



A temporal perspective on aquatic subsidy: Bti affects emergence of Chironomidae

Sara Kolbenschlag^a, Verena Gerstle^a, Julian Eberhardt^a, Eric Bollinger^a, Ralf Schulz^{a,b}, Carsten A. Brühl^a, Mirco Bundschuh^{a,c,*}

^a iES Landau, Institute for Environmental Sciences, University of Kaiserslautern-Landau (RPTU), Fortstr. 7, D-76829 Landau, Germany

^b Eußerthal Ecosystem Research Station, University of Kaiserslautern-Landau (RPTU), Birkenhalstr. 13, D-76857 Eußerthal, Germany

^c Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Lennart Hjelm's väg 9, SWE-75007 Uppsala, Sweden

ARTICLE INFO

Edited by Dr R Pereira

Keywords:

Aquatic insects
Emergence dynamics
Riparian food web
Aquatic-terrestrial linkage
Mosquito control
Floodplain mesocosms

ABSTRACT

Emerging aquatic insects serve as one link between aquatic and adjacent riparian food webs via the flux of energy and nutrients. These insects provide high-quality subsidy to terrestrial predators. Thus, any disturbance of emergence processes may cascade to higher trophic levels and lead to effects across ecosystem boundaries. One stressor with potential impact on non-target aquatic insects, especially on non-biting midges (Diptera: Chironomidae), is the widely used mosquito control agent *Bacillus thuringiensis* var. *israelensis* (Bti). In a field experiment, we investigated emerging insect communities from Bti-treated (three applications, maximum field rate) and control floodplain pond mesocosms (FPMs) over 3.5 months for changes in their composition, diversity as well as the emergence dynamics and the individual weight of emerged aquatic insects over time. Bti treatments altered community compositions over the entire study duration – an effect mainly attributed to an earlier (~10 days) and reduced (~26%) peak in the emergence of Chironomidae, the dominant family (88% of collected individuals). The most reasonable explanation for this significant alteration is less resource competition caused by a decrease in chironomid larval density due to lethal effects of Bti. This is supported by the higher individual weight of Chironomidae emerging from treated FPMs (~21%) during Bti application (April – May). A temporal shift in the emergence dynamics can cause changes in the availability of prey in linked terrestrial ecosystems. Consequently, terrestrial predators may be affected by a lack of appropriate prey leading to bottom-up and top-down effects in terrestrial food webs. This study indicates the importance of a responsible and elaborated use of Bti and additionally, highlights the need to include a temporal perspective in evaluations of stressors in aquatic-terrestrial meta-ecosystems.

1. Introduction

Aquatic and terrestrial ecosystems are linked through fluxes of nutrients, matter and energy (Soininen et al., 2015). These donor-controlled fluxes subsidize the receiving ecosystem and thereby increase productivity (Polis et al., 1997). While the input of leaf litter and invertebrates from terrestrial ecosystems supports freshwater food webs (Cole et al., 2006; Fisher and Likens, 1973), emerging aquatic insects subsidize terrestrial predators (Baxter et al., 2005; Henschel et al., 2001). In fact, emerging insects of aquatic origin are considered high-quality prey for several riparian predators such as bats, birds, arthropods (e.g., spiders) and lizards (Bartels et al., 2012; Gratton and

Vander Zanden, 2009; Henschel et al., 2001; Nakano and Murakami, 2001; Paetzold et al., 2005; Salvarina et al., 2018). Thus, alterations in quantity, nutritious quality and timing of aquatic subsidy may induce changes in recipient terrestrial ecosystems either through bottom-up or top-down cascades (reviewed in Schulz et al., 2015).

Changes in quantitative and qualitative parameters of subsidy are induced by several factors of which seasonal variation is dominant (Baxter et al., 2005; Paetzold et al., 2005). In addition to natural factors, anthropogenic modifications in aquatic ecosystems (e.g., hydro-morphological and hydraulic changes, invasive species and pollution) impact subsidy (Gergs et al., 2014; Greig et al., 2012; Schulz et al., 2015). Emergence of aquatic insects has generally been identified as a

Abbreviations: Bti, *Bacillus thuringiensis* var. *israelensis*; FPM, Floodplain pond mesocosm.

* Corresponding author at: iES Landau, Institute for Environmental Sciences, University of Kaiserslautern-Landau (RPTU), Fortstr. 7, D-76829 Landau, Germany.

E-mail address: mirco.bundschuh@rptu.de (M. Bundschuh).

<https://doi.org/10.1016/j.ecoenv.2023.114503>

Received 16 August 2022; Received in revised form 5 November 2022; Accepted 2 January 2023

Available online 5 January 2023

0147-6513/© 2023 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

sensitive indicator of anthropogenic influences, e.g., chemical stress (Schmidt et al., 2015; Schulz and Liess, 2001). However, many studies only investigated alterations of the total emergence by anthropogenic stressors, while few examples focused on the temporal emergence dynamics (Kotalik, 2020). Indeed, a temporal decoupling between the emergence of aquatic insects and the periods of increased energy demands for terrestrial consumers, e.g., riparian spiders, breeding birds or nursing bats, may have far-reaching ecological consequences (Baxter et al., 2005; Kurta et al., 1989; Twining et al., 2018; Zahn et al., 2007).

One stressor of increasing concern is the mosquito control agent *Bacillus thuringiensis* var. *israelensis* (Bti; Belousova et al., 2021; Brühl et al., 2020). Bti carries parasporal toxins, activated in the alkaline milieu of the larval gut, bind to specific receptors and perforate the midgut epithelium causing substantial damage (Boisvert and Boisvert, 2000). Due to its distinct mode of action, Bti is assumed to act taxa-specifically on several nematoceros dipterans (e.g., mosquito and blackfly larvae). It is, moreover, considered biodegradable with a short half-life (Boisvert and Boisvert, 2000; Federici et al., 2012). These beneficial properties stimulated the global use of Bti as an environmentally friendly biocide at breeding sites of target dipterans, mostly wetlands, to reduce nuisance and transmission of diseases.

Although applied to act specifically on target dipterans, Bti has been found to cause adverse effects on non-target dipterans, especially on non-biting midges (Chironomidae), in a number of laboratory and (semi-)field studies (Allgeier et al., 2019; Charbonneau et al., 1994; Kästel et al., 2017; Liber et al., 1998). Other studies found no substantial impact on non-target dipterans (Lagadic et al., 2016; Lundström et al., 2010; Timmermann and Becker, 2017; Wolfram et al., 2018), however, in some of these studies the efficacy of the Bti treatment was not reported. The indication for non-target effects on Chironomidae, which are phylogenetically closely related to the mosquito target species, is of particular concern, especially against the background of aquatic subsidy of terrestrial food webs. Chironomidae contribute substantially to emergent subsidy, in some cases up to 90% (Armitage et al., 1995; Leeper and Taylor, 1998). At the same time, they are considered easily digestible high-protein prey for a variety of terrestrial predators (Bergeron et al., 1988; de la Noüe and Choubert, 1985). Accordingly, the potential of Bti to impact Chironomidae and their emergence may affect the subsidy for terrestrial predators which might cascade bottom-up or top-down through the terrestrial food web (Henschel et al., 2001; Jakob and Poulin, 2016; Poulin et al., 2010).

We designed a replicated field study using twelve artificial floodplain pond mesocosms (FPMs) harbouring natural communities (Stehle et al., 2022). In contrast to previous (semi-)field studies using enclosures (e.g., Allgeier et al., 2019; Duchet et al., 2015) or individual ponds/wetlands at different locations (e.g., Charbonneau et al., 1994; Lundström et al., 2010), this design allowed comparable and realistic conditions among all FPMs (Stehle et al., 2022), and a field-relevant as well as controlled Bti application (Becker et al., 2018). Half of the FPMs received the maximum field rate of Bti three times during spring while the remaining six FPMs served as unexposed control. We determined the emergence at each FPM in terms of abundance, diversity and weight with a particular emphasis on their dynamics between April and July 2020 (3.5 months). We hypothesized that (1) Bti reduces the emergence of insects, mainly those of the family Chironomidae, with consequences on the composition of the emerging insect communities. Driven by potential sublethal implications in the larval stage of this family we additionally hypothesized (2) a temporal delay of emergence with (3) lower individual weight of successfully emerged insects. The latter hypotheses are informed by the dynamic energy budget theory suggesting the allocation of energy to maintenance protecting against toxic stress which in turn reduces the energy available for development and growth (Kooijman, 2000; Péry et al., 2002).

