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Body stores of emergent aquatic insects are associated with body size, sex, swarming behaviour and dispersal strategies

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Abstract

1. Dispersal is a determining step in the life cycle of insects and a key factor of their energy budget. If the body stores of terrestrial insects are relatively well-documented, those of merolimnic insects (i.e., with aquatic larvae and terrestrial winged adults) remain poorly investigated.
2. We assayed the body stores (protein, carbohydrate and lipid contents) of 18 families of merolimnic insects, encompassing a wide diversity of biological and ecological traits.
3. We highlighted allometry between body stores and mass. The proportion of triglyceride and free carbohydrate relatively decreases with an increase in absolute insect mass, whereas protein and glycogen contents are proportional.
4. We found a significant effect of insect order, Diptera having relatively more proteins than Trichoptera. For swarming taxa, males have relatively more triglycerides than their conspecific female. Passive dispersers are characterised by higher protein and glycogen contents
5. Each body store was associated with specific life-history traits, emphasising the importance of considering each body store independently to better understand the associated functions.
6. Dispersal strategies seem to be the main structuring factor underlying body store patterns of merolimnic insects, being significantly associated with the variation in adjusted triglycerides (30%), proteins (21%), glycogen (29%) and free carbohydrates (17%). However, our study estimated the role of flight only indirectly through the biological traits of insects. Future works should focus on identifying which substrates are used for flight, by comparing the proportion of body stores before and after flight, as well as protein identification to distinguish proteins associated with flight or reproduction. These further studies will help researchers in understanding the link between body stores of emerging merolimnic insects and the various facets of their ecology.

Gerber and Cabon to be considered as joint first author.

KEYWORDS
allometry, aquatic insects, carbohydrates, lipids, proteins

1 | INTRODUCTION

Freshwater ecosystems cover less than 1% of the Earth's surface but host a remarkable biodiversity with more than 140,000 known species, including approximately 88,000 insect species (Balian et al., 2007). Among these insects, 80% are merolimnic––that is their juvenile life stages are confined to freshwater environments while winged adults are terrestrial (Petersen et al., 2004). The flight of imagoes is crucial for the colonisation of new habitats (Kovats et al., 1996; Petersen et al., 2004), maintaining genetic diversity (Didham et al., 2012; Phillipsen et al., 2015) and defining community composition (Bacca et al., 2021; Tonkin et al., 2018). Flight also is important for fitness-related functions such as escaping predators, locating food and reproduction (Amat et al., 2012; Chen et al., 2011; David et al., 2015; Dudley, 2002; Tokeshi & Reinhardt, 1996). Variable flight distances have been reported depending on species, ranging from 160 m for some Ephemeroptera to 17 km for some Chironomidae (Muehlbauer et al., 2014). However, current knowledge on insect flight comes from studies that use heterogeneous methods and target one or a few species at once, leading to limited understanding of flight ability.

One way to approach the flight ability of merolimnic imagoes is to look at their energy content. Flight requires high energy consumption and is a key determinant of the energy budget of insects (Amat et al., 2012). The metabolic rate of insects when in flight can be 50–100-fold higher than their resting metabolic rate, and their flight muscles exhibit the highest metabolic rate of any known animal locomotor tissue (Beenakkers et al., 1984; Downer & Matthews, 1976; Dudley, 2002). Body size is a key constraint for insect metabolism and dispersal strategies. Firstly, the allometric relationship between resting metabolic rate and body mass is widely recognised (Harrison et al., 2014; Maino & Kearney, 2014), and light insects (<10 mg) are expected to consume proportionally less energy during flight than heavy ones (Niven & Scharlemann, 2005). Secondly, light species often are called "passive dispersers" as they are more subjected to wind dispersal (Peredo Arce et al., 2021), when bigger species can fly faster than the wind and choose their flight direction (Compton et al., 2002). Energy comes from the oxidation of one or several energy substrates (i.e., carbohydrates for short flights, to which lipids are added for long flights). Carbohydrates are stored freely in the cytoplasm and in the form of glycogen (Roma et al., 2010). Lipids are stored mostly in the form of triglycerides (Arrese & Soulages, 2010; Winkelmann & Koop, 2007). Some species also oxidise the amino acid proline (Amat et al., 2012; Beenakkers et al., 1984; Tigreros & Davidowitz, 2019). However, this knowledge of insect body stores is based largely on terrestrial species, and studies on emerging merolimnic imagoes are scarce.

Merolimnic insects encompass a large number of species divided into 12 orders (Dijkstra et al., 2014), and represent great variability in terms of phylogenetic relationships, morphology, size and life-history traits. Ephemeroptera, Plecoptera, Trichoptera (EPT) and Diptera account for 85% of all species of merolimnic insects (Dijkstra et al., 2014). Within these orders, Diptera and Trichoptera are phylogenetically the closest and most recent orders, Ephemeroptera is the most ancestral one, and Plecoptera is intermediate (Ishiwata et al., 2011; Yeates et al., 2016). Merolimnic imagoes display variable feeding behaviours, and this may influence energy allocation and the amount of energy stored before their emergence from the water. Diptera and Plecoptera can feed as adults (Armitage, 1995; Brittain, 1990; Burtt et al., 1986; de Figueroa & Sánchez-Ortega, 1999, 2000; Rúa & de Figueroa, 2013), but Trichoptera rarely do so (Morse, 2009; Petersson & Hasselrot, 1994), and Ephemeroptera have no functional mouthparts, relying exclusively on the energy stored during larval development (Jacobus et al., 2019; Sartori & Brittain, 2015). Moreover, merolimnic insects have evolved different mating systems, notably Ephemeroptera and Chironomidae males form swarms (i.e., aggregative flights; Sullivan, 1981) to attract females (Brittain & Sartori, 2009; Harker, 1992; Tokeshi & Reinhardt, 1996). Some Trichoptera families also can swarm (e.g., Hydropsychidae, Leptoceridae) yet this behaviour has never been observed among Plecoptera (Holzenthal et al., 2015). As swarming requires great quantities of energy (Dudley, 2002), males of swarming species could have greater body stores than conspecific females.

In the present work, we studied body store patterns of 18 common families of merolimnic insects belonging to EPT and Diptera. We quantified their free carbohydrate, glycogen, lipid and protein contents, and we searched for variables that might explain variation among species offering a large range of morphology, body size and flying behaviour. The following questions were addressed: Is there allometry between body store and mass of merolimnic species imagoes? Do their body stores vary according to insect order, sex, and/or between passive and active dispersal strategies? We hypothesised that mass strongly influences the quantity of body stores, with small taxa having relatively more stores than big ones. We also hypothesised that flight-related life-history traits (dispersal strategy and mating system) structure most of the pattern in body stores.

2 | MATERIALS AND METHODS

2.1 | Study area and sampling

Samples were taken in Brittany (western France), a region characterised by a patchy landscape of forests, pastures, crop cultures and farming zones under a temperate oceanic climate. Insects were caught using aquatic emergence traps (Cadmus et al., 2016). The traps consisted of a floating pyramidal tent (1 m^2 at its base) made of nylon mesh, with a collecting bottle placed at the top (Raitif et al., 2018). Depending on how the targeted insect groups emerged, the traps were placed either on the water (to catch insects emerging from the water surface such as Chironomidae; Pinder, 1986) or placed half on water and half on riverbanks (to capture insects crawling out of the water to moult such as Plecoptera; Hynes, 1976). The traps were lifted every 24h to minimise energy expenditure after emergence. During the journey back to the laboratory, the insects were placed on ice before freezing.

