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PRIMARY RESEARCH PAPER

Larval growth and metabolic energy storage of *Micropterna lateralis* (Trichoptera: Limnephilidae) in an intermittent stream: glycogen dominates in final instars

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Abstract The caddisfly species *Micropterna lateralis* is an abundant representative of limnephilids in intermittent streams. Yet, its basic life history characteristics and adaptations related to environmental factors, such as stream drying, are comparatively understudied. Here, we investigated larval growth and metabolic energy reserves (glycogen, triglycerides) through development in their natural habitat. We concentrated on the larval development because this period represents the important phase of energy accumulation necessary for growth, metamorphosis and embryogenesis. Besides larval physiology, female adults were studied in terms of ovarian maturation. Our results indicate that adult females lack an imaginal diapause, which is otherwise often observed in intermittent stream-inhabiting Limnephilidae. Further, *M. lateralis* is univoltine and exhibits a relatively fast larval development with five distinct instars, of which four are characterised here (instars II–V). Accrual of biomass occurs in final instars, where a high amount of glycogen is accumulated. Lipid concentrations, on the other hand, are kept constant in final stages and slightly lower than in preceding instars. This dominance of glycogen in final instars found in *M. lateralis* is highly unusual in insects and of potential adaptive significance for the species' ability to exploit intermittent habitats.

Keywords Autecology · Caddisfly larvae · Energetics · Stream drying

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Introduction

The caddisfly, *Micropterna lateralis* (Stephens, 1837), is a species of Limnephilidae distributed across most of central, northern and eastern Europe outside of the Mediterranean Region (Fig. 1). Across its range, *M. lateralis* inhabits eucrenal, hypocrenal and epirithral zones of freshwaters and can be found up to 450 m a.s.l. in low mountain ranges (Graf et al., 2008). The species appears to be an indicator of small, organic streams that may be subject to periodic stream drying (Sommerhäuser, 1998). According to Sommerhäuser (1998), *M. lateralis* seems to be more abundant in intermittent than permanent habitats in the Lower Rhine area.

In order to overcome the waterless summer period in intermittent streams, many limnephilids evolved an imaginal diapause (Novak & Sehnal, 1963; Crichton, 1971; Denis, 1978), where adults emerge with underdeveloped ovaries and aestivate in dark and humid places (e.g., caves) until flow resumes. *M. lateralis* populations from Sweden and Britain show a short flight period in spring and summer (June/July) without diapause (Crichton, 1971; Svensson, 1972). Sommerhäuser (1998) reported adult emergence in May and June, while stating the existence of diapause to be not fully clarified. Longer flight periods are indicated in Tobias & Tobias (1981), lasting from May to October. In France, single imagines were regularly observed in subterranean environments (i.e., natural and artificial cavities; Bouvet, 1976), presenting a characteristic behaviour of diapausing species. Such geographic differences in adaptations have been observed in the congener *Micropterna sequax* McLachlan, 1875, where Swedish populations have no diapause (Svensson,

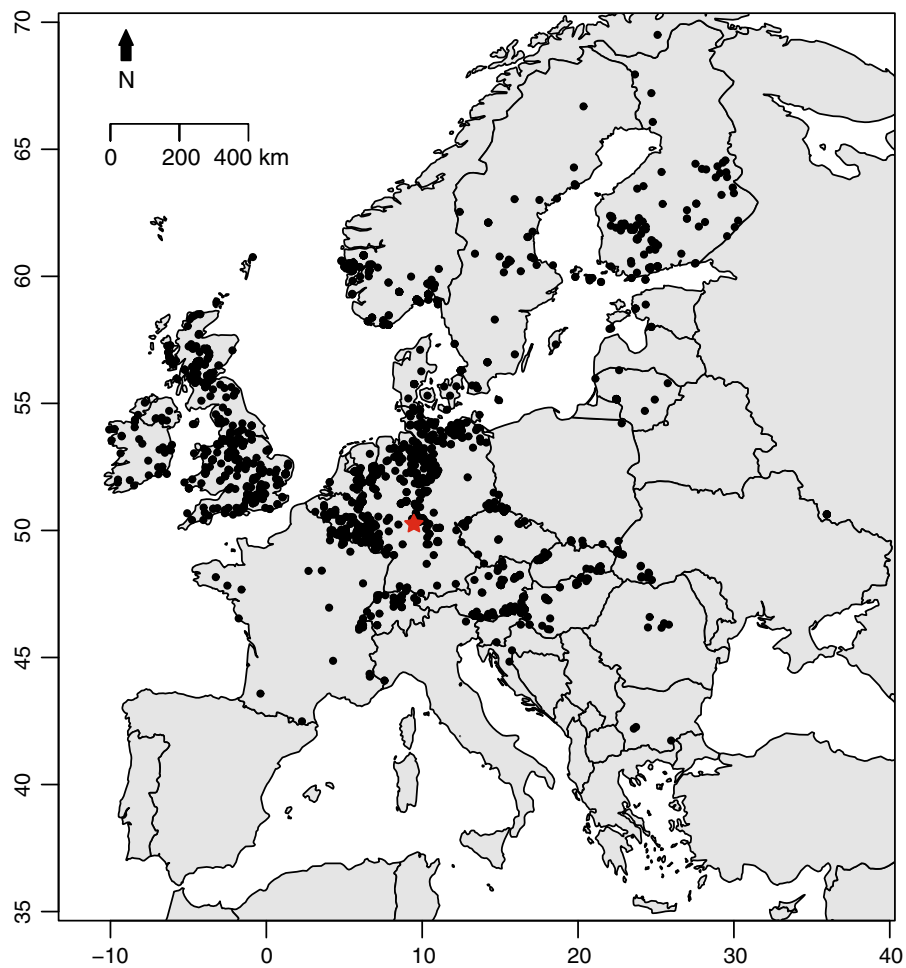

Fig. 1 Distribution of *M. lateralis* across Europe based on data extracted from the data collection "Distribution Atlas of European Trichoptera" (Schmidt-Kloiber et al., 2015, 2017). The approximate location of our study sites is indicated as *red star*

