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**„Diversity, microhabitat distribution and life cycles
of Trichoptera in the Schreierbach (Lunz, Lower
Austria)“**

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Abstract

Trichoptera are among the most diverse and widespread macrozoobenthos taxa in freshwater ecosystems. Their high adaptability enables caddisfly larvae to colonize virtually all types of freshwater habitats, thereby triggering the specific evolution of various feeding strategies, such as shredders, grazers, predators and filter feeders. Therefore, species-specific indicator values can be used to understand river habitat functioning and community composition patterns. Moreover, their high bioindicator potential can be used in ecological assessment procedures addressing anthropogenic pollution and river fragmentation whereas cold-adapted species, e.g., *Drusus* spp., are ideal target species for studying the effects of ongoing global change.

The site of the present study is the Schreierbach, a calcareous mountain stream, which has been a focus of research since the emergence studies of Malicky (1972-1980). This stream with a constant water temperature of about 6°C is a high gradient, oxygen rich, summer-cold headwater. Re-sampling the stream with standardized multi-habitat sampling and DNA barcoding allowed the comparison of the datasets and provided a backbone for future studies. We could show that Trichoptera abundance and community composition is driven mostly by hydraulic forces, whereas limnochemical parameters did not differ significantly between study sections due to buffering by groundwater upwelling. We also recorded species-specific and even instar-specific, choriotope preferences. Most Trichoptera were found in microlithal and macrophytes, with strongly decreasing abundances from megalithal to xylal, mesolithal, macrolithal and akal choriotopes. In general, significant higher abundances were recorded in the upstream section of the Schreierbach. Ecometrics based on the Trichoptera community of the Schreierbach indicate a hypocrenal to epirhithral stream reach lacking any pollution. Since the 1980ies, high stream temperature peaks have become more frequent. This, however, did not affect the Trichoptera species richness as most species were detected again in the present study (2017). At the same time our results suggest that relative species abundances changed drastically. Species with well-synchronised life cycles emerging before the onset of low summer discharge increased in abundance, whereas species with acyclic life cycles, e.g. *Rhyacophila* spp., suffered severe relative abundance losses when compared with the studies conducted in the last century.

1. Introduction

The order of Trichoptera represents the sister taxon of the Lepidoptera within the monophylum of Amphiesmenoptera. As their scientific name suggests (Greek: *trichos* = hair), the wings of Trichoptera (Greek: *pteron*= wing) are covered by fine hairs instead of the scales in Lepidoptera. About 14,500 species have been identified worldwide, and still much more species are expected. In Europe, more than 1700 species are known; in Austria, 310 species are reported so far (Malicky, 2013; Neu *et al.*, 2018).

First paleontological records of caddisflies date back 250 million years. It was not until the Jurassic age (203-135 million years before now) that fossil wing or larval case records disclose their way to freshwater habitats (Wiggins, 2004; Holzenthal *et al.*, 2007). Trichoptera are now among the most diverse and widespread macrozoobenthos taxa in aquatic systems with only few exceptions to aquatic larvae restricted to humid soil habitats (Holzenthal *et al.*, 2007; Malicky, 2013). Due to the special characteristics of water, these habitats are more predictable than terrestrial ecosystems and enhance adaptations of organisms to biotic interactions rather than other habitat types (Wiggins, 2004; Schwoerbel & Brendelberger, 2012).

The presence of caddisflies in almost all freshwater habitats is due to different reasons.

The ability to secrete silk from the tip of the labium allowed Trichoptera to occupy many vacant niches in freshwater ecosystems (Mackay & Wiggins, 1979; Hauer & Lamberti, 1996; Wiggins, 2004). The high adaptability of the larvae conferred through this capacity is one of the reasons that Trichoptera can now be found in all macrohabitats along a freshwater habitat continuum (Malicky, 1987; Hauer & Resh, 1996; Waringer & Graf, 2011; Malicky, 2014b; Wichard, 2015). The proteinaceous silk is used in distinctive ways for each species. However, three major architectonic strategies can be distinguished: case construction (Integripalpia and 2 out of 4 Spicipalpia families), retreat construction (Annulipalpia) and cocoon construction (remaining Spicipalpia families) (Wiggins, 2004; Holzenthal *et al.*, 2007). Furthermore, various feeding strategies, such as shredders, grazers, predators and filter feeders, can be identified in this insect order (Wiggins, 2004). Since Trichoptera species have different case making strategies, they have a wide range of habitat preferences. While some species prefer slow flowing or even stagnant water to feed on filamentous algae, others are adapted to fast flowing cool headwater springs and are rather predacious or grazers of diatoms (Mackay & Wiggins, 1979). In combination with silk secretion, however, also adaptations to cope with dissolved oxygen, nutrients, discharge and water temperatures are important in Trichoptera diversification (Mackay & Wiggins, 1979; Poff, 1997; Wiggins, 2004; Brand & Miserendino,

2012). These abiotic variables are frequently interlinked with each other. Temperature, as a basic habitat variable, affects ecosystems and life on earth at the molecular level, in fluid dynamics, in the solubility of gases and at the organismic level by regulating metabolic rates (Hauer & Lamberti, 1996). In fact, the specialization of species for certain habitats, and diverse feeding strategies can be used for bioindication and assessment procedures in applied ecology (Mackay & Wiggins, 1979; Malicky, 1987; Poff, 1997; Graf *et al.*, 2008; Moog, (Ed.) 2017). Malicky (2013 & 2014b) suggested the possibility to characterize freshwater habitats by solely examining the caddisfly species composition.

Nevertheless, morphological and molecular species level determinations are often problematic, and additional ecological and life cycle information may be helpful (Resh & Rosenberg, 2010). Identification to species level is crucial because it greatly increases information content (Waringer, Graf & Malicky, 2013). Species-specific indicator values are fundamental to fully understand river habitat functioning and community composition patterns. The fitness of single species is of special interest as climatic conditions change rapidly, anthropogenic pollution increases and river fragmentation alters the riverine systems (Barnett, Adam & Lettenmaier, 2005; Brown, Hannah & Milner, 2007; Botter *et al.*, 2013).

Due to a well-established body of literature Trichoptera are reliable bioindicators in all types of aquatic habitats. Moreover, contemporary identification tools for larvae (e.g., Waringer & Graf, 2011) enable bioindication using the pre-imaginal stages, which constitute an important fraction of their life cycle (Wiggins, 2004). Furthermore, studying larvae with only limited dispersal abilities when compared with imagines increases the information yield with respect to habitat preferences (Cantonati, Gerecke & Bertuzzi, 2006). In fact, larvae provide good insights into this multi-dimensional space of microhabitats that changes in the course of seasons and between larval instars (Lampert & Sommer, 2007). Besides hydrological conditions and life cycles, also feeding behavior of single species and biotic interactions are important drivers of species communities (Wallace & Webster, 1996; Poff, 1997; Brown, 2003). Feeding ecology is closely related to the central trophic position of Trichoptera (Hauer & Lamberti, 1996), which covers a wide range within Trichoptera species and, in the case of Drusinae, also within the same genus (Waringer, Graf & Pauls, 2007; Waringer *et al.*, 2010; Pauls *et al.*, 2008; Vitecek *et al.*, 2015). In addition, Trichoptera are important players in the transfer of nutrients from aquatic to terrestrial ecosystems (Wallace & Webster, 1996). For example, dry periods can be avoided by timing emergence and synchronizing life cycles. This is often observed in Trichoptera species worldwide (Resh & Rosenberg, 1989; Brand & Miserendino, 2012).

Trichoptera have been well studied in Europe over long decades and were constantly used for routine river monitoring (Krawany, 1928, 1930; Malicky, 1979, 1980, 2013, 2014a; Waringer, 1986).

When comparing the findings of past decades with contemporary investigations, community shifts and habitat adaptations become obvious. This is of great importance for river monitoring programs and the assessment of river classifications. Moreover, future scenarios can be modeled with regard to the changing climatic conditions. Understanding species migration patterns and habitat requirements is crucial for nature conservation programs (Wessely *et al.*, 2017). For such comparisons pristine ecosystems are of great interest. The Schreierbach, for example, is such a stream situated in the Lunz area, on the western slope of the Großer Hetzkogel in a calcareous catchment. The spring belongs to the type of rheocrenes and opens in a high-gradient, oxygen-rich channel. High current velocities add to the heterogeneity of choriotores, and the significant input of organic materials in the stream creates a multitude of ecological niches (Mackay & Wiggins, 1979; Vannote *et al.*, 1980; Hauer & Lamberti, 1996) which are reflected by a high Trichoptera species richness (Mackay & Wiggins, 1979; Wiggins, 2004; Hannah *et al.*, 2007b; Kløve *et al.*, 2014). Moreover, this stream is classified as a summer-cold headwater and is characterized by a very narrow annual water temperature range of 5-8°C (mean= 6.3°C; (Zwick & Zwick, 2010; Schwoerbel & Brendelberger, 2012; Malicky, 2014b). In springs, water temperature is generally very constant and equivalent to mean annual air temperature of the site. Nevertheless, diurnal and annual temperature oscillations, caused by solar radiation, are important for stream organisms, e.g. for the timing of emergence (Hauer & Lamberti, 1996; Lampert & Sommer, 2007; Schwoerbel & Brendelberger, 2012). Global warming may influence this established mechanisms in a negative way; in fact, water temperatures in the water bodies of the Lunz area, a location with a long tradition in limnological research (Schiemer, 2014), increased rapidly since 1980, especially in summer (Kainz *et al.*, 2017).

For this thesis, the Schreierbach was selected as study site. Whereas many studies on Trichoptera and other aquatic insects were conducted in the Lunz area over the last decades (Krawany, 1928, 1930; Malicky, 1976, 1979, 1980, 1989, 2013, 2014a; Waringer, 1986; Zwick & Zwick, 2010), there also exist reference data for the Schreierbach proper: Malicky (1979, 1980, 2014b) constructed a greenhouse of 4.5 m² over a section of the Schreierbach. The greenhouse permitted samples of emerging caddisflies in regular intervals.

Today, identification based on morphological characters is often supported by DNA barcoding methods (Hebert *et al.*, 2003a). The Trichoptera barcode initiative: a strategy for generating a species-level Tree of Life (Zhou *et al.*, 2016) aims on a combined phylogeny of

caddisflies which is constantly updated, improved, and provided also the basis for subsequent phylogenetic work at subfamily level (e.g., Pauls *et al.*, 2008; Vitecek *et al.*, 2015). Therefore, the Barcode Of Life Data System (BOLD) initiative, a workbench for DNA barcoding records, was founded (Ratnasingham & Hebert, 2007). The branches of this initiative try to record all animals, plants and fungi in a country, like the “Austrian Barcode of Life”-initiative (ABOL; <http://www.abol.ac.at>; Zimmermann *et al.*, 2013; Szucsich, 2015). Barcoding enabled the attempt to reveal investigating caddisfly feeding behavior in the present thesis, and the results were also uploaded to the ABOL database. Unfortunately, this shift from morphological to genetic identification methods goes along with diminishing taxonomical expertise (Boero, 2001, 2010; Resh & Rosenberg, 2010). Experts, however, are urgently needed for supplementing barcoding with morphological identifications to species level (Waringer *et al.*, 2013), although this is possible only in adults and last instar larvae.

In a fast changing world, organisms are heavily influenced, especially by the climate, land cover and land use (Parmesan, 1999; Hering *et al.*, 2004; Barnett *et al.*, 2005; Thomas, 2005; Conrad *et al.*, 2006; Thomas *et al.*, 2009; Bona *et al.*, 2008; Domisch *et al.*, 2011; Suter & Cormier, 2015; Thomas 2016; Hallmann *et al.*, 2017; Wessely *et al.*, 2017; Ulseth *et al.*, 2018). In addition, freshwater organisms will have to cope with shifts in river flow regimes due to altering precipitation patterns and faster snowmelt runoff (Barnett *et al.*, 2005; Botter *et al.*, 2013; Berghuijs, Woods & Hrachowitz, 2014). Resampling the Schreierbach with the standardized multi-habitat-sampling method allows long-term comparisons with data published forty years ago and provide the basis for possible future studies in the stream. The rationale of the present investigation can be summarized, therefore, in the following basic questions:

- 1) Is there a significant impact of hydraulic and limnochemical parameters on caddisfly abundance?
- 2) Is there a difference in larval Trichoptera abundance between and within choriotores of the three sections sampled in the Schreierbach?
- 3) Which stream zonation pattern and which saprobic valencies are indicated by the larval Trichoptera community of the Schreierbach? Which morphological feeding guilds dominate?
- 4) Which lifecycle types can be observed in the most abundant taxa?
- 5) Are there differences between the Trichoptera community composition of the Schreierbach when comparing the actual dataset (2017) with data published by Malicky (1979, 1980)?

2. Methods

2.1 Sampling

The pristine Schreierbach, located at the eastern slope of the Großer Hetzkogel (47°50'10"N; 15°04'11"E; 700 m above sea level), is a tributary of the Upper Seebach. This first-order headwater stream joins the Upper Seebach between two power stations situated between the Mittersee and Untersee at Lunz am See (Lower Austria)(Figure 1).

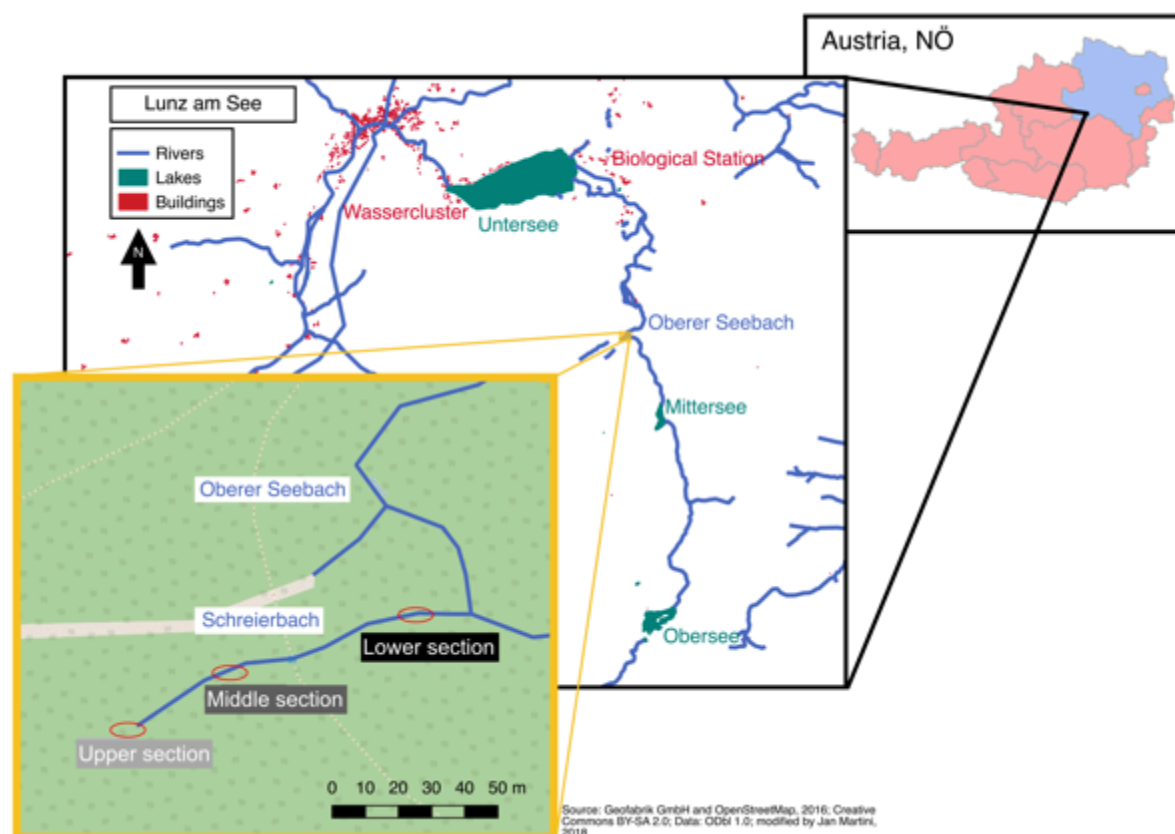


Figure 1: Map of the Schreierbach (Lunz, Lower Austria) and the three sections sampled during 2017.

In order to obtain the caddisfly species inventory and to investigate microhabitats preferences and life history traits, three 10 m sections were chosen as sampling sites for the study. These three sampling sites termed “upper” (728 m a.s.l.), “middle” (708 m a.s.l.) and “lower section” (686 m a.s.l.) were sampled on 12th March, 19th May, 1st July, 28th August, 1st November and 20th December 2017.

At each section and date, twenty biotic samples were taken following the standardised multi-habitat-sampling (MHS) procedure (AQEM Consortium, 2002). Following this guideline, a net (25x25 cm area, 500 µm mesh size) was used to sample each choriotope separately; the number of samples per choriotope reflected its proportional area within each section:

$$\text{Number of samples} = \frac{\text{choriotope percentage area}}{5}$$

Sampling the choriotope individually levels out the overestimations or underestimations of species present in dominant or rare microhabitats. Moreover, for this study a clear differentiation on the choriotope scale enables to assess microhabitat preferences of single species. The choriotope composition was determined once on 12th March using a standard classification scheme by Braukmann (1987) (anorganic choriotope types: Akal [0.2-2 cm], Microlithal [>2-6.3 cm], Mesolithal [>6.3-20 cm], Macrolithal: >20-63cm, Megalithal:>63; organic types Xylal [Twigs, roots, logs >10cm] and Macrophytes [stones covered mostly with mosses or algal mats]) (Table1).

Table 1: Percentage of choriotope composition per section (data from March 2017) within the three sampling sites of the Schreierbach.

Choriotope	section		
	Lower	Middle	Upper
Akal	10%	-	-
Microlithal	20%	30%	10%
Mesolithal	20%	20%	-
Macrolithal	20%	5%	-
Megalithal	-	-	40%
Macrophytes	25%	30%	25%
Xylal	5%	15%	25%

All samples per choriotope were pooled for each section separately. The smaller choriotope (Akal, Microlithal and Mesolithal) were “kick-sampled”. Macrolithal, Megalithal, Xylal and Macrophytes were carefully taken by removing the attached substrate within a 25x25cm surface area with a toothbrush.

The samples were pre-sorted (exclusion of large organic materials and stones > 2cm) prior to bottling of the remaining material. Organisms were preserved in 96% ethanol.

The samples were stored at 1°C until sorting and determination under a stereomicroscope (Nikon SMZU-1000). All organisms were determined to order or higher level (Mollusca, Crustacea, Hydracarina, Collembola, Ephemeroptera, Plecoptera, Diptera, Trichoptera, Coleoptera and Invertebrata varia). Trichoptera, however, were determined to species level whenever feasible, following Waringer & Graf (2011); head width of each individual was measured using an ocular micrometer. Three taxa were subsampled because >500 individuals were present in the samples: *Drusus* juveniles, *Drusus discolor* (Rambur 1842) and

Micrasema morosum (McLachlan 1868); for these three samples, a subsample of 100 individuals was measured.

2.2 Abiotic parameters

Stream velocity (m s^{-1}) was measured with a horizontal-axis Ott current meter at 40% depth within each choriotope type separately. Stream velocity (v) was calculated by using the following calibration equations based on the number of rotations per second (n):

$$v = 0.0586 + 0.0845 * n \quad \text{for } n = 0.22 - 1.21$$

$$v = 0.0374 + 0.102 * n \quad \text{for } n > 1.21$$

Mean stream width (w) was measured with a tape and was based on 5 measurements. The arithmetic mean was then multiplied by mean stream velocity (mean choriotope velocity per section) and mean stream depth (mean choriotope depth per section) to obtain mean stream discharge ($\text{m}^3 \text{s}^{-1}$).

Limnochemical parameters were measured using the following electrodes: Oxygen: WTW, Oxi 3310 IDS, conductivity: WTW, Cond 3310, pH: WTW, pH 3310; two water temperature measurements were taken per section using the electrode's temperature sensor. For continuous water temperature measurements, three data loggers (HOBO Pendant Temp/Light 8K) took data every 12h. The loggers were exposed from 12th March 2017 until 11th April 2018 in the respective sections. The data logger of the lower section was lost in summer 2017 due to a flood event.

2.3 DNA Barcoding

All caddisflies were fixed in 96% Ethanol and identified morphologically using Waringer & Graf (2011) to species level. For DNA barcoding, *Drusus monticola* McLachlan 1876, *Drusus biguttatus* (Pictet 1834), *Metanoea rhaetica* Schmid 1955, *Drusus discolor* and *Drusus chrysotus* (Rambur 1842) were selected as target species. Leg fragments were carefully dissected and used for DNA extraction.

The Drusinae group was chosen because they have very different feeding strategies within the same genus (Pauls *et al.*, 2008; Vitecek *et al.*, 2015). Therefore, we prepared the gut content to get insight into their diet. The Drusinae species chosen were abundant in the Schreierbach. Gut contents of two specimens per species were carefully extracted with tweezers under the stereomicroscope, labelled and frozen until processing.

