J Comp Physiol B (2007) 177:119–128
DOI 10.1007/s00360-006-0114-7

ORIGINAL PAPER

The management of metabolic energy storage during the life cycle of mayflies: a comparative field investigation of the collector-gatherer *Ephemera danica* and the scraper *Rhithrogena semicolorata*

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Received: 5 May 2006 / Revised: 3 August 2006 / Accepted: 4 August 2006 / Published online: 8 September 2006

Abstract The concentration and seasonal dynamics of the major energy storage components, triglycerides and glycogen, were measured in two species of mayfly (*Rhithrogena semicolorata* and *Ephemera danica*) with contrasting life cycle strategies living in a small mountain stream. *E. danica* is a burrowing, semivoltine collector-gatherer; *R. semicolorata* is univoltine and scrapes periphyton from stones. This is the first publication which focuses on the role of metabolic energy sources during the larval life span of two mayfly species until the larvae emerge. Although triglycerides are the major energy reserve in both species (>84% of total energy storage) throughout the whole larval development their seasonal dynamic differed considerably. In *R. semicolorata* the triglyceride concentration declined during the last weeks prior to emergence in both sexes. The same pattern was found in female larvae of *E. danica*, but not in male *E. danica.* It is suggested that females use triglycerides in the last larval stages for egg maturation, which is completed in the last larval instar. In male *E. danica* the triglyceride concentrations remained high until emergence, presumably due to their high energy demands as adults for their swarming flights. Glycogen concentrations did not show such a difference between species and sexes. Its significance as a storage substrate for energy is rather low; however, concentrations decreased in both species and sexes prior to emergence.

Keywords Ecophysiology · Triglycerides · Glycogen · *Ephemera danica* · *Rhithrogena semicolorata*

Communicated by G. Heldmaier

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Introduction

Most of the results in insect physiology reported in the literature are based on the study of only a few insect species, usually those that are easy to rear in the laboratory (Cavanoso et al. 2001). Investigations on aquatic insect species and their ecophysiology are rare, especially within their natural habitat. Mayflies (Ephemeroptera) are hemimetabolic with only a short adult life span. Their life cycle includes four stages: egg, nymph, subimago and imago. The transition from the aquatic nymph to the terrestrial subimago (emergence) is an essential cut in the life history of mayflies. As they do not feed as adults they have to gain and store all necessary energy for reproduction (development and deposition of eggs, male swarm flight) during their larval life. Thus the amount of energy stored by the aquatic larval stages is of crucial importance for the reproductive success of mayflies. However, little is known yet about the details of different strategies of metabolic energy management of aquatic insect larvae and the ecological consequences.

Usually phosphor-L-arginine quickly provides ATP for locomotion in the first instance, while triglycerides and glycogen are the basic storage substrates for further ATP supply (Grieshaber et al. 1994; Gewecke 1995; Nation 2001). Glycogen provides substrate and

energy for the carbohydrate metabolism even under anoxic conditions, whereas the mobilisation of ATP out of triglycerides is slower, but more efficient (Grieshaber et al. 1994). The energy management, therefore, can be measured as the amount and variability of these major storage components at different times during the development of animals. Both substrates, triglycerides and glycogen, mainly result from dietary lipids and carbohydrates. In most insects they are transferred from midgut to the fat body, where triglycerides can make up more than 90% of the total lipid content (Beenakkers et al. 1985). Thus the amount of energy that can be stored largely depends on the quality and quantity of the available food resources. At which time and in which quantity energy is spent during the development of insects, or stored for other processes like reproduction, can be studied well in mayflies because all energy has to be stored by the larvae, prior to emergence and reproduction.

The purpose of this study is to provide insight into the energy metabolism of mayflies by measuring the storage components, triglycerides and glycogen, in the larvae during their aquatic life span in their natural habitats with a special focus on the last weeks before emergence. Therefore we compared the univoltine, grazing March Brown mayfly *Rhithrogena semicolorata* (Curtis 1834, Heptageniidae) and the semivoltine, burrowing Green Drake *Ephemera danica* (Müller 1764, Ephemeridae). The main hypothesis is that energy storage in larvae is focused on emergence. Further questions are: (1) Do male and female larvae use different management strategies of energy storage? (2) Do environmental factors in the habitat such as food resources and predation pressure affect the storage of energy reserves in the larvae? This might provide an insight into energy allocation as a tool to maximize ecological fitness and as adaptation to environmental factors.

Thus, the management of energy storage in both species is compared with respect to their different life histories, different micro-habitats and their feeding and mating behaviour.

Methods

Study site and organisms

The animals were collected in the lower section (at 3–3.5 km) of a small second-order mountain stream (Gauernitzbach, length 4.6 km) draining into the river Elbe approximately 15 km downstream of the city of Dresden (Saxony, Germany). The catchment area is dominated by agriculture. In the lower part, where the larvae were collected, the stream flows through a deciduous woodland valley (mainly alder, maple and oak trees). In the sampling area the stream has a mean width of 1.5 m and a mean discharge of $55 \pm 46\,l\,s^{-1}$. Water temperature ranges between 0 and 17°C with highest values in August. Other abiotic environmental factors do not show strong seasonal trends (means ± SD 2002–2004: pH 8.4 ± 0.2, $n = 57$; electrical conductivity 888.5 ± 70, $n = 66$; oxygen saturation 98 ± 12%, $n = 55$).