2. Material and methods

2.1. Study sites

The study was conducted from April to July 2020 in twelve artificial floodplain pond mesocosms (FPMs; Fig. 1) at the Eußerthal Ecosystem Research Station (EERES) of the University of Kaiserslautern-Landau, Germany, located in the Palatinate Forest. The FPM system was constructed in 2017 and the ecological development as well as the natural colonization of the FPMs, were monitored for more than two years as described in detail by Stehle et al. (2022). The initial insect communities of the FPMs consisted mainly (< 99%) of Chironomidae, Baetidae, and Coenagrionidae (Stehle et al., 2022). To regulate the water level, every FPM is equipped with an adjustable water supply from a small stream as well as a closable outlet. The structural and ecological quality of the supplying stream is classified as high with negligible anthropogenic influences (Stehle et al., 2022). Since one shore is very shallow, the FPM surface area changes with increasing water level. At application time (see Bti application), every FPM covered a surface area of approx. 104 m² (~20 m x 5.2 m). The vegetation in the FPMs included waterweeds and coontails, green algae as well as emergent plants such as bulrushes and rushes. Over the whole study duration, temperature and dissolved oxygen were monitored with data loggers, and pH was measured manually (Fig. S1).

2.2. Bti application

Between mid of April and the end of May 2020, the water levels of the FPMs were raised from 30 cm to 50 cm three times to mimic flooding. Floods usually trigger the hatching of target mosquito larvae, mainly the floodwater mosquito *Aedes vexans*, and thus the application of Bti in the study region, the Upper Rhine Valley (Becker et al., 2018). Here, the application frequencies can vary between one and eleven times per year with an average of five applications, usually between April and September (Becker et al., 2018; Brühl et al., 2020). On the third day of each flooding (i.e., 14th April, 4th May, 25th May), the mosquito control agent VectoBac WDG (Valent BioSciences, Illinois, USA), containing *Bacillus thuringiensis* var. *israelensis* (Bti), was applied to six FPMs. In Germany, the application rate and method depend on the water depth (i.e., below or above 10 cm) as well as the density and developmental stage of mosquito larvae (BAuA, 2018). To treat the FPMs with the highest recommended application rate (i.e., 2.88×10^9 ITU/ha), a suspension of VectoBac WDG was evenly applied to the FPM surface using a conventional knapsack sprayer (prima 5, GLORIA, Germany). To prevent cross-contamination of control ponds, the spraying was exclusively done on calm days and when no gusts appeared. One week after each

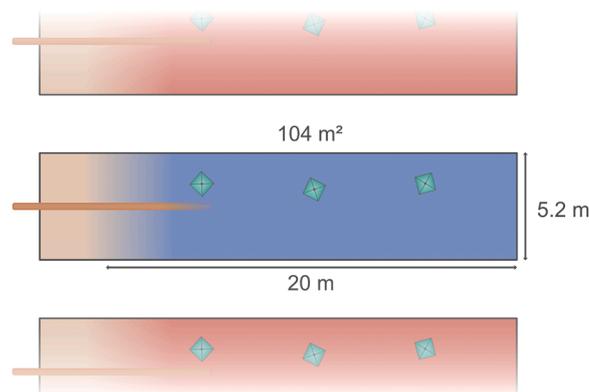


Fig. 1. Schematic figure of a control FPM (blue) and the neighbouring Bti-treated FPMs (red) with dimensions. Light blue squares represent the emergence traps (each 0.33 m²). The floodplain area at the left shore is implied by a fawn gradient.

application, excess water was released to obtain initial water levels (i.e., 30 cm water height) as a precondition for the next application. A detailed time schedule is provided in the [Supplementary material \(Table S1\)](#).

To verify the efficiency of Bti treatments, a biotest was conducted in parallel to every application since no analytical method was available to determine the concentration of Bti toxins. Thus, twelve buckets with filtered FPM water (mesh filter, pore size: 55 µm) and a known number of local mosquito larvae (*Culex sp.*) were prepared in the morning of each application day. One open bucket was placed in every treatment and control FPM over the course of each application leading to the exact same treatment of FPMs and buckets. Subsequently, the number of dead and emerged mosquitoes in every bucket was ascertained daily for seven days. All treatments were considered successful since the mortality of mosquitoes in treated buckets reached at least 90% compared to control mosquitoes which is consistent with official mortality rates achieved by Bti (Becker, 2003, 1997).

2.3. Emergent insect collection and identification

Three floating pyramidal emergence traps (Cadmus et al., 2016) covering a total area of 1 m² were installed at each FPM. The traps were equipped with collection bottles filled with ethylene glycol to catch and preserve emerging insects. From mid-April until mid-June, all bottles were emptied and replaced twice a week being continued with a reduced frequency (i.e., once per week) until the end of July (Table S1). Every insect was determined to family level using a stereomicroscope (SZX 9, Olympus) with a transmitted brightfield illumination base (SZX-ILLB200, Olympus) and taxonomical keys (Klausnitzer, 2011; Köhler et al., 2015). Subsequently, all insects were stored in 70% ethanol for biomass quantification. Organisms without an aquatic life stage and those with a total abundance of only one were considered bycatch and excluded from further analyses.

2.4. Biomass determination of emergent insects

The weight of individuals of three families, namely Chironomidae, Baetidae and Coenagrionidae, was determined after drying at 60 °C for at least 48 h and used to calculate the biomass of the respective family. Those families have been selected as they dominated the community composition either by numbers or size. Baetidae and Coenagrionidae were weighed to the nearest 0.01 mg, while Chironomidae were instead sorted into four size classes based on length and body shape resulting in an average weight per specimen as prerequisite for further analyses.

2.5. Calculations and statistics

The abundances of all collected and determined families were cumulated to obtain the total number of emerged insects. Using the total number of emerged insects as well as subsets for Chironomidae and Baetidae, fluxes as emerged individuals per day and m² were calculated. EmT50 values (Time until 50% of the individuals emerged) were calculated from cumulative abundance data by dose-response modelling using the package “drc” (Ritz et al., 2015). To investigate differences in the diversity of communities, Shannon diversity index *H'* as well as taxa richness *S* and evenness *E* were calculated for every time point using the package “vegan” (Oksanen et al., 2020). The average dry weight (*dw*) of Chironomidae at each time point was calculated from the following equation

$$dw = \frac{1}{n_{total}} \sum_{i=1}^4 n_i \cdot dw_i \quad (1)$$

where *n*_{total} is the sum of individuals, *i* is the size class (1–4), *n*_{*i*} is the number of individuals of size class *i* and *dw*_{*i*} is the average dry weight per individual of size class *i*.

To assess the impact of Bti treatments over the whole study on total abundances, average individual weight, and diversity endpoints, pairwise comparisons between Bti-treated and control FPMs were performed via t-tests after checking for normality and homoscedasticity. To investigate the influence of Bti treatments on the emergence of Chironomidae and Baetidae as well as on the diversity of communities and the weight of emerged individuals over time, autoregressive mixed effect models (LME) were used with treatment, time and their interaction as fixed effects and FPM as a random effect to account for repeated measurements and autocorrelation. For these analyses, fluxes and weight were log₁₀-transformed to fulfil assumptions (i.e., normality and homoscedasticity). Statistical significance of factors in these models was tested using an analysis of variance (ANOVA) with an alpha level of 0.05. Multiple comparisons among factor combinations were done using a Tukey-adjusted post-hoc test based on least-squared means (SAS Institute Inc, 2012).

To further investigate Bti-induced changes in community composition, permutational multivariate analysis of variance (PERMANOVA) in 9999 permutations was performed on Bray-Curtis dissimilarities with treatment and time as predictors. To account for repeated measurements, FPM ID was set as strata. Homogeneity of group dispersion was confirmed using the “betadispr” function (Oksanen et al., 2020). To reduce the influence of dominant species, data were square-root transformed and standardized using the “decostand” function (method: “total”; Oksanen et al., 2020). Additionally, community composition during Bti application (April – May) and afterwards (June – July) were compared. Non-metric multidimensional scaling (NMDS) ordination plots were used for visualization.

All calculations, statistics and data visualizations were conducted with R (4.1.2, R Core Team, 2021) using the packages “dplyr” (Wickham et al., 2021), “plyr” (Wickham, 2011), “tidyr” (Wickham, 2021), “tidyverse” (Wickham et al., 2019), “vegan” (Oksanen et al., 2020), “nlme” (Pinheiro et al., 2021), “emmeans” (Lenth, 2021), “ggpubr” (Kassambara, 2020), “scales” (Wickham and Seidel, 2020), “patchwork” (Pedersen, 2020) and “ggplot2” (Wickham, 2016).