Molecular genetic analyses and their gut content were conducted by Mag. Dr. Brigitte Gottsberger from the Division of Tropical Ecology and Animal Biodiversity of the University of Vienna. Standard forward (LepF: TTCAACCAATCATAAAGATATTGG) reverse (LepR: AACTTCTGGATGTCCAAAAAATC) primers were used. Both bind to a region of the mitochondrial gene cytochrome oxidase c subunit I (COI), which was selected as marker gene and amplify a 664bp mitochondrial DNA fragment. For the PCR (Polymerase Chain Reaction), the mastermix of Table 2 was used. The protocols of the Analytik Jena innuPrep DNA Micro Kit were followed. The PCR reaction was programmed with the cycles listed in Table 2 and carried out with the Eppendorf Master Cycler.

Agarose gel electrophoresis was performed using a 1 % agarose gel (1.7 g agarose and 1.7 µl GelRed™ Nucleic Acid Gel Stain, 10,000x BIOTIUM in 170 ml TAE (Tris Acetate-EDTA)). Nine out of 14 PCRs were successful fragments and further purified. The amplified reverse and forward DNA fragments were then sequenced with the 48-capillary 3730 DNA Analyser (Thermo scientific, Applied Biosystems) following the Sanger sequencing method by Dr. Michael Barfuss. In Table 2 the sequencing reactions and sequencing programs are listed.

In addition, we used the standard 16S primers (16S0008F: AGAGTTTGATCCTGGCTCAG; 16S1512R: CAGCAGCCGCGGTAATAC) to detect and determine bacteria, which may be present in the biofilm community. The protocol of the Analytik Jena innuPrep DNA Micro Kit was followed. For the PCR reaction the mastermix and adaptation of Table 2 was used and carried out using an Eppendorf Master Cycler. Agarose Gel Electrophoreses and sequencing procedures were the same as with the COI DNA fragments (Table 2). Again, the Sequencing was performed by Dr. Michael Barfuss.

Table 2: Information on the COI and 16S RNA PCR reaction mastermixes, the recipes and the programmes used. Sequencing reagents and sequencing programs were the same for the COI and 16S RNA sequences.

Mastermix (COI)		Mastermix (16S RNA)		PCR- programm (COI)			PCR- Programm (16S RNA)			Sequencing- reagent		Sequencing- programm	
Reagent	µl	Reagent	µl	Cycle	(°C)	h	Cycle	(°C)	h	Reagent	µl	Cycle	h
ddH ₂ O	6.2	Aqua bidest	13	1x	94	04:00	1x	95	03:00	Big Dye	0.5		01:00
10xPCR buffer	1	Solis Firepol	4		94	01:00		95	00:30	Sequencing buffer	1.75		00:10
MgCl ₂ (25 mM)	1	16S0008F [10 pmol µl ⁻¹]	1	5x	44	01:30	40x	56	00:30	ddH ₂ O	3.35	35x	00:05
dNTP (10 mM)	0.2	16S1512R [10 pmol µl ⁻¹]	1		72	01:30		72	00:45	Primer LepF/LepR	0.4		03:00
LepF [10 pmol µl ⁻¹]	0.2				94	01:00	1x	72	10:00	Trehalose	2		
LepR [10 pmol µl ⁻¹]	0.2			35x	49	01:15				DNA (exo)	2		
BSA	0.1				72	01:15							
Taq (5 U µl ⁻¹)	0.1			1x	72	07:00							
DNA extract	1												

The forward and reverse output strands were aligned with SeqMan (DNASTAR, Lasergene), and the sequence was corrected manually for ambiguities. Further comparisons and corrections were made with BioEdit v7.0 (Hall, 2004). The sequences were then compared in GenBank of NCBI nucleotide BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

2.4 Data analysis

While data were sorted and organised with Microsoft Excel 2010, statistical analysis and plots were done in R (R Core Team, 2016).

For life cycle analysis five separate data frames were constructed, including only the respective instars of the nine most abundant species (total abundance >1%). *Micrasema morosum* (24%), *Drusus discolor* (21%), *Drusus monticola* (4%), *Tinodes dives* (Pictet 1834) (2.6%), *Rhyacophila glareosa* McLachlan 1867 (2%), *R. stigmatica* Kolenati 1859 (1%) and *R. tristis* Pictet 1834 (1%). In addition, *Halesus rubricollis* (Picet 1834) (0.6%) and *Acrophylax zerberus* Brauer 1867 (0.5%) were selected in order to include taxa of subfamily Limnephilinae. The rare species were neglected for this part of the study because specimens of single or multiple larval instars were missing. For the abundant species growth plots were constructed by log-transforming the head width (HW) of the caddisflies. By plotting HW against the larval instar, a linear increase in HW is predicted by Dyar's rule (1890). Life cycles of these species were plotted with the package “vioplot” using the row head width data (Adler, 2005).

To assess the impact of abiotic parameters in the Schreierbach at section and choriotope scale we used ANOVA as implemented in R package “stats”(R Core Team, 2016). For stream velocity, variances were compared on the choriotope level, for other abiotic factors (pH, conductivity, temperature, O₂), within-section variances were too small and therefore comparisons were calculated at section level.

Abiotic variables were used to model and analyse the microhabitat preferences and species composition of the caddisflies in the Schreierbach. For evaluating Trichoptera abundance on the section scale we log - transformed the data for a linear mixed effect model (Pinheiro *et al.*, 2017). This lme function allows using abiotic factors as fixed factors and sampling date as random factors. Moreover, the implemented corCAR function outlevels the autocorrelation effects of the repeated sampling design. We also implemented a biotic interaction variable in

the model by calculating non-metric multidimensional scaling (nMDS (Oksanen *et al.*, 2017)). We used the nMDS axis scores of the macrozoobenthos species composition in the stream as proxy of the biotic interaction variable in the lme model.

For assessing species diversity in the Stream and on the choriotope scale we calculated Shannon diversity indices and consulted ordination analysis with the R package “vegan” (Oksanen *et al.*, 2017). The analyses were carried out separately for three different levels (choriotope, choriotope*date, choriotope*date* head width). As in the lme procedure, Canonical Correspondence Analysis (CCA) enabled us to set sampling date as condition to overcome autocorrelation effects. To assess habitat preference and the influence of abiotic factors we made two different CCAs, one with the choriotopes as explaining variables and the other with the abiotic factors implemented. Moreover, the habitat preferences based on species abundance per m² for each choriotope was calculated; choriotopes with highest abundance were defined as preferred habitats.

To calculate the abiotic, biotic and habitat effects on a given instar three CCAs (Oksanen *et al.*, 2017) per instar were performed. The abiotic variables were scaled.

For abiotic factors we used the relationship:

$$\text{CCA-abiotic} = \text{species (x)} \sim \text{abiotic factors, condition (date + section),}$$

for choriotopes:

$$\text{CCA-choriotope} = \text{species (x)} \sim \text{choriotopes, condition (date + section),}$$

with x indicating larval instars 1-5.

The explained variation of the constrained variables was then plotted to compare the influence of the abiotic, choriotope and biotic interaction variables within each larval instar.

Finally, to assess the influence of abiotic factors on the community, a CCA model regarding the single larval instars was performed. In this case the model was simplified by excluding insignificant variables via the step function implemented in the vegan package.

Long-term comparisons with data published by Malicky (1979, 1980) were done by evaluating stream zonation, saprobic valencies and feeding guilds of the Trichoptera

community in 1978, 1979 and 2017 according to 'Fauna Aquatica Austriaca' (Moog, (Ed.) 2017). The two dataframes by Malicky (1979, 1980), due to little differences, were pooled. Moreover, relative species abundances were compared. In this case the shift of the most abundant species was used to assess possible shifts in species community.

3. Results

3.1 Abiotic variables

Conductivity ($\mu\text{S cm}^{-1}$), pH and oxygen (%) changed significantly (O_2_{Date} : $\chi^2=44.23$; $\text{DF}=5$; $p<0.001$; pH_{Date} : $\chi^2=62.08$; $\text{DF}=5$; $p<0.001$; $\text{conductivity}_{\text{Date}}$: $\chi^2=87.57$; $\text{DF}=5$; $p<0.001$) between sampling dates. Oxygen (%) varied significantly between sections ($\text{O}_2_{\text{Section}}$: $\chi^2=39.38$; $\text{DF}=2$; $p<0.001$). Conductivity and pH were insignificantly different between sections ($\text{conductivity}_{\text{Section}}$: $\chi^2=0.91$; $\text{DF}=2$; $p>0.05$; $\text{pH}_{\text{Section}}$: $\chi^2=0.36$; $\text{DF}=2$; $p>0.05$)(Table 3). Overall, the abiotic parameters of the water body of the Schreierbach were found to be quite homogenous.

Table 3: Abiotic parameters (pH, conductivity and O_2) in the three sections (upper, middle and lower) of the Schreierbach in 2017.

Sampling dates	ph			Conductivity ($\mu\text{S cm}^{-1}$)			O_2 (%)		
	Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
12 Mar 2017	8.5	8.5	8.6	209	210	211	101.1	101	100.5
19 May	8.5	8.4	8.3	182	182	182	101.7	102	100.9
01 Jul	8.3	8.5	8.4	202	204	205	104.2	103.8	101.2
28 Aug	8.3	8.3	8.2	228	228	229	103	103.6	101.1
01 Nov	8.4	8.4	8.6	212	212	212	101.9	102.1	101
20 Dec	8.8	8.5	8.5	216	216	216	101.6	101.2	100.4

Mean discharge changed significantly between upper and the middle and lower sections (ANOVA: $\text{DF}=2$; $\text{SS}=1533$; $\text{MSS}=766.4$, $F=6.133$; $p<0.01$) and sampling dates. We noticed a significant decrease in summer and high discharge due to snowmelt in May (ANOVA: $\text{DF}=5$; $\text{SS}=2450$; $\text{MSS}=489.9$, $F=3.92$; $p<0.01$) (Figure 2).

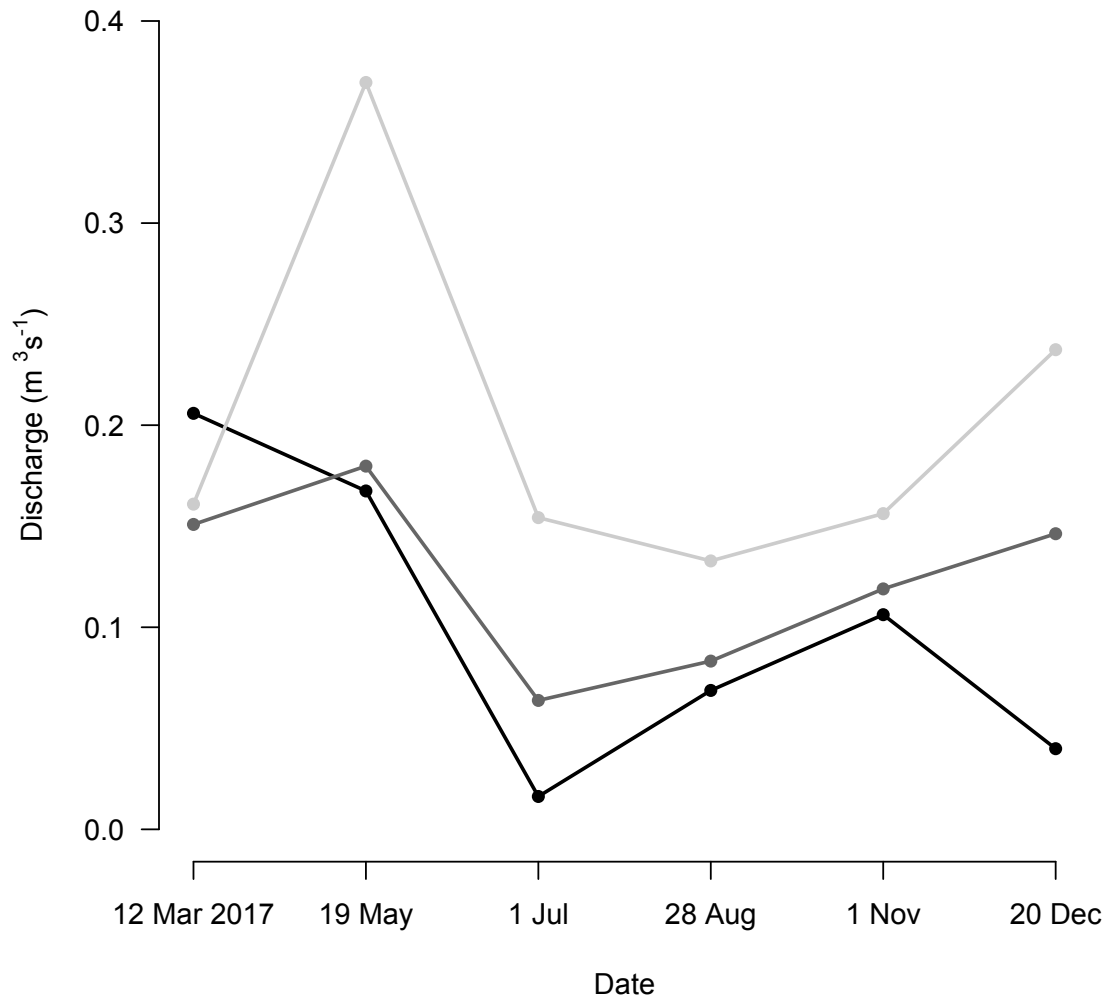


Figure 2: Discharge in the three sampling sections of the Schreierbach (black: lower section, dark grey: middle section, light grey: upper section).

A significantly higher discharge was observed in the upper reach, indicating downwelling further downstream. Minimum discharge was observed in July.

In accordance with discharge, mean flow velocity per choriotope type significantly decreased in summer (ANOVA: $DF=5$; $SS=0.76$; $MSS=0.15$, $F=3.87$; $p<0.001$). Stream velocities differed significantly between choriotopes, in particular between mesolithal and macrolithal (ANOVA: $DF=6$; $SS=4.1$; $MSS=0.68$, $F=17.3$; $p<0.0001$). In akal and microlithal choriotopes velocities were lowest, in xylal, meso- and macrolithal intermediate. In macrophytes (= moss) and megalithal choriotopes, stream velocities were significantly ($p<0.05$) higher (Figure 3).

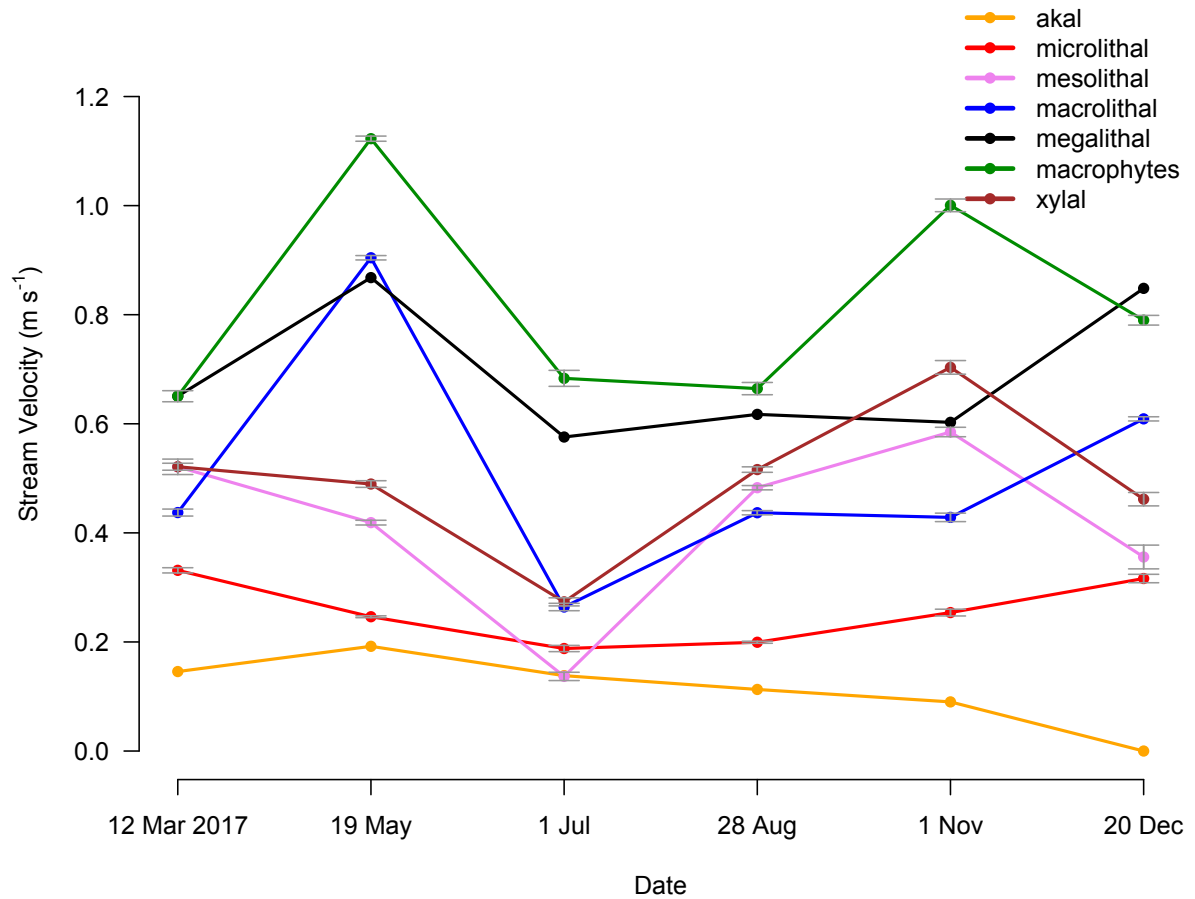


Figure 3: Stream velocity (40% depth from bottom; arithmetic mean $\pm$ 95% confidence interval) in each choriotope type of the Schreierbach in 2017. Stream velocity was significantly different between microhabitats and showed a significant drop in July.

Stream temperature varied only slightly during the year (Figure 4).

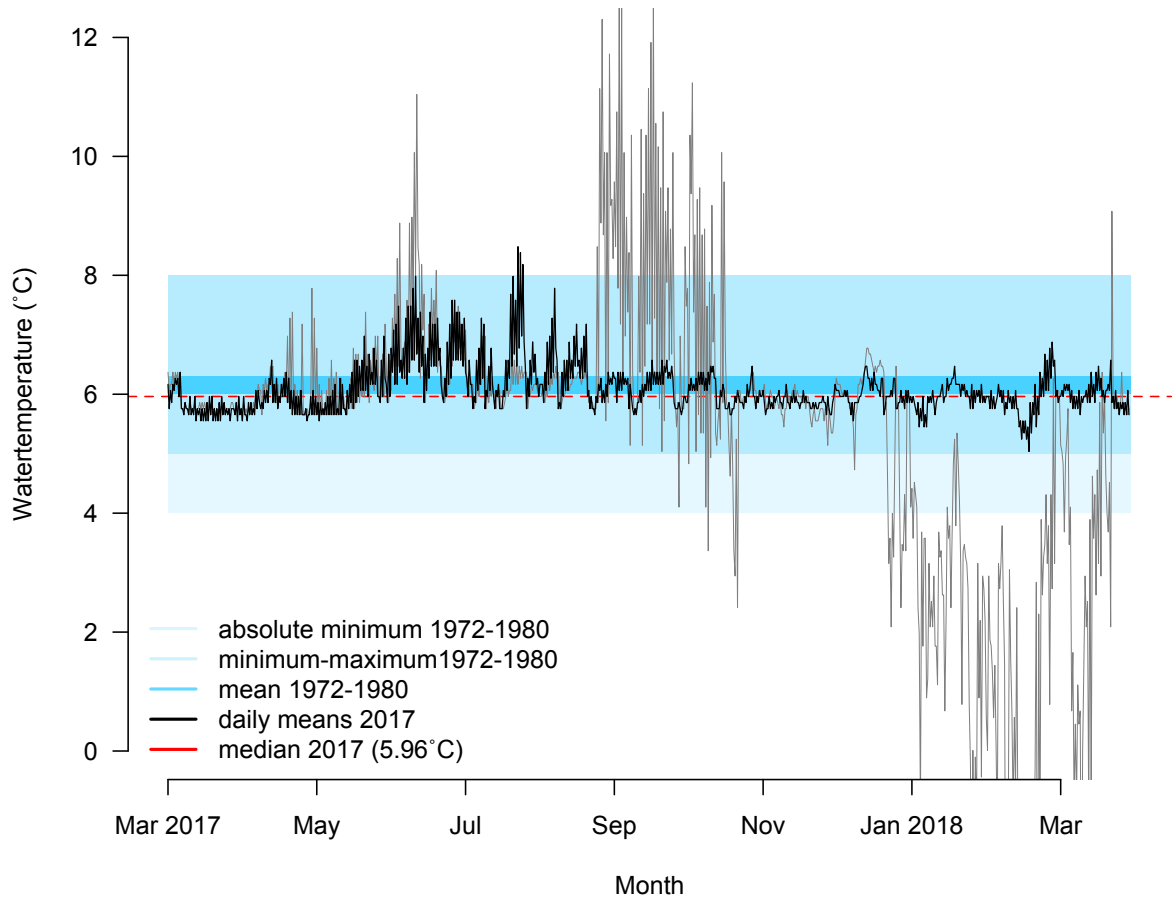


Figure 4: Water temperatures in the middle (black) and upper (grey) sampling section of the Schreierbach from March 2017 to April 2018. Peaks in the upper reach are air temperatures because data loggers emerged due to low water level. Blue: water temperature data of Malicky (1972-1980).