1972) but southern European populations do (Denis, 1976). Based on experimental evidence from *Limnephilus rhombicus* (Linnaeus, 1758), this may be explained by the differences in photoperiod length between higher and lower latitudes (Denis, 1978). Whether a similar geographic pattern is present in *M. lateralis* remains to be investigated.

M. lateralis larvae are mainly grazers and shredders, but also show predatory behaviour (Graf et al., 2008). They are expected to develop through five distinct instars within a year, yet relatively little is known about the size ranges of individual larval stages. The larvae are the main feeding stages and accumulate the necessary energy reserves for metamorphosis, as well as sustenance and reproduction of adults. The central energy storage in insects is the fat body, an organ unique to insects (Law & Wells, 1989). In the fat body, reserves are primarily stored as glycogen, triglycerides and proteins (Harrison et al., 2012). The amount of stored lipids is usually higher than that of carbohydrates across insects (reviewed in Arrese & Soulages, 2010), while the total amount of accumulated reserves varies between species. Proteins only present a minor metabolic energy storage component (e.g., in the caddisfly *Sericostoma vittatum* Rambur, 1842; Campos et al., 2016). The ability to store energy is an important ecophysiological trait fundamental in insect lives, yet autecological studies of growth-related energetics within natural habitats are rare.

Thus, our primary objective in this study was to provide an overview of life history characteristics that could affect the ability of *M. lateralis* to exploit intermittent habitats. Specifically, we aimed to clarify the existence of an imaginal diapause in *M. lateralis* by determining ovarian maturation of newly emerged adults. Notes on other caddisfly species present at the study streams will be given for comparative reasons. Further, we aimed to differentiate *M. lateralis* instars based on head capsule width and characterize growth, energy storage management and case material during larval development. In order to assess the adaptive potential of these traits for intermittent stream caddisflies, the characteristics are compared with those of two other intermittent stream caddisflies (*M. sequax* and *Stenophylax permistus* McLachlan, 1895).

Methods

Study site and field sampling

We studied the intermittent sections of the Auerbach (50°16′34.2″N, 9°25′58.7″E) and Klingbach (50°14′57″N, 9°26′23″E). Both are lower mountain streams (around 200–430 m a.s.l.) situated in the Rhine-Main-Observatory LTER-D site (Haase et al., 2016) in the Hessian Spessart (Germany) in mixed deciduous forest (dominant taxa: *Fagus sylvatica* L., *Urtica dioica* L. and *Bryophyta*). An approximate location of those sites is indicated in Fig. 1 showing the European distribution of *M. lateralis* based on occurrence data extracted from the "Distribution Atlas of European Trichoptera" (Schmidt-Kloiber et al., 2015, 2017). Sampling point coordinates were used to generate a map using R statistical software (R Core Team, 2016).

At the Auerbach, the summer dry period starts around mid-June to mid-July and lasts for two to four months, but is interrupted by several, precipitation-driven, short flow events (years of observation: 2014–2016). Water temperature ranges between 1.1 (01/19/16) and 9.8°C (04/05/16), based on weekly measurements from October to April (2015 and 2016). Other environmental factors are stable over the flow period (pH 7.1 ± 0.1; electrical conductivity 158.4 ± 8.8 µS cm^{-1}; oxygen concentration 11.3 ± 0.4 mg l^{-1}; mean ± se October–April 2015/16, $N = 17$). Hydrological patterns are more variable at Klingbach. Drying starts between April and mid-July and lasts for five to eight months (years of observation: 2014–2016). During our study period, drying was not interrupted by intermediate flow events. Weekly measured water temperatures show little fluctuations ranging from 7.0 to 9.1°C, while other factors remained stable during the flow period (pH 5.9 ± 0.04; electrical conductivity 99.5 ± 0.9 µS cm^{-1}; oxygen concentration 11.3 ± 0.1 mg l^{-1}; mean ± se October–April 2015/16, $N = 15$).

At both streams, emergence traps were operated from April/May until September/October in 2014 to 2016. Specimens were preserved in ethanol and identified using Malicky (2004).

Larval specimens of *M. lateralis* were collected from mid-October 2015 until early April 2016 at

Auerbach. Sampling was performed at a variable interval of one to three weeks from October to December, biweekly in February and weekly in March and April (five specimens per sampling occasion). In addition, four specimens of *S. permistus* and *M. sequax* were sampled in March (Klingbach) and April (Klingbach/Auerbach), respectively. In the field, animals were hand-picked from leaf packs, removed from their case and flash frozen in liquid nitrogen. In the laboratory, larvae were identified using DNA barcodes (mtCOI; Hebert et al., 2003).

Imaginal ovarian maturation and larval growth

In the laboratory, we dissected adult *M. lateralis* females caught in emergence traps (2014–2016; $N = 49$) to determine ovarian maturation, following the four-stage classification of Novak and Sehnal (1963): 'A': immature female; 'B': maturing female; 'C': mature female; and 'D': female after oviposition. Caddisfly species other than *M. lateralis* were also assessed for comparative purposes ($N = 38$).