Emerging merolimnic insects were collected during two sampling campaigns in 2018 (4–25 April; for Leuctridae only) and 2020 (12 March–10 July). Traps were regularly moved along the sampling area to catch species from various microhabitats and were checked nearly every day at the same time. They were carefully emptied of all insects when the sampling could not be done the day before. Emergence traps were settled in four sites close to our institutions: Chênelais (48.503093, −1.598211), Guyoult (48.470309, −1.628374), Petit Hermitage (48.488179, −1.571154) and Gueuche (48.614058, −1.008783). In addition, a citizen science-based sampling was organised to collect insects from four distant sites: Elorn (48.408397, −4.08789), Ruisseau de Condat (47.700761, −2.631534), Arz (47.713978, −2.403896) and Le Loc'h (47.761264, −3.505502). All emergence traps were settled in agricultural landscapes, except Le Petit Hermitage (forested area), and in small, 1–2 order (Strahler's stream order) headwater streams (except a pond at Le Loc'h).

2.2 | Assessment of body stores

2.2.1 | Sample preparation

Insects were sorted, sexed and identified at the family, genus or even species level when possible. For each taxonomic group (i.e., the lowest level of identification for each taxon), body store determination was performed on at least seven replicates and when the sampling allowed it, individuals were taken from different sites. The protocol was designed to maximise the number of species, and therefore intertaxa comparisons. The number of individuals *per* replicate depended on the average mass of the taxon. When insects were too light to be assayed individually (all of the Diptera, Baetidae and Leptophlebiidae), two to 10 individuals of the same sex were pooled in each replicate (Table 1). Each replicate was freeze-dried for at least 24 h and weighed (XP2U Mettler Toledo microbalance, ±0.01 mg) to provide the dry mass.

2.2.2 | Body store assays

The insects were placed in phosphate buffer (300 and 600 μl for replicates below and above 500 μg, respectively), homogenised in a bead-beating tube for 1 min 30 s at 25 Hz, and centrifuged for 5 min at 500 ***g*** and 4°C. Protein and glycogen assays were realised according to Andrade et al. (2020). The total protein content was assayed with the Bradford method (Bradford, 1976) using bovine serum albumin as a standard. The free carbohydrate and glycogen contents were assayed with the Anthrone method. Additionally to Andrade et al. (2020), we assayed the free carbohydrates as suggested by Foray et al. (2012). Briefly, we collected 150 μl of the initial supernatant of each insect sample into a microplate well and evaporated the well contents for 120 min at room temperature until a volume of approximately 10 μl was reached. Then, 240 μl of anthrone reagent was added to each well and the plate was incubated for 15 min at room temperature and then heated 15 min at 90°C in a water bath, after which the absorbance of the samples was read at 625 nm using D-glucose as the standard. Triglycerides were assayed using the EnzyChrom™ Triglyceride Assay Kit (cat. no. ETGA-200), following the manufacturer's instructions.

We expected that body stores will not be proportional to insect mass (i.e., allometric relationships between body stores and mass). Therefore, data analyses were performed on the body store mass (mg) adjusted by allometric relationships computed for each type of body stores (proteins, free carbohydrates, glycogen, and triglycerides). For pooled replicates (i.e., small species), the mean individual body store mass was used.

3 | DATA ANALYSES

In our analyses, each body store was considered individually or pooled by fuels (glycogen, triglyceride and free carbohydrate) or total body store (fuels + proteins). As proteins are the main constituent of insect muscles (Vigoreaux, 2006) and flight muscles can constitute up to 65% of the body mass (Marden, 2000), we used protein content as a proxy for flight muscle. A larger quantity of flight muscle is associated with a better flight endurance (Marden, 2000).

Three explanatory factors of body stores were considered: (1) insect order; (2) mating system, including sex and swarming behaviour; and (3) dispersal strategy, including passive and active dispersal according to the DISPERSE database (Sarremejane et al., 2020). We ran tests to check for a possible effect of sampling sites on the quantity of body stores (see Appendix S1).

3.1 | Allometric analyses

Analysis of allometry followed the procedure proposed by Warton et al. (2006). To determine the relationship between body stores and body mass, we conducted standardised major axis (SMA) regression (model 2 regression) with the "smatr" package "sma" function (Warton et al., 2012). SMA is a least squares method (the regression line is estimated by minimising the sum of squares of residuals) but unlike linear regressions, the direction in which residuals are measured is not vertical. The SMA is the line that minimises the sum of squares of the shortest distances from the data points to the line, calculated on standardised data, then rescaled to the original axes (Warton et al., 2006). To use independent data, the analyses were carried out between the body store categories as response variables and the remaining body mass (i.e., the individual dry mass minus the measured body store mass) as explanatory

TABLE 1 Results of the sampling effort showing the level of taxonomic identification and the sampling design for each taxon

Order	Family	Genus	Species	Replicates per sites	Replicates per sex, ♀:♂	Individuals per replicate	Dispersal strategies	Swarming behaviour	References for swarming behaviour
Diptera	Chironomidae	*Conchapelopia*	-	Guy (8)	4:4	2	Weak	Swarm	Bay, 1993; Vallenduuk & Pillot, 2007
	Chironomidae	*Micropsectra*	-	Loc (20)	10:10	5	Passive	Swarm	Bay, 1993; Vallenduuk & Pillot, 2007
	Chironomidae	*Phaenopsectra*	-	Guy (20)	9:11	5	Passive	Swarm	Bay, 1993; Vallenduuk & Pillot, 2007
	Chironomidae	*Polypedilum*	-	Loc (8)	4:4	2	Passive	Swarm	Bay, 1993; Vallenduuk & Pillot, 2007
	Chironomidae	*Rheotanytarsus*	-	Her (9)	5:4	10	Passive	Swarm	Bay, 1993; Vallenduuk & Pillot, 2007
	Empididae		-	Loc (9) Guy (8)	4:13	2/5[a]	Weak	Swarm	Downes, 1969; Courtney & Cranston, 2015
	Sphaeroceridae	-	-	Guy (2) Herm (5) Mix (3)	4:6	4	NA	NA	-
Epheme-roptera	Baetidae	*Baetis*	-	Che (2) Her (6) Mix (2)	7:3	2	Active	Swarm	Jackson, 1988
	Ephemerellidae	*Serratella*	*ignita*	Che (1) Elo (6) Con (2)	5:4	1	Active	Swarm	Brittain & Sartori, 2009
	Leptophlebiidae	*Habrophlebia*	-	Guy (10)	4:6	2	Weak	Swarm	Brittain & Sartori, 2009; Martynov, 2016
Plecoptera	Chloroperlidae	-	-	Elo (4) Con (3)	7:0	1	Weak	No swarm	Harrison & Dobson, 2008
	Perlodidae	*Isoperla*	*grammatica*	Arz (6) Gue (2) Elo (1)	6:3	1	Weak	No swarm	Harrison & Dobson, 2008
	Leuctridae	*Leuctra*	-	Her (7) Con (1)	4:3	1	Active	No swarm	Harrison & Dobson, 2008
	Nemouridae	*Nemoura*	-	Guy (10)	9:1	1	Weak	No swarm	Harrison & Dobson, 2008
Trichoptera	Glossosomatidae	*Agapetus*	-	Che (6) Gue (2) Her (2)	6:4	1	Weak	No swarm	Nijboer, 2004
	Hydropsychidae	*Hydropsyche*	-	Gue (1) Elo (1) Her (9)	6:5	1	Active	Swarm	Malik et al., 2008; Müller-Peddinghaus, 2011
	Lepidostomatidae	*Lepidostoma*	*hirtum*	Her (9) Con (1)	4:6	1	Active	No swarm	Gullefors & Petersson, 1993
	Limnephilidae	*Limnephilus*	-	Arz (1) Loc (6)	5:2	1	Active	No swarm	Gullefors & Petersson, 1993
	Polycentropodidae	*Polycentropus*	-	Elo (3) Her (3)	6:0	1	Active	Swarm	Müller-Peddinghaus, 2011
	Psychomyiidae	*Lype*	-	Her (10)	5:5	1	Weak	Swarm	Müller-Peddinghaus, 2011
	Rhyacophilidae	*Rhyacophila*	*dorsalis*	Arz (1) Che (1) Elo (7) Her (1)	6:4	1	Active	No swarm	Gullefors & Petersson, 1993
	Sericostomatidae	*Sericostoma*	-	Che (6) Her (4)	4:6	1	Weak	No swarm	Gullefors & Petersson, 1993

Note: The column “Swarming behaviour” refers to whether insects undergo swarming behaviour before mating (swarm) or not (no swarm). The column “Replicates per site” indicates which site was sampled, and the numbers in brackets indicate how many replicates per site. Che, Chênelais; Guy, Guyoult; Her, Petit Hermitage; Gue, Gueuche; Elo, Elorn; Con, Ruisseau de Condat; Arz, Arz; Loc, Le Loc'h; Mix, pooled replicates of individuals from different sites. The column “Dispersal strategies” is based on the DISPERSE database (see Sarremejane et al. (2020) for details). Rows correspond to the taxa considered in analyses. “-” means identification was not performed.