Water temperatures slightly increased from June to September. One data logger positioned in the upper section was not submerged continuously, as is evident from temperature records below 0°C in winter and unexpected high temperatures in summer. Nevertheless, median temperatures of both curves (middle section: 5.96 ± 0.44 °C; upper section 5.86 ± 2.71 °C) were not significantly different and representative for the water temperature regime of the stream. Additional spot temperature measurements confirmed this temperature increase in summer, especially in the lower reach where the highest (summer, 8.0°C) and the lowest (winter, 5.0°C) temperatures were recorded.

3.2 Macrozoobenthos diversity and abundance

Throughout the sampling period, on average 3315 ± 69 macrozoobenthic organisms were sampled per square meter. The most abundant taxa (mean $\pm$ 95% CL) were Chironomidae (1542 ± 4.3 ; 46.5%), Trichoptera (628 ± 6.5 ; 18.9%), Plecoptera (628 ± 1.5 ; 18.9%). Ephemeroptera (125 ± 0.5 ; 3.8%), Diptera non-Chironmidae (124 ± 3.7 %), Coleoptera (97 ± 0.4 ; 2.9%), Tubellaria (74 ± 0.3 ; 2.2%), Collembola (39 ± 0.1 ; 1.2%), Mollusca (26 ± 0.1 ; 0.8%) and Hydracarina (17 ± 0.4 ; 0.5%). Crustacea (*Gammarus* sp. and *Niphragus* sp.) were extremely rare (15 ± 0.1 ; 0.5%).

3.3 Trichoptera diversity and abundance

In total, 22 Trichoptera species were found in the Schreierbach. Trichoptera diversity varied slightly over sampling dates and choriotores. Overall, juveniles (instar 1) of Limnephilidae, subfamily Drusinae, which were too small for identification to species level, were the most abundant Trichoptera taxon observed (Figure 5). Other dominant species were *Micrasema morosum* and *Drusus discolor*. *Drusus monticola*, *Tinodes dives*, *Rhyacophila glareosa*, *R. stigmatica*, *R. tristis*, and *Metanoea rhaetica* were frequent with all larval instars present in the samples. However, the majority of species were rare (< 1% of total abundance), and even if species were collected throughout the sampling period, not all larval instars were sampled.

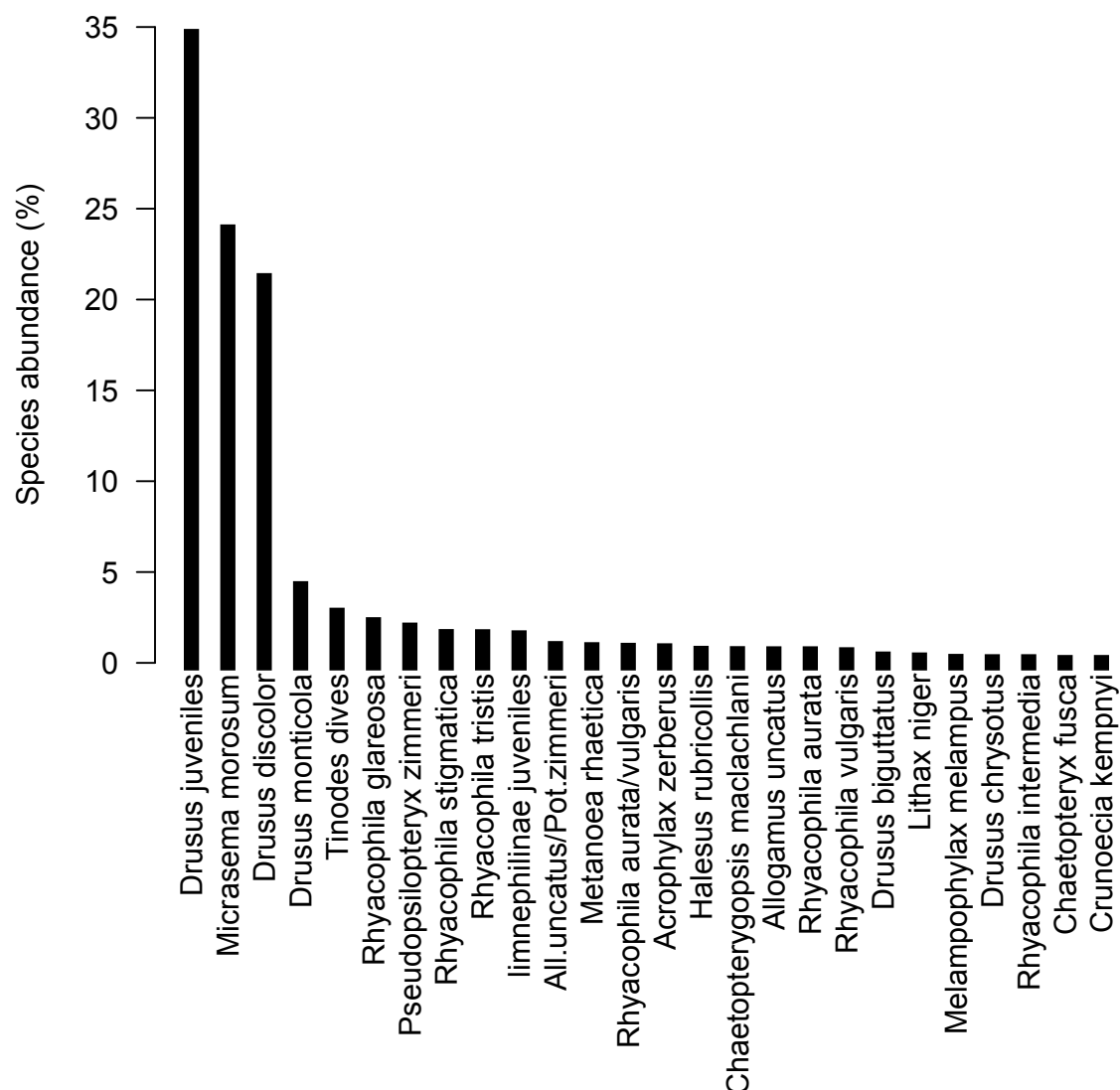


Figure 5: Total species abundance of the Trichoptera community of the Schreierbach. The abundant *Drusinae* juveniles, *Micrasema morosum* and *Drusus discolor* made up 79% of the community. All other species occurred less frequently or only occasionally throughout the sampling period.

The identification of *Limnephilinae* juveniles or the differentiation of small larvae of the pair *Allogamus uncatus* and *Pseudopsiloptyx zimmeri*, or the pair *Rhyacophila aurata* and *R. vulgaris*, was difficult as well. Early instars of *Micrasema morosum*, *Drusus discolor*, *D. chrysotus*, *Lithax niger* (Hagen 1859) or *Tinodes dives* could be clearly identified from the first instar on. However, this was not the case for the remaining *Drusinae* species, *Limnephilinae*, or *Rhyacophilidae*. Other species such as *Melampophylax melampus* (McLachlan 1867), *Chaetopteryx fusca* (Brauer 1857), *Crunoecia kempnyi* (Morton 1901) or

Chaetopterygopsis machlachlani (Stein 1874) were only collected occasionally with early instars lacking

Trichoptera species richness did not differ between sampling sections (upper 19 species, middle 19 species, lower 20 species). Differences are due to the rare species. *Chaetopteryx fusca* and *Crunoecia kempnyi* were found once in the lower section. *Rhyacophila aurata* (Brauer 1857) and *Drusus chrysotus* could only be found further upstream. *Rhyacophila intermedia* (McLachlan 1868) were found occasionally in the upper and lower section. The rare *Melampophylax melampus* was not found in the upper section of the Schreierbach. All other species, however, were collected in the three sampled sections at least once during the sampling period.

With respect to abundance, 1900 ± 1039 individuals m^{-2} ($\pm$ standard deviation) were sampled in the upper section. When compared with the middle (205 ± 23 individuals m^{-2}) and the lower section (141 ± 22 individuals m^{-2}), we collected about ten times more specimens upstream, i.e. Trichoptera abundance was significantly higher at the upper reach when compared with the middle and lower reach (Linear mixed-effects model: DF=74 Estimate= -40.6; SE=7.5; $t=-5.4$; $p<0.0001$) (Figure 6).

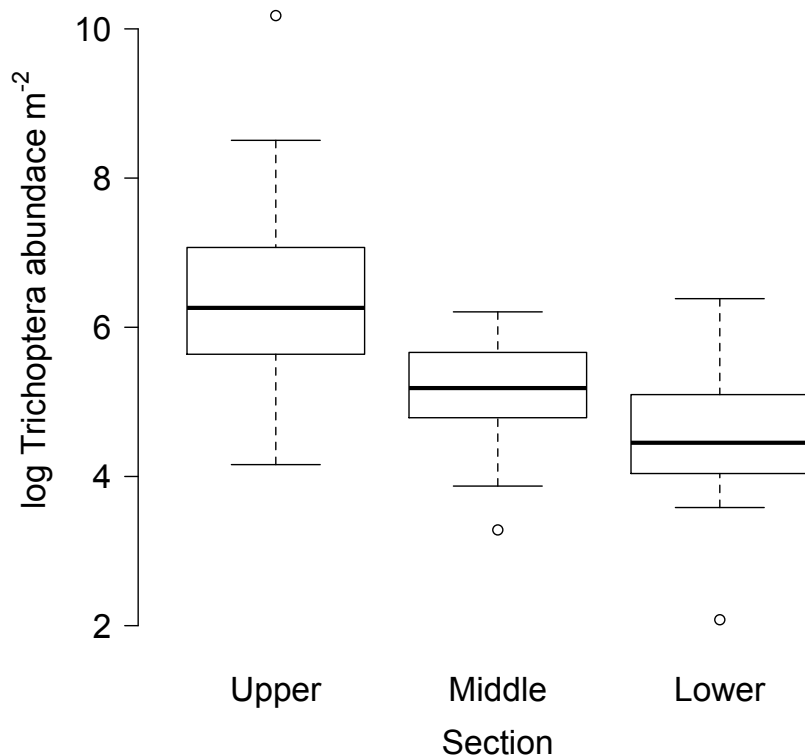


Figure 6: Boxplots showing log-transformed Trichoptera abundance (individuals m^{-2}) in the upper, middle and lower sections of the Schreierbach (median with quartiles and 1.5 times interquartile range).

With respect to choriotoxes, Trichoptera abundances were not significantly different within each sampling section (Figure 7) (ANOVAs: Upper: DF= 3, SS= 11.86, MS= 3.953, F= 3.021, $p>0.05$; Middle: DF=4, SS= 3.234, MS= 0.8084, F= 2.16, $p> 0.05$; Lower: DF= 5, SS= 3.879, MS= 0.7758, F= 0.792, $p> 0.05$).

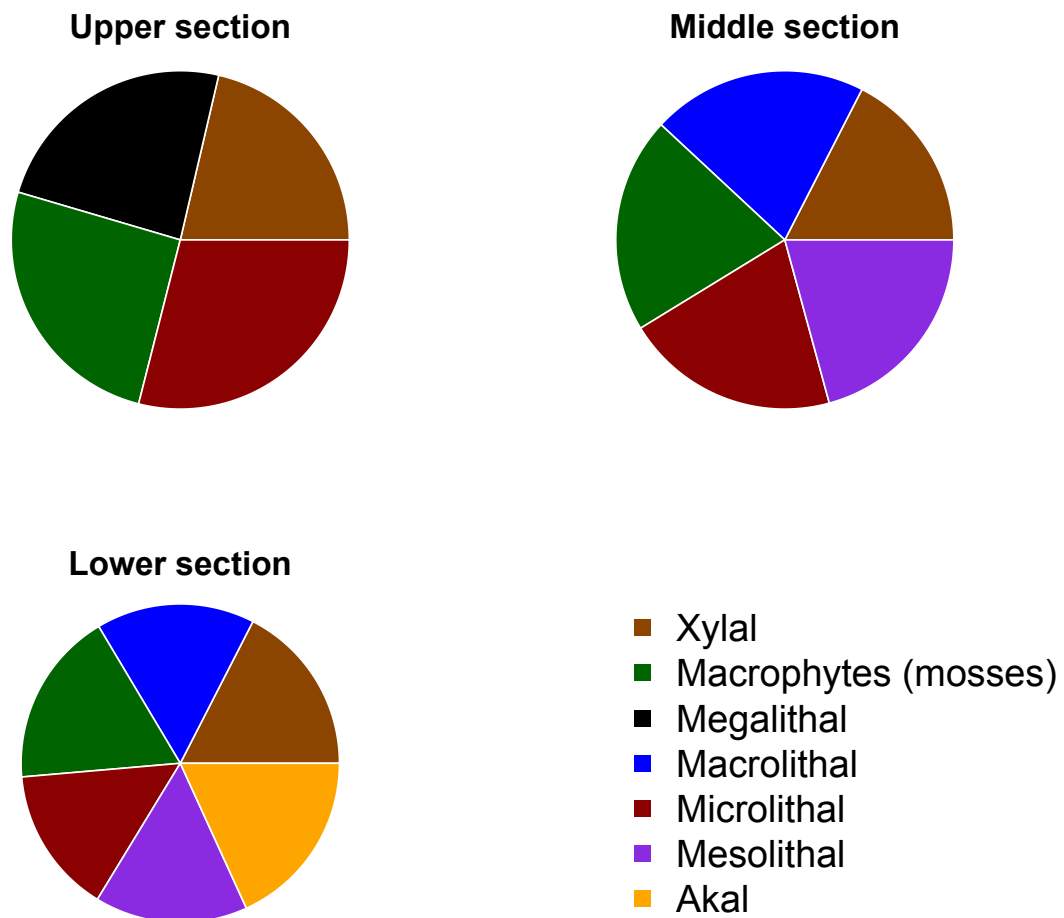


Figure 7: Percentage of Trichoptera abundance in each choriotope of the sampled Schreierbach sections.

To assess habitat preferences of Trichoptera, Shannon diversity indices were calculated for each choriotope, both for the whole study period and for single sampling dates. As shown in Figure 8, there are significant differences ($p< 0.05$) between choriotoxes over the sampling period of one year (ANOVA: DF=76; Estimate=0.61; SD=0.18; $t=3.39$; $p<0.001$); abundance was highest in microlithal and lowest in akal habitats.

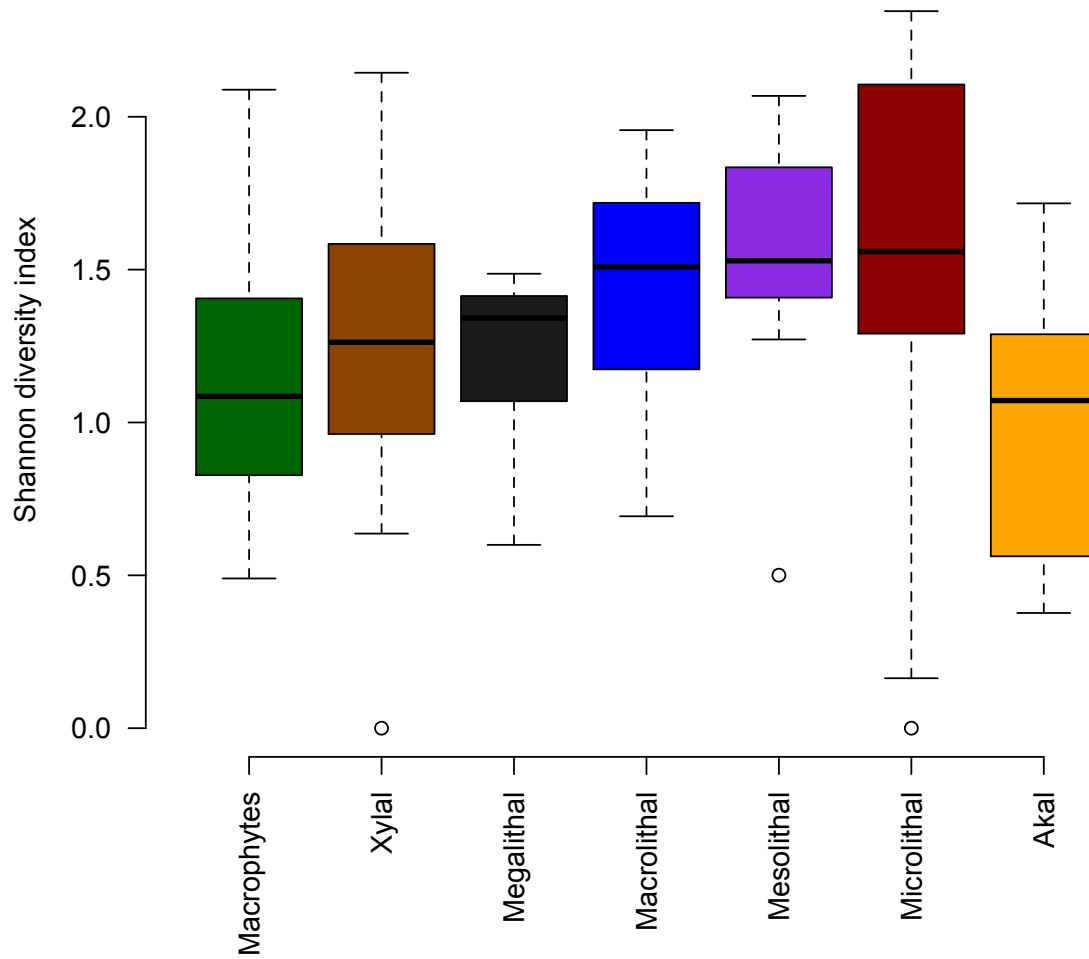


Figure 8: Shannon diversity indices for Trichoptera per choriotope (median with quartiles and 1.5 times interquartile range, circles refer to outliers) in the Schreierbach in 2017.

Differences in Trichoptera diversity become even more evident by comparing individual sampling dates (Figure 9), indicating a diversity shift within the study period. In March species diversity was highest in microlithal and became more homogenous throughout the choriotope in May. In summer species diversity of the meso-, microlithal and akal decreased, before higher diversities in these microhabitats were recorded in fall and winter. Species diversity of xylal and macrophytes (mosses) did not vary as much as the abiotic choriotope. A little increase is observed in the July xylal samples. Species diversity of megalithal and akal varied strongly as only one sample was taken per sampling date.

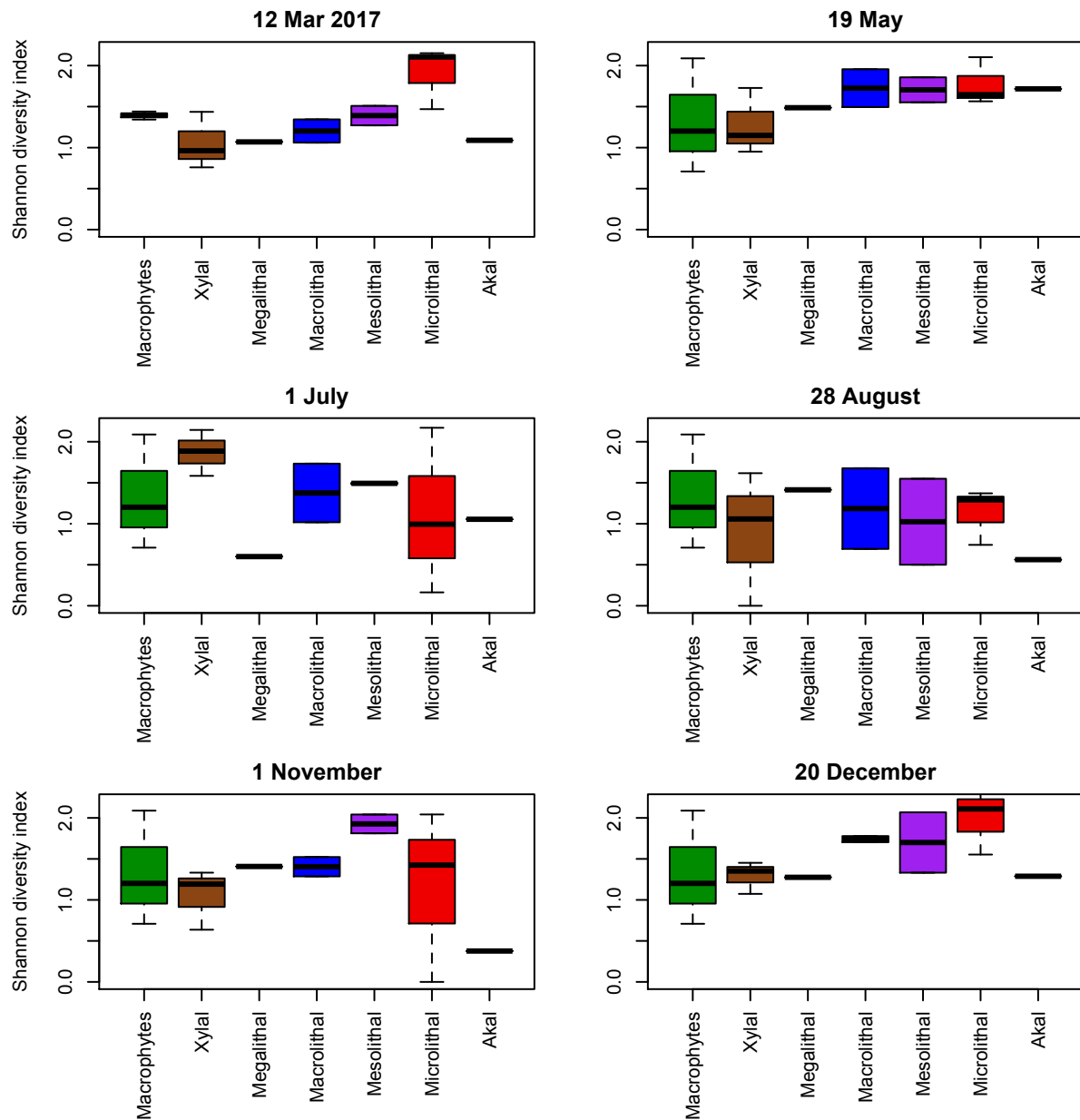


Figure 9: Shannon diversity indices of Trichoptera per choriotype and at different sampling dates (median with quartiles and 1.5 times interquartile range).