To determine larval stages, heads of all collected larvae of *M. lateralis* ($N = 83$), *M. sequax* ($N = 4$) and *S. permistus* ($N = 4$) were cut off in order to measure head capsule width (HCW) as the maximum width of the head capsule using a binocular (Olympus SZX 16, Frankfurt, Germany). During head preparation, larval bodies were kept frozen and later freeze-dried and weighed to the nearest mg. Caddisfly cases were air-dried and assigned to three types of building material: organic, mineral and mixed organic/mineral.

Energy storage assay and analysis

Homogenized freeze-dried larval bodies were used to determine glycogen and triglyceride contents by enzymatic assays. Because of the minimal amount of tissue required for the enzymatic analysis, larvae were allotted to glycogen and triglyceride analysis based on their dry weight: individuals lighter than 1 mg were used in glycogen analysis and individuals of 1–2 mg in triglyceride analysis, whereas in individuals heavier than 2 mg both storage compounds were analysed. We used all collected specimens per sampling date in case of smaller instars (II, III) and three specimens each for instars IV and V (exception: $N_{IV} = 4$ at 01/05/2016).

For analysis of glycogen concentrations, samples were extracted in perchloric acid (PCA). After neutralization with $KHCO_3$ (2.0 M), glycogen was hydrolysed using amyloglucosidase (Sigma-Aldrich, Steinheim, Germany) during a 2-hour incubation (40°C) in acetate buffer (0.2 M; pH 4.8). The enzyme was thermally immobilised (5 min at 100°C), the samples were centrifuged (10 min at 18,600×*g* and 4°C, 200R, Hettich, Tuttlingen, Germany) and the supernatant was photometrically (Specord 200 Plus, Analytik Jena, Jena, Germany) analysed following Keppler & Decker (1984) in a modified enzymatic assay described by Becker et al. (2013).

To extract triglycerides, ice-cold hexane was added to the samples prior to 10-minute incubation on ice, followed by a centrifugation step (10 min at 19,900×*g* and 4°C) using a cooled micro-centrifuge (200R, Hettich, Tuttlingen, Germany). The supernatant was transferred into glass vials and placed under the fume hood to evaporate the solvent. Enzymatic analysis of triglyceride concentration was performed using a commercial kit (Triglyceride FS, DiaSys Diagnostic Systems GmbH, Holzheim, Germany) and a spectral photometer (Specord 200 Plus, Analytik Jena, Jena, Germany; for details see Winkelmann & Koop, 2007). Glycogen and triglyceride concentrations were expressed as µmol g^{-1} animal dry weight and % of animal dry weight, while energy content was expressed as kJ g^{-1} animal dry weight. Energy stored in glycogen and triglycerides was calculated using an energy content of 15.6 kJ g^{-1} glucose (180 g mol^{-1}) and 37.3 kJ g^{-1} tripalmitin (807 g mol^{-1}; Wieser, 1986), respectively. Tripalmitin is the dominant compound of storage lipids (Wieser, 1986). The total amount of stored energy is referred to as the sum of the energy stored as glycogen and triglycerides measured in the same individual and does not account for additional energy stored in the form of proteins. In instars III, glycogen and triglycerides could not be measured from the same individual (see above). Thus, the total stored energy is calculated from the average of energy stored in glycogen plus the average of energy stored in triglycerides (note that the standard errors were also summed).

Data analysis

To detect differences in dry mass or energy storage between instars, one-way ANOVAs with the factor 'instar' and the dependent variables dry weight, triglyceride concentration or glycogen concentration

were performed. To achieve the normal distribution and the best approximation to variance homogeneity, data were transformed after analysing the optimal exponent for transformation via the 'boxcox' function in R (exponents 0.15 for dry weight and glycogen, no transformation for triglycerides). Differences in energy content were analysed using a *t* test, because sufficient data were available only for the fourth and fifth instars. We employed a piecewise linear regression analysis (Crawley, 2007) to detect potential break points in the seasonal dynamics of energy storage components. This analysis allows fitting separate regression slopes to characterize the relationship between two variables. The resulting model was compared to a simple exponential curve fitting. All statistical analyses were done in R statistical software (R Core Team, 2016).

Results

Emergence and ovarian maturation

Adult *M. lateralis* were caught in emergence traps from mid-May to August under wet and dry hydrological conditions in both studied streams. The species was found together with caddisflies that are typical of intermittent streams (Auerbach: *M. sequax*, *Limnephilus* cf. *sparsus* Curtis, 1834, *Plectrocnemia conspersa* Curtis, 1834, *Rhyacophila praemorsa* McLachlan, 1879, *R. philopotamoides* McLachlan, 1879, *Beraea pullata* Curtis, 1834, *Synagapetus moselyi* Ulmer, 1938, *Wormaldia occipitalis* Pictet, 1834; Klingbach: *M. sequax*, *L.* cf. *sparsus*, *L. bipunctatus* Curtis, 1834, *P. conspersa*, *Sericostoma flavicorne* Schneider, 1845, *Stenophylax permistus*, *S. mitis* McLachlan, 1875). A list of Plecoptera species is available in Supplementary Material Table S1.

Dissection of female *M. lateralis* revealed that specimens emerged with ovaries in stages B ($N = 23$), C ($N = 9$) or D ($N = 17$), but not in the immature stage A. However, freshly emerged females in ovarian development stage A were found in *M. sequax*, *Limnephilus* cf. *sparsus*, *L. bipunctatus* and *S. mitis* (Table S2).