[a] Number of individuals per replicate can be two or five depending on the replicate.

variable, both logarithmically transformed. The "sma" function allows us to calculate the regression lines between body stores and the remaining body mass for taxonomic groups. As the data are logarithmically transformed, the slope *b* of the regression line is equal to the allometric coefficient (see Peig & Green, 2009, for details). If the slope $b = 1$, the link between body stores and mass is isometric (i.e., increasing proportionally with the absolute increase in size). If $b > 1$, light individuals have relatively less body stores than heavy individuals (hyper-allometry). If $b < 1$, light individuals have relatively more body stores than heavy individuals (hypo-allometry).

In order to investigate the allometry intertaxa, a first series of regressions was performed on the mean by taxonomic groups to get the coefficient *b* for each body stores. The overall body store slope was compared to metabolic rate coefficients (2/3 and 3/4) by testing the correlation between residual and fitted values. Then, in a second series of regressions, we tested for differences in slopes between orders (using the mean of body stores by taxonomic groups) and between sexes (using the mean of body stores by taxonomic groups AND sex), using the Bartlett-corrected likelihood ratio statistic. When differences were detected, pair-wise comparison tests were run. When no differences were detected (i.e., there is a common slope), between-order and between-sex differences were tested by calculating the differences between the regression lines for elevations (i.e., if significant, regression lines are separated by a gap indicating differences in body store quantity for a same body mass) and for shifts (i.e., if significant, regression lines are separated along a parallel axis and indicate a difference in the body store quantity due to a difference in body mass; see Warton et al., 2006 for details). Taxa with only one male or one female (Table 1; Nemouridae and Chloroperlidae among Plecoptera; Polycentropodidae among Trichoptera) were excluded from the sex analyses. We also explored the allometry intrataxa by conducted a third series of SMA regression within the taxonomic groups (Table 2).

Data on each body store category were allometrically adjusted as follows, considering the mean of each body store by taxonomic group ($n = 22$; Table 1), or by taxonomic group and sex, when investigating the mating system. The allometrically adjusted ratio used is $\bar{Y}_i = Y_i / X_i^b$ (Albrecht et al., 1993): for each individual i, $\bar{Y}_i$ is the allometrically adjusted body store ratio (which will be used in the further analyses); Y_i, the mass of the considered body store and X_i the body mass. The allometric coefficient *b* (i.e., slope) was obtained from the first series of SMA regressions.

TABLE 2 Coefficients of standardised major axis (SMA) regression between body stores and the remaining mass for each taxonomic group

				Overall body stores		Fuel		Proteins	
Order	**Family**	**Genus**	**Species**	**Slope**	***p***	**Slope**	***p***	**Slope**	***p***
Diptera	Chironomidae	*Conchapelopia*	-	0.70	0.004	−0.5	0.964	1.34	0.02
	Chironomidae	*Micropsectra*	-	1.21	0.001	−0.33	0.369	2.01	0
	Chironomidae	*Phaenopsectra*	-	0.55	0.112	0.29	0.912	2.29	0.122
	Chironomidae	*Polypedilum*	-	0.54	0.008	−0.55	0.228	1.62	0.037
	Chironomidae	*Rheotanytarsus*	-	0.55	0.19	0.28	0.256	2.18	0.05
	Empididae	-	-	1.02	0.034	0.84	0.144	1.7	0.692
	Sphaeroceridae	-	-	0.8	0.381	0.53	0.794	2.49	0.213
Ephemeroptera	Baetidae	*Baetis*	-	NA	NA	−0.62	0.24	NA	NA
	Ephemerellidae	*Serratella*	*ignita*	NA	NA	NA	NA	−1.79	0.881
	Leptophlebiidae	*Habrophlebia*	-	0.48	0.171	0.39	0.842	1.92	0.461
Plecoptera	Chloroperlidae	-	-	NA	NA	NA	NA	2.21	0.29
	Perlodidae	*Isoperla*	*grammatica*	2.1	0.695	−0.38	0.071	3.73	0.671
	Leuctridae	*Leuctra*	-	1.1	0.006	0.32	0.011	2.82	0.019
	Nemouridae	*Nemoura*	-	0.71	0.001	0.14	0.064	1.58	0.004
Trichoptera	Glossosomatidae	*Agapetus*	-	0.7	0.605	0.61	0.115	2.13	0.877
	Hydropsychidae	*Hydropsyche*	-	0.56	0.019	0.78	0.214	0.64	0.021
	Lepidostomatidae	*Lepidostoma*	*hirtum*	2.37	0.401	1.1	0.804	5.58	0.258
	Limnephilidae	*Limnephilus*	-	NA	NA	NA	NA	−0.23	0.843
	Polycentropodidae	*Polycentropus*	-	0.82	0.018	0.23	0.746	1.33	0.016
	Psychomyiidae	*Lype*	-	NA	NA	0.19	0.942	NA	NA
	Rhyacophilidae	*Rhyacophila*	*dorsalis*	1.5	0.017	1.3	0.014	1.83	0.017
	Sericostomatidae	*Sericostoma*	-	1.26	0.139	0.86	0.013	2.18	0.425

Note: The columns "slope" represent allometric coefficient of the regression. The columns "*p*" give the significance of the regressions. Numbers in green represent significant linear regressions (i.e., $p < 0.05$).

3.2 | Principal component analysis of body stores

A principal component analysis (PCA) was performed on adjusted ratios, with free carbohydrate, glycogen and triglyceride and proteins as variables using the "FactomineR" package (Lê et al., 2008). Five missing data (NA) were treated by the "estim_npcPCA" function of the "missMDA" package (Josse & Husson, 2016). The npc value (i.e., the number of components to be retained for the PCA) given by this function was 0, meaning that NAs had to be substituted by the mean value of each variable. As the data were normalised, NAs were replaced by zeros. The coordinates of the first two principal components were used as response variables, and the factors of the PCA projections (order and dispersal) were used as explanatory variables. Active and passive dispersals were combined in three modalities: active dispersal (affinity to active dispersal = 2 or 3, eight taxa), passive dispersal (affinity to passive dispersal = 2 or 3, four taxa) and weak dispersal (affinity to active and passive dispersals = 0 or 1, eight taxa). Shapiro–Wilk tests and Bartlett's tests were conducted to evaluate the assumptions of normality and homoscedasticity. When heteroscedasticity or non-normality were detected, Kruskal–Wallis tests were performed and when assumptions were met, ANOVA tests were used. When necessary, Dunn's *post hoc* tests were performed using the "FSA" package to test pairwise differences (Ogle et al., 2021). The alpha levels were adjusted using the Holm–Bonferroni correction to correct for multiple comparisons.

3.3 | Effect of mating system on body store patterns

We tested the differences of body stores between sexes for swarming taxa and for non-swarming taxa with Wilcoxon–Mann–Whitney tests for paired sample. The same tests were used to compare body stores between sexes for Diptera and Trichoptera as the number of replicates for each order is low (>10). Ephemeroptera and Plecoptera did not have enough replicates (more than five) to make reliable between-sex comparisons. We also compared variation in body stores between males and females in the orders Diptera and Trichoptera.