3.4 Effects of environmental parameters on Trichoptera community structure

3.4.1 Choriotores

For detecting habitat preferences in the most abundant caddisfly species of the Schreierbach a Canonical Correspondence Analysis (CCA) was used (Figure 10).

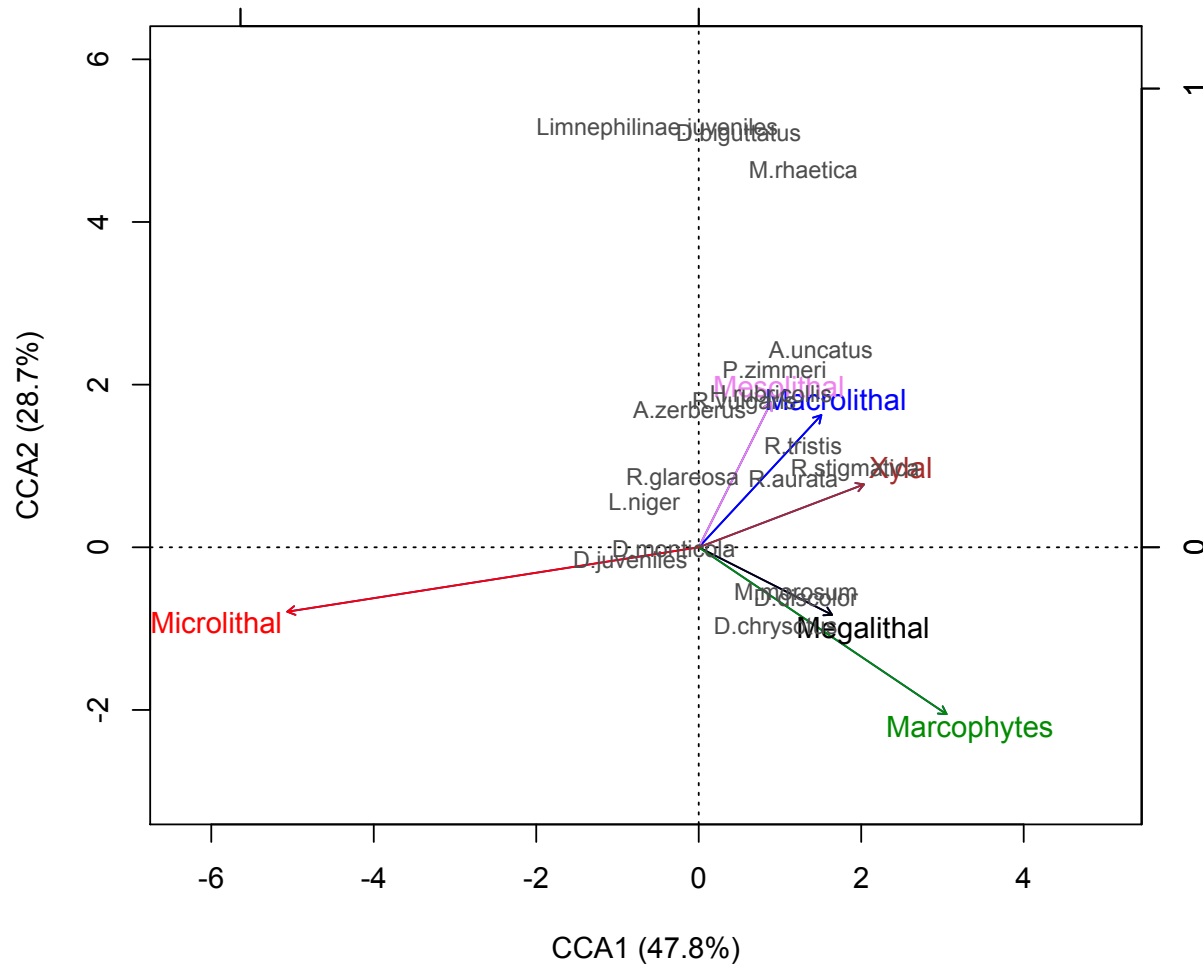


Figure 10: CCA plot of the Trichoptera species community in the Schreierbach using choriotores as constrained variables.

In total, 24.4% of the variances of Trichoptera species abundance in the Schreierbach were explained by the CCA when using choriotores as constrained variables. Most dominant species were positively correlated with macrolithal, mesolithal and xylal habitats within the ordination space. Megalithal and macrophyte habitats, however, were significantly associated with *Drusus discolor* and *Micrasema morosum*, the most abundant taxa in the stream. Juvenile Drusinae were closely associated with microlithal habitats (Figure 10).

When comparing habitat preferences for each species and instar, it became obvious that species frequently remained in the same or in similar habitats during larval development. Choriotores associated with low stream velocity, such as microlithal and akal habitats, remained the preferential choriotope for *R. glareosa*, *H. rubricollis*, *A. zerberus* and *D. monticola*. These choriotores also had slightly higher temperatures and higher oxygen saturation. Megalithal, macrolithal, macrophyte and xylal habitats characterized by higher stream velocities were preferred by later instars of *M. morosum*, *D. discolor*, *T. dives* and *R. stigmatica*. However, while fifth instars of *R. stigmatica* preferred xylal habitats, *M. morosum*, *T. dives* and *D. discolor* were most abundant in megalithal and macrolithal habitats throughout all larval instars (Table 4).

Table 4: Habitat preferences (based on specimens m⁻²) of the most abundant Trichoptera species in the Schreierbach, broken down to larval instars. Choriotopes with the highest relative abundance were color-coded as preferred habitats.

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3.4.2 Hydraulic and limnochemical parameters

A combination of stream velocity, oxygen saturation and water temperature explained 21.4% of the variance of caddisfly species composition (Figure 11). High velocity patches were significantly associated with *Micrasema morosum* and *Drusus discolor*. Most species are placed within the 6°C gradient line due to the very constant water temperature of the Schreierbach. O₂ saturation is negatively correlated with stream velocity as in summer discharge, and therefore stream velocity was low, but O₂ slightly increased due to better insolation. Drusinae juveniles chose warmer and lenitic patches at the streambed whereas Limnephilinae species such as *Acrophylax zerberus*, *Allogamus uncatus* (Brauer 1857) and *Pseudopsilopteryx zimmeri* (McLachlan 1876) were abundant in warmer microhabitats with high O₂ saturation.

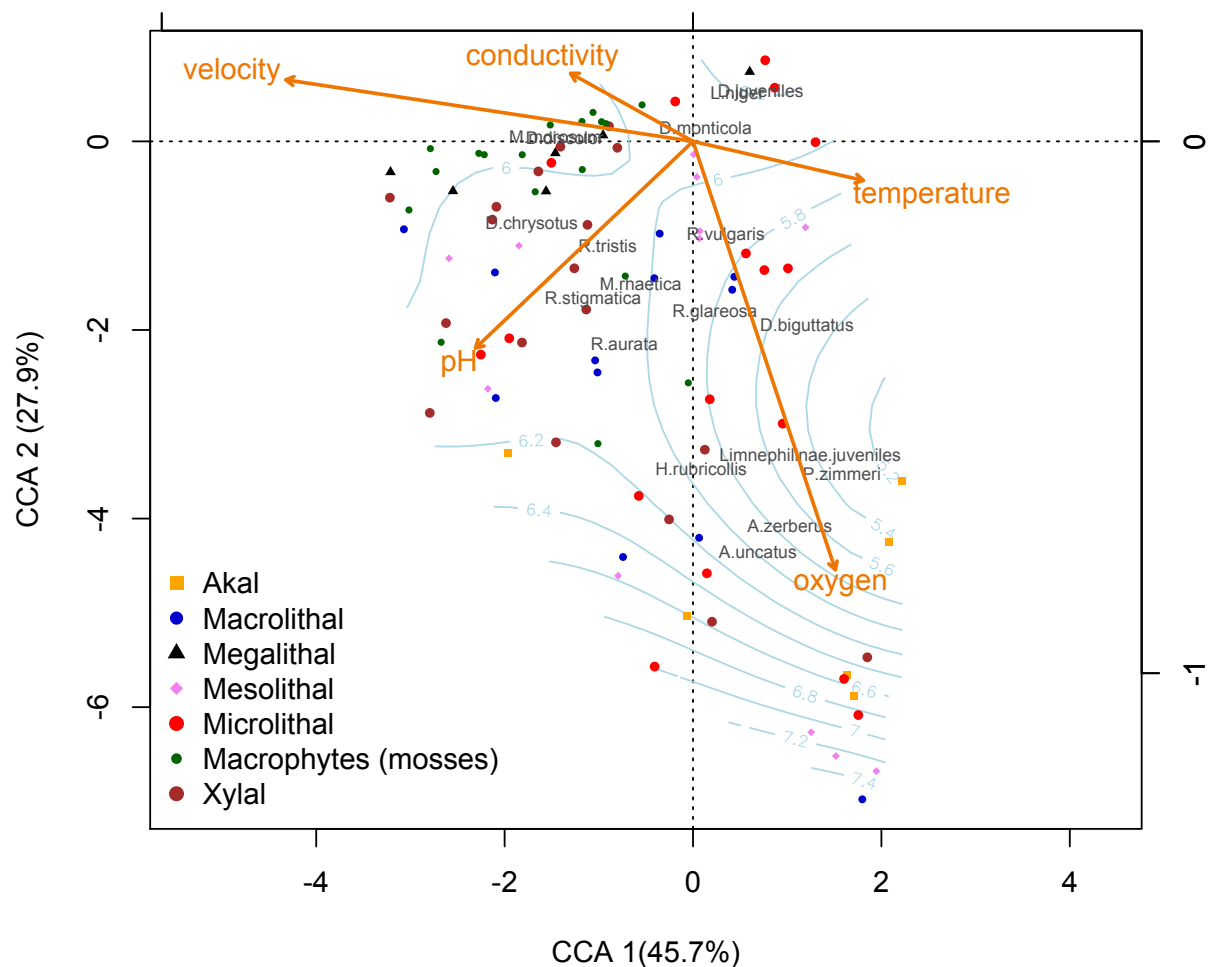


Figure 11: CCA plot of the Trichoptera species community in the Schreierbach using mean temperature (°C), mean stream velocity (ms⁻¹) and oxygen saturation (%) as constrained variables.

When correlating species abundance at instar level with abiotic parameters (stream velocity, oxygen saturation, water temperature, conductivity, pH), CCA models explained 21.4% of the total variation. Abiotic variables affected each instar community slightly differently (Figure 12). While stream velocity was a major variable for each larval instar the effects of temperature and oxygen decreased from the first to the second and third instar and gained importance in the fourth and fifth stages. Conductivity and pH showed the opposite tendency. In the first, fourth and fifth instars the influence was quite low. These variables affected strongly the second instar. The third instar community was affected mainly by pH, velocity and temperature.

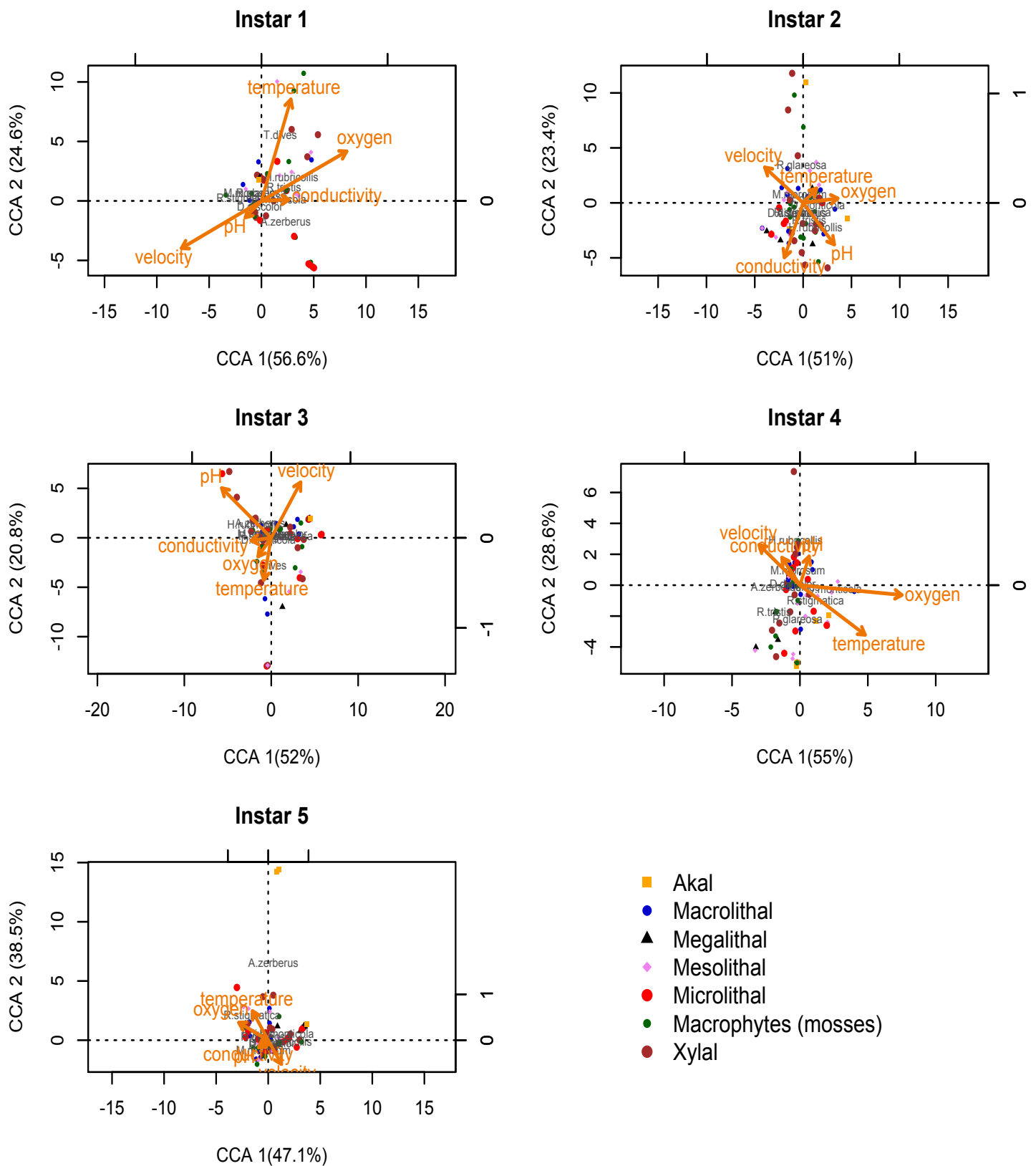


Figure 12: CCA plots of the Trichoptera community of the Schreierbach, broken down to instar level (pooled data of abundant species).

With respect to the variances explained, abiotic factors explained 7-15% of species composition throughout the larval development. In comparison, the significance of habitat was highest in young larvae and decreases rapidly in the following larval stage. The habitat influence on the larvae then increased again in the fifth instar (Figure 19).

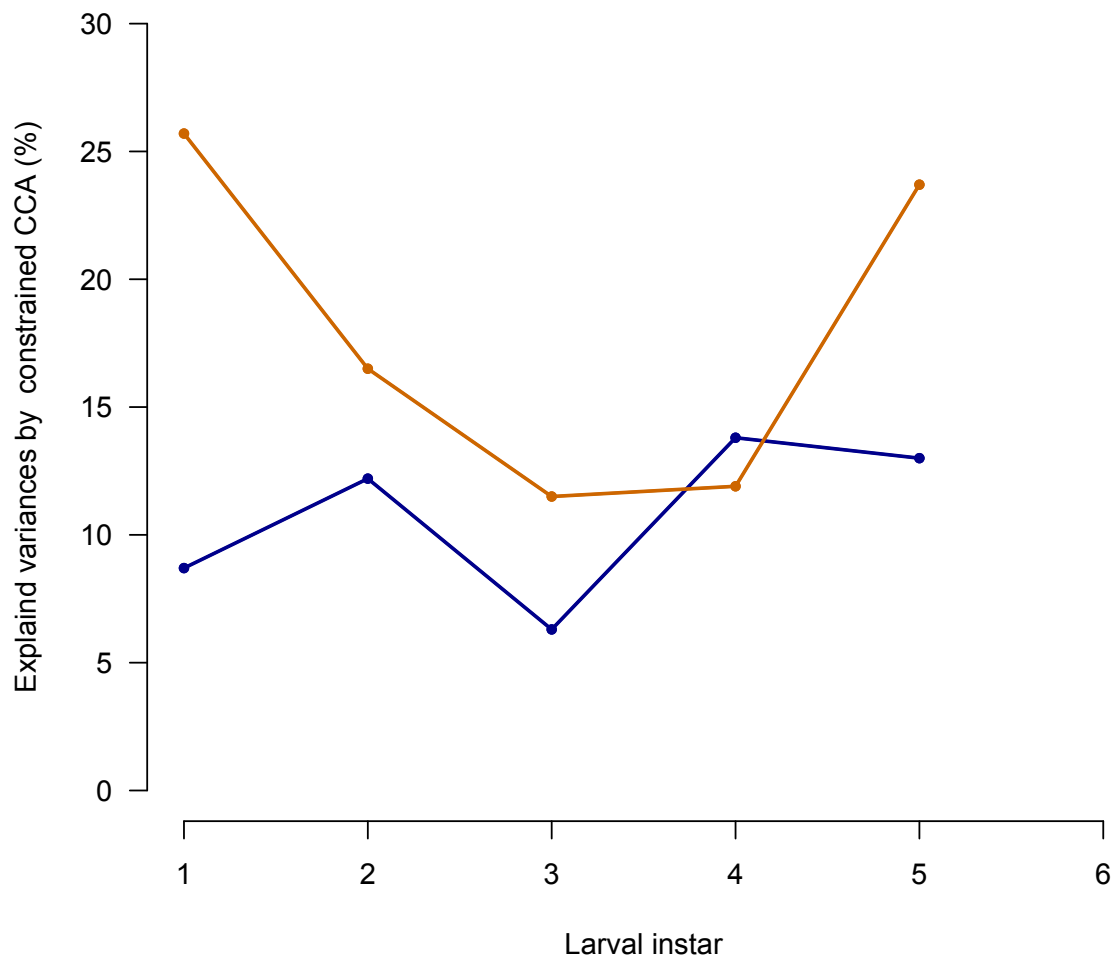


Figure 13: Explained variances for the constrained abiotic and biotic effects per larval instar of the Trichoptera community of the Schreierbach (brown= choriotope, blue= abiotic effects).

3.5 Phenology

3.5.1 Larval instars in time

Based on pooled data of all Trichoptera taxa, the abundance of a given instar changed over the year. While most first instar larvae were collected in September, second larval instars were most frequent in November. At the end of December, third larval instars and in March and May larval instars 4 and 5 dominated. Interestingly, maximum abundance decreased with increasing instar, reflecting population losses in time (Figure 14).