Larval growth

Four *M. lateralis* instars were clearly distinguished within our samples, showing no overlap in head capsule width between subsequent instars (Table 1). The highest values matched those reported for final instars V in Waringer & Graf (2011) indicating the presence of instars II–V within our samples: instar II occurring in October ($N = 4$), instar III from October to December ($N = 15$), instar IV from November to January ($N = 22$) and instar V from November to April ($N = 42$). Mean weights of early instars II and III were similar, while a significant increase in weight occurred from instar III to V (Fig. 2a; Table 2). Most accrual of biomass happened in the long-lived instar V.

The case building material in *M. lateralis* differed between instars and was characterized by a shift from purely mineral cases (instars II & III) to mixed mineral/organic cases (IV) to purely organic cases in the last instars (V). Final instars of *S. permistus* used organic case material, while *M. sequax* had mixed cases at the Auerbach and organic cases at the Klingbach.

Metabolic energy storage

In *M. lateralis*, we observed a significant increase in glycogen concentration towards the fifth instar, while triglyceride concentrations did not change significantly (Tables 1, 2). Triglycerides were the main energy storage component in instars III and IV accounting for more than 59.5 ± 7.2% (mean ± standard error, $N = 18$) of total stored energy. In the last instar (V), however, triglycerides represented only 42.2 ± 2.8% (mean ± standard error, $N = 32$) of the stored energy and glycogen became the more important energy storage component. The weight-specific amount of total stored energy showed a significant difference between instars IV and V. Glycogen accounted for ~2% of total dry weight in instars II to IV and increased to ~10% in final instars. Triglyceride content slightly decreased with instar succession from 4.6 (III) to 2.8% (V) of total dry weight (Table 1).

The seasonal dynamics of metabolic energy storage in *M. lateralis* differed between glycogen and triglycerides. Glycogen concentrations increased over time and closely followed larval weight increase within instar V (Fig. 2b). This increase was steeper in spring compared to autumn and winter based on the segmented linear regression analysis (Fig. S1; better fit of linear than exponential curve). On the other hand,

Table 1 Summary of growth and energy measurements expressed as mean ± SE for instars II–V of *M. lateralis* (number of samples given in brackets) and instar V of *M. sequax* ($N = 4$) and *S. permistus* ($N = 4$)

Species	*M. lateralis*				*M. sequax*	*S. permistus*
Instar	II	III	IV	V	V	V
Head capsule width (mm)	0.59 ± 0.01 (4)	0.86 ± 0.01 (15)	1.36 ± 0.02 (22)	2.00 ± 0.01 (42)	1.99 ± 0.02	2.18 ± 0.01
Dry weight (mg)	0.38 ± 0.04 (4)	0.85 ± 0.12 (15)	5.16 ± 0.34 (22)	34.02 ± 2.95 (42)	58.42 ± 4.88	59.74 ± 2.78
Concentration (μmol g^{-1} dry weight)						
Glycogen	128.73 ± 14.20 (4)	90.41 ± 9.86 (11)	178.63 ± 37.45 (14)	526.31 ± 41.10 (32)	525.84 ± 93.26	725.16 ± 116.58
Triglycerides	–	57.17 ± 23.11 (4)	43.16 ± 8.19 (14)	34.97 ± 2.53 (32)	39.77 ± 3.54	25.82 ± 1.41
Energy content (kJ g^{-1} dry weight)						
Glycogen	–	0.25 ± 0.03 (11)	0.50 ± 0.11 (14)	1.48 ± 0.12 (32)	1.48 ± 0.26	2.04 ± 0.33
Triglycerides	–	1.78 ± 0.72 (4)	1.35 ± 0.26 (14)	1.09 ± 0.08 (32)	1.24 ± 0.11	0.80 ± 0.04
Total	–	2.04 ± 0.75 (*)	1.85 ± 0.26 (14)	2.57 ± 0.16 (32)	2.72 ± 0.19	2.84 ± 0.32
Concentration (% of dry weight)						
Glycogen	2.32 ± 0.26 (4)	1.63 ± 0.18 (11)	3.22 ± 0.67 (14)	9.48 ± 0.74 (32)	9.47 ± 1.68	13.06 ± 2.10
Triglycerides	–	4.61 ± 1.87 (4)	3.48 ± 0.66 (14)	2.82 ± 0.20 (32)	3.21 ± 0.29	2.08 ± 0.11

* Total energy content (kJ g^{-1} dry weight) for instars III is calculated as the average of energy stored in glycogen plus the average of energy stored in triglycerides (note that the standard errors were also summed) because glycogen and triglycerides were not measured in the same individuals