3.4 | Contribution of insect order, sex and dispersal on body store patterns

In order to determine the relative contribution of each explanatory factor (order, mating system and dispersal strategy) to body store patterns, partitions of variance were performed using the "varpart" function in the "vegan" package (Oksanen et al., 2015). We used three explanatory matrices––one for each factor. As the "varpart" function does not deal with NAs and as flight ability data were not available for Sphaericidae (Diptera), this taxon was removed from the variance partitioning. A total of six partitions of variance were performed, one for each body store, one by combining fuels and one combining all body stores. The analyses were conducted on the mean by taxa and sex. Significance of each explanatory factor was checked using ANOVA tests.

All statistical analyses were performed with R software v.4.1.1 (R Core Team, 2021).

4 | RESULTS

We sampled 556 merolimnic imagoes, representing 22 taxa of 18 families and four orders (Diptera, Ephemeroptera, Plecoptera and Trichoptera; Table 1).

4.1 | Allometry of body stores

By considering all body stores together (sum of glycogen, triglycerides, free carbohydrates and proteins), a significant hypo-allometry was detected (b = 0.70; Figure 1a). Light individuals had relatively more body stores than heavy individuals. The allometry coefficients did not differ from the 3/4 nor 2/3 metabolic rates (p = 0.418, r = 0.19, df = 15 and p = 0.481, r = 0.21, df = 15, n = 17, respectively).

Owing to the small number of Ephemeroptera (three taxa), their allometric coefficients could not be statistically compared with other orders (i.e., Diptera, Plecoptera and Trichoptera). For Plecoptera, only proteins were significantly correlated with the remaining mass of individuals. Consequently, the protein allometric coefficients of Plecoptera, Diptera and Trichoptera were compared, but the fuels (the sum of glycogen, triglycerides and free carbohydrates) and overall body store coefficients were compared between Diptera and Trichoptera only.

For both overall body stores and fuels, Diptera and Trichoptera shared the same allometric relationships (common slope b = 0.86; p = 0.608, likelihood ratio statistic = 0.99, df = 2, n = 13 and b = 0.63; p = 0.196, likelihood ratio statistic = 3.26, df = 2, n = 14, respectively). Diptera had more overall body stores for a given mass than Trichoptera (higher elevation: p = 0.044, Wald statistic = 4.05, df = 1; lower shift: p = 0.001, Wald statistic = 10.88, df = 1; Figure 1a). No difference in elevation was detected for fuels (p = 0.685, Wald statistic = 0.16, df = 1, n = 14), but Trichoptera had an absolute higher fuel content than Diptera, as a consequence of their higher mass, (higher shift, p < 0.001, Wald statistic = 12.45, df = 1, n = 14; Figure 1b).

For proteins, Diptera, Trichoptera and Plecoptera had the same allometric relationships (common slope b = 1.05, p = 0.411, likelihood ratio statistic = 1.78, df = 2, n = 18; Figure 1c). Differences in elevation were detected, together with a shift between Trichoptera and Diptera only. Diptera had more proteins for a given mass than Trichoptera (higher elevation: p = 0.029, pairwise test statistic = 2.59; lower shift: p = 0.004, pairwise test statistic = 10.99).

When investigating allometry intrataxa, nearly half of the taxonomic groups exhibited significant linear regressions for the overall

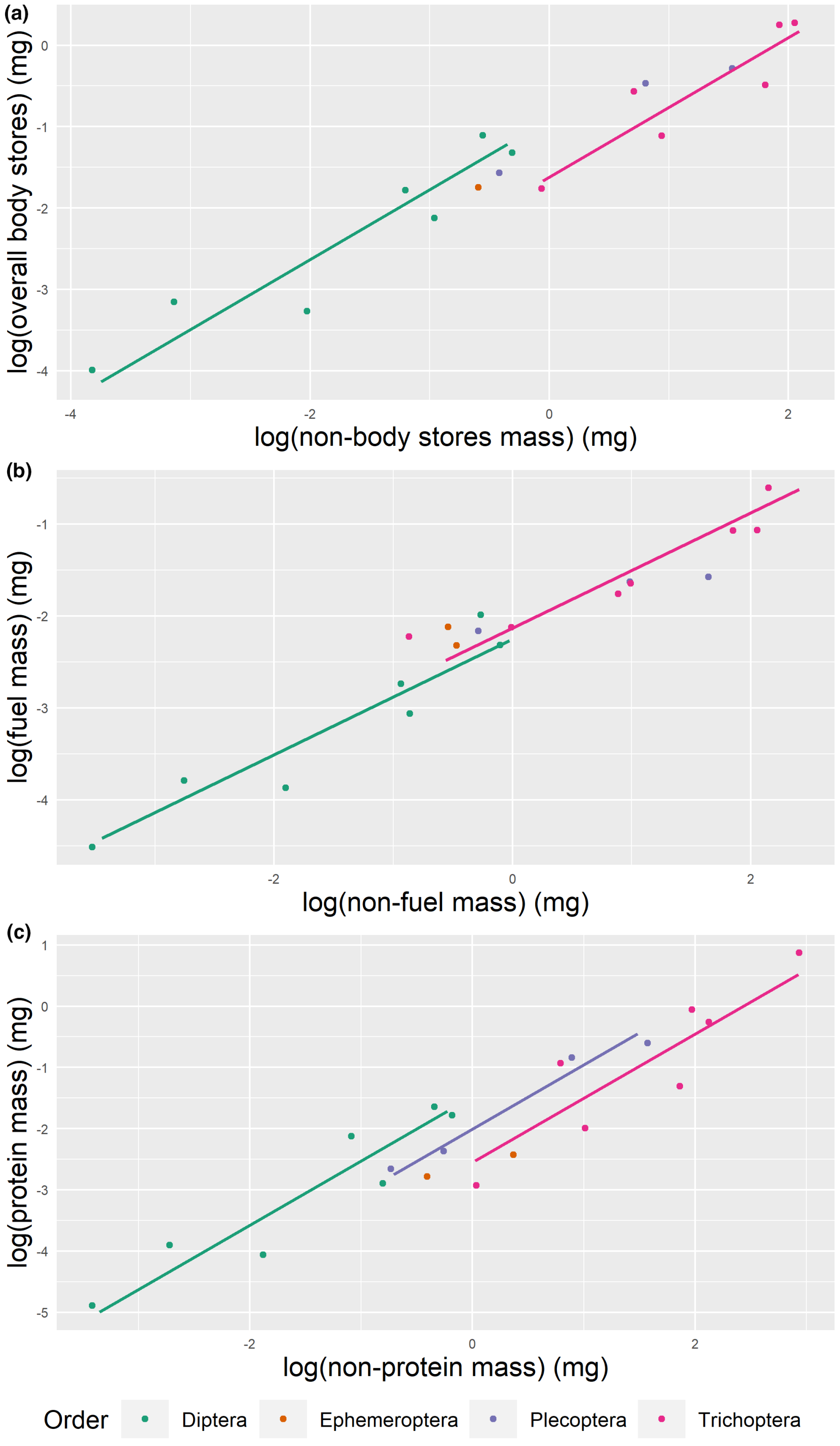

FIGURE 1 Standardised major axis (SMA) regression by order between the logarithms of body stores (mg) and the remaining body mass for the mean of each taxa. (a) SMA regression on all body stores, with the coloured lines for each order. Diptera and Trichoptera share a common slope ($b = 0.86$) and they differ in elevation ($p = 0.044$) and in shift ($p = 0.001$). (b) SMA regression on fuel content (i.e., sum of mass of glycogen, free carbohydrates and triglycerides). Diptera and Trichoptera share a common slope ($b = 0.63$) and differ in shift ($p < 0.001$). (c) SMA regression on proteins. Diptera, Trichoptera and Plecoptera share a common slope ($b = 1.05$). Diptera and Trichoptera differ in elevation ($p = 0.016$) and in shift ($p = 0.03$). No significant correlations are observed for Ephemeroptera and for Plecoptera for regressions on the overall body stores and the fuels; therefore, the regression lines are not shown.

body stores and the proteins, whereas we found only three taxa with significant allometry for the fuel ($p < 0.05$; Table 2). For the overall body stores, no allometric pattern was visible (i.e., the slope indicated either hypo-, iso- or hyper-allometry depending on the taxa). For the proteins, seven of eight taxa with significant repressions exhibited a hyper-allometry (i.e., light individuals have relatively less proteins than heavy individuals).