3. Results

3.1. Collected insects

Between mid of April and the end of July, we collected a total of 34,855 emerged insects of aquatic origin belonging to 24 families within five orders: 31,243 flies (Diptera), 3,333 mayflies (Ephemeroptera), 163 dragon- and damselflies (Odonata), 112 caddisflies (Trichoptera) and four beetles (Coleoptera). The most abundant families were Chironomidae (Diptera) and Baetidae (Ephemeroptera) which accounted for 87.8% and 9.4% of the collected insect specimens, respectively. Every other family constituted less than 0.5% (Table S2).

3.2. Effects of Bti on community structure

Total abundance of emerged insects from FPMs treated with Bti was reduced by 11.7% relative to the control. Proportions of all insect families were in equal ranges with small differences between treatments (< 3%; Fig. 2a, c). Consequently, we did not find statistically significant effects of Bti on the total number of collected insects (t.test, df = 9.850, p = 0.485) nor of collected Chironomidae (t.test, df = 9.764, p = 0.405) or Baetidae (t.test, df = 9.936, p = 0.619). Quantities of mosquitoes (Culicidae) were also in equal ranges (Table S2) as they did not occur in the FPMs during the months of Bti application but afterwards, i.e., June and July. Since their developmental time during summer is usually less than two weeks (Clements, 1992), the present individuals were not affected by Bti. Analyses of insect families with a proportion of at least 0.1% revealed a statistically significant difference only for one family of caddisflies, that is Limnephilidae (t.test, df = 7.965, p = 0.022). However, Bti-related differences, positive as well as negative, were also

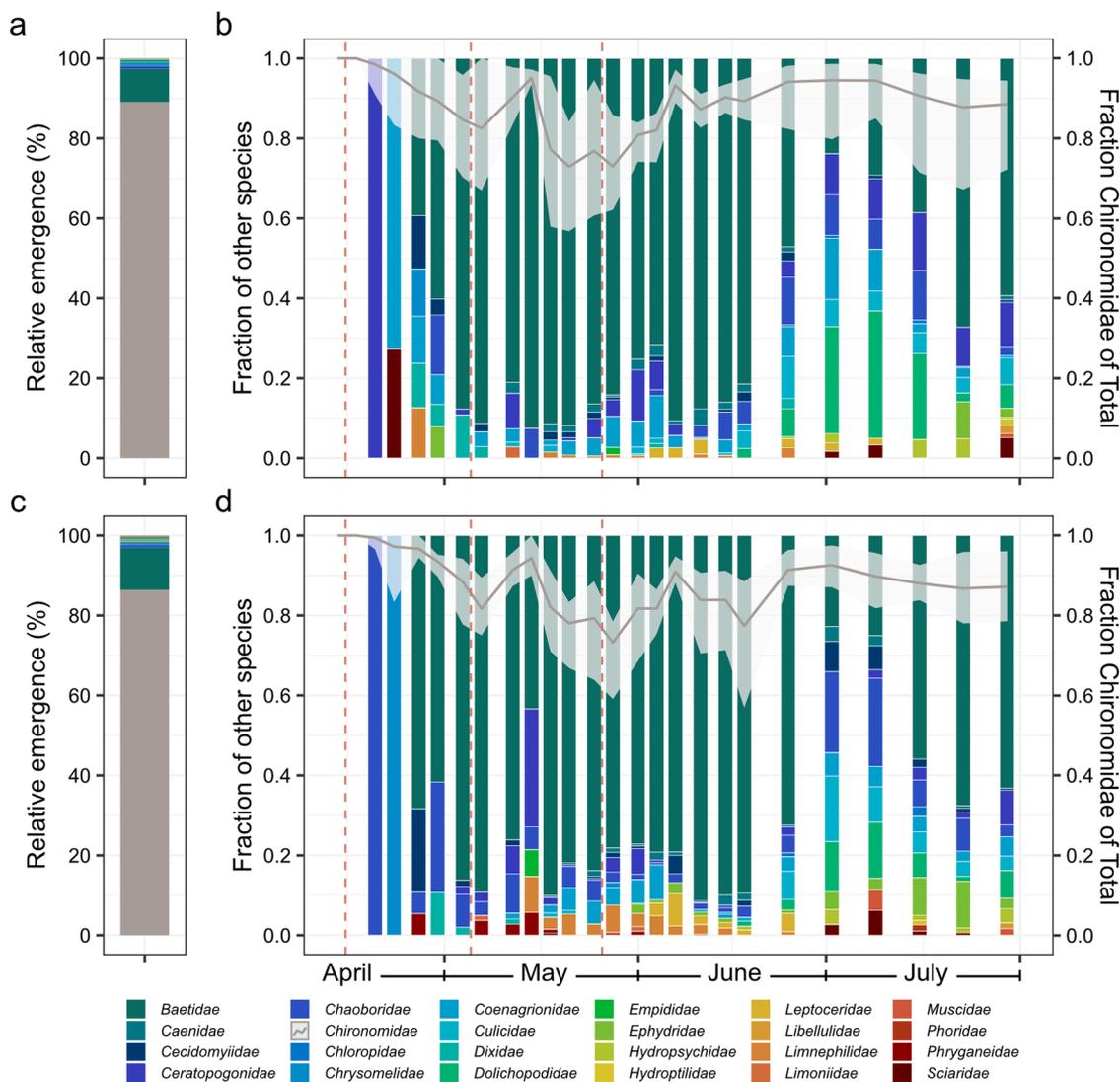


Fig. 2. Left: Composition of total collected insects from a) control FPMs ($n = 6$) and c) FPMs treated with Bti ($n = 6$). Right: Composition of the collected insects for every sampling time point from b) control FPMs ($n = 6$) and d) FPMs treated with Bti ($n = 6$) three times (dashed red lines). Fractions of Chironomidae are displayed on the right and are represented by the grey line. Fractions of other families on the remain are displayed on the left and shown as bars.

observed in other families (Table S2) but due to low abundances and high variability in the data those differences are difficult to interpret and did not turn out statistically significant. Thus, we refrain from a detailed discussion due to substantial uncertainty.

Investigating the community composition (Fig. 2b, d), we found a significant Bti effect over the whole study duration (PERMANOVA, $F_{1,308} = 2.719$, $p = 0.0001$). We further compared the communities during (April – May; Fig. 3a) and following Bti application (June – July; Fig. 3b; PERMANOVA, $F_{1,308} = 2.875$, $p = 0.034$) and found communities after Bti application were more dissimilar than before. Additionally, we detected differences in the temporal dynamics of total emerging insects between treatments (Fig. 4a, ANOVA, $F_{25,249} = 1.751$, $p = 0.0174$). Both results were related to Chironomidae constituting almost 90% of the emerged organisms, thus their dynamics (Fig. 4b; ANOVA, $F_{25,249} = 1.817$, $p = 0.012$) dominated changes on community level (SIMPER, Chironomidae: 79.9%, Baetidae: 14.6%) and also the emergence dynamics of all insects (Fig. 4a, b).

During the period of Bti application, the emergence of Chironomidae in Bti-treated FPMs was on average 1.7 times higher relative to their untreated counterparts (least-square means, $p = 0.016$). Moreover, the peak of Chironomidae emergence was about 10 days earlier under Bti exposure (i.e., beginning of June) with an approximately 26% lower

abundance compared to the peak of the control (i.e., mid of June). Consequently, Chironomidae emergence from treated FPMs reached 50% 8.5 days earlier than from control FPMs ($EmT50_{Bti} = 46.9$ d, $EmT50_{Control} = 55.4$ d). In the period following Bti applications, numbers of emerging Chironomidae from treated FPMs were on average a factor of 1.3 below the control (least-square means, $p = 0.037$). We could not confirm a similar pattern or any other Bti-related difference between treatments for Baetidae (Fig. 4c; ANOVA, $F_{21,191} = 0.250$, $p = 0.999$).