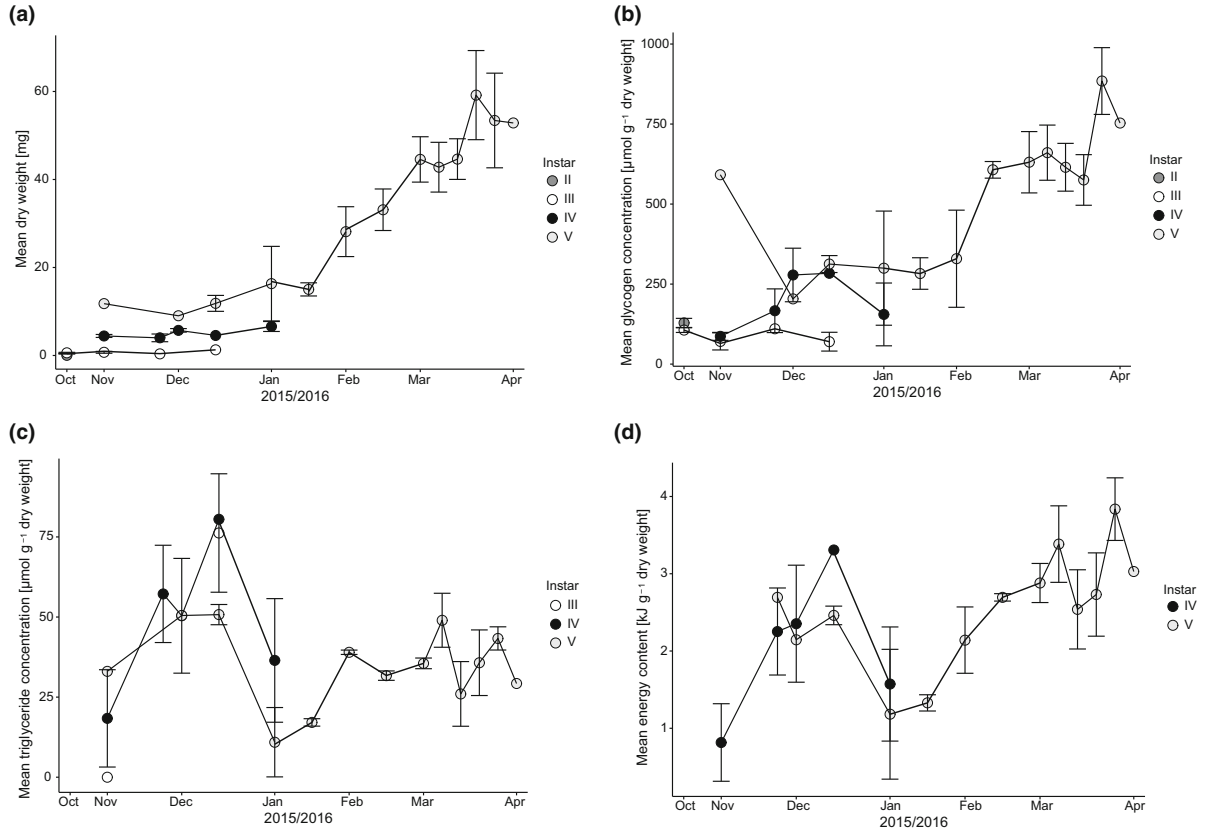

Fig. 2 Seasonal changes in the dry weight, concentrations of glycogen and triglycerides and the amount of stored energy of *M. lateralis* during larval development (mean ± SE)

Table 2 Results of ANOVAs and post hoc tests of differences in dry weight and energy storage substrates between instars of *M. lateralis* (*N* as indicated in Table 1)

	Global test			Pairwise tests		
	Df	Sum Sq	*P*	Instar II vs III	Instar III vs IV	Instar IV vs V
Dry weight						
Instar	3	7.2623	**<0.001**	0.773	**<0.001**	**<0.001**
Residuals	79	1.2156				
Glycogen						
Instar	3	3.7694	**<0.001**	0.709	0.243	**<0.001**
Residuals	57	2.3624				
Triglycerides						
Instar	2	2083.2	0.152	–	–	–
Residuals	47	24961.0				

Significant *P* values are indicated using bold font

concentration of triglycerides showed no systematic seasonal change (Fig. 2c). The seasonal dynamics of total energy content resembled the pattern of triglycerides from November until early February (Fig. 2d). After that, the curve was more similar to the course of glycogen concentrations over time. The total

amount of energy per individual followed a linear relationship with total dry weight, as did the amount of energy stored as glycogen and triglycerides per individual (Fig. 3). The increase is higher for glycogen than triglycerides, again showing that larvae tended to store more energy as glycogen compared to triglycerides.

In comparison, final instars of the species *M. sequax* ($N = 4$) had similar glycogen concentrations and energy stored as glycogen as *M. lateralis*, while the amount of energy stored in triglycerides was somewhat higher (Table 1). In *S. permistus*, the difference in glycogen and triglyceride concentrations, and consequently the amount of energy stored as glycogen or triglycerides, was even more pronounced (Table 1). In *M. sequax* and *S. permistus,* glycogen accounted for 53.3 ± 6.1 and 70.5 ± 4.3% (mean ± standard error, $N = 4$) of total stored energy, respectively.

Discussion

In this study, we characterize growth and energetics through four instars of *M. lateralis* in their natural habitat. Overall, developmental patterns are as expected showing a relative increase in head capsule width of approximately 1.5 following Dyar's rule of a constant size ratio between moults (Dyar, 1890). A