4.2 | Site effect

The site effect for overall body stores, fuels, proteins, triglycerides and free carbohydrates was tested as random term. For glycogen, the site effect was added directly as explanatory factor. Sites did not have a significant effect on body stores (Table 3). Although there is no overall effect, trends are observed on the dotplots (Figure 2),

TABLE 3 Significance of the site effect for each body stores

	Overall body stores	Fuels	Proteins	Triglycerides	Free carbohydrates	Glycogen
p-values	0.08	0.06	0.16	0.06	0.46	0.96
Likelihood ratio statistic	3.13	3.41	2.02	3.5	0.53	1.93

Note: Mixed-effects models were used with adjusted body stores were used as response variables, taxa, sex and their interactions as explanatory variables, and site as random effect. For Glycogen, we used a generalised linear model with taxa, sex and their interactions, and site as explanatory variables.

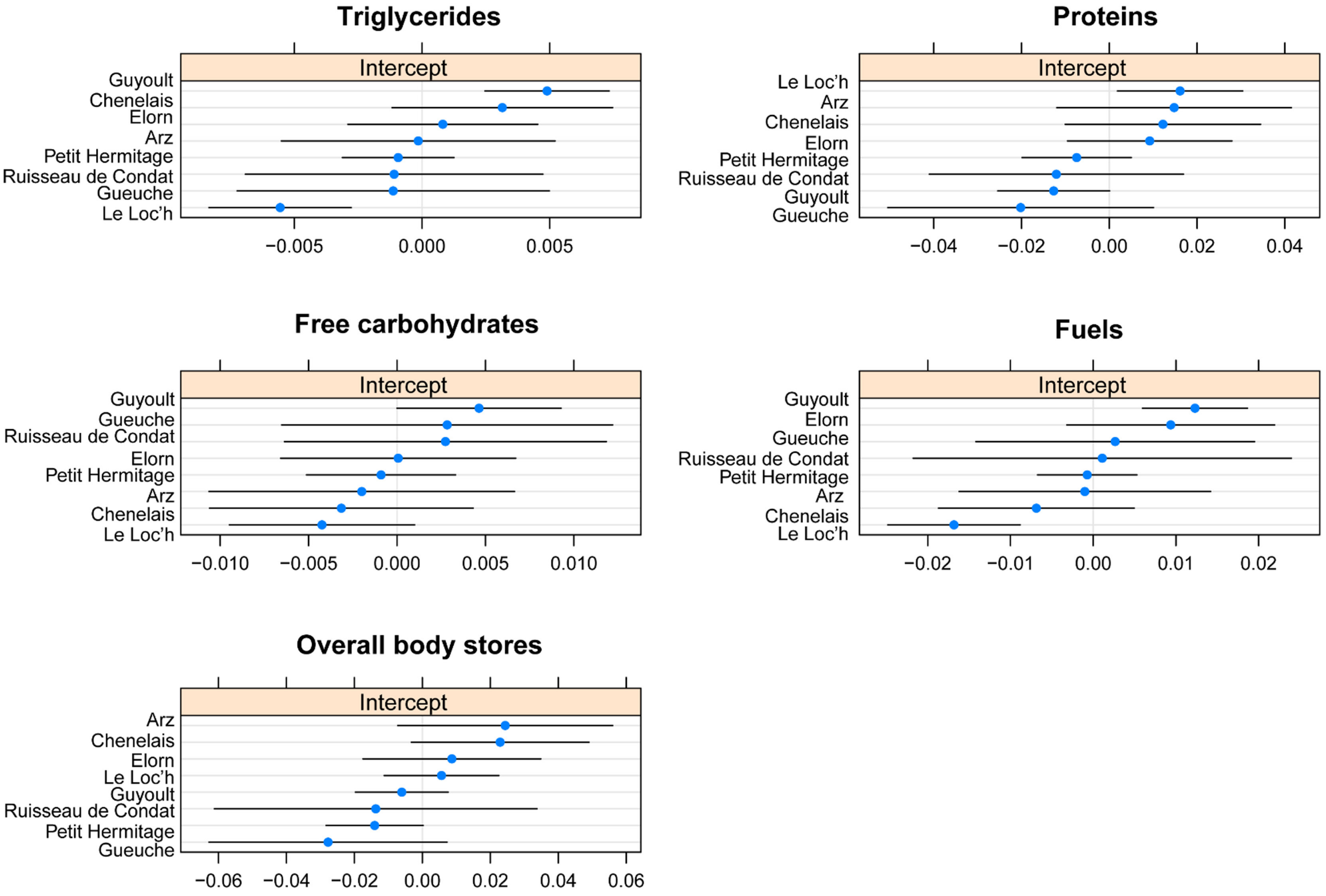

FIGURE 2 Dotplot of the conditional modes of site effect as random term for each body stores. Conditional mode is the difference between the average predicted response and the response predicted for a particular individual. Blue dots, conditional values; black lines, conditional SDs.

especially for Triglycerides and Fuels. In particular, the "Le Loc'h" site differs from the "Guyoult" site. These differences could be explained by variations in taxonomic composition.

4.3 | Relationship between body stores and insect order

The first three principal components of the PCA (PC1, PC2 and PC3) explained 41.7%, 29.1% and 22.9% (respectively) of the total variance of body stores (Figure 3a). Free carbohydrate content contributed mainly to PC1 (34.3%). Adjusted triglyceride contributed mainly to PC2 (54.3%), and adjusted glycogen to PC1 (23%), PC2 (29.8%) and PC3 (25.6%). Adjusted proteins contributed at 26.8% to PC1 and 62.4% to PC3. Diptera, Ephemeroptera, Trichoptera and Plecoptera separated along PC1, but only Diptera and Ephemeroptera differed significantly on PC1 (pairwise comparison Dunn test: $p = 0.026$, Z-statistic $= 2.85$; Figure 3b).

4.4 | Relationship between body stores and dispersal

Passive and weak dispersal significantly differed on PC1 (pairwise comparison Dunn test: $p = 0.005$, $Z = 3.12$; Figure 3c).