3.3. Effects of Bti on diversity

To analyse the diversity of communities, we compared Shannon diversity indices of Bti-treated FPMs ($H' = 0.54 \pm 0.07$, mean \pm 95% CI) and control FPMs ($H' = 0.47 \pm 0.08$; ANOVA, $F_{1,10} = 0.139$, $p = 0.717$) and their dynamics (ANOVA, $F_{23,205} = 1.031$, $p = 0.429$). Although Bti had no statistically significant effect on those indices, diversity in Bti-treated FPMs was by tendency higher than in control FPMs in the period following Bti application (June – July, Fig. 5a). Investigating the evenness of taxa ($E_{Bti} = 0.19 \pm 0.02$, $E_{Control} = 0.18 \pm 0.03$; Fig. 5b) revealed no significant changes over time (ANOVA, $F_{23,205} = 1.249$, $p = 0.207$). However, evenness was higher in control FPMs during Bti

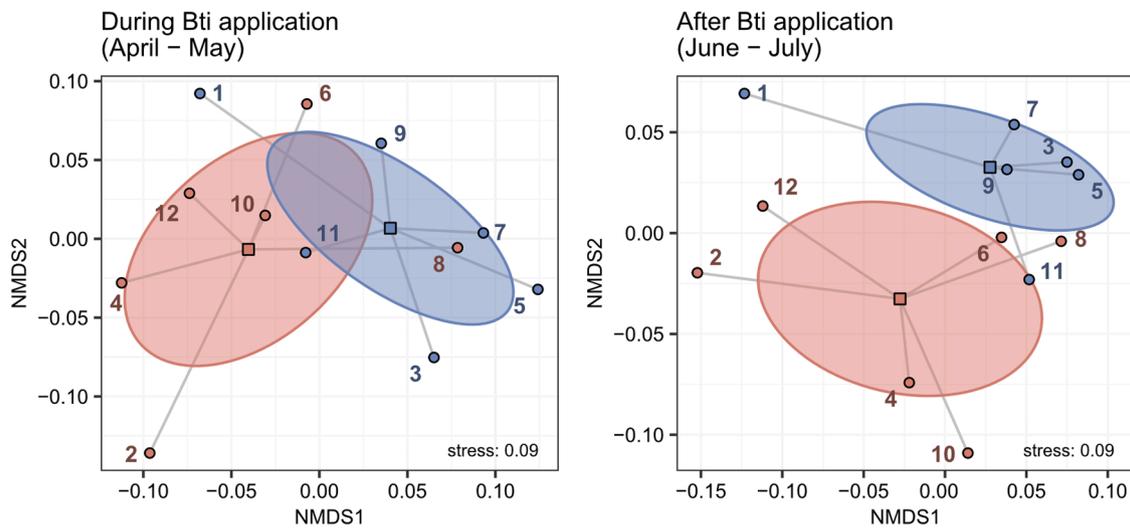


Fig. 3. Non-metric multidimensional scaling (NMDS) ordination for the composition of insect communities from Bti-treated (red; $n = 6$) and control FPMs (blue; $n = 6$) during Bti application (left) and afterwards (right). The stress values are stated as a “goodness-of-fit” measure, indicating a good ordination for values below 0.1 (Clarke, 1993).

application followed by a higher evenness in treated FPMs, which is an inversion of the emergence pattern of Chironomidae (cf. Figs. 4 and 5). We further compared the average number of collected taxa (richness S) between treatments. In total, we collected slightly more taxa in Bti-treated FPMs ($S = 16.67 \pm 1.20$) than in control FPMs ($S = 14.17 \pm 1.71$) which seemed to be confined to the period of Bti application (Fig. 5c). On average, the number of collected taxa per FPM was 7% higher in Bti-treated FPMs than in control FPMs (ANOVA, $F_{1,10} = 1.033$, $p = 0.333$) and changed equally over time (ANOVA, $F_{25,249} = 0.517$, $p = 0.974$; Fig. 5c). Compared to the dominance of Chironomidae, the additional taxa have less influence on the diversity index.

3.4. Effects of Bti on the weight of individuals

Chironomidae from Bti-treated FPMs were 11% heavier than control individuals (weight_{Bti} = 0.23 mg, weight_{Control} = 0.21 mg) though not statistically significant over the entire study duration (ANOVA, $F_{1,10} = 1.902$, $p = 0.198$) or between time points (ANOVA, $F_{25,247} = 0.978$, $p = 0.497$). During the period of Bti application, the average dry weight of Chironomidae in both Bti-treated and control FPMs decreased from ~0.45 mg to ~0.2 mg, with individuals from the Bti treatment being on average 21% heavier (Fig. 6). Due to the method of size sorting, these observations can be attributed to differences in the body condition but also to changes in the dominant species. Following the Bti application period, the average weight remained stable and comparable among both treatments.

Similarly, the average dry weight of Baetidae was not impacted by Bti treatment (t.test, $df = 7.482$, $p = 0.705$). Nonetheless, we observed a general decrease in the individual dry weight of Baetidae over the course of the season (Fig. 6). It was not possible to analyse the dynamics of the weight of Coenagrionidae since numbers were too low. However, dry weight of Coenagrionidae emerging from Bti-treated and control FPMs differed on average by only 0.2% (t.test, $df = 7.512$, $p = 0.976$). Overall, weight changes were mainly related to seasons instead of Bti, thus the total biomass of the assessed families collected over 3.5 months did not significantly differ between treatments (Fig. 6, right; t.test_{Chironomidae}, $df = 8.684$, $p = 0.723$; t.test_{Baetidae}, $df = 9.599$, $p = 0.675$; t.test_{Coenagrionidae}, $df = 7.892$, $p = 0.373$). Consequently, the dynamics of emerged biomass of Chironomidae as well as Baetidae showed nearly the same pattern as the emerged individuals (Fig. S2).

4. Discussion

4.1. Effects on emerging insects

By including a temporal perspective, we could show that even with no significant effects of Bti treatments on the total number of emerging aquatic insects – which was also observed by other studies (reviewed in Brühl et al., 2020) – Bti affected the community composition as well as the number of emerging insects over the course of the study. This points towards alterations in the temporal emergence dynamics of exposed systems. Similarly, diversity indices indicate consequences of Bti mainly for the post-application period (i.e., June – July). As most of the identified taxa contributed less than 0.5% to the emergence, these shifts in diversity and fluxes are mostly driven by implications on the most abundant taxa, Chironomidae and Baetidae, justifying a focus on these in the following.

Emergence dynamics of Chironomidae from Bti-treated and control FPMs differed by a main point: the emergence was shifted by about 10 days leading to an earlier and reduced peak associated with higher emergence during April and May and fewer during June and July. This highly dynamic pattern is, to the best of our knowledge, not yet described as an impact caused by Bti. However, changes in the temporal emergence dynamics of aquatic subsidy were previously observed for other aquatic contaminants, e.g., nanoparticles, pyrethroids or metals, in terms of a delay or a shortening of the emergence peak (Bundschuh et al., 2019; McCahon and Pascoe, 1991; Schulz and Liess, 2000) but also described earlier emergence (Dewey, 1986; Gruessner and Watzin, 1996). Explanations for the observed pattern were mostly contaminant-specific but reasons for earlier emerging insects remained mainly unclear. Our observations may be explained by local chironomid communities assembled from various species comprising a wide range of breeding cycles (voltinism) and feeding strategies (Armitage et al., 1995). Chironomidae species are known to be variably susceptible to Bti which can be partly attributed to the feeding strategy (Ali et al., 1981; Kondo et al., 1995, 1992; Theissinger et al., 2019). Collecting-gathering, grazing or filter-feeding chironomids, for instance, are more likely to directly ingest Bti and thus might be compromised to a higher extent than predatory representatives (Liber et al., 1998; Pillot, 2014a, 2014b). The variations in the susceptibility to Bti of Chironomidae species with different breeding cycles may translate to an altered emergence dynamic of the whole family as observed here. A shift towards more resistant species of larger size could further explain the