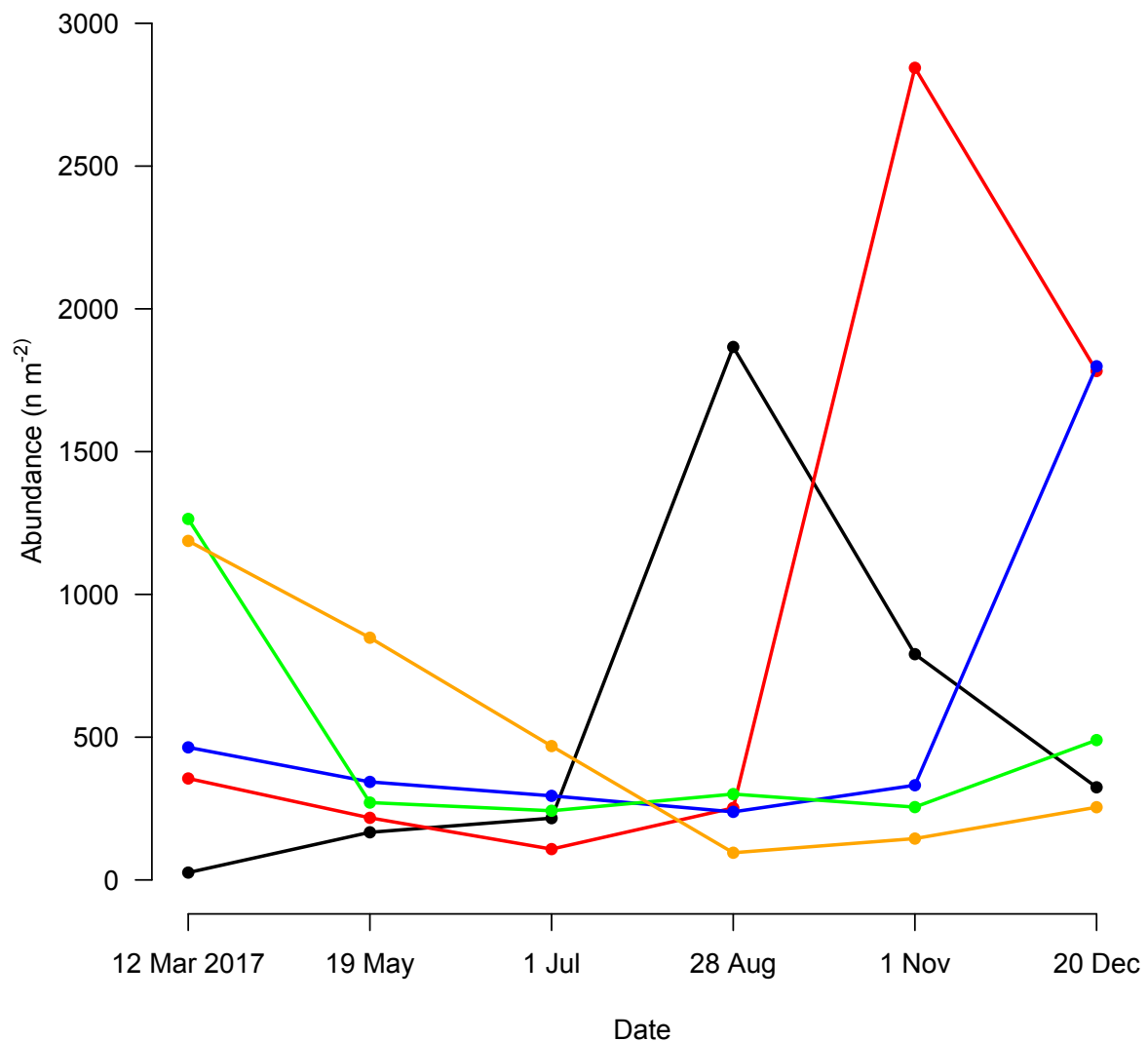


Figure 14: Phenology of larval instars of Trichoptera larvae in the Schreierbach in time (pooled data); showing the abundance ($n\ m^{-2}$) on each sampling date (instars: black= first, red= second, blue= third, green= fourth, yellow= fifth instar).

3.5.2. Life cycles

Head width-frequency histograms for the most abundant Trichoptera species ($\geq 1\%$ of total abundance and *Halesus rubricollis*) of the Schreierbach are shown in Figures 15-17. Generally, larval instars showed five distinct peaks, thereby defining instars 1-5. Driven by population losses during larval development, the number of specimens with increasing head width decreased. For many species, however, the first larval instar frequency was lower than the abundance of second instar larvae. Separation of first and second instars was difficult, as head width increases were only marginally, especially for small species. For *Drusus monticola* and *Rhyacophila stigmatica*, e.g., only a few specimens could be identified to species level. Moreover, first instars of small species such as *Tinodes dives*, *Micrasema morosum* or *Rhyacophila stigmatica* were possibly underestimated. Generally, head widths were measured to the nearest 0.03mm.

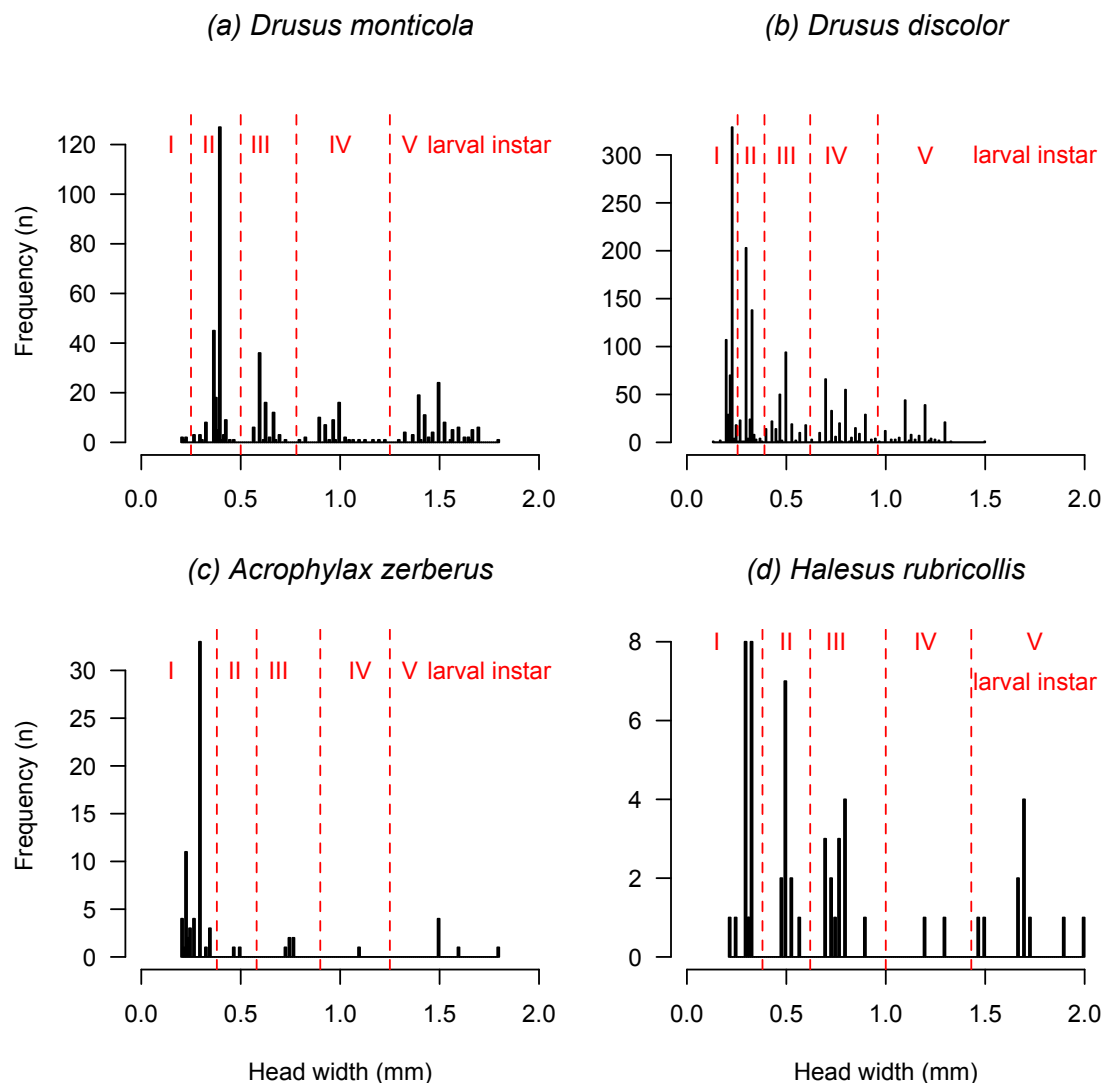


Figure 15: Size-frequency plots of the Limnephilidae species *Drusus monticola* (a), *Drusus discolor* (b), *Acrophylax zerberus* (c) and *Halesus rubricollis* (d). Instar numbers are indicated in red.

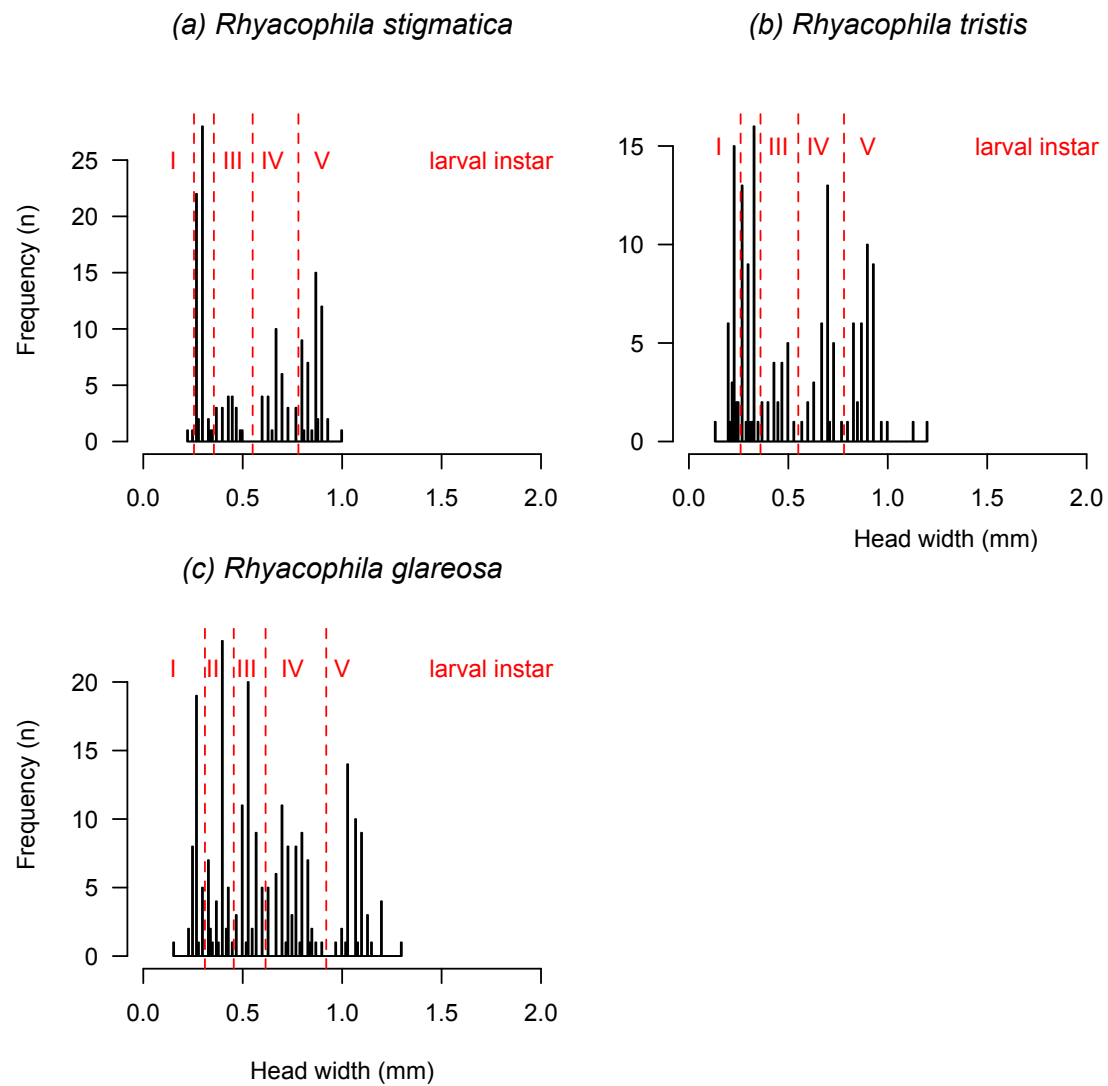


Figure 16: Frequency plots of the Rhyacophilidae species *Rhyacophila stigmatica* (a), *Rhyacophila tristis* (b) and *Rhyacophila glareosa* (c). Instar numbers are indicated in red.

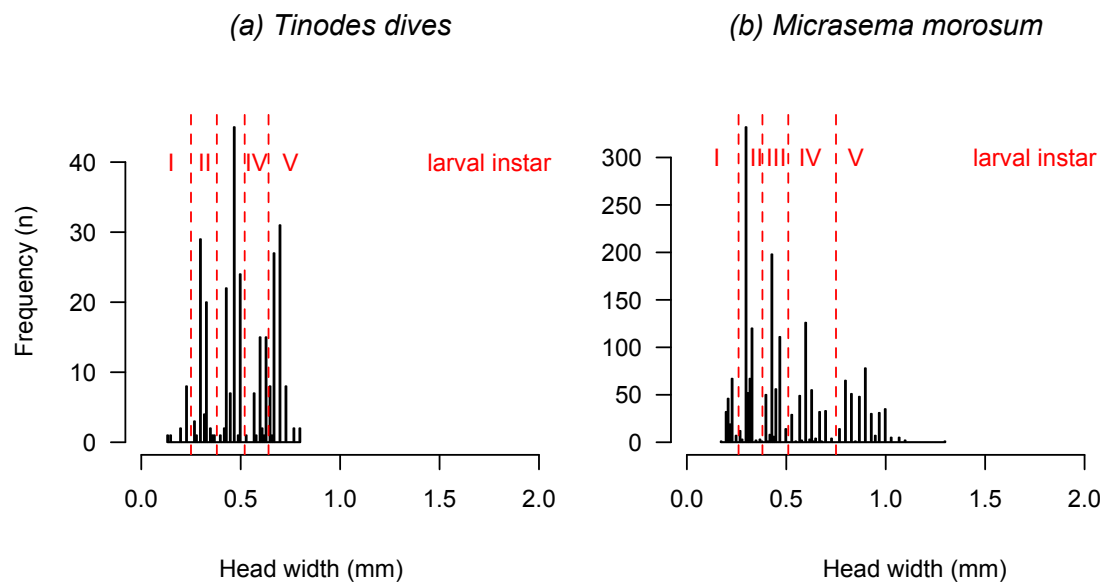


Figure 17: Frequency plots of the species *Tinodes dives* (a) and *Micrasema morosum* (b). Instar numbers are indicated in red.

By plotting the natural logarithm of head width versus larval instars, the associated regression line is linear, indicating an exponential increase of head width per moult and demonstrating the applicability of Dyar's rule (Dyar, 1890) for the species studied in detail (Figures 18-20). For all abundant species this rule was confirmed, suggesting an exponential larval growth.

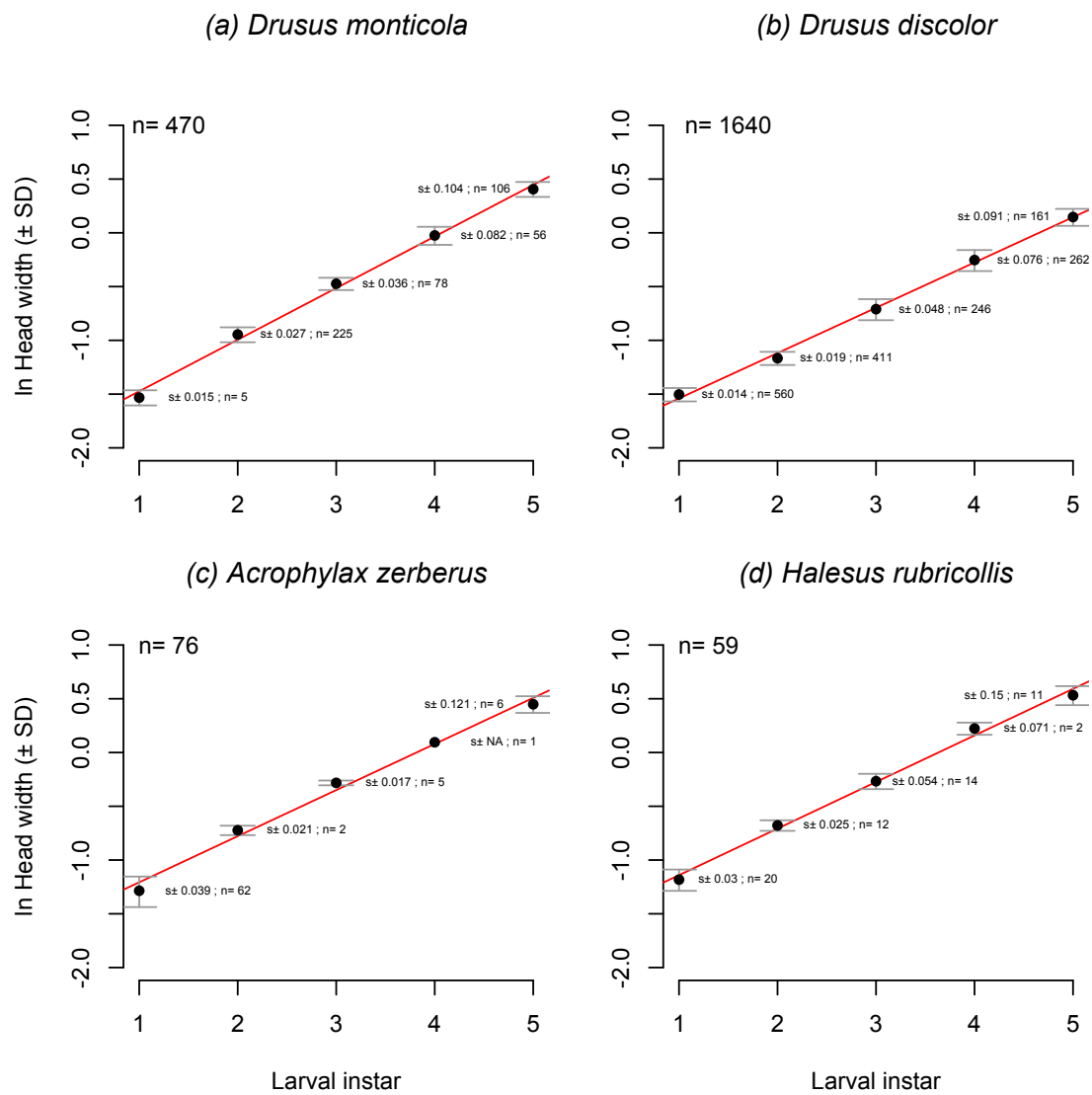


Figure 18: Relationship between instar number and head width (ln mm $\pm$ standard deviation) of the Limnephilidae species *Drusus monticola* (a), *Drusus discolor* (b), *Acrophylax zerberus* (c) and *Halesus rubricollis* (d). N = number of specimens measured.

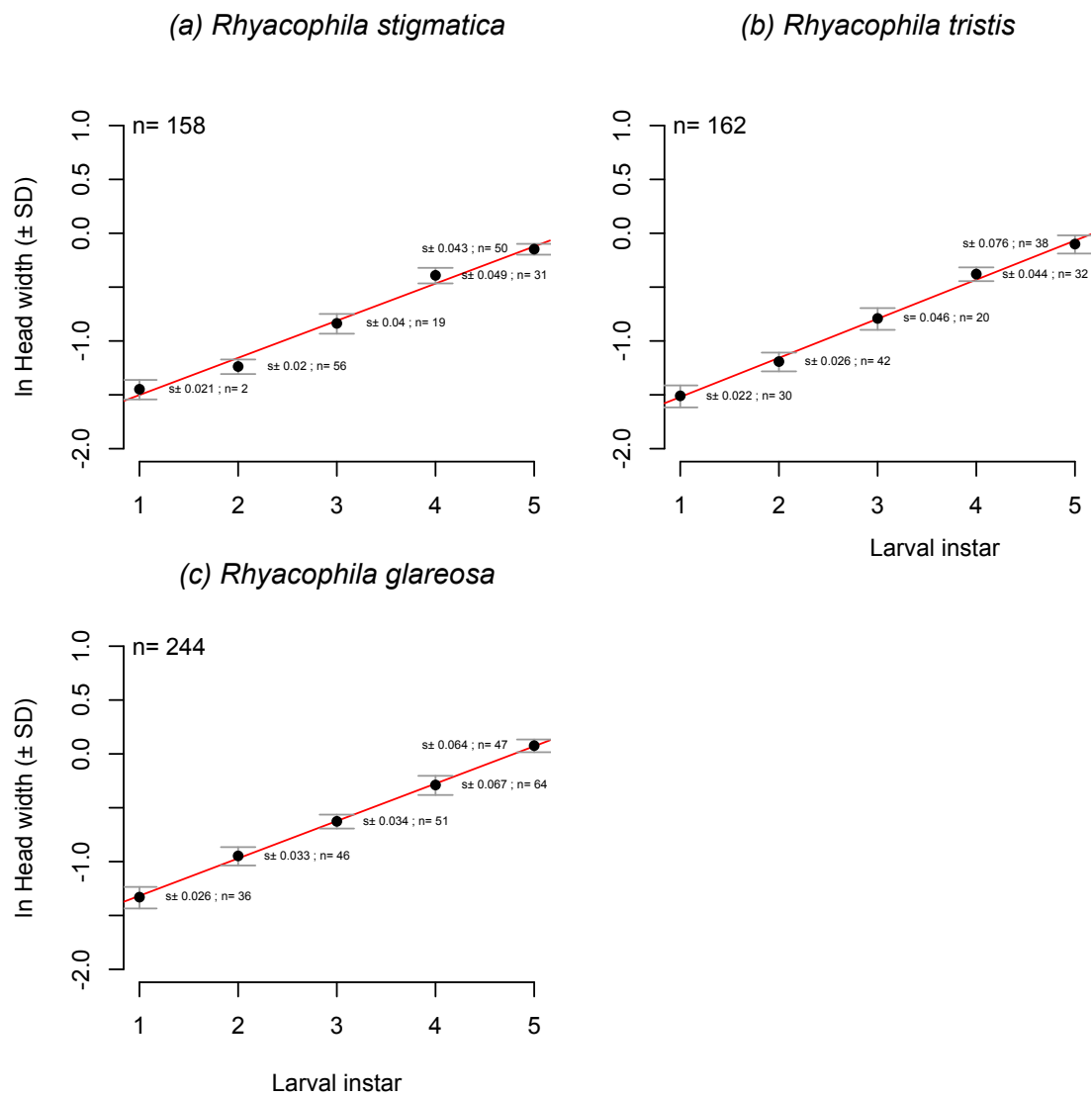


Figure 19: Relationship between instar number and head width (ln mm $\pm$ standard deviation) of the five larval instars of the Rhyacophilidae species *Rhyacophila stigmatica* (a), *Rhyacophila tristis* (b) and *Rhyacophila glareosa* (c). N = number of specimens measured.