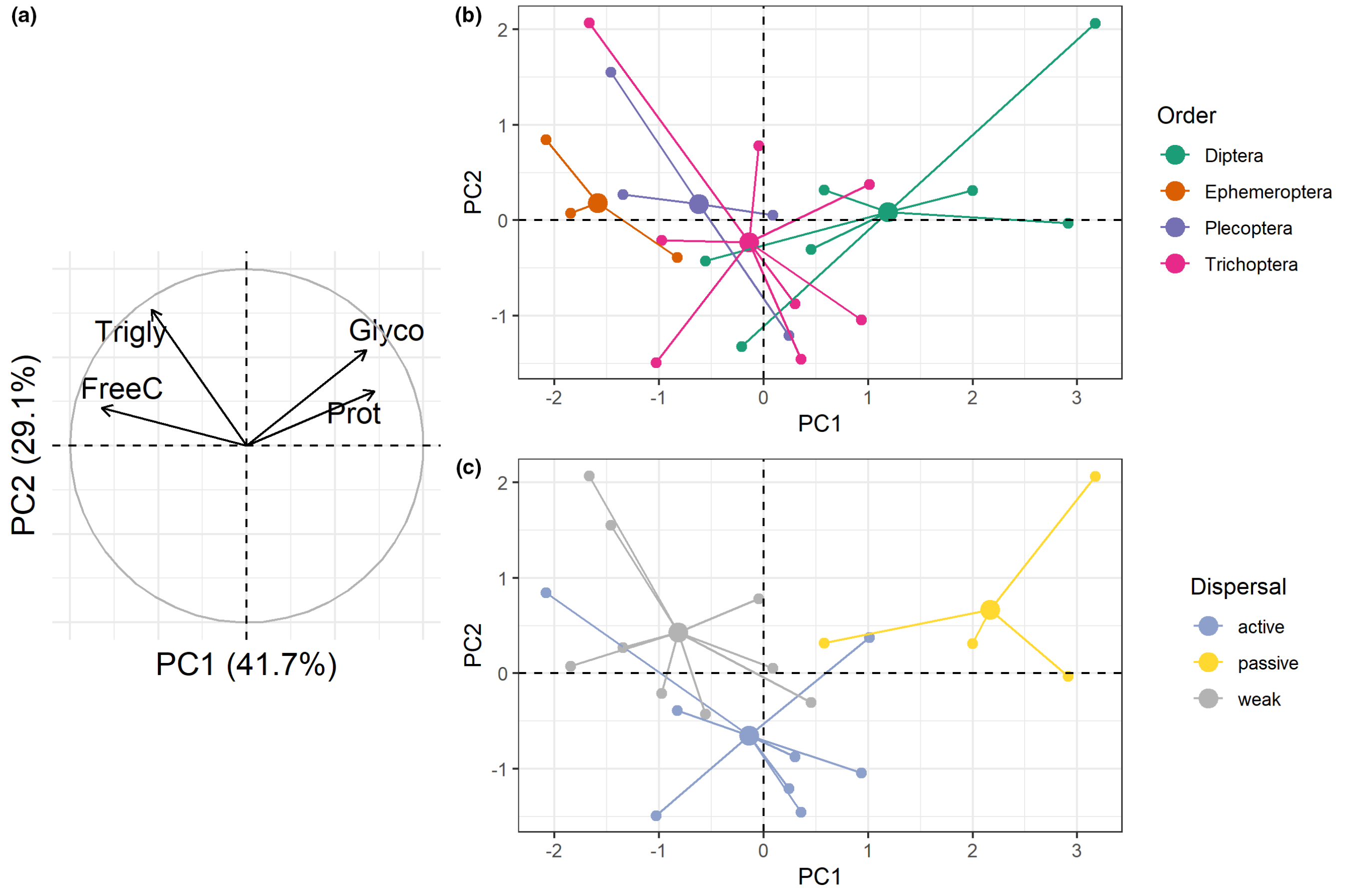

FIGURE 3 Principal component analysis (PCA) of the merolimnic insect body stores. (a) Correlation circle showing correlations among body stores (Prot, proteins; Glyco, glycogen; free C., free carbohydrates; Trigly, triglycerides) and between body stores and PCA axes. Projections of insects on PCA axes according to (b) Insect orders (Diptera; Ephemeroptera; Plecoptera; Trichoptera) and (c) Dispersal affinity. Body stores are allometrically adjusted.

By testing the influence of dispersal strategies on each body store, we found that passive dispersers had significantly lower adjusted triglycerides than weak disperser (pairwise comparison Dunn test: $p = 0.050$, $Z = -2.40$). Active and weak dispersers had less adjusted glycogen than passive dispersers (pairwise comparison Dunn test: $p = 0.013$, $Z = -2.85$ and $p = 0.053$, $Z = 2.22$, respectively). Passive dispersers tended to have more proteins than active ones (pairwise comparison Dunn test: $p = 0.053$, $Z = -2.22$). However, when considering directly the adjusted overall body stores, fuels and free carbohydrates, no difference between dispersal strategies were detected.

4.5 | Relationship between sex and body stores

When considering the allometry between males and females, no significant differences in slope nor shift were detected for total body stores (Figure 4a). Overall, males had more fuel and triglycerides than females (difference in elevation: $b = 0.64$, $p < 0.001$, Wald statistic = 11.72, $df = 1$ [Figure 4b]; $b = 0.59$, $p = 0.019$, Wald statistic = 5.51, $df = 1$, $n = 38$ [Figure 4e], respectively). By testing swarming and non-swarming taxa separately, we found that males of a given taxon had more free carbohydrates and fuel than their conspecific female, independently of their swarming behaviour (Wilcoxon–Mann–Whitney tests for paired sample, $p < 0.05$). However, for swarming taxa only, males of a given taxon had more triglycerides than their conspecific females (Wilcoxon–Mann–Whitney tests for paired samples, $p = 0.002$, V-statistic = 1).

Between-sex differences also were mediated by insect order. For Diptera and Trichoptera, females had less adjusted fuel, and free carbohydrates than males (Wilcoxon–Mann–Whitney tests for paired samples: $p < 0.05$). However, female Diptera also had more adjusted proteins and less triglyceride than males (Wilcoxon–Mann–Whitney tests for paired sample, $p < 0.031$).

4.6 | Contribution of insect order, mating system and dispersal strategy

For the adjusted overall body stores, none of the explanatory factors were significant (ANOVA, $p > 0.05$, $n = 28$). Thus, their relative contributions could not be interpreted. Mating system significantly explained 26% of the fuel variation (ANOVA: $p = 0.013$, $F = 4.29$, $df = 3$, $n = 28$; Figure 5a). Dispersal strategy is the only significant factor for explaining variation of adjusted triglycerides (30% of

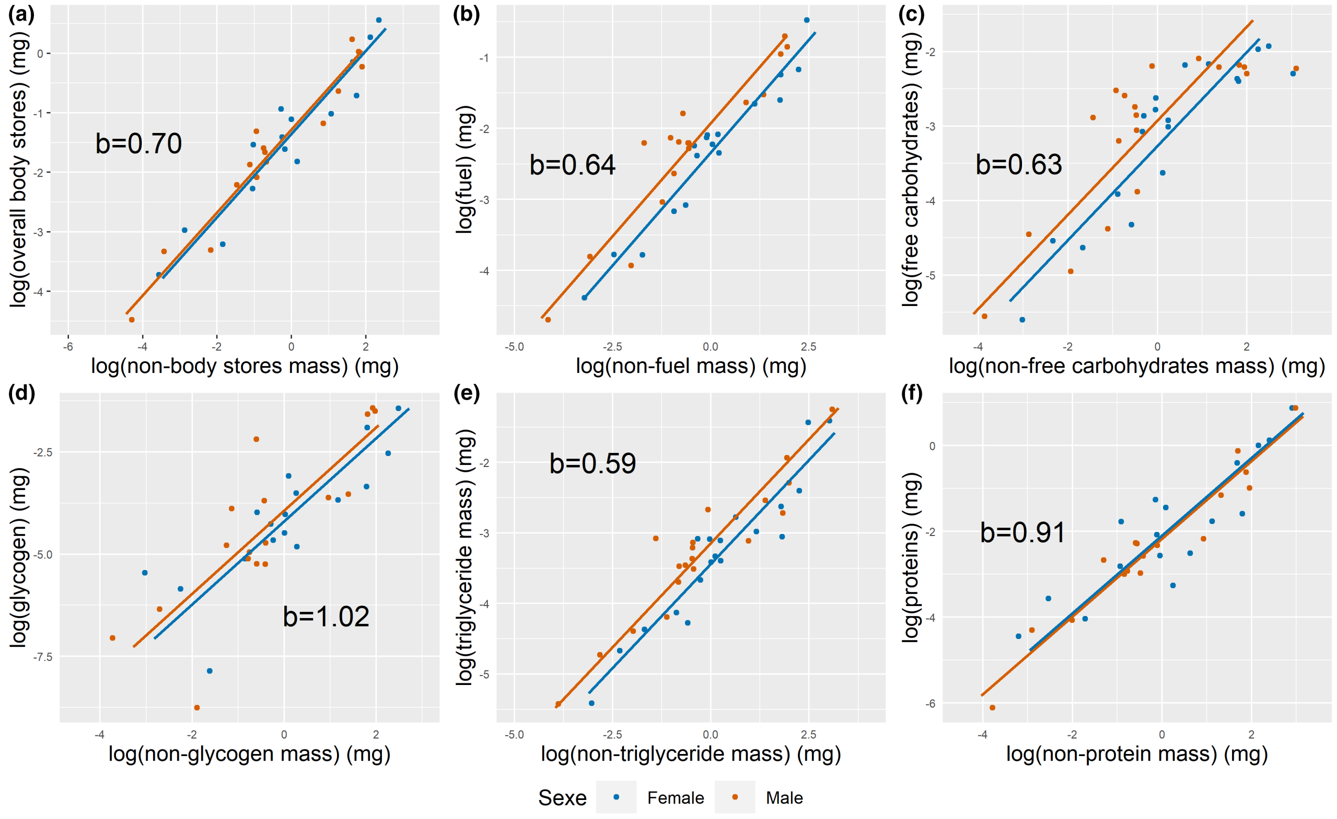

FIGURE 4 Standardised major axis (SMA) regressions by sex between the logarithms of body stores (mg) and the remaining body mass for each individual. (a) Sum of all body stores; (b) Fuels––male and female differ in elevation ($p < 0.001$); (c) Free carbohydrates; (d) Glycogen; (e) Triglycerides–male and female differ in elevation ($p = 0.019$); (f) Proteins. No differences in slope nor shift