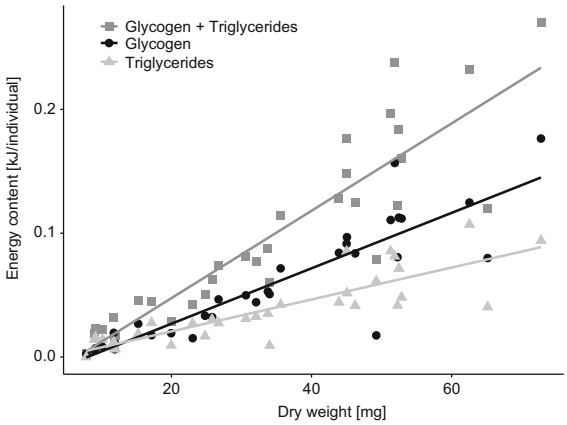

Fig. 3 Linear relationships of individual dry weight of final instars of *M. lateralis* against energy content per individual. Three regression lines are shown for the total amount of energy content as well as separate amount for glycogen and triglycerides. Coefficients for each model are as follows: Glycogen + Triglycerides: $y = -0.023 + 0.00353 \times x$, $R^2 = 0.816$; Glycogen: $y = -0.0179 + 0.00224 \times x$, $R^2 = 0.789$; Triglycerides: $y = -0.0051 + 0.00129 \times x$, $R^2 = 0.719$

major finding is the accrual of glycogen reserves in the last instars, while lipid contents are more or less constant. More specifically, the species shifts from a period where energy is stored primarily as triglycerides to predominant glycogen storage before emergence. This is both surprising and highly unusual, as lipids typically are the major storage component throughout larval development as well as in adult stages of insects (reviewed by Arrese & Soulages, 2010). Among aquatic insects, this is, for instance, described in mayflies (Winkelmann & Koop, 2007) and mosquitoes (Timmermann & Briegel, 1999). In experimentally held caddisflies, two studies report a lower weight-specific glycogen than lipid content in final instars of *L. rhombicus* (Mondy et al., 2011) and *S. vittatum* (Campos et al., 2016). Most of the earlier work on caddisfly development primarily focuses on lipids rather than carbohydrates: lipids account for up to 14, 22 and 18% of individual dry weight in larval *Potamophylax cingulatus* (Stephens, 1837), *P. nigricornis* (Pictet, 1834) and *Parachiona picicornis* (Pictet, 1834), respectively (Sehnal, 1963; Otto, 1974). In *M. lateralis*, proportions of triglycerides are markedly lower (up to 8.5% of individual dry weight) and stabilize at roughly 3% in final instars, while glycogen represents around 10% of individual dry weight, indicating the high importance of glycogen as an energy storage compound in *M. lateralis* compared to the abovementioned limnephilids. This is also true for final stages of *S. permistus* and *M. sequax*. Independent of the specific energy-storing components, the total energetic content of *M. lateralis* individuals increases linearly with the accumulation of larval weight. A high amount of available energy at the end of the last instar is crucial for successful metamorphosis and to support life and reproduction as adults (Ziegler, 1991). Therefore, lipid reserves supply energy and fatty acids needed for the production of eggs (e.g., in mosquitoes; Van Handel, 1993) as well as energy for flight (reviewed in Beenakkers et al., 1985). Likewise, glycogen can be converted to trehalose as a substrate for insect flight as was shown in *Anopheles gambiae* Giles, 1902 (Kaufmann & Brown, 2008) or *Locusta migratoria* (Linnaeus, 1758) (Van der Horst et al., 1980). Glycogen is further used during pupation, e.g., in the form of glucose for the synthesis of chitin (e.g., in *Phormia regina* Meigen, 1826; Tate & Wimer, 1971). Other than that, the high glycogen content that we observe in *M. lateralis* and

also *M. sequax* and *S. permistus* could be of adaptive significance in intermittent stream species. This hypothesis is based on the fact that glycogen can be converted to trehalose and sugar alcohols under drought stress (e.g. in *Polypedilum vanderplanki* Hinton, 1951; Watanabe et al., 2002) and binds bulk water as well, thus increasing the total pool of available water. Desiccation-resistant selection lines of *Drosophila melanogaster* Meigen, 1830 store high levels of carbohydrates that provide additional energy for dehydration acclimation and recovery (Chippindale et al., 1998; Djawdan et al., 1998). Similarly, *L. rhombicus*, a caddisfly species inhabiting small ponds that may be subject to drying, shows high carbohydrate contents (but not glycogen; Mondy et al., 2011). Further, a positive correlation between glycogen levels and desiccation resistance is reported for *Aedes* mosquitoes (Sawabe & Mogi, 1999). Thus, glycogen in final *M. lateralis* instars is potentially accumulated in large quantities to serve as nutrient store and desiccation protectant of larvae, pupae or egg masses.

In this study, we focus on larval stages for energy storage analyses and do not include pupal or adult specimens as well as eggs. Thus, the exact fate of energy reserves and the allocation of resources remains unknown. Generally, resource allocation at metamorphosis depends on the life history strategy and the future requirement of the species (Boggs, 1981). For example, the adult life span determines the amount of energy resources used for somatic investment compared to reproduction (Karlsson & Wickman, 1989). In a study on adult limnephilid caddisflies, Stevens (2000) tested this idea by investigating male thorax (somatic investment) and abdomen (reproductive investment) dry mass in species with different life histories. He found variation in the change of dry mass among the studied species, which was, however, more related to mating system (i.e., monandry or polyandry) than flight period length (Stevens, 2000).

