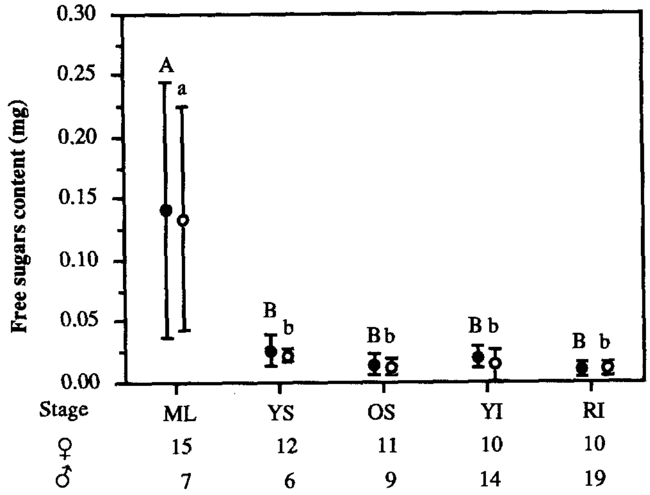

Fig. 5. Mean free sugar content of males (*open circles*) and females (*filled circles*) in the five stages examined. For explanations see Fig. 1

amount of fat during the young imago stage (2.76 ± 0.55 and 2.57 ± 0.61, in males and females, respectively; $F_{1,26} = 0.71$, NS). It is probable that this difference in the amount of fat used arose from the size difference between sexes since the large females had higher metabolic needs. The most striking difference between the sexes occurred during the imaginal stage when females used virtually no fat between the young and reproductive imago stages, whereas males used 52% (Scheffe F-test = 6.01, $P < 0.001$) of their fat reserves during the same period. This indicates that males, in contrast to females, used a significant amount of fat reserves as fuel for flying. It is noteworthy that reproductive male imagoes exhibited great variation in their fat content (Fig. 3). It should be mentioned that reproductive imagoes were collected at any age during their mating flight. Some of them had just started to fly, whereas others had been flying for several hours. Therefore the high variance in male fat content may reflect age differences, and older males should have lower fat reserves. Interestingly, females exhibited only small variation in fat reserves, as compared to males (Fig. 3). This is consistent with females using no or only a small amount of fat as fuel for flying.

Both males and females contained relatively low amounts of glycogen and free sugars (Figs. 4 and 5). Carbohydrate (glycogen and free sugars) content expressed as a percentage of dry weight never exceeded 2.5%. Glycogen content decreased in both sexes during the ontogeny ($F_{4,50} = 17.19$, $P < 0.001$, and $F_{4,55} = 10.91$, $P < 0.001$, for males and females, respectively). In males the decrease was regular between all stages, whereas females mostly used glycogen between the mature larval and young subimago stages. In males there was a slight decrease in reserves of this substrate, although the difference was not significant, between the young and reproductive imago stages (Scheffe F-test = 1.833, NS), suggesting that males possibly use some glycogen during the swarm flight. In contrast, there was no change in the female glycogen reserves between the young and reproductive imago stages (Scheffe F-test = 0.006, NS), suggesting they use only very small quantities, if any, of glycogen during their mating flight.

Males and females exhibited a similar pattern in their use of soluble sugar reserves (Fig. 5). Individuals of both sexes used most of their free sugar before their emergence from water (males 84%, Scheffe F-test = 9.61, $P < 0.001$ and females 82%, Scheffe F-test = 7.53, $P < 0.001$). Later, the content of free sugar remained very low, and it did not vary significantly in either sex (Fig. 5). Therefore, this substrate is unlikely to be a significant source of energy for flying.

Discussion

Comparisons of the carbohydrate and fat content of males just before and during the swarm flight showed that they used significant amounts of fat and virtually no glycogen and free sugars during this period. Thus fat seems to be the main source of energy used by males for flying. The use of the fat reserves was associated with a significant loss in dry weight during the swarming. Since males were collected during flight and not when they had finished swarming, it was not possible to estimate accurately the total amount of fat reserves used. Nevertheless our data showed that males collected when they were still flying had already used, on average, half of their fat reserves; some of them (probably the older ones) had used up nearly all their fat reserves.

In contrast to males, females did not exhibit any decrease either in weight or in fat content during flight. Furthermore, we detect any decrease in the glycogen and free sugar reserves during flight. Since imagoes cannot feed, these findings demonstrated that females only used a small fraction of their energy reserves, suggesting that they do not undertake long flights. This conclusion is supported by field observations indicating that some males may fly continuously for several hours while waiting for females, whereas females normally only spend a few minutes flying before mating and ovipositing (Brittain 1982; Sartori pers. obs.).

Sex differences in the energetics of flight have also been observed in the silkmoth *Hyalophora cecropia* (Gilbert and Schneiderman 1961). Males have a high lipid content, and they fly long distances in search for virgin females, while females only have a low fat content permitting short flights. It should be noted that the use of fat as flight energy source is typical of long-distance fliers like certain lepidopterans or orthopterans, whereas short-distance fliers primarily use carbohydrates (Beenakkers 1969; Downer and Matthews 1976; Kammer and Heinrich 1978; Beenakkers et al. 1985).

Our data show that both females and males have only small glycogen and free sugar reserves. Both sexes seem to utilise most of their free sugar and a significant proportion of their glycogen content between the mature larval and young subimaginal stages. Thus these energy sources are used during the processes of emergence, i.e. swimming near the surface, moulting and flying to the riparian vegetation. This is not surprising since carbohydrates have been shown to be an efficient source of energy for short-term efforts (Kammer and Heinrich 1978; Wheeler 1989).

In contrast to the imago stage, no significant differences were detected between the sexes with respect to energy use during the process of emergence. This is consistent with the fact that there is no apparent difference between the sexes in the process of emergence (Sartori, pers. obs).

Finally, the high variance between males in their fat reserves raises the question of whether differences in their fat reserves might be associated with differences in their mating success. In *S. aestivalis*, females apparently accept mating with the first male to grasp them (Sartori, unpublished data). Similar behaviour is common in most mayfly species (Sartori, pers. obs.) and has also been reported for other swarming insects such as the caddis fly *Mystacides azurea* (Petersson 1987). It may be that male mating success is age-associated: younger males with higher fat content are more successful than older ones with lower fat content. Such a correlation between age and mating success has been shown in three caddis fly species studied by Petersson (1989). It is also noteworthy that adult caddisflies can only ingest fluids (Richards and Davies 1977) and males also exhibit an age-related loss of fat (Petersson 1989).

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