variance explained, ANOVA: $p = 0.004$, $F = 6.84$, $df = 2$, $n = 28$; Figure 5b) and the variation of adjusted glycogen (29% of variance explained, ANOVA: $p = 0.007$, $F = 6.59$, $df = 2$, $n = 28$; Figure 5e). It also explained 17% of the free carbohydrate variation (ANOVA: $p = 0.033$, $F = 3.83$ $df = 2$, $n = 28$; Figure 5d). Insect order explained 38% of the free carbohydrate variation (ANOVA: $p = 0.038$, $F = 6.61$ $df = 3$, $n = 28$).

Altogether, the three factors significantly explained the adjusted proteins (Figure 5c). Mating system explained 36% (ANOVA: $p = 0.002$, $F = 6.11$ $df = 3$, $n = 28$), dispersal strategies explained 30% (ANOVA: $p = 0.018$, $F = 4.55$ $df = 2$, $n = 28$) and order explained 20% (ANOVA: $p = 0.014$, $F = 4.95$ $df = 3$, $n = 28$). Nevertheless, a large part of the variance remained unexplained (from 32% [Figure 5b] to 82% [Figure 5e]). Variation jointly explained by two or three factors (i.e., where the circles overlap) were not considered here and can be positive or negative, which explains why the sum of the variations for each factor do not always match with the total variance explained.

5 | DISCUSSION

In this study, we present a novel dataset on body stores of proteins, carbohydrates and triglycerides from >550 imagoes belonging to 22 taxa and four major orders of merolimnic insects, Diptera, Ephemeroptera, Plecoptera and Trichoptera. Firstly, we found that, according to their mass, small-bodied insect taxa stored a higher proportion of fuelling substrates than heavier insects, highlighting allometry between body stores and dry mass. This was particularly true for Trichoptera and Plecoptera, for which a hypo-allometry relationship was observed, indicating that light insects had relatively more energy stores than heavier insects. Previous studies stated that light insects consumed less energy during flight than heavy insects because they have a lower mass-specific flight metabolic rate (i.e., the rate of energy consumption in flight divided by the body mass; Niven & Scharlemann, 2005). Based on maximum power production relative to the power required to maintain steady flight (Hepburn et al., 1998), recent studies on bees showed that light individuals had more available power during flight than large ones (Duell, 2018; Grula et al., 2021; Helm et al., 2021), suggesting that body stores of light insects might be reduced without affecting their flight ability. Yet, we observed the opposite pattern, suggesting that allometric variation in overall body stores is not only linked to the energetic cost of flight for merolimnic taxa.

When considering each body store independently, light insects exhibited relatively more free carbohydrates and triglycerides than heavy insects. For glycogen and proteins, the relationship between body stores and size was close to isometric: glycogen and proteins increase proportionally with body size. Generally, fuel stored is greater for small insects and the quantity of muscle (i.e., proteins) remained proportional to the size. Interestingly, the resting metabolic rate (i.e.,

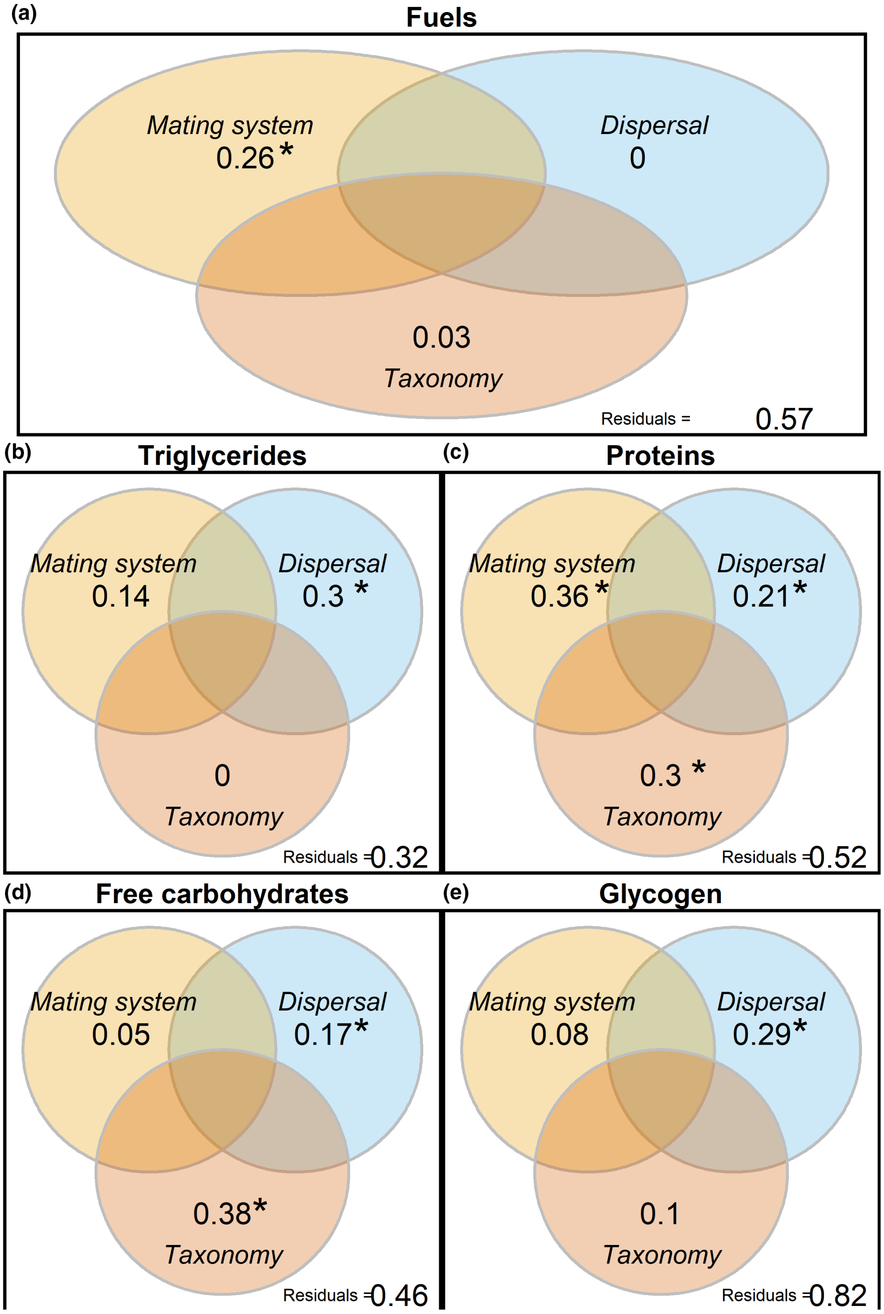

FIGURE 5 Analyses of variance partitioning from allometrically adjusted body stores showing the relative contribution of the effect of the mating system (in yellow), dispersal mode by flight (i.e., active or passive) (in blue) and order (in orange) in explaining body store strategies. Values are the proportion of variance explained by each factor, including the fractions shared with other factors (i.e., where the circles overlap). Values of the fractions shared with other factors are not shown. Residuals represent the unexplained variation. (a) Fuels, (b) Triglycerides, (c) Proteins, (d) Free carbohydrates and (e) Glycogen. Each number on the coloured section indicates the explained variance (%) by each combination of variables. Residuals show the unexplained variance. Values lower than 0% are considered as 0. Note: *When ANOVA tests are significant ($p < 0.05$).

the rate of energy consumption at rest) has long been recognised to scale hypo-allometrically with mass. For insects, the resting metabolic rate scales to mass$^{0.66–0.9}$, depending on the metabolic models (Harrison et al., 2014). Again, these results suggested that the relative increase of fuel in a light insect is not linked to the cost of flight, but rather to the relative increase of their resting metabolic rate.