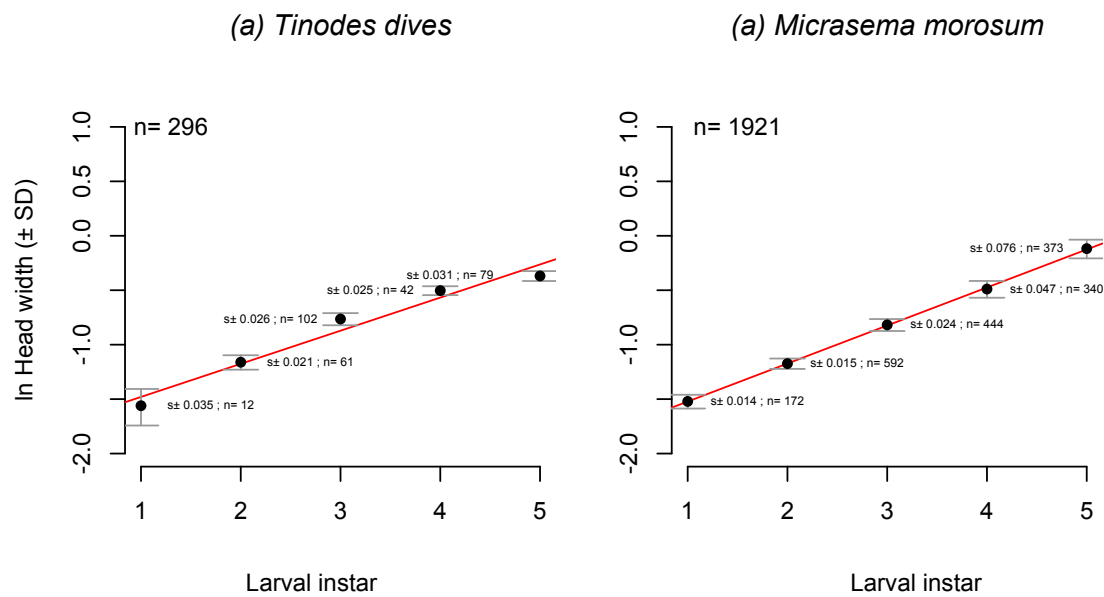


Figure 20: Relationship between instar number and head width (ln mm $\pm$ standard deviation) of the five larval instars of *Tinodes dives* (a), and *Micrasema morosum* (b). N = number of specimens measured.

The combination of larval growth (expressed as head width) with time enables a reconstruction of the life cycles of the dominant species. In all species, the life cycle was univoltine (Figures 21-23). In *D. monticola*, *Drusus discolor* and *Halesus rubricollis* first larval instars were observed in August with growth during the winter month being slow. In spring, however, growth accelerated until the onset of emergence (Figures 21 a, b, d). For *Acrophylax zerberus* the pattern is unclear, because the species was quite rare and some instars were only sampled sporadically (Figure 21 c).

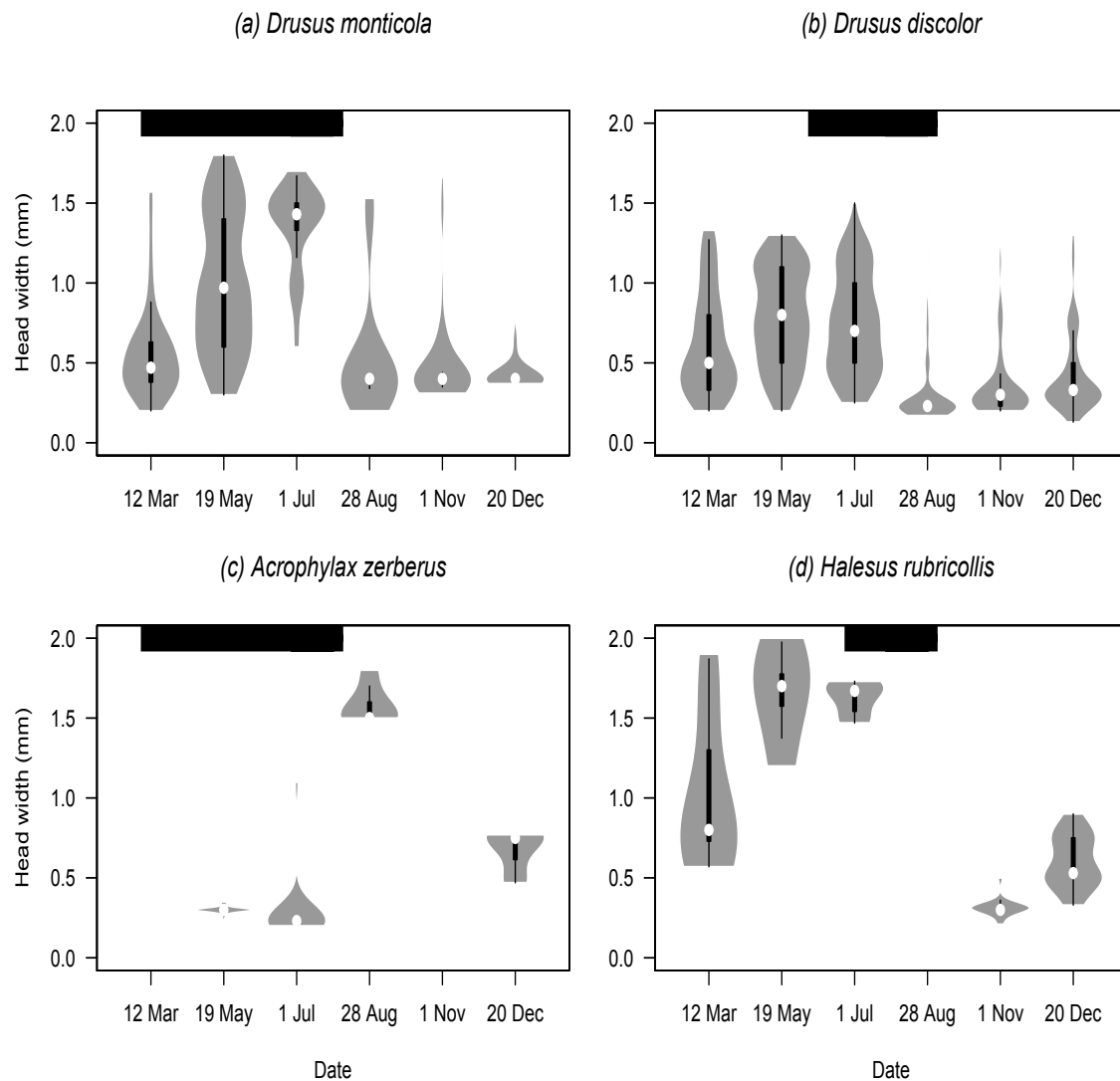


Figure 21: Life cycle diagram of *Drusus monticola* (a), *Drusus discolor* (b), *Acrophylax zerberus* (c) and *Halesus rubricollis* (d). White dots represent median abundance, black boxes the upper and lower quartiles. Black lines represent the 1.5 times interquartile range. The grey background indicates the frequency of specimens per head width. Black bar= flight period.

Life cycles of the *Rhyacophila* species are asynchronized. The majority of first and second instars of *Rhyacophila stigmatica* and *Rhyacophila tristis* were observed during November and December. The larvae grew exponentially from December to March. During spring and summer the median head width suggest that most specimens emerged and new larvae hatched, with generally all instars present in this period (Figure 22 a, b). Similarly, *Rhyacophila glareosa* showed a slow growth in summer and fall, and an accelerated growth phase during winter. The species mainly emerged during the end of spring and the beginning of summer. In this species also many different larval instars could be found simultaneously (Figure 22 c).

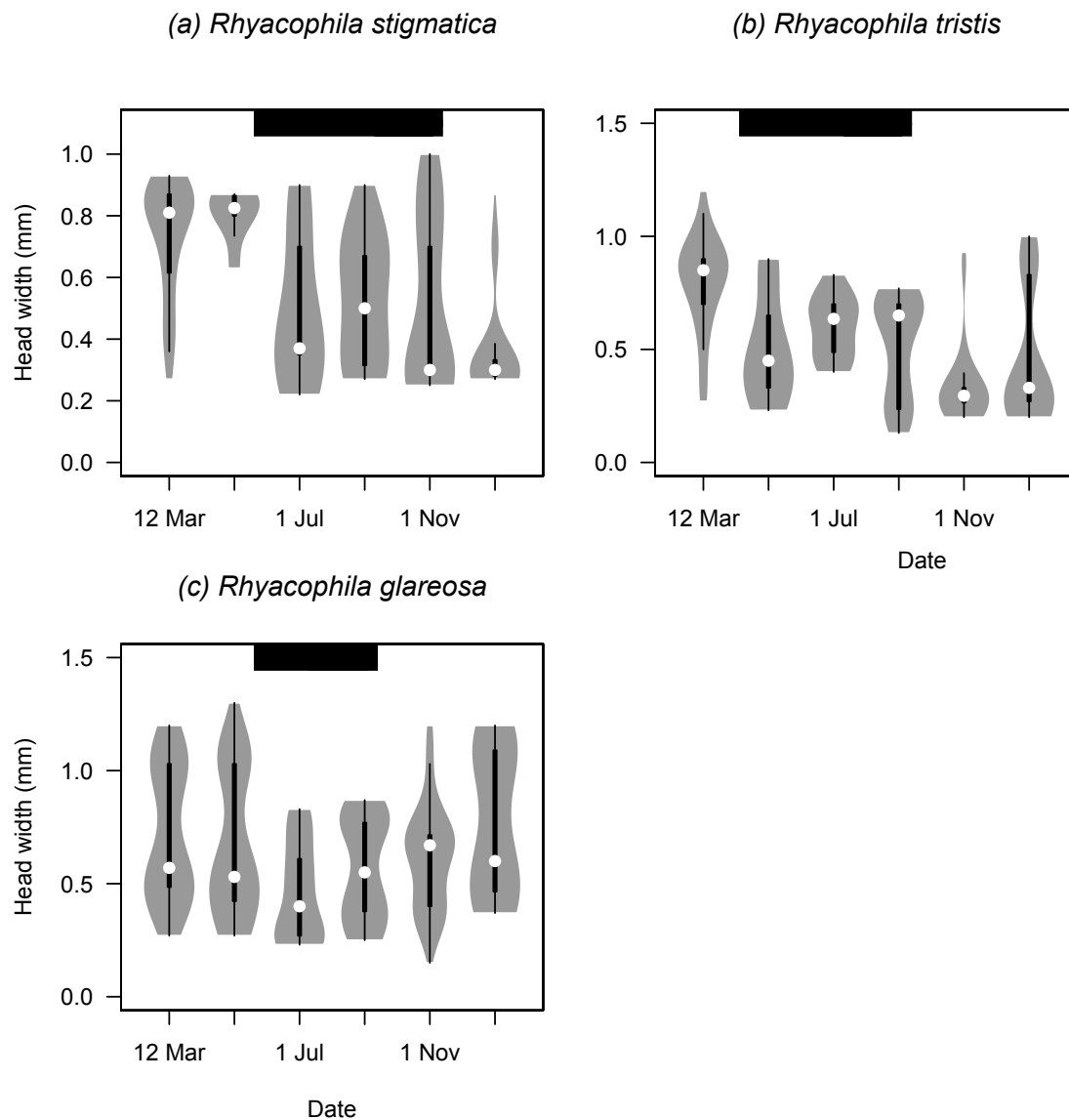


Figure 22 Life cycle diagram of *Rhyacophila stigmatica* (a), *Rhyacophila tristis* (b), and *Rhyacophila glareosa* (c). White dots represent median abundance, black boxes the upper and lower quartiles. Black lines represent the 1.5 times interquartile range. The grey background indicates the frequency of specimens per head width. Black bar= flight period.

In *Tinodes dives* mean head width slightly decreased in July and August with later instars observed in spring. However, the median head width was very similar throughout the sampling period. This pattern indicates a univoltine but asynchronous life cycle (Figure 23 a). In contrast, a perfectly synchronic life cycle has been observed in the abundant *Micrasema morosum*. With the end of the emergence period in July, head width abruptly dropped, followed by a steady growth phase in fall. During winter the species accelerated growth until reaching the final instar in May (Figure 23 b).

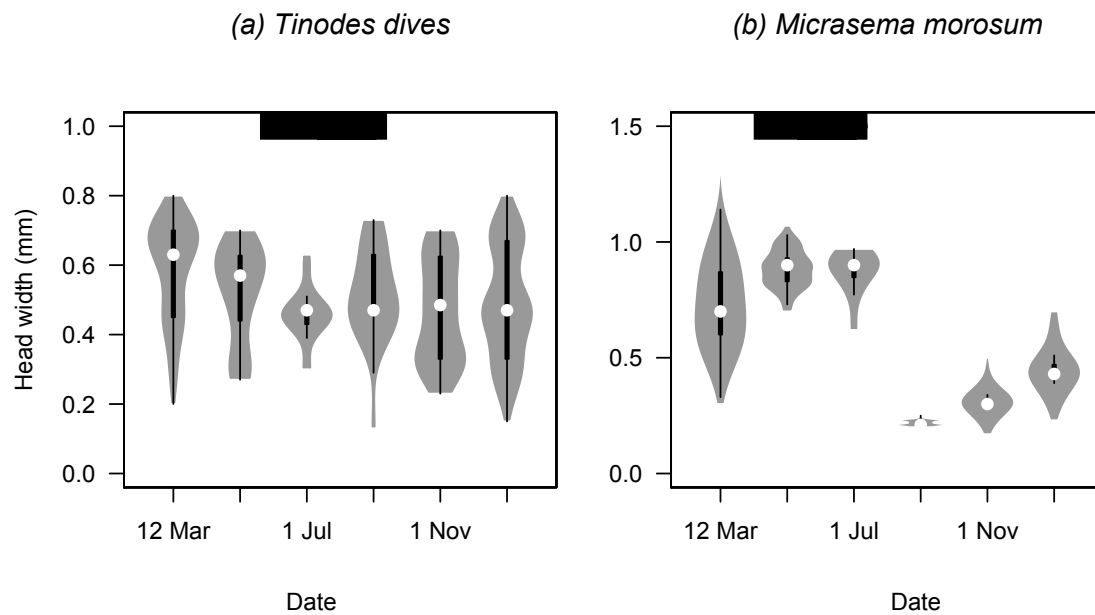


Figure 23: Life cycle diagram of *Tinodes dives* (a), and *Micrasema morosum* (b). White dots represent median abundance, black boxes the upper and lower quartiles. Black lines represent the 1.5 times interquartile range. The grey background indicates the frequency of specimens per head width. Black bar= flight period.

3.6 The Trichoptera species inventory of the Schreierbach then and now

Almost 40 years have passed since Hans Malicky conducted his Trichoptera studies using greenhouse emergence traps at the Schreierbach (Malicky, 1979, 1980, 1981). When comparing the Trichoptera inventory then and now, some interesting changes with respect to the most important ecometrics were observed. Although species diversity basically remained constant, there are shifts in proportions and in species evenness (Figure 24).

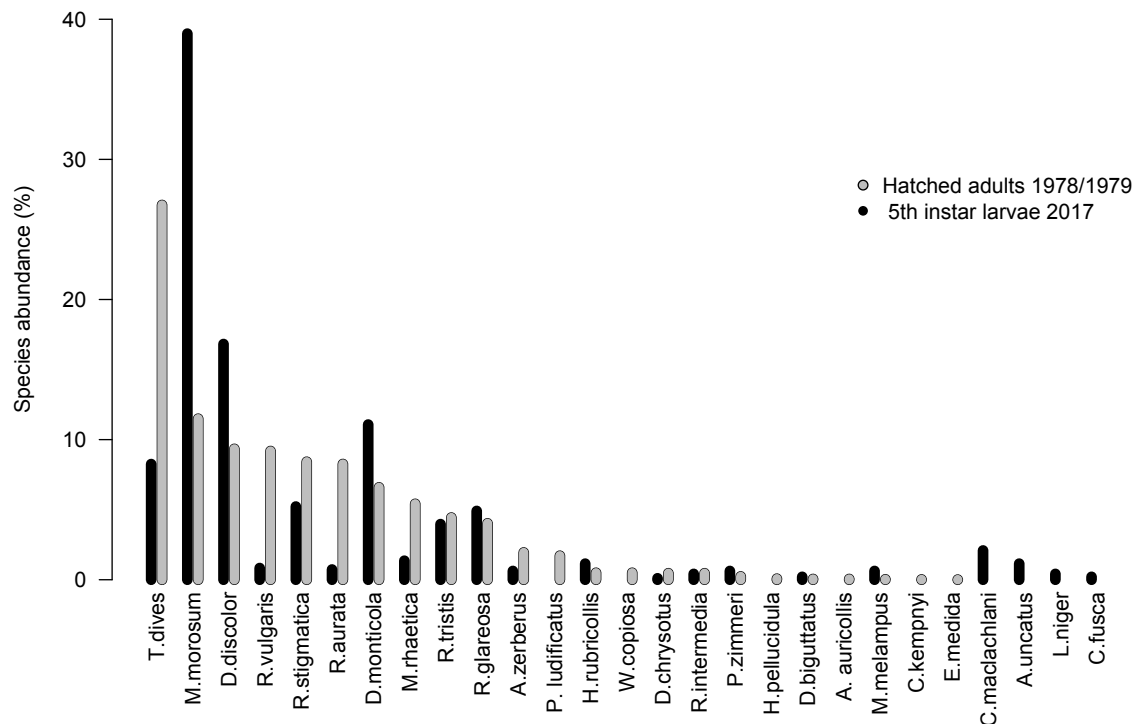


Figure 24: Comparison of the species inventory and the relative abundances of the Trichoptera of the Schreierbach 40 years ago (Malicky, 1978, 1979) and now (present study).

While relative abundancies of *Micrasema morosum*, *Drusus discolor* and *D. monticola* increased, a decrease was observed in *Tinodes dives*, *Rhyacophila vulgaris*, *R. tristis*, *R. stigmatica*, *R. aurata*, *Methanoea rhaetica* and *Acrophylax zerberus*. Some subdominant and rare species (e.g., *Wormaldia copiosa* McLachlan 1868, *Crunoecia kempnyi*, *Ecclisopteryx madida* McLachlan 1867 and *Hydropsyche pellucidula* Curtis 1834) were lacking in the samples of 2017. In contrast, *Chaetopterygopsis maclachlani*, *Allogamus uncatus*, *Chaetopteryx fusca* and *Litax niger* were not recorded by Malicky in 1978 and 1979.

With respect to stream zonation patterns, eucrenal, metarhithral and hyporhithral valencies increased, whereas hypocrenal and epirhithral valencies decreased in terms of relative species abundances. In the proportion of feeding guilds, a shift from grazers, predators and gathering collectors to shredders and passive filter feeders is evident.

Saprobic valencies slightly shifted from beta-mesosaprobic and oligosaprobic to xenosaprobic (Figure 25).