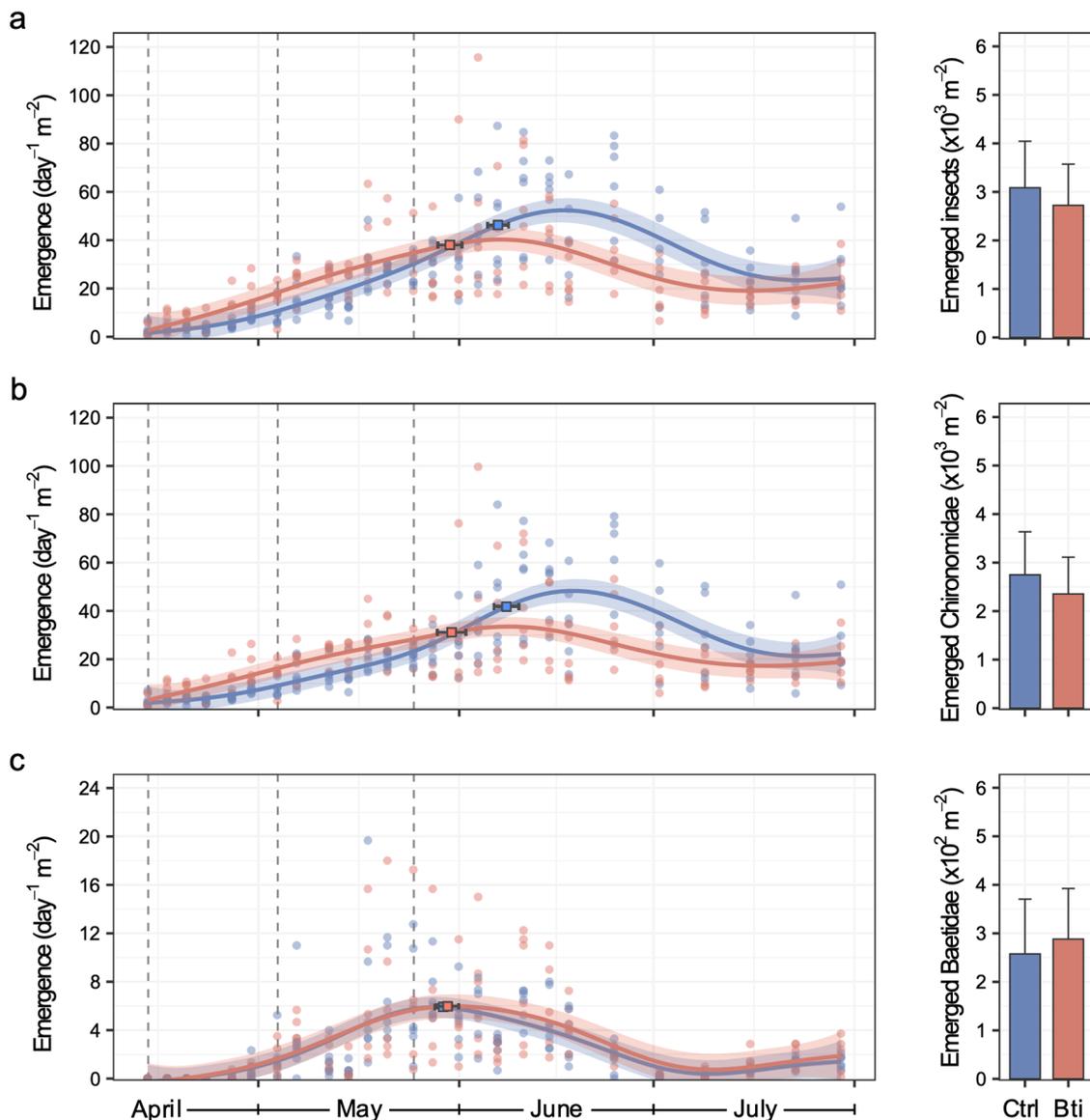


Fig. 4. Number of emerged individuals per day and m^2 over 16 weeks (left) and mean (with 95% CI) total emergence (right) of a) all insects, b) the most abundant insect family: Chironomidae and c) the second most abundant insect family: Baetidae. Six out of twelve FPMs were treated with Bti (red line; control: blue line) three times (dashed lines). The time points of 50% emerged specimens are indicated with squares (with 99% CI).

increased individual weight of emerged Chironomidae from treated FPMs during April and May. Analysing the community composition of the Chironomidae collected in the present study on a lower taxonomic level by using metabarcoding and considering ecological traits such as feeding mode, could give a deeper insight into the underlying mechanisms of taxa-specific Bti susceptibility. It would also provide information on the number and abundance and thus the importance of multivoltine chironomid species (species with more than one generation per year; Pinder, 1986) equally ovipositing in FPMs of both treatments which could have mitigated the observed effects of Bti. Additionally, larvae from the same species can be at different developmental stages in an FPM and thus vary in their sensitivity to Bti (Armitage et al., 1995). In fact, the sensitivity of larvae to Bti decreases with increasing developmental stage (Kästel et al., 2017; Ping et al., 2005). Hence, it may be well feasible that individuals at initial developmental stage have been strongly negatively affected by Bti during its application, which may have translated to a reduced emergence of Chironomidae during the post-application phase (i.e., June and July). This is further supported by a simultaneous investigation of benthic communities in the FPMs during June which found Bti-induced reductions in larval chironomid

abundances by on average 41% (Gerstle et al., 2022). Since the average developmental time of Chironomidae larvae is between two and four months (Pillot, 2014a, 2014b), an extended study duration could have revealed further consequences of Bti on the dynamics of Chironomidae emergence.

A lower abundance of early-stage larvae or highly susceptible species reduces the pressure of resource competition for further developed and less sensitive specimens. Consequently, these organisms may have been able to acquire nutrients and energy more efficiently leading to a quicker development and thus earlier emergence as indicated by the emergence pattern of Chironomidae (Fig. 4; Oliver, 1971; Péry et al., 2002). Also, other species or families of emerging insects could have benefitted from lower densities of chironomid larvae since they could expand their trophic niches leading to higher portions in Bti-treated FPMs (i.e., Limnephilidae; Fig. 2). An increased availability of energy resources is further underlined by heavier Chironomidae emerging from Bti-treated FPMs during the application period. We initially hypothesized lighter individuals due to stress-induced energy allocation from storage to increased maintenance costs and repair mechanisms (Kooijman, 2000; Saraiva et al., 2020; Sokolova et al., 2012). In fact, several studies

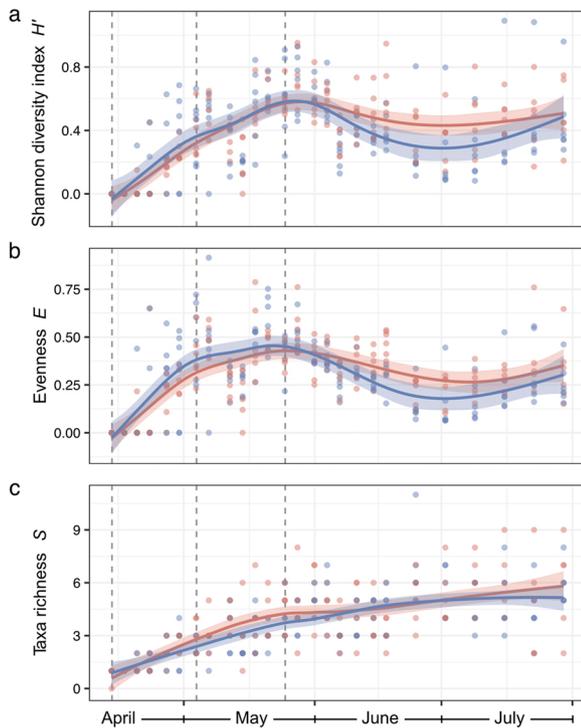


Fig. 5. Diversity endpoints for insect communities from control FPMs (blue; n = 6) and FPMs treated with Bti (red; n = 6) three times (dashed lines). All indices are based on family level.

documented higher energetic costs as a consequence of immunological or biochemical stress responses leading to retarded growth and delayed emergence of individual species (Bordalo et al., 2020; Saraiva et al., 2017; Silvestre Pereira Dornelas et al., 2020). Thus, increased resource availability in a much more complex benthic and in particular chironomid community within our FPMs could have concealed potential energy-demanding effects of Bti. Beyond that, a higher energy supply can accelerate larval growth and development and, in some species, influence adult weight (Hooper et al., 2003; Ristola et al., 1999)

explaining the unexpected results in our study. A closer look into underlying processes, namely the energetic budget of the emerged organisms, might help to further explain our observations, especially concerning reported higher protein content as a consequence of Bti exposure (Bordalo et al., 2020). Additionally, it could improve the assessment of qualitative changes in aquatic subsidy, i.e., in which form energy is transferred, for the nutrition of riparian predators. Given the known differences between sexes in adult weight and emergence time (“Protandry”; Armitage et al., 1995), investigating sex-specific emergence patterns could further deepen the understanding of complex communities.