Rhithrogena semicolorata prefers riffle sections of streams and rivers. There they feed mainly on periphyton by scraping it from the substrates or gather fine particulate organic detritus from the surface of the sediment (Elliot et al. 1988). The nymphs usually swim in short bursts, interspersed with periods of clinging to benthic substrates like stones and wood. Larval development is completed within 1 year reaching fresh mass of 14.1 ± 6.2 mg (mean ± SD, $n = 17$, 10.5.2004–17.5.2004). In contrast, the Green Drake *E. danica* mainly lives in lakes and in the pool sections of streams and rivers with a sandy and gravel bottom (Elliot et al. 1988; Wesenberg-Lund 1943). There nymphs form a tubular burrow and use their prominent gills to circulate the water. *E. danica* larvae are collector-gatherers, sometimes also referred to as deposit feeder, which feeds on fine particulate organic detritus. *E. danica* usually has a 2-year life cycle reaching fresh mass of 99.6 ± 30.7 mg (mean ± SD, males, $n = 12$) and 147 ± 13 mg (females, $n = 12$, 22.5.2001–25.5.2001). In warmer waters the species is able to complete its life cycle within 1 year.

Field sampling and environmental factors

Ephemera danica was sampled mostly weekly from January to July 2001 by passing sediment through a sieve and picking large larvae from the remainder (three intervals of 3 weeks in January, February and April). *R. semicolorata* was sampled from October 2003 to March 2004 every second week by collecting single individuals from stones. Shorter sampling intervals were chosen prior to emergence (every week in April, 3–4 days during May and June).

Because reproduction period of the two species lasts at least 6 weeks and larval development is not entirely synchronized different larval stages could occur in the stream on each sampling occasion. If the content of glycogen and triglycerides changes during larval development, sampling of different developmental stages at one date would lead to a high variability of the values. To minimize this variability, we always chose the

respective largest and furthest developed larvae. Thus, on every sampling occasion we sampled animals from the same or a similar developmental stage. In the case of *E. danica* only larvae from the second year of their development cycle were selected. Animals were quickly transported to the laboratory in a cool box (4–8°C). Body length, mass and sex of the larvae were determined. Male larvae were determined by the visible first segment of the forceps (male genitalia) on the abdomen. This was always possible for *E. danica* but only for the more developed larval stages of *R. semicolorata* (from April onwards). Thereafter the animals were frozen in liquid nitrogen (−196°C). Close to emergence period additionally large larvae were sampled and conserved in 80% ethanol to verify the egg maturation. The timing of emergence and the number of emerged subimagoes were measured by using emergence traps of the type "week" (LeSage and Harrison 1979). The traps swim on the water surface and catch continuously all emerging animals over the covered surface (base 40 × 40 cm^2; 0.16 m^2) in a 1% formaldehyde solution. Along a stream stretch of about 1,000 m length a total of six emergence traps in 2001 and three traps in 2003/2004 were installed in the stream. The traps were emptied every second week during early spring but at least weekly prior to and during the period of emergence of the studied species. At each sampling event water temperature and sediment temperature in a depth of 3 cm (habitat of *E. danica*), pH, electrical conductance and concentration of dissolved oxygen were measured (LF196, pH196, Oxi96, WTW Weinheim).

The food supply for *E. danica* was calculated by determining the percentage of fine particulate organic material in the fraction of fine sediment (FPOM, grain size < 1 mm). For that purpose, at each sample point five small sediment cores (core diameter 3 cm, core depth 5 cm) were sampled. The cores were transported to the lab in a cool box (4–8°C). Afterwards the sediment cores were dried for 12 h at 80°C and weighed. Thereafter, all organic components of the dried sediment samples were burned for 4 h at 550°C and the mass difference between the dried and burnt samples was calculated as ash-free dry mass. To determine the available food source for *R. semicolorata* at each sample point three stones were randomly taken from the streambed. The periphyton (biofilm) was removed from the stones by brushing every stone carefully. Ash-free dry mass of the biofilm was determined by drying the brushed material for 12 h at 80°C, weighing and burning it for 4 h at 550°C. The stone surface was estimated by covering it with foil and weighing the removed foil (after Doeg and Lake 1981). The amount of biofilm was reported as ash-free dry mass per area (mg cm^{-2}).

Estimation of egg maturation

The egg number of last instar female larvae (black wing pads) was counted by removing the eggs from the abdomen and separating them by short ultrasonic pulses (UW 70, Badelin electronic, Berlin). Afterwards, the homogenous egg suspension was filtered (cellulose acetate, 0.45 μm). The filter was slightly stained by a drop of ink and all eggs of at least three out of eight parts of the filter were counted under a dissecting microscope (mean number of eggs per part filter tissue 230).

Analyses of energy storage components

Triglycerides and glycogen contents of individual larvae (whole animal) were determined by enzymatic assays. First in 2001 the method described below was adapted to measure triglycerides in single individuals of *E. danica* larvae. In 2003 we intended to use the same method to measure triglycerides in *R. semicolorata*. However, because of the considerably lower biomasses of *R. semicolorata* and to maintain the measurement of single individuals the method was adapted. Therefore it differed slightly from the method used for *E. danica*. Thus in this study our emphasis lies rather on the comparison of the seasonal pattern of the triglyceride concentration than on the direct comparison of the absolute concentrations between the two species.