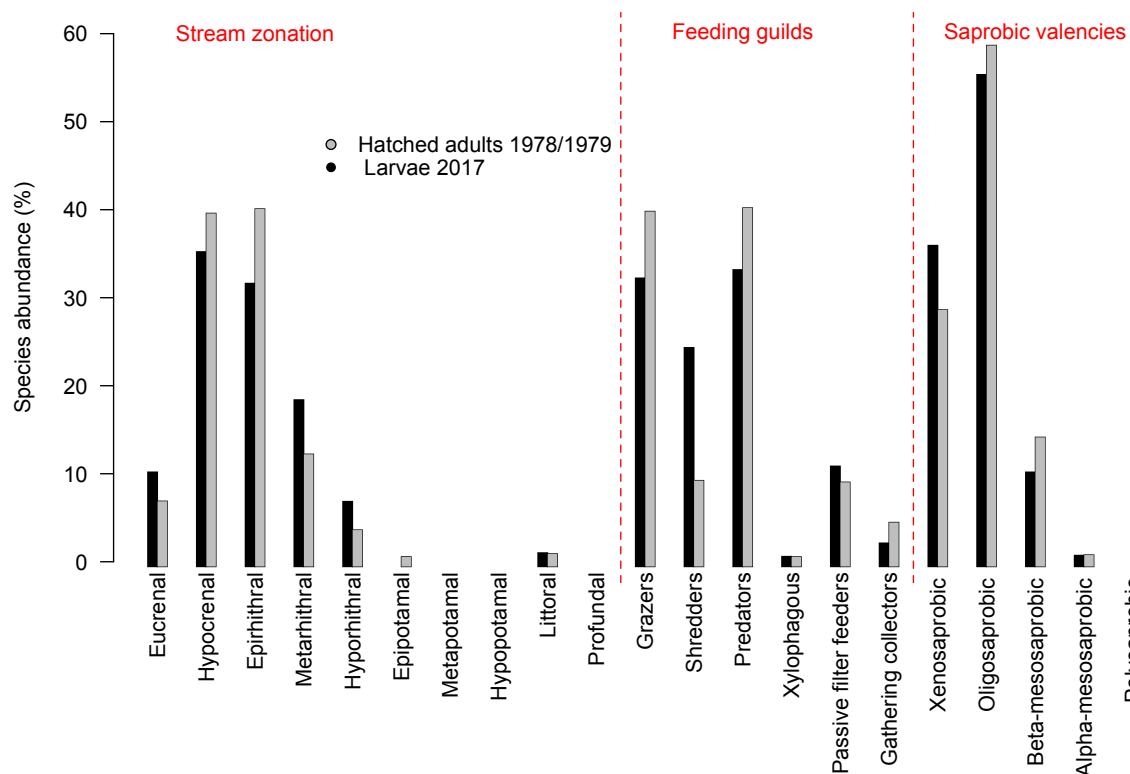


Figure 25: Comparison of stream zonation patterns, feeding guild composition, and saprobic valencies based on emergence data of Malicky (1979, 1980) and larval data of the present study.

3.7 DNA Barcoding and gut contents

In the framework of this thesis, 28 samples from 10 specimens of subfamily Drusinae were sequenced. In total, five species and nine gut contents were prepared. Five out of 14 COI amplification attempts did not produce PCR products and could not be analysed further, and 4 sequences did not match reference sequences. All sequences of the gut contents were problematic, and effectively, no prey organism could be determined.

Regarding the 14 sequences of 16S RNA, 6 were not amplified by the PCR and 2 sequences did not match. The sequences of *Drusus discolor* and *D. chrysotus* contained some Bacillariophyceae taxa (*Asterionella* sp., *Diatoma* sp., *Amphora* sp.) or the cyanobacterium *Tychonema bourrellyi* (*D. chrysotus*). In both cases, the sequenced organisms belong to the biofilm community and presumably were caught between the setae of the caddisfly legs.

Gut sequences of *D. chrysotus* and *D. biguttatus* included gammaproteobacteria (*Wohlfahrtiimonas* spp., *Koukoulia* spp.). In addition, *D. biguttatus* gut sequences matched with a *Staphylococcus* sp. (*Capitis* sp.) reference. The *Metanoea rhaetica* gut sequence matched with Bradyhizobiaceae, presumably of genus *Bosea* sp. (Table 5).

Table 5: Results of querying obtained COI and 16S rRNA barcoding sequences against existing reference libraries.

Nr.	Species	Content	Results COI	Match %	Results 16S
1	<i>Drusus discolor</i>	Specimen	<i>Drusus discolor</i>	100	<i>Asterionella</i> , <i>Diatoma</i> , <i>Amphora</i> (Bacillariophyceae)
2	<i>Drusus discolor</i>	gut	no PCR product		no PCR product
3	<i>Drusus discolor</i>	gut	no PCR product		no PCR product
4	<i>Drusus chrysotus</i>	Specimen	<i>Drusus chrysotus</i>	99.85	<i>Tychonema bourrellyi</i> (Cyanobacteria)
5	<i>Drusus chrysotus</i>	gut	<i>Drusus chrysotus</i>	99.85	<i>Wohlfahrtiimonas</i> spp., <i>Koukoulia</i> spp.
6	<i>Drusus chrysotus</i>	gut	no PCR product		no PCR product
7	<i>Drusus biguttatus</i>	Specimen	No match		„ <i>Wohlfahrtiimonas</i> sp.“
8	<i>Drusus biguttatus</i>	gut	No match		<i>Staphylococcus</i> sp., <i>Capitis</i> sp.
9	<i>Metanoea rhaetica</i>	Specimen	<i>Metanoea rhaetica</i>	99.85	no match
10	<i>Metanoea rhaetica</i>	gut	No match		no PCR product
11	<i>Metanoea rhaetica</i>	gut	No match		<i>Bosea</i> spp. (Bradyrhizobiaceae)
12	<i>Drusus monticola</i>	Specimen	<i>Drusus monticola</i>	99.24	no match
13	<i>Drusus monticola</i>	gut	no PCR product		no PCR product
14	<i>Drusus monticola</i>	gut	no PCR product		no PCR product

4. Discussion

4.1 Abiotic variables

Water temperature has been identified as one of the key drivers for macrozoobenthos communities (e.g., Resh & Rosenberg 1984; Ward 1992). With respect to this basic abiotic parameter, only very little variation has been observed in the Schreierbach at Lunz. Malicky (1976; 1980; 1981) and Zwick & Zwick (2010) report constant water temperatures of about 6°C throughout the year. In comparison, the mean water temperature of the middle section of the present study in 2017/18, situated close to the measuring site of Malicky (1976; 1980; 1981) was $6.09 \pm 0.43^\circ\text{C}$. The median temperature (5.96°C), however, suggests that stream temperature in 2017/18 was slightly below 6°C in autumn, winter and spring. Data logger data of summer 2017 yielded mean temperatures of $6.43 \pm 0.56^\circ\text{C}$ and median temperatures of 6.3°C. The highest temperatures (8.5°C) and increasing day/night oscillations were recorded in the summer months from June to September. In total, there were three days with temperatures above 8°C; 33 days were recorded with a water temperature above 7°C with 26 of them in summer. These high temperature peaks are in line with water temperature trends of the Lunzer Untersee by Kainz *et al.* (2017). The authors suggest that the highest temperature increase of the Lunz area took place in-between 2000-2015, especially in summer. The temperature increase of the Schreierbach is presumably caused by increased solar radiation and due to the fact that there was low vegetation cover over the stream, in combination with low discharge in summer. Malicky (1976) reported similar temperature peaks of 8°C between 1972 and 1974 during low discharge and prolonged sunny periods. In contrast, the lowest measured temperature in 1972-1974 was 4°C, recorded after strong rainfall in spring and the beginning of summer. Such low temperatures were not recorded in 1981 and in 2017/18. In the present study, the lowest measured temperature was 5.04°C in March 2018.

Other abiotic factors, such as pH, O₂ and conductivity, changed slightly in the course of the sampling period and were similar over the study sections. This suggests a low impact of the catchment area and a buffering of limnochemical parameters by groundwater.

Discharge changed significantly throughout the year 2017. While discharge was low in summer, it increased from fall to spring. In fact, such alternating discharge regimes are expected during future climate change scenarios (Barnett *et al.*, 2005; Botter *et al.*, 2013; Berghuijs *et al.*, 2014).

4.2 Trichoptera diversity and abundance

Trichoptera species were among the most abundant macrozoobenthos taxa found throughout the year in the Schreierbach. In fact, only Chironomids and stoneflies were more abundant. Almost all species found in the Schreierbach occur mainly in the Alps and in adjoining ecoregions, and many of them have upper temperature thresholds of approximately 10°C (Malicky 2014). Exceptions to this distribution pattern are *Tinodes dives* and *Rhyacophila tristis*. These species have a wider distribution in Europe, including plains. *Tinodes dives* is also recorded in Ireland and Great Britain (Waringer & Graf, 2011; Neu *et al.*, 2018). Abundances, however, changed throughout the sampling year. In general, higher caddisfly abundances were recorded in July and August. In general, abundance decreased with increasing age of the caddisfly larvae (Figures 15-17), illustrating drift and predation losses. Drift caused by hydraulic stress plays a significant role for the macrozoobenthos community (Bacher & Waringer, 1996). Drift is the consequence of accidents caused by three hydraulic stressors: hydrodynamic lift, turbulent pressure drag and viscous drag (Waringer, 1989, 1992; Hauer *et al.*, 2012). Hydrodynamic lift forces can be largely neglected for cylindrical bodies and the typical cylindrical cases constructed by Drusinae species and other cased Trichoptera species of the Schreierbach. In addition, caddisflies increase their drift resistance exponentially due to growth which increases their submerged weight. Nevertheless, in spring regions with relatively high discharge, many individuals drift frequently over short distances; the decreasing abundance of mature Trichoptera larvae can therefore be partially explained by the increasing drift rates, which are caused by increasing pressure forces for bigger individuals (Waringer, 1993). In contrast, most first and second instars were collected during the summer months, where low discharge was measured. The sampling sections in the Schreierbach were situated close to the spring which significantly lowers immigration from upstream; therefore, lower macrozoobenthos densities should be expected at the upper section. However, we observed significantly higher densities at the upper section with a linear decrease towards the middle and lower section. This may be due to the fact that most individuals were first instar larvae in the upper section which can be partly explained by compensation flights of females (Lehmann, 1970; Waters, 1972; Müller, 1982). However, the compensation flight theory is discussed highly controversial and highlights only one aspect of high upstream abundance (Light & Adler, 1983; Kopp, Jeschke & Gabriel, 2001). Another important aspect in the Schreierbach is bed morphology: the three sampling sections strongly differed in water level due to a large rock blocking the upper section, thereby causing extremely low water levels in the middle and lower sections during low summer discharge

when most individuals were sampled. As water level is a major factor for creating colonization patches at the choriotope level (Hynes, 1970; Feminella, 1996) and also influences biofilm formation (Singer *et al.*, 2005; Singer *et al.*, 2010; Ceola *et al.*, 2013; Niederdorfer *et al.*, 2016), extremely low water levels at the lower section might have induced upstream migration.

4.3 Effects of environmental parameters on Trichoptera community structure

4.3.1 Choriotopes

Species diversity in the different choriotopes changed over the year. While we observed the highest diversity in the microlithal during winter, a more homogeneous distribution during summer was documented. These variances can be explained by different abiotic conditions. Abiotic variable shifts, trophic relationships and feeding strategies affect habitat selection of each species individually (Cummins & Klug, 1979; Light & Adler, 1983; Wallace & Webster, 1996). However, most Trichoptera species seem to prefer certain microhabitats and maintain their chosen choriotope or a similar one during larval development (Table 4). In addition, with respect to functional feeding types, the four most abundant species differ in type: *Micrasema morosum* is a shredder-grazer, *Drusus discolor* a carnivorous filtering-collector, and *Drusus monticola* and *Tinodes dives* are mainly grazers. The latter two species, however, have different feeding strategies (Waringer & Graf, 2011). While *Drusus monticola* prefers microlithal and macrophyts, *Tinodes dives* tends to avoid these habitats and was found predominantly on megalithal and macrolithal because they need large, stable platforms for fixing their silk galleries and for optimizing biofilm growth. Similarly, *Drusus discolor* and the predaceous Rhyacophilidae species differed greatly in their choriotope chosen. *Rhyacophila glareosa* had a clear preference for microlithal, *Rhyacophila stigmatica* could be found in most cases on xylal and macrolithal, and *Rhyacophila tristis* prefers macro- and megalithal. Megalithal and macrophytes were the preferred choriotopes of *Drusus discolor*, a carnivorous passive filter feeder. With respect to shredders, *Micrasema morosum* prefers macrophytes, while *Acrophylax zerberus* and *Halesus rubricollis* preferred mostly macrolithal, akal and xylal. These examples highlight the fact that species sharing the same functional feeding type use different choriotopes in order to minimize competition (Cummins & Klug, 1979).

4.3.2 Hydraulic and limnochemical parameters

The environmental impact on Trichoptera larvae changed during their life cycle. The fact that adults are expected to choose suitable microhabitats for oviposition implies that this choice has a great impact for the first larval instar (Müller, 1982; Light & Adler, 1983). In the later instars, larvae move freely, selecting the choriotope which optimizes feeding and reduces hydraulic stress. For the 5th instar, habitat selection even increases in importance, as species have to make the right microhabitat choice for pupation and emergence. In fact, Jannot *et al.* (2007) suggest that resource conditions affect the allocation pattern for metamorphosis and potentially the mating success. Pupation of caddisflies last for about four weeks and the pupae is anchored on the stream bottom by silk strands. Every species, however, has individual strategies (Füreder *et al.*, 2005; Malicky, 2000; Waringer & Graf, 2011).

Abiotic conditions explained about 10% of variances in abundance for each species and instar. This relatively low impact illustrates the high mobility of the larvae shifting habitats when abiotic conditions become unfavourable.

The constrained variables of the CCA explained about 15% of variance in species abundance. While megalithal, xylal and macrophytes dominated in high stream velocity, mesolithal, microlithal and akal was positively linked to O₂ saturation, higher temperatures and slower stream velocities. *Micrasema morosum*, *Tinodes dives*, *Rhyacophila stigmatica*, *Rhyacophila tristis* and *Drusus discolor* colonized lotic habitats, while *Drusus monticola*, *Acrophylax zerberus* and *Rhyacophila glareosa* was linked to microlithal.

Conductivity and pH were not significantly different between sections. Erman & Erman (1995) suggest that higher Calcium, alkalinity and pH concentrations could positively affect species richness in springs. The authors, however, conclude that persistence of water, constancy of temperature and the presence of macrophytes are of even greater importance.

4.4 Phenology

For abundant species (>1% of total abundance), size-frequency plots were constructed (Figures 15-17). In most cases, a clear separation of the 5 larval instars was possible. For less abundant species, such as *Pseudopsilopteryx zimmeri*, *Allogamus uncatus*, *Chaetopterygopsis machlachlani*, *Metanoea rhaetica*, *Rhyacophila aurata*, *Rhyacophila vulgaris*, *Drusus bigguttatus*, *Lithax niger*, *Melampophylax melampus* and *Drusus chrysotus*, determination of

juveniles was either difficult or it was not possible to obtain all larval instars. In all species studied in detail, the average head width increment per moult was proportionately constant, and therefore Dyar's rule was applicable.

In the Schreierbach, we observed synchronized life cycles in *Drusus monticola*, *Micrasema morosum*, *Halesus rubricollis* and *Drusus discolor* where instars chronologically followed each other, whereas in *Rhyacophila tristis*, *Rhyacophila stigmatica* and *Tinodes dives* different instars were present in the stream at the same time, suggesting an asynchronic life cycle (Richardson, 2001; Brand & Miserendino, 2011, 2012). Waringer (1986) reports *Rhyacophila* hatches from April to November in the Oberer Seebach. Emergence, as a consequence, is expected to be synchronised in more predictable environments (Lytle & Poff, 2004). Life cycle timing, however, is a consequence to lifelong selection pressures operating differently at each life cycle stage (Richardson, 2001).

4.5 The Trichoptera species inventory of the Schreierbach then and now

Since the emergence studies of Malicky (1979 & 1980; area sampled= 4.5 m²), the median of stream zonation in the present study (area sampled= 22 m²) shifted slightly in an upstream direction. Similarly, also the xenosaprobic valencies increased.

Although two different sampling methods were used (emergence trap, multi habitat sampling), the two investigations resulted in very similar species diversity patterns. New species recorded in the stream are *Lithax niger* and *Allogamus uncatus*, not recorded but expected, by Malicky (1978, 1979, 2014). In addition, by sampling adults there might be an immigration of species from the Oberer Seebach (Malicky, 2013; Rosati *et al.*, 2016). *Allogamus auricollis* and *Hydropsyche pellucidula* were found rarely by Malicky and can both be seen as such immigrants, as *Allogamus auricollis* is very abundant in the Oberer Seebach (Waringer, 1986). *Philopotamus ludificatus* and *Wormaldia copiosa* reached significant abundances in the Schreierbach during the years 1978 and 1979 and were not found in 2017. Regarding the feeding types, much more shredders were found in the stream in 2017. The increase of *Micrasema morosum* was a reason for this shift. Comparing the species community, drastic changes in the most abundant species were noticed. *Rhyacophila* species, *Tinodes dives*, *Acrophylax zerberus* and *Metanoea rhaetica* decreased whereas other species such as *Micrasema morosum*, *Drusus discolor* and *D. monticola* increased. Comparing the losses and gains of relative species abundance with their life cycle data, it was observed that species with a synchronised life cycle increased in relative abundance. In future scenarios

even higher temperature maxima, uncertain stream flow and more frequent droughts are expected (Barnett *et al.*, 2005; Hannah *et al.*, 2007a; Berghuijs *et al.*, 2014).

Another important impact on the Schreierbach community was a recent landslide, which affected most of the surrounding vegetation in the upper and middle sections, causing a loss of canopy shading and leaf litter input.

This, combined with increasing water temperature during summer and altered discharge regimes, might be factors provoking significant shifts in relative species abundance, illustrating the vulnerability of this ecosystem.

4.6 DNA Barcoding and gut contents

Many studies have been carried out concerning Trichoptera DNA barcodes and the primers used (mostly COI) with protocols well established for most taxa (Hebert, Ratnasingham & de Waard, 2003b; Geraci, Al-Saffar & Zhou, 2011; Zhou *et al.*, 2016). Species identification is very secure also through high and accurate reference data in the DNA- barcoding libraries. The increasing availability of sequence data allow the application of DNA-based species identification over all biogeographical regions (Huemer *et al.*, 2018). However, the importance of an integrative taxonomic approach in a growing field of DNA barcoding is strongly supported (Boero, 2001, 2010; Terlizzi *et al.*, 2003; Meyer & Paulay, 2005; Collins & Cruickshank, 2013; Huemer *et al.*, 2018).

In this study, we also attempted to sequence the gut contents of different feeding types within the subfamily Drusinae. Unfortunately only a few sequences matched. For gut content sequences of grazing and shredding Drusinae, algal and fungal primers were not available. Nevertheless, the DNA barcode method is a powerful tool for ecologist and the sequencing of environmental or fecal samples (Valentini, Pompanon & Taberlet, 2009; Hebert *et al.*, 2016). For further studies, accurate preparations of specific gut regions and the usage of multiple primers and protocols or even a meta- barcoding approach are suggested.

The matched sequences indicated bacteria such as *Wohlfahrtiimonas spp.* (Tóth *et al.*, 2008). The authors describe *Wohlfahrtiimonas chitiniclastica* as colonizing the gut of *Wohlfahrtia magnifica* (Diptera: Sarcophagidae). Little is known about the impact on biofilm ecosystems by *Bosea sp.* (Marcondes de Souza, Jackson *et al.*, 2014; Anderson-Glenna *et al.*, 2008). More studies are available on *Staphylococcus sp.* as an important player within biofilm formations (Götz, 2002; Branda *et al.*, 2005; Abee *et al.*, 2011; Solano, Echeverz & Lasa, 2014).

The 16S RNA sequences of *Drusus discolor* suggest the presence of Diatoms on the filtering setae of their legs (Waringer & Graf, 2011). This result is supported by the fact that Diatoms dominate the Schreierbach algal community (Vasselon *et al.*, 2017). Similarly, the detected Cyanobacteria on *Drusus chrysotus* were probably attached to the body surface of the larvae. The results have shown that specific primers for many algal taxa, fungi and other groups are still missing (Le Gall & Saunders, 2010; Vasselon *et al.*, 2017). Ultimately, since habitat preferences, functional traits and indicator values are known on the species level (Waringer *et al.*, 2013; Jackson *et al.*, 2014), DNA barcoding, will provide a cheap and fast monitoring tool which might be crucial in fast-changing ecosystems and their associated foodweb structure (Stein *et al.*, 2014; Bartley *et al.*, 2015).

5. Conclusio

- 1) Variations of limnochemical parameters were strongly buffered by the groundwater input of the stream. Nevertheless, we could show that hydraulic parameters are the main drivers for caddisfly community composition in the Schreierbach. While limnochemical variables affected all larval instars, hydraulic stressors differed in their impact on different larval stages.
- 2) Differences of Trichoptera larval abundance in time were significantly higher between sections than within a given section. As the choriotope area percentage was different in the three sections, we conclude that species with similar functional feeding types and, hence, choriotope preference, shifted sections in order to minimize competition.
- 3) Based on its Trichoptera community, the three study sections of the Schreierbach are classified as a hyporenal to epirhithral stream reach lacking any pollution (xeno- to oligosaprobic). With respect to functional feeding guilds, grazers, shredders and predators dominate.
- 4) All caddisfly species in the Schreierbach were univoltine; the dominant taxa emerged in summer and had a rapid growth phase in winter and spring. Life cycles were either well synchronized (Limnephilidae, *Micrasema morosum*) or acyclic (*Rhyacophila* spp.).
- 5) Comparing the data of the present thesis with the records of Malicky (1979, 1980), little has changed in terms of species richness, but differences in evenness became apparent. Species with well-synchronised life cycles increased in terms of relative abundance. We also recorded a higher percentage of the shredder fraction in the stream, mainly due to an increasing abundance of *Micrasema morosum*.