No impact of Bti on the emergence dynamics or the weight of Baetidae was observed, which is in line with our expectations and informed by the following considerations: The phylogenetic distance between Baetidae (Ephemeroptera) and the Bti target organisms mosquitoes (Culicidae, Diptera) leads to missing prerequisites in their gut for Bti to act (Boisvert and Boisvert, 2000). Thus, the possibility of negative effects is limited which is confirmed by studies reporting no Bti-induced effects on Baetidae (cf. Boisvert and Boisvert, 2000). Consequently, the present study provides further evidence for the prevailing assumption that especially Chironomidae are among the non-target species most likely directly affected by Bti under natural conditions. Nevertheless, the complex interrelations in aquatic insect communities also hold the potential for indirect effects on other taxa. As mentioned above, lower competitive pressure can positively affect population development of other consumers. Furthermore, the decrease of one taxon, especially of a highly abundant one such as Chironomidae, can induce a restructuring of the ecological niches between its competitors but also predators leading to both negative and positive indirect effects on their abundance (Gerstle et al., 2022; Giller, 2012). In fact, differences in the abundance data of a few taxa could further point to an alteration in the species interactions within the assessed communities, even though not statistically significant.

4.2. Consequences of altered dynamics of aquatic subsidy

The shift in the emergence dynamics of Chironomidae, which are the main constituent (e.g., 88%) of the assessed and other natural communities and drive aquatic subsidy, can lead to temporal (un)availability of prey (Allgeier et al., 2019; Hershey et al., 1998). This can be as limiting

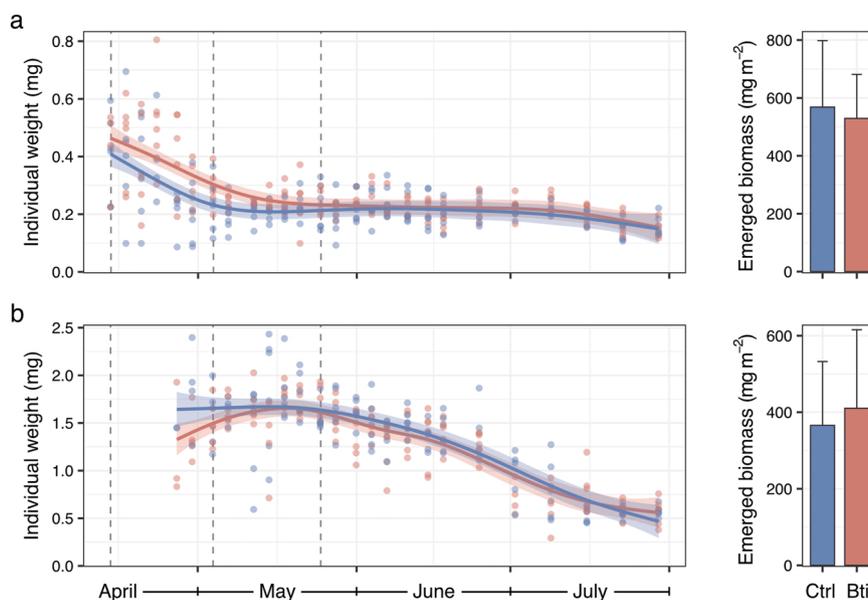


Fig. 6. Mean individual weight in mg over 16 weeks (left) and mean (with 95% CI) total biomass in mg per m² (right) of a) the most abundant insect family: Chironomidae and b) the second most abundant insect family: Baetidae in control FPMs (blue; n = 6) and FPMs treated with Bti (red; n = 6) three times (dashed lines).

to consumers as lower overall abundances since predators may be behaviourally and physiologically adapted to cycles of emergence and thus timing of energy fluxes (Armstrong et al., 2016). Furthermore, fluxes of prey subsidy from aquatic to terrestrial food webs and vice versa can be seasonally asynchronous depending on the productivity of the donor habitat (Nakano and Murakami, 2001). In fact, the in situ prey biomass in some terrestrial habitats (e.g., forests in continental climate) is lowest during winter and spring, and peaks in late summer. Reversely, the flux of aquatic prey to terrestrial systems is highest between spring and early summer, as observed in our systems, buffering for the low availability of prey of terrestrial origin (Nakano and Murakami, 2001). Additionally, insects of aquatic and terrestrial origin feature different nutrient compositions regarding fatty acids. Aquatic insects contain up to 34 times higher amounts of omega-3 long-chain polyunsaturated fatty acids which are essential for several terrestrial consumers since they are unable to synthesize them themselves leading to even higher importance of aquatic subsidy (Shiple et al., 2022). Consequently, the observed shifts in aquatic subsidy may have implications for temporarily dependent terrestrial predators (Polis et al., 1997; Schindler and Smits, 2017). Indeed, the density of terrestrial spiders, particularly horizontal orb-weavers (Tetragnathidae), follows the dynamics of aquatic emergence driven by the high degree of dependency on aquatic subsidy (Kato et al., 2003; Krell et al., 2015; Marczak and Richardson, 2007). Moreover, subsidy-induced changes in spider abundances can cause top-down effects on terrestrial prey as observed by Henschel et al. (2001). Besides spiders, vertebrate consumers such as insectivorous bats and birds show seasonal dependences on aquatic subsidy mainly due to limited terrestrial prey during spring (Altringham, 1996; Nakano and Murakami, 2001; Vaughan, 1997; Zahn et al., 2007). During this season energy demands of both bats and birds are elevated due to reproduction (Arlettaz et al., 2001; Bryant and Westerterp, 1983; Fukui et al., 2006; Zahn et al., 2007). Additionally, the end of hibernation of bats as well as the return of migrant birds during spring are further examples pointing to the importance of well-timed aquatic prey (Gray, 1993; Zahn et al., 2007). Since the application of Bti reduces mosquitoes intentionally by up to 90% (Becker, 1997), additional changes in the temporal availability of other aquatic subsidy, as indicated by our results, could lead to a lack of appropriate nutrition during sensitive time periods (e.g., reproduction phases). In fact, a field study by Poulin et al. (2010) supports this assumption showing negative effects on chick survival of breeding house martins which the authors linked to a Bti-induced reduction in aquatic subsidy. Hence, side effects of Bti on non-target Chironomidae in terms of shifted emergence dynamics may affect the survival as well as the reproductive success of temporal-dependent predators and lead to consequences in other parts of the linked terrestrial systems. Therefore, we suggest to re-assess the use of Bti considering alternative mosquito control measures (e.g., traps or natural predators; Acquah-Lamprey and Brandl, 2018; Poulin et al., 2017) in sensitive natural areas and ecologically valuable wetlands (such as protected areas for nature conservation; BMUV, 2022) or during vulnerable seasons.

5. Conclusion

Our results show that Bti has the potential to change the emergence dynamics of insect families that are closely related to target mosquitoes. If those insects are an important subsidy for adjacent terrestrial food webs, such as Chironomidae, a time-shifted emergence can translate to effects at higher trophic levels. Seasonal dynamics in the availability of in situ, as well as allochthonous prey, cause a temporal dependence of terrestrial predators on aquatic subsidy to cover energy demands. Thus, altered emergence dynamics could lead to a lack of appropriate prey inducing a shift in the diet – if other prey organisms are available – which holds the potential for further indirect effects. As terrestrial predators themselves function as consumers but also as prey within the terrestrial ecosystem, their diet and abundance can affect higher as well

as lower trophic levels. Consequently, changes in the temporal emergence dynamics of aquatic subsidy could alter the whole food web of linked terrestrial ecosystems.

Funding

This study was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – 326210499/GRK2360.

CRediT authorship contribution statement

Sara Kolbenschlag: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Verena Gerstle:** Methodology, Writing – review & editing. **Julian Eberhardt:** Investigation, Writing – review & editing. **Eric Bollinger:** Formal analysis, Visualization, Writing – review & editing. **Ralf Schulz:** Conceptualization, Writing – review & editing. **Carsten A. Brühl:** Methodology, Writing – review & editing. **Mirco Bundschuh:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

The authors thank Lea Bosch and Lukas Beyer for laboratory assistance. The constant support by the team of the Eußerthal Ecosystem Research Station (EERES) is much appreciated without whom this study would not have been feasible. Sincere thanks to the Struktur- und Genehmigungsdirektion Süd (SGD), Neustadt, Germany, for sampling permissions.

Appendix A. Supporting information

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.114503.