The studied population of *M. lateralis* shows a flight period length of over three months (mid-May to August) without an imaginal diapause. Thus, our results support the classification of *M. lateralis* into a group of species characterized by "a shorter fight period, without a diapause, in spring and summer, and sometimes extending into autumn" following Crichton's (1971) assessment of populations in Great Britain. Consequently, there is no evidence for a geographic difference in adult adaptation between Swedish (Svensson, 1972), British (Crichton, 1971) and German (this study) populations. Regarding other caddisfly species present at the study streams (Table S2), both an extended flight period into autumn and an imaginal diapause were confirmed in *M. sequax* (Denis, 1976), *L.* cf. *sparsus* (Novak & Sehnal, 1963; Svensson, 1972) and *S. mitis* (Bouvet, 1978). In line with lacking a diapause, adults of *M. lateralis* must lay their eggs well before stream flow resumes. As shown in other temporary-water limnephilids, we expect eggs to be laid in dry substrates or damp vegetation, where first instars develop within gelatinous masses. Likely, the larvae quiesce for several months (Stevens, 2000) and actively leave the gelatin following long-lasting rainfall or total submersion in water (e.g. Novak & Sehnal, 1963). Once they enter the water, they are expected to show fast larval development as we observed in the remaining four instars.

In nature, both larvae and pupae experience and resist drought, as stream drying does not terminate emergence of imagines in *M. lateralis* and other caddisflies (also see Bohle, 2000). Another life history characteristic of potential importance is the observed shift from mineral to organic case material in final instars. While this behaviour is not unique, organic cases can hold water more efficiently than mineral cases (Zamora-Muñoz & Svensson, 1996). Hence, the shift in building material could increase the species' ability to exploit intermittent habitats and present an advantage for both final instars and pupae. This also applies to the final instars of *S. permistus* and *M. sequax* that use organic or mixed mineral/organic cases.

In conclusion, our results show that the studied limnephilid caddisfly species *M. lateralis* as well as *M. sequax* and *S. permistus* present an exceptional case of resource accumulation that differs from the commonly observed pattern in both terrestrial and aquatic insects. This result highlights the importance of species-specific investigations of energy storage management during insect development in order to capture the full diversity of energetic strategies. Moreover, our findings represent a promising baseline for future comparative studies in order to understand the link between energy storage and drought adaptation. We further emphasize the need

for continued studies of the complex life histories of insects inhabiting highly dynamic systems such as intermittent streams.

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References

Arrese, E. L. & J. L. Soulages, 2010. Insect fat body: energy, metabolism, and regulation. Annual Review of Entomology 55: 207–225.

Becker, J., C. Ortmann, M. A. Wetzel, C. Winkelmann & J. H. E. Koop, 2013. Mate guarding in relation to seasonal changes in the energy reserves of two freshwater amphipods (*Gammarus fossarum* and *G. pulex*). Freshwater Biology 58: 372–381.

Beenakkers, A. M., D. J. Van der Horst & W. J. Van Marrewijk, 1985. Insect lipids and lipoproteins, and their role in physiological processes. Progress in Lipid Research 24: 19–67.

Boggs, C. L., 1981. Nutritional and life-history determinants of resource allocation in holometabolous insects. American Naturalist 117(5): 692–709.

Bohle, H. W., 2000. Anpassungsstrategien ausgewählter Organismen an temporäre Wasserführung-Insekten periodischer Fließgewässer Mitteleuropas. Gewässer ohne Wasser 5: 53–71.

Bouvet, Y., 1976. Ecologie et reproduction chez les Trichoptères cavernicoles du groupe de *Stenophylax* (Limnephilidae, Stenophylacini). In Proceedings of the first International Symposium on Trichoptera. Springer, Netherlands: 105–109.

Bouvet, Y., 1978. Adaptations physiologiques et camportementales des *Stenophylax* (Limncphilidae) aux eaux temporaires. In Proceedings of the 2nd International Symposium on Trichoptera. Springer Netherlands: 177–179.

Campos, D., C. Gravato, C. Quintaneiro, O. Koba, T. Randak, A. M. Soares & J. L. Pestana, 2016. Are insect repellents toxic to freshwater insects? A case study using caddisflies exposed to DEET. Chemosphere 149: 177–182.

Chippindale, A. K., A. G. Gibbs, M. Sheik, K. J. Yee, M. Djawdan, T. J. Bradley & M. R. Rose, 1998. Resource acquisition and the evolution of stress resistance in *Drosophila melanogaster*. Evolution 52(5): 1342–1352.

Crawley, M. J., 2007. The R book. Wiley, New York: 425–430.

Crichton, M. I., 1971. A study of caddis flies (Trichoptera) of the family Limnephilidae, based on the Rothamsted Insect Survey, 1964-68. Journal of Zoology 163(4): 533–563.

Denis, C., 1976. Données sur la chronologie de la maturation ovarienne et sur la diapause de *Micropterna sequax* Mc Lachlan (Trichoptera: Limnephilidae). Bulletin de la Societe Scientifique de Bretagne XLIX: 125–129.

Denis, C., 1978. Larval and imaginal diapauses in Limnephilidae. In Proceedings of the 2nd International Symposium on Trichoptera. Springer Netherlands: 109–115.

Djawdan, M., A. K. Chippindale, M. R. Rose & T. J. Bradley, 1998. Metabolic reserves and evolved stress resistance in *Drosophila melanogaster*. Physiological and Biochemical Zoology 71(5): 584–594.

Dyar, H. G., 1890. The number of molts of lepidopterous larvae. Psyche: A Journal of Entomology 5(175–176): 420–422.

Graf, W., J. Murphy, J. Dahl, C. Zamora-Munoz & M. J. López-Rodríguez, 2008. Distribution and ecological preferences of European freshwater organisms. Volume 1. Trichoptera (Vol. 1). Pensoft Publishing.

Haase, P., M. Frenzel, S. Klotz, M. Musche & S. Stoll, 2016. The Long-term Ecological Research (LTER) network: relevance, current status, future perspective and examples from marine, freshwater and terrestrial long-term observation. Ecological Indicators 65(1): 1–3.