6. References

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7. Zusammenfassung

Köcherfliegen (Trichoptera) sind in limnischen Ökosystemen weit verbreitet und zählen zu den häufigsten und diversesten Macrozoobenthostaxa. Die Köcherfliegenlarven gelten als sehr anpassungsfähig, wodurch sie in allen denkbaren Süßwasserhabitaten vorkommen können. Sehr unterschiedliche Ernährungstypen, wie z.B. Weidegänger, Zerkleinerer, Prädatoren oder passive Filtrierer, sind dadurch im Laufe der Evolution entstanden. Zum Verständnis von Biozönosen und Ökosystem-Funktionen werden häufig artspezifische Zeigerwerte dieser Insektenordnung herangezogen. Durch ihr hohes Bioindikatorpotential werden mit Hilfe der Köcherfliegen die Auswirkungen anthropogener Verschmutzung, von Flussverbauungsmaßnahmen und die Einflüsse des Klimawandels auf aquatische Ökosysteme erforscht. Diesbezüglich sind kalt adaptierte Arten (z.B. *Drusus* spp.) eine ideale Zielgruppe, um die Reaktionen der Artgemeinschaften auf die sich schnell verändernden Umweltparameter zu untersuchen.

Am Schreierbach, einem Bergbach in den Lunzer Kalkalpen, wurden schon viele Studien durchgeführt. Vor allem die langjährigen Emergenzstudien von Malicky (1972-1980) stellen eine wertvolle Grundlage für die vorliegende Arbeit da. Der Schreierbach zeichnet sich durch eine konstante Wassertemperatur von ungefähr 6°C das ganze Jahr hindurch aus. Neue Untersuchungen im Rahmen der vorliegenden Arbeit am Schreierbach mit standardisierten Methoden wie „Multi Habitat Sampling“ und DNA-Barcoding ermöglichen Langzeitvergleiche und legen einen wichtigen Grundstein für zukünftige Studien.

Mit der vorliegenden Studie konnte gezeigt werden, dass die Trichopterenabundanz stark von hydraulischen Kräften geformt und beeinflusst wird, während sich limnochemische Faktoren in den untersuchten Flussabschnitten nicht signifikant unterscheiden.

Des Weiteren konnten, artspezifische und auch stadienspezifische Choriotoptoppräferenzen nachgewiesen werden. Höchste Individuendichten konnten im Mircolithal festgestellt werden. Hier wurde auch die höchste Biodiversität beobachtet. In Macrophytenbeständen und, in abnehmender Reihenfolge in Megalithal Xylal Mesolithal, Microlithal und Akal wurden signifikant weniger Trichopterenlarven festgestellt. Bemerkenswert ist die sehr hohe Abundanz der Köcherfliegenlarven im quellnächsten Abschnitt im Vergleich zu den mittleren und unteren Abschnitten.

Die artspezifischen Zeigerwerte der Köcherfliegengemeinschaft weisen den Schreierbach als unverschmutztes Segment am Übergang des Hypocrenals zum Epirhithral aus. Seit den 1980er Jahren konnten häufiger auftretende Wassertemperaturmaxima aufgezeichnet werden. Trotzdem wurden die meisten Köcherfliegenarten der Aufnahme von 1980 wieder gefunden.

Gleichzeitig nahmen Arten mit synchronisiertem Lebenszyklus an relativer Häufigkeit zu, während azyklische Arten, (z.B. *Rhyacophila* spp) im Vergleich zu den Studien des letzten Jahrhunderts an relativer Häufigkeit abnahmen.

8. Appendices

Appendix A

Appendix A: Trichoptera abundance per m² in the Schreierbach 2017

Section	Chorotope	Date	<i>Acrophylax zerberus</i>	<i>Allogamus uncatus</i>	<i>All. uncatus/Pes. zimneri</i>	<i>Chaetopterygopsis maclachlani</i>	<i>Chaetopteryx fusca</i>	<i>Crinoecia kempnyi</i>	<i>Drusus biguttatus</i>	<i>Drusus chrysotus</i>	<i>Drusus discolor</i>	<i>Drusus juveniles</i>	<i>Drusus monticola</i>	<i>Halesus rubricollis</i>	<i>Limnephiliinae juveniles</i>	<i>Lithax niger</i>	<i>Melampophylax melampus</i>	<i>Metanoea rhaetica</i>	<i>Microsema morosum</i>	<i>Pseudopsilopteryx zimneri</i>	<i>Rhyacophila aurata</i>	<i>Rhyacophila aurata/vulgaris</i>	<i>Rhyacophila glareosa</i>	<i>Rhyacophila intermedia</i>	<i>Rhyacophila stigmatica</i>	<i>Rhyacophila tristis</i>	<i>Rhyacophila vulgaris</i>	<i>Tinodes dives</i>
Upper	Megalithal	1		26					2	164	24		10						628		14					2		54
Upper	Macrophytes	1		38					3	170			144						246						29			
Upper	Microlithal	1		72									112			8			32		8							48
Upper	Xylal	1		13									3						173						19	6	3	13
Middle	Macrophytes	1									35		11						99		5		5		11	8		
Middle	Macrolithal	1									64								128							96		
Middle	Mesolithal	1									52		4	4			4	12					16					12
Middle	Microlithal	1		32					3		27		13	3		3		5	19		3		48			29		43
Middle	Xylal	1																	48				21		21			5
Lower	Macrophytes	1		13							19		13					3	10						58			
Lower	Macrolithal	1		32														28	8				8			4		
Lower	Mesolithal	1		72			4				8		4					48							4	4		
Lower	Microlithal	1		32			4				28		20	12		4		48	16			4	4		32	4		
Lower	Akal	1		16														48					8		8			
Lower	Xylal	1									32							16							128			
Upper	Megalithal	2									116		4						40	6		16	2			14		32
Upper	Macrophytes	2		3	6								32						32						3			
Upper	Microlithal	2		8	40								368		16				24	264	8		16				40	80
Upper	Xylal	2									35		42			3			90	3		10	13		13	13		
Middle	Macrophytes	2									24		5						80									
Middle	Macrolithal	2									48								32	16						16		16
Middle	Mesolithal	2	8		8						64		28				4	36				4	28					16
Middle	Microlithal	2	80	11	16						45		48			3	3	21	16	19			107					37
Middle	Xylal	2									11		5						27	5								
Lower	Macrophytes	2		6	3						10		3						10	10		3	6			3		
Lower	Macrolithal	2		24							8		8					8	4	4			4	4		4		
Lower	Mesolithal	2		40	4						8		12	4					44				8					
Lower	Microlithal	2	32	20	32						12		4						20									
Lower	Akal	2		16	56								8	24		8		80		80			8					
Lower	Xylal	2									48									16					16			
Upper	Megalithal	3									14	514	20			2			2	2	6				4	4		18
Upper	Macrophytes	3									147	186	42	3								6	3					
Upper	Microlithal	3										25640	272	8	24					96		96	120			24		40
Upper	Xylal	3									16		16							3				3	6	19		
Middle	Macrophytes	3		3							147		3						13			5						
Middle	Macrolithal	3		16									16						16	32		32			16			
Middle	Mesolithal	3	16	36							28			4			20			164		16	4				4	
Middle	Microlithal	3	35	13	3						29		8	3						24	3		27			5	5	19
Middle	Xylal	3	11										5						11	11	11	5	11		5			5
Lower	Macrophytes	3			13			3			70								10			3		3				
Lower	Macrolithal	3	12	24	16								4							144		4						
Lower	Mesolithal	3		12	24									4			4	4		92			8	4		4		4
Lower	Microlithal	3	4									4								40			4	4				
Lower	Akal	3			64															32							8	8
Lower	Xylal	3		16		32					32			16			48			96				16	48			
Upper	Megalithal	4									162		6	14					54		6		2		4	12	4	18
Upper	Macrophytes	4									454	90	80						6		3				6			
Upper	Microlithal	4									320	984	24		32				472		8	16	56			40	8	
Upper	Xylal	4									256	13	3						10		6	10	3		13	35		

Middle	Macrophytes	4				131			3			5		5		3
Middle	Macrolithal	4				16		16				48		16	16	32
Middle	Mesolithal	4				44	96					8	8	12	4	20
Middle	Microlithal	4	3			3	29	3						29		5
Middle	Xylal	4				101		5	11			27		27	27	32
Lower	Macrophytes	4				6		13	19		10				10	
Lower	Macrolithal	4						4						4		
Lower	Mesolithal	4	12						48							
Lower	Microlithal	4	4						120	4				36		
Lower	Akal	4							48					16		
Lower	Xylal	4													48	
Upper	Megalithal	5				32		2			142		14		10	18
Upper	Macrophytes	5				3	560	6	10	3		1226	6	3	3	10
Upper	Microlithal	5				8	672	304		16		192		48	8	8
Upper	Xylal	5				118	3	3				150	6		6	19
Middle	Macrophytes	5				13	3	155		8	11			5	8	5
Middle	Macrolithal	5						16				16		32	128	48
Middle	Mesolithal	5						80	56	44	28		24	4	4	44
Middle	Microlithal	5	3			5	45	88	64		32	3	37	3	96	5
Middle	Xylal	5						5						11		5
Lower	Macrophytes	5				10		42		3	13	13		64		19
Lower	Macrolithal	5				8		8		4				12		4
Lower	Mesolithal	5						28	24	8	12		4		4	8
Lower	Microlithal	5							4							
Lower	Akal	5					56							8		
Lower	Xylal	5					16									32
Upper	Megalithal	6						354	4	8	10		590	12		22
Upper	Macrophytes	6				3	2547	13	13				2282		16	13
Upper	Microlithal	6	16					24	352	536	88		120	8	8	8
Upper	Xylal	6				6		182	10	10	3		243	3		10
Middle	Macrophytes	6						120	13		5		251		5	8
Middle	Macrolithal	6							32				80	16		64
Middle	Mesolithal	6						56	28	4	4		8	16	24	12
Middle	Microlithal	6				5		3	24	13	5		8	5		13
Middle	Xylal	6					5		11	5	5			107		11
Lower	Macrophytes	6				3		218	22		10		3	304		6
Lower	Macrolithal	6						12		16	12			76		4
Lower	Mesolithal	6							28	12			8		4	
Lower	Microlithal	6				4		4	8	16	24		24		20	4
Lower	Akal	6							64	8	360		8	88		8
Lower	Xylal	6						16		64	80					16

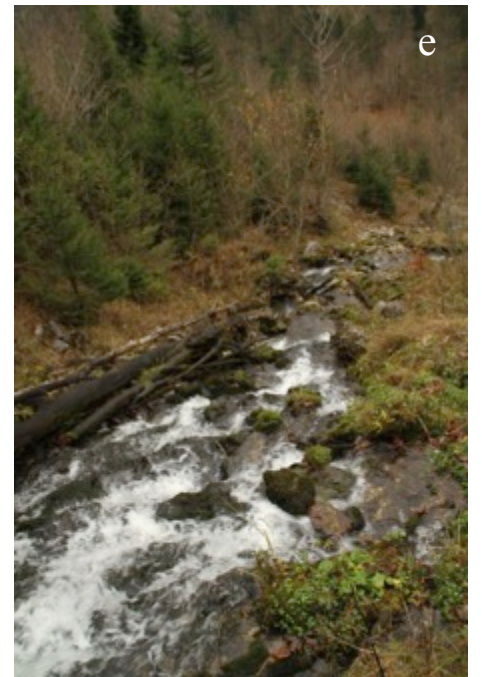
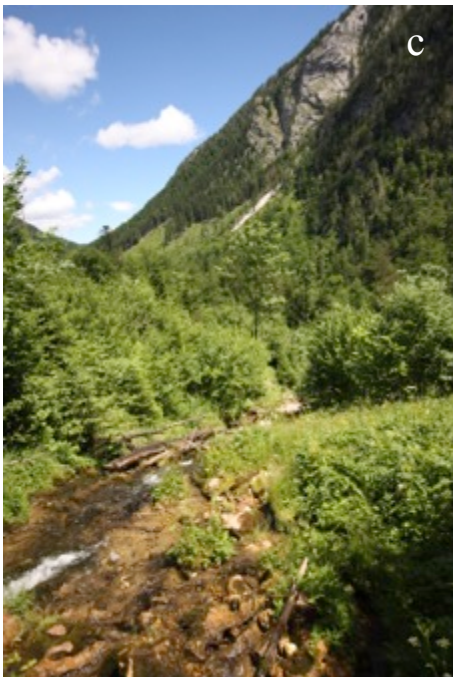
Appendix B

Appendix B: Macrozoobenthos taxa in the Schreierbach 2017 per m⁻².

Section	Choriotope	Date	Trichoptera	Chironomidae	Plecoptera	Ephemeroptera	Collembola	Diptera	Elmids	Gastropoda	Turbellaria	Hydrachnidia	Invertebrata varia	Total (ind m ⁻²)
Upper	Microlithal	1	280	424	264	56	8	24	32	16	96	0	0	1200
Upper	Macrophytes	1	630	822	1667	0	10	285	490	42	109	26	13	4093
Upper	Megalithal	1	924	422	986	0	6	8	28	8	14	2	0	2398
Upper	Xylal	1	230	186	381	6	0	45	35	6	42	0	0	931
Middle	Microlithal	1	227	219	901	325	61	99	421	69	96	11	5	2435
Middle	Macrolithal	1	288	3184	544	16	16	272	32	0	0	0	0	4352
Middle	Mesolithal	1	104	644	168	4	12	32	36	128	28	4	0	1160
Middle	Macrophytes	1	173	347	381	0	80	1363	27	8	27	3	8	2416
Middle	Xylal	1	117	805	373	16	21	389	21	5	11	5	0	1765
Lower	Akal	1	80	56	344	144	32	40	8	56	32	0	56	848
Lower	Mesolithal	1	144	48	380	68	0	28	12	72	16	0	16	784
Lower	Macrolithal	1	80	248	240	4	8	52	12	4	0	4	12	664
Lower	Xylal	1	176	2176	608	0	0	128	16	0	0	0	0	3104
Lower	Macrophytes	1	115	205	125	0	3	394	3	6	3	0	13	867
Lower	Microlithal	1	60	64	224	168	4	8	8	0	8	4	0	548
Upper	Microlithal	2	832	1848	1152	248	0	40	344	128	152	0	24	4768
Upper	Macrophytes	2	77	589	234	0	0	77	19	3	3	0	3	1005
Upper	Megalithal	2	230	2472	84	2	0	18	4	6	2	0	0	2818
Upper	Xylal	2	221	1619	608	3	3	157	48	3	115	0	45	2822
Middle	Macrolithal	2	128	7808	400	0	0	848	16	16	32	0	32	9280
Middle	Microlithal	2	405	1117	760	512	5	85	376	165	232	0	43	3701
Middle	Mesolithal	2	196	1648	704	64	8	152	92	28	60	0	8	2960
Middle	Macrophytes	2	109	1261	197	0	11	240	19	3	16	0	3	1859
Middle	Xylal	2	48	2197	165	0	43	176	27	5	197	0	32	2891
Lower	Akal	2	280	64	552	432	0	48	16	136	56	0	24	1608
Lower	Mesolithal	2	120	512	264	160	4	80	8	16	24	0	4	1192
Lower	Macrolithal	2	68	2368	320	0	24	224	16	4	4	0	44	3072
Lower	Xylal	2	80	2432	592	64	0	384	16	0	16	0	64	3648
Lower	Macrophytes	2	54	2867	656	6	16	419	6	19	0	0	118	4163
Lower	Microlithal	2	120	360	780	348	8	48	4	16	0	0	4	1688
Upper	Microlithal	3	26320	3624	2176	152	312	72	872	184	904	80	40	34736
Upper	Macrophytes	3	387	3050	806	3	134	26	342	0	80	13	0	4842
Upper	Megalithal	3	586	2856	124	0	80	10	52	12	24	2	2	3748
Upper	Xylal	3	64	2003	243	0	48	16	90	0	42	0	10	2515
Middle	Microlithal	3	173	1152	685	320	101	56	355	27	115	19	19	3021
Middle	Macrolithal	3	128	10352	80	16	48	48	0	16	32	0	0	10720
Middle	Mesolithal	3	292	2444	588	116	44	60	172	8	100	4	0	3828
Middle	Macrophytes	3	171	2771	835	0	29	64	109	0	45	5	0	4029
Middle	Xylal	3	75	3803	203	5	59	48	11	0	192	5	11	4411
Lower	Akal	3	112	912	1248	256	88	184	144	16	48	104	40	3152
Lower	Mesolithal	3	40	220	100	196	12	16	4	0	4	16	4	612
Lower	Macrolithal	3	204	148	76	0	0	12	0	0	0	4	0	444
Lower	Xylal	3	304	4576	832	64	96	112	0	16	48	0	0	6048
Lower	Macrophytes	3	99	5370	1322	10	48	243	26	3	16	26	10	7171
Lower	Microlithal	3	56	152	308	296	16	16	20	4	28	4	8	908
Upper	Microlithal	4	1960	912	784	40	40	32	312	64	200	104	32	4480
Upper	Macrophytes	4	643	2211	1203	0	141	48	106	45	45	22	10	4474
Upper	Megalithal	4	282	2402	106	0	4	24	58	4	20	30	0	2930
Upper	Xylal	4	349	2266	413	0	10	38	51	0	51	10	0	3187
Middle	Microlithal	4	72	275	195	235	35	16	85	19	123	5	8	1067
Middle	Macrolithal	4	144	8528	80	64	0	48	96	16	144	144	0	9264
Middle	Mesolithal	4	196	1676	176	184	32	12	216	4	60	72	8	2636
Middle	Macrophytes	4	147	1531	309	5	27	67	109	3	85	32	3	2317
Middle	Xylal	4	229	3600	187	16	5	101	139	0	245	48	0	4571
Lower	Akal	4	64	288	560	680	112	8	40	0	216	64	8	2040
Lower	Mesolithal	4	60	164	152	596	16	48	0	4	24	12	4	1080
Lower	Macrolithal	4	8	664	20	24	32	4	0	0	0	48	4	804
Lower	Xylal	4	48	3008	448	16	144	512	32	0	48	0	16	4272
Lower	Macrophytes	4	58	1072	358	6	51	173	10	6	26	22	22	1805
Lower	Microlithal	4	164	304	304	604	88	28	28	16	48	16	12	1612
Upper	Microlithal	5	1336	864	1296	456	104	32	592	368	872	24	240	6184
Upper	Macrophytes	5	1850	2227	1421	0	6	48	58	6	48	0	0	5664
Upper	Megalithal	5	284	572	116	0	8	4	6	2	8	36	0	1036
Upper	Xylal	5	310	1254	710	0	3	38	35	0	16	6	6	2381
Middle	Microlithal	5	400	403	1205	1275	123	176	640	61	211	13	24	4531
Middle	Macrolithal	5	240	1568	48	16	0	48	16	0	0	0	0	1936
Middle	Mesolithal	5	308	576	496	220	8	136	344	48	120	28	16	2300
Middle	Macrophytes	5	363	2109	880	13	0	675	131	8	45	11	0	4235

Middle	Xylal	5	27	320	107	5	16	21	27	0	5	11	0	539
Lower	Akal	5	64	8	264	416	40	32	8	8	32	0	24	896
Lower	Mesolithal	5	92	132	300	224	12	40	8	12	12	0	8	840
Lower	Macrolithal	5	36	64	68	24	4	84	0	4	0	24	0	308
Lower	Xylal	5	48	64	48	16	0	64	0	0	0	16	0	256
Lower	Macrophytes	5	163	1123	954	3	26	179	10	0	26	10	3	2496
Lower	Microlithal	5	4	12	28	52	8	4	4	4	0	4	0	120
Upper	Microlithal	6	1224	720	1856	432	192	72	528	200	280	48	144	5696
Upper	Macrophytes	6	4944	4717	4672	0	16	112	144	29	166	16	0	14816
Upper	Megalithal	6	1130	1280	568	6	16	38	6	6	8	10	2	3070
Upper	Xylal	6	467	493	605	0	45	26	3	3	19	0	6	1667
Middle	Microlithal	6	120	107	499	168	53	32	40	16	21	5	11	1072
Middle	Macrolithal	6	496	3872	352	32	16	128	96	0	16	32	16	5056
Middle	Mesolithal	6	184	352	792	336	24	52	72	16	40	32	4	1904
Middle	Macrophytes	6	405	1075	1024	3	35	136	45	0	29	5	3	2760
Middle	Xylal	6	192	251	389	5	5	96	59	5	133	16	0	1152
Lower	Akal	6	592	184	1680	408	24	64	80	40	56	32	0	3160
Lower	Mesolithal	6	56	68	232	88	44	28	4	4	4	16	0	544
Lower	Macrolithal	6	264	1800	1248	20	84	164	0	4	4	20	0	3608
Lower	Xylal	6	192	176	672	64	32	96	32	0	16	48	0	1328
Lower	Macrophytes	6	570	864	2963	3	285	208	29	6	58	48	0	5034
Lower	Microlithal	6	116	184	1060	436	80	24	8	8	4	36	0	1956

Appendix C



Schreierbach in 2017: 12 March (a), 19 May (b), 1 July (c), 28 August (d), 1 November (e) and 20 December (f).
Fotos: Jan Martini.

Appendix D



Trichoptera larvae in the Schreierbach: (a) *Drusus monticola* in the wave, (b) *Lithax niger* on a stone, (c) Larvae on stone, (d) *Drusus discolor* on macrophytes, (e) *Drusus monticola* grazing, (f) *Limnephilidae* spp. feeding on a stone. Fotos: Jan Martini