The animals were lyophilised for 12 h (*R. semicolorata*) or 24 h (*E. danica*) and carefully homogenised with a glass stick in clean vials (polyethylene vials with a volume of 2 ml for *R. semicolorata*, 10 ml glass vials for *E. danica*). Triglycerides were extracted by adding 1–2 ml HIP (hexanol:isopropanol mixture, 3:2, v:v, Hara and Radin 1978). All working steps with the extraction solvent were conducted on ice to prevent evaporation of the solvent. The homogenate was mixed well for some seconds (Vortex shaker) and short ultrasonic pulses (UW 70, Badelin Electronic) were applied to separate the particles from each other. Samples were left for 5 min on ice for triglyceride extraction. Subsequently, tissue was separated from the solvents by centrifugation (cooled micro-centrifuge, 25,000 × g, Eppendorf, Germany). For *E. danica* triglycerides were split into glycerol and free fatty acids by saponification (70°C) and the liberated glycerol was measured enzymatically (Kreutz 1962; Pinter et al. 1967) using a commercial triglyceride assay (Triglycerides 320-A, Sigma Diagnostics, St Louis, USA) and a spectral photometer (Lambda12, Perkin

Elmer, Überdingen, Germany). For *R. semicolorata* the extract was transferred into glass vials and lyophilised for 12 h to eliminate the solvent. Triglycerides were split into free glycerol and fatty acids enzymatically by lipoprotein lipase. For enzymatic determination a commercial assay for analysing triglycerides in human serum (Dr. Lange Test LCN 351, GPO-PAP-Method, Berlin, Germany) and a spectral photometer (Lambda12, Perkin Elmer) was used. The Sigma Diagnostics Triglyceride Calibrator set (No. T2772) was used for calibration.

It cannot be ruled out that differences in triglyceride concentrations between the two species may result from the different analytical approaches. However, we avoid direct comparison of single values between the species. The purpose of this study is to compare seasonal changes or sex-depended differences between the species. Thus only relative differences were of interest.

The glycogen content of whole larvae was analysed enzymatically as described in Bergmeyer (1985). Prior to the enzymatic hydrolysis the glycogen in *E. danica* was cleaned up by hot KOH extraction and precipitation by saturated Na_2SO_4 solution. For *R. semicolorata* the internal enzymes of the larval tissue were thermally immobilised (vials with single animals for 10 min in 100°C water bath). For enzymatic hydrolysis samples were incubated for 3 h at 37°C in acetate buffer (2 mol l^{-1}, pH 4.8) with amyloglucosidase (Sigma Aldrich, Steinheim, 3 U for *R. semicolorata*, 14 U for *E. danica*). After thermal enzyme immobilisation the homogenate was centrifuged (cooled micro-centrifuge, 25,000 × g, Eppendorf) and the supernatant was analysed for free glucose enzymatically using a commercial glucose assay (Nobima-Hit I Glucose-HK, Hitado Diagnostic Systems, Möhnesee, Germany or Infinity Glucose-Reagent 17-UV, Sigma Diagnostics) and a spectral photometer (Lambda12, Perkin Elmer).

Data analysis

The total accumulated energy (kJ g^{-1} dry mass) in glycogen or in triglyceride storages of larvae of both species (whole animals) was calculated as described in Wieser (1986). The energy content of triglycerides is assumed to be 31,503 kJ mol^{-1} (palmitic acid 9,948 kJ mol^{-1}; glycerol 1,659 kJ mol^{-1}). The energy content of glycogen was calculated with 2,813 kJ mol^{-1} glucose (Wieser 1986). Because of methodical differences in the analyses of triglycerides and glycogen a transformation between dry and fresh masses was necessary. The transformation factor was obtained by a linear fresh mass–dry mass correlation from the analysed animals (*E. danica* slope 0.286, $n = 110$, $R^2 = 0.755$, $P < 0.001$; *R. semicolorata* slope 0.362, $n = 117$, $R^2 = 0.87$, $P < 0.001$). Based on these values the energy content accumulated in the glycogen and in the triglyceride storages of the analysed larvae was calculated and expressed as kJ g^{-1} animal dry mass.

For comparisons of mass, energy content, concentrations of triglycerides and glycogen between the sexes or species *t* tests were used (calculated in R 2.0). As the concentrations of glycogen showed a trend during the sampling period a paired *t* test was used to compare the sexes over the whole sampling period. Variance asymmetry occurred in the case of energy content, thus the Welch test was performed here. Despite most multicellular organisms *E. danica* and *R. semicolorata* showed no decrease of growth rates during the later larval development. Thus the rates of mass increase for the two species were obtained by fitting a linear model to the field data instead of an allometric function ($y = a - bx$, Sigma Plot 8.0). In the case of *R. semicolorata* mass increase showed two distinct phases. Thus two separate curves were fit. To describe the decrease of triglycerides and glycogen also a linear model was used, as it fitted well and there is no reason to expect another function. The parameters and plots are shown only for the significant regressions.