References

- Acquah-Lamprey, D., Brandl, R., 2018. Effect of a dragonfly (*Bradinopyga strachani* Kirby, 1900) on the density of mosquito larvae in a field experiment using mesocosms. *Web Ecol.* 18, 81–89. <https://doi.org/10.5194/we-18-81-2018>.
- Ali, A., Baggs, R.D., Stewart, J.P., 1981. Susceptibility of Some Florida chironomids and mosquitoes to various formulations of *Bacillus thuringiensis* serovar. *israelensis*. *J. Econ. Entomol.* 74, 672–677. <https://doi.org/10.1093/jee/74.6.672>.
- Allgeier, S., Kästel, A., Brühl, C.A., 2019. Adverse effects of mosquito control using *Bacillus thuringiensis* var. *israelensis*: reduced chironomid abundances in mesocosm, semi-field and field studies. *Ecotoxicol. Environ. Saf.* 169, 786–796. <https://doi.org/10.1016/j.ecoenv.2018.11.050>.
- Altringham, J.D., 1996. *Bats: Biology and Behaviour*. Oxford University Press.
- Arlettaz, R., Christe, P., Lugon, A., Perrin, N., Vogel, P., 2001. Food availability dictates the timing of parturition in insectivorous mouse-eared bats. *Oikos* 95, 105–111. <https://doi.org/10.1034/j.1600-0706.2001.950112.x>.
- Armitage, P.D., Pinder, L.C., Cranston, P.S., 1995. *The Chironomidae: Biology and Ecology of Non-biting Midges*. Springer Science & Business Media.
- Armstrong, J.B., Takimoto, G., Schindler, D.E., Hayes, M.M., Kauffman, M.J., 2016. Resource waves: phenological diversity enhances foraging opportunities for mobile consumers. *Ecology* 97, 1099–1112. <https://doi.org/10.1890/15-0554.1>.
- Bartels, P., Cucherousset, J., Steger, K., Eklöv, P., Tranvik, L.J., Hillebrand, H., 2012. Reciprocal subsidies between freshwater and terrestrial ecosystems structure consumer resource dynamics. *Ecology* 93, 1173–1182. <https://doi.org/10.1890/11-1210.1>.
- BaAuA, 2018. Zusammenfassung der Eigenschaften des Biozidproduktes VectoBac WG. Zulassungsnummer DE-0011520–18.