We also looked at variation of allometry within each taxon. Even if the sampling did not enable us to highlight systematic allometric relationships among the body stores within our taxonomic groups, the results suggest that, within species, the protein contents increased relatively with size. As shown in different taxonomic groups (Green et al., 2001), the intraspecies allometry for proteins seemed to differ from the interspecies allometry.

When removing the allometry effect, we showed that Diptera had relatively more overall body stores, in particular more adjusted proteins, than Trichoptera. Insect order partly explains the body store patterns. It can be hypothesised that between-order variations result from the diversification of feeding habits from the ancestral group of Ephemeroptera, for which imagoes do not feed, to the more recently evolved Diptera that present a wide range of feeding habits (Courtney et al., 2017). Johnson et al. (2018) showed that diversification of feeding habits may strongly contribute to the diversification of insects, especially in Paraneoptera. The diversification of feeding resources may dramatically change the nutritional quality of food (Arrese & Soulages, 2010) and consequently change the body store patterns of merolimnic insects. Variations among taxonomic groups also could be explained by environmental conditions. Two studies emphasised the potential role of environmental variation on triglycerides and glycogen contents for three species of Ephemeroptera (Koop et al., 2008; Winkelmann & Koop, 2007). In particular, water temperature, which is known to influence the size of the emerging adult, should be considered in further study of body store patterns (Lytle, 2002; Sweeney & Vannote, 1978).

We found differences in adjusted body stores between males and females. At comparable size, males contained more fuels, specifically more triglycerides, than females. Differences between males and females have been reported already in the literature. For example, Sartori et al. (1992) showed that males of *Siphlonurus aestivalis* (Ephemeroptera, Siphlonuridae) consumed lipids for their swarming flights whereas females did not use lipids for flight. Indeed, males of swarming species fly considerably longer than females, resulting in a greater energy consumption (Dudley, 2002; Lancaster & Downes, 2013). We found that males of swarming taxa contained more triglycerides than their conspecific females, contrary to non-swarming taxa. This suggests that the energy substrates used for the swarm would be mainly triglycerides. In particular, within Diptera males had more long-flight energy substrates (i.e., fuels, triglycerides) and energy availability (i.e., free carbohydrates circulating in the haemolymph directly available; Amat et al., 2012). Therefore, males should be able to fly longer since long flights first demand glycogen to initiate flight, then only lipids are subsequently required (Beenakkers et al., 1984; Chapman et al., 2013; Tigreros & Davidowitz, 2019). This ability to maintain flight longer could be used for swarming, as all the Diptera in our study can swarm. The high energy content found in males of swarming taxa and the high energy cost of maintaining flight suggest that body stores may be strongly driven by sexual selection. Thus, swarming behaviour could structure a part of the body stores, especially the triglycerides.

In our study, passive dispersers had relatively more proteins and glycogen (a fuel for flight) than weak dispersers and active dispersers. Mostly represented by small chironomids in our study, passive dispersers need to constantly beat their wings to maintain flight (i.e., they cannot glide to save energy power; Bhat et al., 2019). The highest proportion of glycogen (i.e., a common flight fuel for Diptera; Dudley, 2002) could help chironomids to extended flight and dispersal. According to Armitage (1995), the action of wind on swarming males may disperse populations away. In addition to reproductive success, we can hypothesise that the higher quantity of triglycerides in male Chironomidae could improve their dispersal ability. Nevertheless, chironomids colonise new habitats predominantly through the passive dispersal of fertilised females (Armitage, 1995). This result is corroborated by McLachlan (1985) who showed that females have wider wings than males, allowing extended flight. This is consistent with the high protein content found in chironomid females, associated with more muscle (Vigoreaux, 2006) and flight endurance (Marden, 2000).

We expected active dispersers to have more fuel than weak dispersers, but weak dispersers had relatively more triglycerides. Potentially, triglycerides are allocated to other functions such as reproduction, but this has yet to be investigated. The typology of the dispersal strategies used here allowed us to analyse general trends of dispersal. However, these categories can be questioned on several aspects; for example, dispersal can vary within the same species depending on the population (Bonada & Dolédec, 2018). Even though these hypotheses need to be consolidated, our results highlight that physiological studies can help understand the conclusions of studies based solely on insect dispersal distances.

Furthermore, our study shows how body storage strategies could result in a trade-off between insect order, mating systems and dispersal. These factors did not explain the variation of the overall body stores. But each adjusted body store was partly explained by at least one factor (i.e., insect order, mating system and dispersal), thereby reinforcing the link between physiology and life-history traits. Interestingly, adjusted flight fuels, glycogen and triglycerides are well-explained by dispersal, which is in line with the importance of flight in the energy stores of insects (Amat et al., 2012). Moreover, we do not find the same patterns between overall flight fuels and each constituent (i.e., triglycerides, free carbohydrates and glycogen), suggesting the use of different types of body stores for reproduction depending on the species. Adjusted proteins are well-explained by combination of the mating system, the dispersal strategy and the insect order. It is congruent with the diversity of protein use, either fuel for flight (Bursell, 1963), muscle components (Marden, 2000) or fuel for reproduction (Chapman et al., 2013; Hahn et al., 2008). For example, we found that Diptera females had more adjusted proteins than males, highlighting an important role of proteins for reproduction (Chapman et al., 2013). Future work should be planned to identify the type of proteins and ascertain whether proteins serve as energy reserves for flight or reproduction or as constituents of muscle tissue.

Finally, we showed that a large part of variance of body stores remained unexplained (from 32% to 82% according to the type of body store), indicating that there are other influential variables that were not accounted for in this study. For instance, merolimnic insects differ in terms of voltinism (Bonada & Dolédec, 2018), life duration, period of emergence in the year (Kjær et al., 2021) and morphological traits, all of which can influence body stores. In addition to life-history traits, environmental factors might influence body store patterns within taxa (Shipley et al., 2012; Slansky Jr. & Haack, 1986), but also between taxa (Koop et al., 2008; Mathieu-Resuge et al., 2021). Very little information is available on the biology and ecology of merolimnic imagoes especially at the local scale where variability among populations can be high (Bonada & Dolédec, 2018).

6 | CONCLUSIONS

Our results on body storage strategies of emerging imagoes belonging to a large set of merolimnic insect families indicate that the body stores scaled allometrically with mass. This could be explained by the resting metabolic rate as it scales with insect body mass at the same magnitude. We also highlight a differentiation of body stores depending on order and sex, overall males had a greater amount of body stores than females. Finally, each body store is explained by a different life-history trait emphasising the need to choose which body store to measure depending on the question being addressed. Flight-related factors (i.e., mating system through swarm and dispersal) structured most of the body stores. To better investigate the body storage strategies of insects, future works should focus on identifying flight substrates and protein identification. Linking body stores of emerging merolimnic insects to the various facets of their ecology also is challenging.

AUTHOR CONTRIBUTIONS

Conceptualisation: BB, JM, CP. Developing methods: RG, BB, JM, CP. Conducting the research: LC, RG, CP. Data analysis: LC, RG. Data interpretation LC, RG, BB, JM, CP. Preparation figures & tables: LC, RG. Writing LC, RG, BB, JM, CP.

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DATA AVAILABILITY STATEMENT

Data are available from the authors upon reasonable request.

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