Results

Environmental factors

The seasonal dynamics of food supply differed for the two species. *E. danica* larvae were able to use a relatively stable food supply during their larval development. The mean proportion of fine particulate organic matter (FPOM, grain size < 1 mm) in the sediments on each sampling date ranged between 5.6 and 7.2% and did not show a consistent seasonal trend (Fig. 1). On the contrary, *R. semicolorata* larvae which feed on periphyton had to face seasonal changes in food supply. During winter periphyton biomass remained permanently low (November–February 0.3 ± 0.2 mg cm^{-2}, mean ± SD, $n = 7$), whereas in spring and summer food supply was better reaching a maximum of 1.2 mg cm^{-2} (March–July 0.75 ± 0.41, $P < 0.001$, $n = 20$, *t* test). Especially in March there was a sudden increase from 0.2 up to 1.1 mg cm^{-2} within only 17 days (27.2.2004–15.3.2004, Fig. 1).

Larval development

The mean biomass of *E. danica* larvae at the last sampling date before emergence differed significantly between the sexes (males 64 mg, females 157 mg,

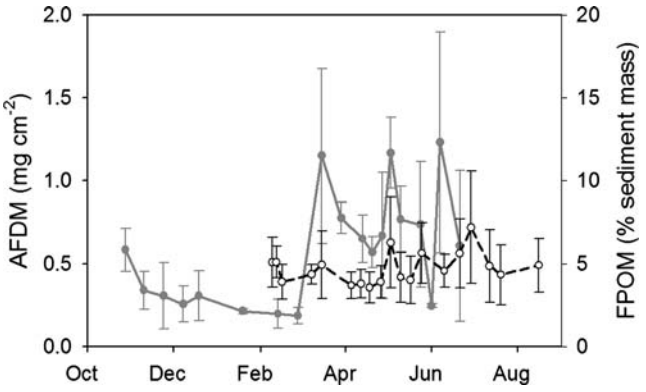

Fig. 1 Mean concentrations of food supply (± standard error, $n = 3$ on each sampling occasion) in the natural habitats of *E. danica* and *R. semicolorata* during the season. Fine particular organic matter (*FPOM*) is used by *E. danica*. Periphyton, measured as ash-free dry mass (*AFDM*) is used by *R. semicolorata*. *Grey circles* AFDM, *open circles* FPOM

$P < 0.001$, $n = 6$, t test). The biomass of *R. semicolorata* larvae was considerably lower (14 mg prior to emergence) and the mature larvae of *R. semicolorata* showed no difference in body mass between the sexes ($P = 0.48$, $n = 6$, t test). Somatic growth of the two species throughout the year could be described with linear models (Fig. 2, Table 1). Female larvae of *E. danica* larvae showed a faster growth than male larvae (slope of linear mass increase: females 0.44, males 0.13). *R. semicolorata* larvae showed a relatively slow growth rate during winter (slope of linear mass increase: 0.03) and a higher rate during spring (slope of linear mass increase 15.3.2004 until emergence: females 0.38, males 0.32). Egg maturation was completed in the last larval instar (black wing pads) immediately before emergence in both species. In that stage the whole abdomen was filled with eggs and intestines were empty and collapsed. Female larvae of *E. danica* contained more eggs than *R. semicolorata* (*E. danica*: 4,740 ± 570, $n = 3$; *R. semicolorata*: 1,529 ± 420, $n = 34$, mean ± SD). The emergence period of *E. danica* started at the end of May and lasted for at least 8 weeks (Fig. 3). *R. semicolorata* emerged earlier in May and emergence period was slightly shorter (Fig. 4).

The concentration of energy storage components in the animals

Triglycerides were the main energy storage component for both species. Glycogen accounted for only 2.8 ± 3.2% of total stored energy for *E. danica* and 16 ± 7.8% for *R. semicolorata* (mean value of the whole study period ± SD). The seasonal dynamics of triglycerides differed between the two studied organisms. Mean concentration of triglycerides in male larvae of *E. danica* during their second year was 169 ± 35 μmol g^{-1} DM (mean ± SD, $n = 52$, Fig. 3). During the final larval development (last 50 days before the start of emergence) triglyceride storage was kept nearly constant and therefore showed no apparent change with time left until emergence (Fig. 5a). During winter and spring female larvae stored similar amounts of triglycerides (January–May 176 ± 31 μmol g^{-1} DM, mean ± SD, $n = 32$, Fig. 3a). However, 2 weeks before the start of the emergence period triglyceride concentrations declined rapidly to a minimum value of