Harrison, J. F., H. A. Woods & S. P. Roberts, 2012. Ecological and environmental physiology of insects, Vol. 3. Oxford University Press, Oxford.

Hebert, P. D., A. Cywinska & S. L. Ball, 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London B: Biological Sciences 270(1512): 313–321.

Kaufmann, C. & M. R. Brown, 2008. Regulation of carbohydrate metabolism and flight performance by a hypertrehalosaemic hormone in the mosquito *Anopheles gambiae*. Journal of Insect Physiology 54(2): 367–377.

Karlsson, B. & P. G. Wickman, 1989. The cost of prolonged life: an experiment on a nymphalid butterfly. Functional Ecology 3: 399–405.

Keppler, D. & K. Decker, 1984. Glycogen. In Bergmeyer, H. U. (ed.), Methods of enzymatic analysis, Vol. 6. Verlag Chemie, Weinheim: 11–17.

Law, J. H. & M. A. Wells, 1989. Insects as biochemical models. Journal of Biological Chemistry 264(28): 16335–16338.

Malicky, H., 2004. Atlas of European Trichoptera/Atlas der Europäischen Köcherfliegen/Atlas des Trichoptères d'Europe. Springer, Dordrecht: 359.

Mondy, N., E. Cathalan, C. Hemmer & Y. Voituron, 2011. The energetic costs of case construction in the caddisfly *Limnephilus rhombicus*: direct impacts on larvae and delayed impacts on adults. Journal of Insect Physiology 57(1): 197–202.

Novak, K. & F. Sehnal, 1963. The development cycle of some species of the genus *Limnephilus* (Trichoptera). Casopsis Ceskoslovenske Spolecnosti Entomologicke 60: 68–80.

Otto, C., 1974. Growth and energetics in a larval population of *Potamophylax cingulatus* (Steph.)(Trichoptera) in a south Swedish stream. Journal of Animal Ecology 43(2): 339–361.

R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Sawabe, K. & M. Mogi, 1999. Differences in energy metabolism and adult desiccation resistance among three *Aedes*

(*Stegomyia*) species (Diptera: Culicidae) from South Sulawesi, Indonesia. Journal of Medical Entomology 36(1): 101–107.

Schmidt-Kloiber, A., P. J. Neu & W. Graf, 2015. Metadata to the distribution atlas of European Trichoptera. Freshwater Metadata Journal 9: 1–6.

Schmidt-Kloiber, A., P. J. Neu, M. Malicky, F. Pletterbauer, H. Malicky & W. Graf, 2017. Aquatic biodiversity in Europe: a unique dataset on the distribution of Trichoptera species with important implications for conservation. Hydrobiologia, 1–17.

Sehnal, F., 1963. Gesamtstoffwechsel der Insekten. 15. Sauerstoffverbrauch und Änderung der chemischen Zusammensetzung während der larvalen Entwicklung von zwei Köcherfliegenarten (Trichoptera). Acta Societatis Zoologicae Bohemicae 27: 185–201.

Sommerhäuser, M., 1998. Limnologisch-typologische Untersuchungen zu sommertrockenen und permanenten Tieflandbächen am Beispiel der Niederrheinischen Sandplatten (Doctoral dissertation. Rheinische Friedrich-Wilhelms-Universität zu Bonn), Essen: 256.

Stevens, D.J., 2000. Developmental Trade-offs and Resource Allocation in Caddis Flies. University of Glasgow, p. 160 (Unpublished PhD thesis).

Svensson, B. W., 1972. Flight periods, ovarian maturation, and mating in Trichoptera at a South Swedish stream. Oikos 23(3): 370–383.

Tate, L. G. & L. T. Wimer, 1971. Carbohydrate changes during metamorphosis of the blowfly *Phormia regina*. Insect Biochemistry 1(2): 199–206.

Timmermann, S. E. & H. Briegel, 1999. Larval growth and biosynthesis of reserves in mosquitoes. Journal of Insect Physiology 45(5): 461–470.

Waringer, J. & W. Graf, 2011. Atlas der mitteleuropäischen Köcherfliegenlarven—Atlas of Central European Trichoptera Larvae. Erik Mauch Verlag, Dinkelscherben: 468.

Watanabe, M., T. Kikawada, N. Minagawa, F. Yukuhiro & T. Okuda, 2002. Mechanism allowing an insect to survive complete dehydration and extreme temperatures. Journal of Experimental Biology 205(18): 2799–2802.

Wieser, W., 1986. Bioenergetik. Energietransformation bei Organismen. Georg Thieme Verlag, Stuttgart.

Winkelmann, C. & J. H. Koop, 2007. 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*. Journal of Comparative Physiology B 177: 119–128.

Van der Horst, D. J., N. M. D. Houben & A. T. Beenakkers, 1980. Dynamics of energy substrates in the haemolymph of *Locusta migratoria* during flight. Journal of Insect Physiology 26(7): 441–448.

Van Handel, E., 1993. Fuel metabolism of the mosquito (*Culex quinquefasciatus*) embryo. Journal of Insect Physiology 39(10): 831–833.

Zamora-Muñoz, C. & B. O. Svensson, 1996. Survival of caddis larvae in relation to their case material in a group of temporary and permanent pools. Freshwater Biology 36(1): 23–31.

Ziegler, R., 1991. Changes in lipid and carbohydrate metabolism during starvation in adult *Manduca sexta*. Journal of Comparative Physiology B 161(2): 125–131.