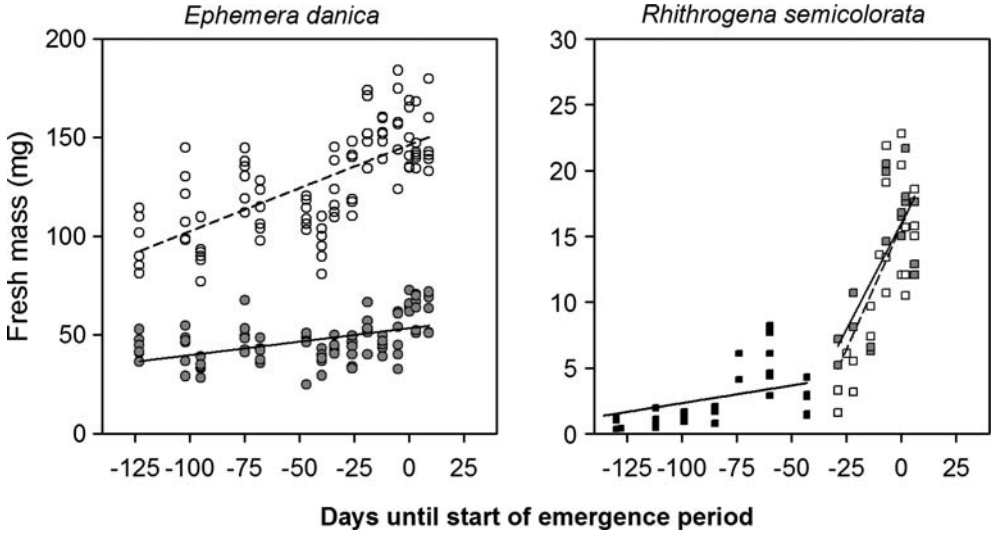

Fig. 2 Somatic growth of the two species *E. danica* and *R. semicolorata* calculated using a linear model ($y = a - bx$) for data of mass increase during the developmental time (measured as days before the start of emergence period). Parameters, sample size and R^2 are listed in Table 1. *Open symbols* female larvae, *grey symbols* male larvae, *black symbols* indeterminate sex

Table 1 Parameters, R^2, sample size and significance levels of regression models for growth over the season and energy storage components ($y = a - bx$) during the last 50 days before emergence of male and female *E. danica* and *R. semicolorata* larvae

	a	*b*	*n*	R^2	*P*
Fresh mass					
R. semicolorata					
Female	15.8	0.375	41	0.56	<0.001
Male	16.01	0.321	18	0.52	<0.001
Both sexes	5.02	0.027	41	0.397	<0.001
E. danica					
Female	146.4	0.44	90	0.48	<0.001
Male	53.5	0.136	90	0.25	<0.001
Triglycerides					
R. semicolorata					
Female	25.8	0.63	24	0.49	<0.001
Male	29.8	0.72	24	0.19	0.03
E. danica					
Female	92	2.16	33	0.69	<0.001
Male	165.9	0.42	32	0.07	0.144
Glycogen					
R. semicolorata					
Female	23.9	0.59	27	0.65	<0.001
Male	24.4	0.15	42	0.1	0.04
E. danica					
Female	31.5	0.42	44	0.31	<0.001
Male	19.5	0.15	45	0.18	0.004

44.3 µmol g^{-1} DM (mean value on 5.6.2001, $n = 6$, Fig. 3b). In females there was a significant correlation between triglycerides concentrations and days left until the start of emergence (last 50 days, Fig. 5a, Table 1). The following increase of triglycerides during June may result from a second cohort sampled after the first one left the stream. This is supported by the two clearly distinct peaks in emergence abundance (Fig. 3a). Triglyceride concentrations of *R. semicolorata* larvae ranged from 10 to 60 µmol g^{-1} DM (39.4 ± 21 µmol g^{-1} DM, mean ± SD, $n = 123$). No differences in triglyceride concentrations between the sexes could be shown ($P = 0.11$, $n = 14$, paired *t* test, Fig. 4). However, both sexes showed a significant decline of triglyceride concentrations within the last 50 days before emergence (Fig. 5b, Table 1).

Glycogen concentrations were in the same magnitude for the two studied species and ranged between 20 and 100 µmol g^{-1} (Figs. 3, 4). No sex-dependent difference over the whole sampling period could be shown for *R. semicolorata* ($P = 0.1$, $n = 12$, paired *t* test) but for *E. danica* glycogen concentrations were consistently higher in female larvae ($P = 0.0036$, $n = 20$, paired *t* test). Starting with the highest values in winter *E. danica* showed a steady decrease of glycogen concentration within the second year of their larval development

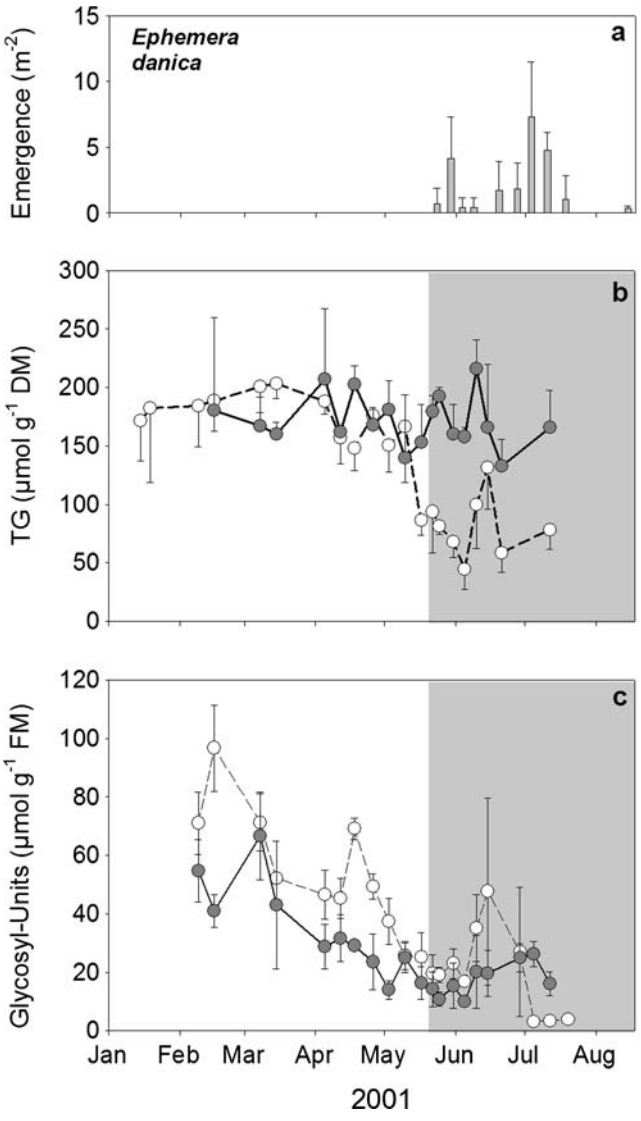

Fig. 3 Timing of larval development shown as mean emergence abundance per week (± standard error, $n = 3$ on each sampling occasion) and mean concentration of triglycerides and glycogen of second year *E. danica* larvae during the larval development (means ± standard error, $n \geq 3$ on each sampling occasion). The *grey parts* of the graphs indicate emergence period. *FM* fresh mass, *DM* dry mass, *open symbols* female larvae, *grey symbols* male larvae

(Figs. 3, 5c, Table 1). The glycogen concentrations of *R. semicolorata* showed a peak in mid-December and a significant decline within the last 50 days prior to emergence (Figs. 4, 5d, Table 1).

The amount of stored energy on the last sampling date before emergence differed significantly between the sexes for *E. danica* ($P < 0.001$, males 4.4 kJ g^{-1} DM, $n = 6$, females 2.4 kJ g^{-1}, $n = 9$, Welch test, 21.6.2001) but not for *R. semicolorata* larvae ($P = 0.174$, $n = 6$, males 1.8 kJ g^{-1}, females 1.2 kJ g^{-1}, Welch test, Fig. 6, 13.5.2004 and 17.5.2004). An estimation of energy content at the start of emergence (day 0) based on the

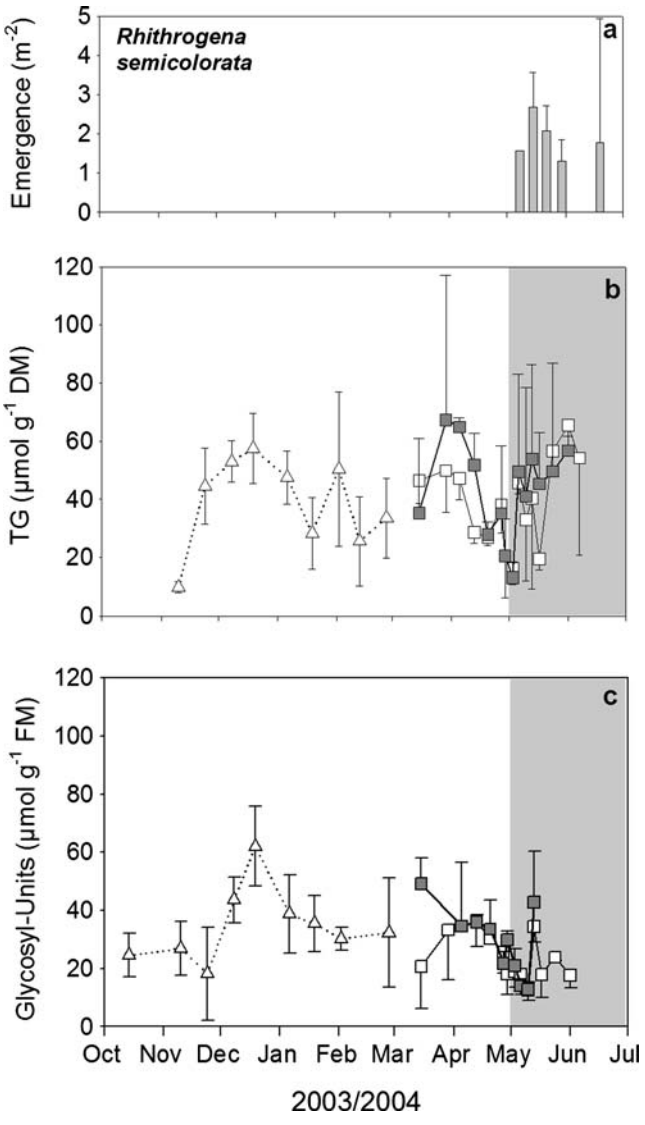

Fig. 4 Timing of larval development shown as mean emergence abundance per week (± standard error, $n = 3$ on each sampling occasion) and mean concentration of triglycerides and glycogen of *R. semicolorata* larvae during the larval development (means ± standard error, $n \geq 3$ on each sampling occasion). The *grey parts* of the graphs indicate emergence period. *FM* fresh mass, *DM* dry mass, *open squares* female larvae, *grey squares* male larvae, *open triangles* larvae with indeterminate sex

linear regressions of triglycerides and glycogen does not produce a different result (*E. danica*: males 5.5 kJ g^{-1}, females 3.1 kJ g^{-1}; *R. semicolorata*: males 1.1 kJ g^{-1}, females 1.0 kJ g^{-1}.

Discussion

We were able to show characteristic and ecologically important patterns of the energy storage management for two different mayfly species during their larval development within their natural habitat. The content of glycogen and triglycerides characterizes and integrates the gain or the loss of energy surpluses by the animals in the course of the previous days, weeks and months of their larval development. Especially the content of triglycerides seems to be an evident trait of the population fitness because reproduction is paid for out of this energy storage.

Our hypothesis was that energy storage strategy focuses on the time of emergence. As the aerial stages of mayflies (subimago, imago) do not feed, we expected an accumulation of energy storage components during larval development. The imagoes utilize these larval energy reserves for all their activity such as swarming flights, mating and egg deposition flights of the females. Because triglycerides are the most efficient energy storage component and a main fuel for flight energetics (Beenakkers 1969; Sartori et al. 1992) we expected the concentration of triglycerides to rise continuously until the start of emergence. This was not true for the two studied species. Only male *E. danica* larvae fit into our expectations by having high triglyceride concentrations until the emergence period. However, no intensive accumulation resulting in a prior-emergence increase could be observed. We assume that this is due to the observed intensive somatic growth until emergence. In contrast to other organisms growth rate did not decrease with increasing body size. One part of the obtained energy is used for growth and another to store energy for the reproductive period. However, as both processes occur simultaneously the absolute amount of stored energy in each individual increases towards emergence even if the concentration measured per unit mass remains constant.

We suggest that female larvae of the two species use triglycerides for the energy-demanding synthesis of vitellogenin, the most important yolk protein. Within the last weeks before the start of the emergence period a strong decline in triglyceride concentration and the development of eggs in the larval abdomen were observed. All eggs were fully developed in the last larval stage, which is easily recognized by black wing pads. The use of stored lipids for egg production seems logical from an ecological point of view and has been observed for other non-aquatic insects (Warburg and Yuval 1996; Murata and Tojo 2002; Horton et al. 2005).

In the case of *E. danica* the differences in the total energy content between the sexes on the last sampling date before emergence might be explained by the time of energy investment in reproduction. Males of this species perform long and energy-demanding flights as adults (swarming) in contrast to females, which invest in eggs during their final larval development and perform only short flights for mating and oviposition

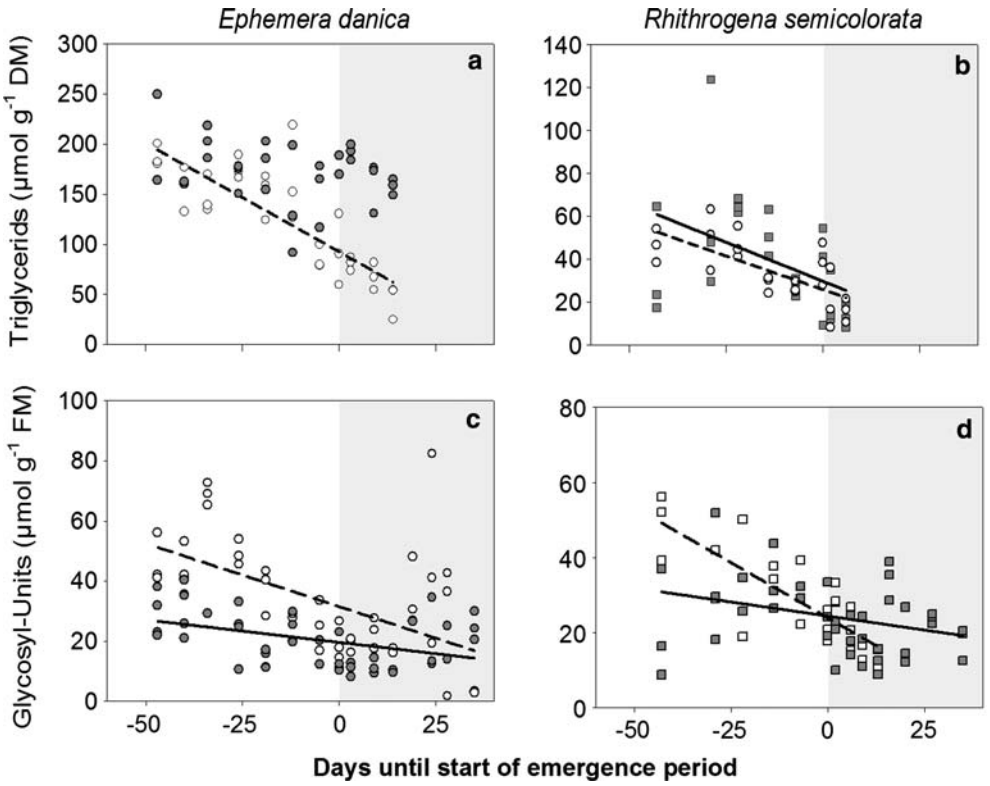

Fig. 5 Linear regressions of glycogen and triglycerides concentrations of the two species *E. danica* and *R. semicolorata* versus the developmental time as days before the start of emergence period during the last weeks of larval development (lines drawn only for significant correlations). Parameters, sample size and R^2 are listed in Table 1. *FM* fresh mass, *DM* dry mass, *open symbols and broken lines* female larvae, *grey symbols and solid lines* male larvae

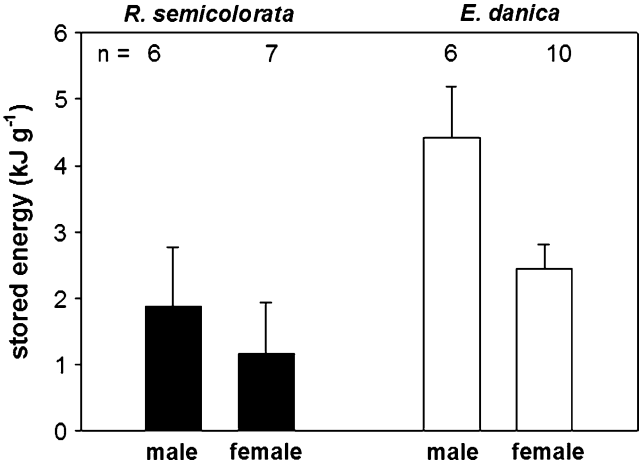

Fig. 6 Amount of the stored energy per dry mass (mean ± standard deviation) at the last sampling before the start of emergence (*E. danica*: 21.6.2001, *R. semicolorata*: 13.5.2004 and 17.5.2004) calculated as sum of the energy content of the stored triglyceride (31,503 kJ mol^{-1}) and glycerine (2,813 kJ mol^{-1}). Number of samples is indicated in the plot

(Harker 1992). Thus on the last sampling date before emergence, only females have already invested large parts of the stored energy in reproduction (eggs). Thus our results for *E. danica* support the presumption of Sartori et al. (1992) that sex-specific energy contents are related to the different mating strategies of the sexes of this species, which they postulated for *Siphonurus aestivalis*. Similar patterns of energy management were also found for *Hexagenia* spp. in two lakes (Cavaletto et al. 2003). Even though Cavaletto et al. (2003) did not report sex-dependent triglyceride concentrations for the larvae, they found significant differences in the subimagoes.

The decline of glycogen at the end of the larval development seems to be a general pattern at least for Ephemeroptera. Besides our data of *E. danica* and *R. semicolorata* this pattern was already described for *Hexagenia* spp. (Cavaletto et al. 2003) and *S. aestivalis* (Sartori et al. 1992). We suppose that adult mayflies do not need much glycogen, as their main energy-demanding activity (flight) is paid for by the breakdown of triglyceride reserves (Beenakkers 1969; Sartori et al. 1992).

Our second question was whether environmental factors in the habitat such as food resources could possibly affect the storage of energy reserves of mayfly larvae. The differences in triglyceride dynamics

could be interpreted as effects of environmental factors on energy metabolism in case of *R. semicolorata* larvae while *E. danica* seemed to be less affected. *E. danica* larvae live covered by sediment and therefore the risk of predation by benthivorous fish is relatively low. Further, nutrient supply does not change remarkably throughout the year (Fig. 1). Thus energy management seems to be optimised with respect to energy demand of reproduction only. Males emerge smaller than females because reproductive success is positively correlated to female body size (Honèk 1993) but have significant higher energy concentrations because of the swarm flight. In contrast, the development of the *R. semicolorata* larvae seems to be more affected by environmental factors. *R. semicolorata* larvae are confronted with predation (Winkelmann et al., unpublished results) and have to deal with considerable changes of food supply throughout the season (Fig. 1). The life cycle of *R. semicolorata* could be regarded as a trade-off between maximal body length to maximize female fecundity (Scrimgeour and Culp 1994; Honèk 1993; Kosnicki and Burian 2003) and early emergence avoiding predation (Peckarsky et al. 2001). Somatic growth of *R. semicolorata* larvae is divided into two phases. During winter the mass increase is remarkably slower than during spring, when water temperature and food concentration rise. Unlike *E. danica* the final larval stages of *R. semicolorata* do not show sex-dependent differences in body mass or triglyceride concentration (Figs. 2, 5). Both sexes seem to use up large parts of their triglycerides storage in the last 4 weeks prior to emergence even though males have to perform mating flights. This could be explained by an accelerated larval growth during spring in order to reduce the time of high predation risk at the end of larval development caused by the higher body mass of the late larval stages. This is supported by the coincidence of fast somatic growth, decrease of triglyceride concentrations and high food concentrations in the habitat. Thus for the fast somatic growth of the larvae energy could be provided by triglyceride break down and the necessary substrate by food uptake. On the other hand, it seems possible that reproductive success is positively correlated with body size not only for females but also for males. This was observed for *Epeorus longimatus* (Flecker et al. 1988) and is suggested for *Heptagenia lateralis* and *Ecdyonurus dispar*, as these species jostle frequently while swarming (Harker 1992). In this case males would rather invest in body growth and store less triglycerides for swarming, which then would have to be shorter than the swarm flight of *E. danica*.

Conclusion

Measurements of energy storage components of mayflies in their natural habitats revealed different strategies of energy management which might be explained by life cycle, habitat selection and may be the effects of environmental factors. If mechanisms behind these management strategies become clear during future investigations, the concentration of triglycerides at certain points in the life cycle (for instance shortly prior to emergence) could be used as a trait for population fitness in ecological field experiments in animals that do not feed as adults. Such traits measuring the physiological status of organisms are not yet common but necessary for investigation of sublethal effects of environmental factors on individuals, populations or whole food webs.

Acknowledgments We would like to thank Gabriele Egerer and Claudia Hellmann for their assistance in field and laboratory. We are also grateful for the very helpful comments of Christian Ortmann and three anonymous reviewers, which clearly improved our manuscript. Parts of this work were financially supported by the German research foundation (grant BE 1671/9-1). All our experiments comply with the current laws of Germany.

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