- Baxter, C.V., Fausch, K.D., Saunders, W.C., 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. *Freshw. Biol.* 50, 201–220. <https://doi.org/10.1111/j.1365-2427.2004.01328.x>.
- Becker, N., 1997. Microbial control of mosquitoes: Management of the upper rhine mosquito population as a model programme. *Parasitol. Today* 13, 485–487. [https://doi.org/10.1016/S0169-4758\(97\)01154-X](https://doi.org/10.1016/S0169-4758(97)01154-X).
- Becker, N., 2003. Ice granules containing endotoxins of microbial agents for the control of mosquito larvae - a new application technique. *J. Am. Mosq. Control Assoc.* 19, 63–66.
- Becker, N., Ludwig, M., Su, T., 2018. Lack of resistance in *Aedes vexans* field populations after 36 years of *Bacillus thuringiensis* subsp. *israelensis* applications in the Upper Rhine Valley, Germany. *J. Am. Mosq. Control Assoc.* 34, 154–157. <https://doi.org/10.2987/17-6694.1>.
- Belousova, M.E., Malovichko, Y.V., Shikov, A.E., Nizhnikov, A.A., Antonets, K.S., 2021. Dissecting the environmental consequences of *Bacillus thuringiensis* application for natural ecosystems. *Toxins* 13. <https://doi.org/10.3390/toxins13050355>.
- Bergeron, D., Bushway, R.J., Roberts, F.L., Kornfield, I., Okedi, J., Bushway, A.A., 1988. The nutrient composition of an insect flour sample from Lake Victoria, Uganda. *J. Food Compos. Anal.* 1, 371–377. [https://doi.org/10.1016/0889-1575\(88\)90038-5](https://doi.org/10.1016/0889-1575(88)90038-5).
- BMUV, 2022. Gesetzentwurf eines Dritten Gesetzes zur Änderung des Bundesnaturschutzgesetzes: BNatSchG.
- Boisvert, M., Boisvert, J., 2000. Effects of *Bacillus thuringiensis* var. *israelensis* on target and nontarget organisms: a review of laboratory and field experiments. *Biocontrol Sci. Technol.* 10, 517–561. <https://doi.org/10.1080/095831500750016361>.
- Bordalo, M.D., Gravato, C., Beleza, S., Campos, D., Lopes, I., Pestana, J.L.T., 2020. Lethal and sublethal toxicity assessment of *Bacillus thuringiensis* var. *israelensis* and *Beauveria bassiana* based bioinsecticides to the aquatic insect *Chironomus riparius*. *Sci. Total Environ.* 698, 134155. <https://doi.org/10.1016/j.scitotenv.2019.134155>.
- Brühl, C.A., Després, L., Frör, O., Patil, C.D., Poulin, B., Tetreau, G., Allgeier, S., 2020. Environmental and socioeconomic effects of mosquito control in Europe using the biocide *Bacillus thuringiensis* subsp. *israelensis* (Bti). *Sci. Total Environ.* 724, 137800. <https://doi.org/10.1016/j.scitotenv.2020.137800>.
- Bryant, D.M., Westerterp, K.R., 1983. Time and Energy Limits to Brood Size in House Martins (*Delichon urbica*). *J. Anim. Ecol.* 52, 905. <https://doi.org/10.2307/4463>.
- Bundschuh, M., Englert, D., Rosenfeldt, R.R., Bundschuh, R., Feckler, A., Lüderwald, S., Seitz, F., Zubrod, J.P., Schulz, R., 2019. Nanoparticles transported from aquatic to terrestrial ecosystems via emerging aquatic insects compromise subsidy quality. *Sci. Rep.* 9, 15676. <https://doi.org/10.1038/s41598-019-52096-7>.
- Cadmus, P., Pomeranz, J.P.F., Kraus, J.M., 2016. Low-cost floating emergence net and bottle trap: comparison of two designs. *J. Freshw. Ecol.* 31, 653–658. <https://doi.org/10.1080/02705060.2016.1217944>.
- Charbonneau, C.S., Drobney, R.D., Rabeni, C.F., 1994. Effects of *Bacillus thuringiensis* var. *israelensis* on nontarget benthic organisms in a lentic habitat and factors affecting the efficacy of the larvicide. *Environ. Toxicol. Chem.* 13, 267–279. <https://doi.org/10.1002/etc.5620130211>.
- Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. *Austral Ecol.* 18, 117–143. <https://doi.org/10.1111/j.1442-9993.1993.tb00438.x>.
- Clements, A.N., 1992. *The Biology of Mosquitoes. Volume 1: Development, Nutrition and Reproduction*. Chapman & Hall.
- Cole, J.J., Carpenter, S.R., Pace, M.L., van de Bogert, M.C., Kitchell, J.L., Hodgson, J.R., 2006. Differential support of lake food webs by three types of terrestrial organic carbon. *Ecol. Lett.* 9, 558–568. <https://doi.org/10.1111/j.1461-0248.2006.00898.x>.
- Dewey, S.L., 1986. Effects of the herbicide atrazine on aquatic insect community structure and emergence. *Ecology* 67, 148–162. <https://doi.org/10.2307/1938513>.
- Duchet, C., Franquet, E., Lagadic, L., Lagneau, C., 2015. Effects of *Bacillus thuringiensis israelensis* and spinosad on adult emergence of the non-biting midges *Polypedium nubifer* (Skuse) and *Tanytarsus curticornis* Kieffer (Diptera: Chironomidae) in coastal wetlands. *Ecotoxicol. Environ. Saf.* 115, 272–278. <https://doi.org/10.1016/j.ecoenv.2015.02.029>.
- Federici, B.A., Lüthy, P., Ibarra, J.E., 2012. Parasitoid Body of *Bacillus thuringiensis israelensis*. In: Barjac, H., Sutherland, D.J. (Eds.), *Bacterial Control of Mosquitoes and Black Flies Biochemistry, Genetics and Applications of Bacillus Thuringiensis israelensis and Bacillus Sphaericus*. Springer, Netherlands, Dordrecht, pp. 16–44. https://doi.org/10.1007/978-94-011-5967-8_3.
- Fisher, S.G., Likens, G.E., 1973. Energy flow in bear brook, new hampshire: an integrative approach to stream ecosystem metabolism. *Ecol. Monogr.* 43, 421–439. <https://doi.org/10.2307/1942301>.
- Fukui, D., Murakami, M., Nakano, S., Aoi, T., 2006. Effect of emergent aquatic insects on bat foraging in a riparian forest. *J. Anim. Ecol.* 75, 1252–1258. <https://doi.org/10.1111/j.1365-2656.2006.01146.x>.
- Gergs, R., Koester, M., Schulz, R.S., Schulz, R., 2014. Potential alteration of cross-ecosystem resource subsidies by an invasive aquatic macroinvertebrate: implications for the terrestrial food web. *Freshw. Biol.* 59, 2645–2655. <https://doi.org/10.1111/fwb.12463>.
- Gerstle, V., Manfrin, A., Kolbenschlag, S., Gerken, M., Ul Islam, A.S.M.M., Entling, M.H., Bundschuh, M., Brühl, C.A., 2022. Benthic macroinvertebrate community shifts based on Bti-induced chironomid reduction also decrease Odonata emergence. *Environ. Pollut.* 120488. <https://doi.org/10.1016/j.envpol.2022.120488>.
- Giller, P., 2012. *Community Structure and the Niche*. Springer Science & Business Media.
- Gratton, C., Vander Zanden, M.J., 2009. Flux of aquatic insect productivity to land: comparison of lentic and lotic ecosystems. *Ecology* 90, 2689–2699. <https://doi.org/10.1890/08-1546.1>.
- Gray, L.J., 1993. Response of insectivorous birds to emerging aquatic insects in riparian habitats of a tallgrass prairie stream. *Am. Midl. Nat.* 129, 288. <https://doi.org/10.2307/2426510>.
- Greig, H.S., Kratina, P., Thompson, P.L., Palen, W.J., Richardson, J.S., Shurin, J.B., 2012. Warming, eutrophication, and predator loss amplify subsidies between aquatic and terrestrial ecosystems. *Glob. Change Biol.* 18, 504–514. <https://doi.org/10.1111/j.1365-2486.2011.02540.x>.
- Gruessner, B., Watzin, M.C., 1996. Response of aquatic communities from a Vermont stream to environmentally realistic atrazine exposure in laboratory microcosms. *Environ. Toxicol. Chem.* 15, 410–419. <https://doi.org/10.1002/etc.5620150402>.
- Henschel, J.R., Mahsberg, D., Stumpf, H., 2001. Allochthonous aquatic insects increase predation and decrease herbivory in river shore food webs. *Oikos* 93, 429–438. <https://doi.org/10.1034/j.1600-0706.2001.930308.x>.
- Hershey, A.E., Lima, A.R., Niemi, G.J., Regal, R.R., 1998. Effects of *Bacillus thuringiensis israelensis* (Bti) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. *Ecol. Appl.* 8, 41–60. [https://doi.org/10.1890/1051-0761\(1998\)008\[0041:EBOBTB\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1998)008[0041:EBOBTB]2.0.CO;2).
- Hooper, H.L., Sibby, R.M., Hutchinson, T.H., Maund, S.J., 2003. The influence of larval density, food availability and habitat longevity on the life history and population growth rate of the midge *Chironomus riparius*. *Oikos* 102, 515–524. <https://doi.org/10.1034/j.1600-0706.2003.12536.x>.
- Jakob, C., Poulin, B., 2016. Indirect effects of mosquito control using Bti on dragonflies and damselflies (Odonata) in the Camargue. *Insect Conserv Divers* 9, 161–169. <https://doi.org/10.1111/icad.12155>.
- Kassambara, A., 2020. ggpubr: 'ggplot2' Based Publication Ready Plots.
- Kästel, A., Allgeier, S., Brühl, C.A., 2017. Decreasing *Bacillus thuringiensis israelensis* sensitivity of *Chironomus riparius* larvae with age indicates potential environmental risk for mosquito control. *Sci. Rep.* 7, 13565. <https://doi.org/10.1038/s41598-017-14019-2>.
- Kato, C., Iwata, T., Nakano, S., Kishi, D., 2003. Dynamics of aquatic insect flux affects distribution of riparian web-building spiders. *Oikos* 103, 113–120. <https://doi.org/10.1034/j.1600-0706.2003.12477.x>.
- Klausnitzer, B. (Ed.), 2011. *Stresemann - Exkursionsfauna von Deutschland, Band 2: Wirbellose Insekten*, 11. Aufl. 2011. ed. Spektrum Akademischer Verlag, Heidelberg.
- Köhler, G., Müller, H.J., Bährmann, R. (Eds.), 2015. *Bestimmung wirbelloser Tiere: Bildtafeln für zoologische Bestimmungsübungen und Exkursionen*, 7., überarbeitete und ergänzte Auflage. ed. Springer eBook Collection. Springer Spektrum, Berlin and Heidelberg. <https://doi.org/10.1007/978-3-642-55395-0>.
- Kondo, S., Ohba, M., Ishii, T., 1992. Larvicidal activity of *Bacillus thuringiensis* serovar *israelensis* against nuisance chironomid midges (Diptera: Chironomidae) of Japan. *Let. Appl. Microbiol* 15, 207–209. <https://doi.org/10.1111/j.1472-765X.1992.tb00764.x>.
- Kondo, S., Ohba, M., Ishii, T., 1995. Comparative susceptibility of chironomid larvae (Dipt., Chironomidae) to *Bacillus thuringiensis* serovar *israelensis* with special reference to altered susceptibility due to food difference. *J. Appl. Entomol.* 119, 123–125. <https://doi.org/10.1111/j.1439-0418.1995.tb01256.x>.
- Kooijman, S.A.L.M., 2000. *Dynamic Energy and Mass Budgets in Biological Systems*. Cambridge University Press.
- Kotalik, C.J., 2020. Mesocosms to Evaluate Aquatic-Terrestrial Contaminant Linkages Using Aquatic Insect Emergence: Utility for Aquatic Life Criteria Development. In: Romano, Kraus (Ed.), *Contaminants and Ecological Subsidies*. Springer International Publishing, pp. 253–278. https://doi.org/10.1007/978-3-030-49480-3_11 ([S.I.]).
- Krell, B., Röder, N., Link, M., Gergs, R., Entling, M.H., Schäfer, R.B., 2015. Aquatic prey subsidies to riparian spiders in a stream with different land use types. In: *Limnologia*, 51, pp. 1–7. <https://doi.org/10.1016/j.limno.2014.10.001>.
- Kurta, A., Bell, G.P., Nagy, K.A., Kunz, T.H., 1989. Energetics of pregnancy and lactation in freeranging little Brown Bats (*Myotis lucifugus*). *Physiol. Zool.* 62, 804–818. <https://doi.org/10.1086/physzool.62.3.30157928>.
- Lagadic, L., Schäfer, R.B., Roucaute, M., Szócs, E., Chouin, S., Maupeou, J., Duchet, C., Franquet, E., Le Hunsec, B., Bertrand, C., Fayolle, S., Francés, B., Rozier, Y., Foussadier, R., Santoni, J.-B., Lagneau, C., 2016. No association between the use of Bti for mosquito control and the dynamics of non-target aquatic invertebrates in French coastal and continental wetlands. *Sci. Total Environ.* 553, 486–494. <https://doi.org/10.1016/j.scitotenv.2016.02.096>.
- Leeper, D.A., Taylor, B.E., 1998. Insect emergence from a South Carolina (USA) temporary Wetland Pond, with Emphasis on the Chironomidae (Diptera). *J. North Am. Benthol. Soc.* 17, 54–72. <https://doi.org/10.2307/1468051>.
- Lenth, R.V., 2021. emmeans: Estimated Marginal Means, aka Least-Squares Means.
- Liber, K., Schmude, K.L., Rau, D.M., 1998. Toxicity of *Bacillus thuringiensis* var. *israelensis* to chironomids in pond mesocosms. *Ecotoxicology* 7, 343–354. <https://doi.org/10.1023/A:1008867815244>.
- Lundström, J.O., Schäfer, M.L., Petersson, E., Persson Vinnersten, T.Z., Landin, J., Brodin, Y., 2010. Production of wetland Chironomidae (Diptera) and the effects of using *Bacillus thuringiensis israelensis* for mosquito control. *Bull. Entomol. Res.* 100, 117–125. <https://doi.org/10.1017/S0007485309990137>.
- Marczak, L.B., Richardson, J.S., 2007. Spiders and subsidies: results from the riparian zone of a coastal temperate rainforest. *J. Anim. Ecol.* 76, 687–694. <https://doi.org/10.1111/j.1365-2656.2007.01240.x>.
- McCahon, C.P., Pascoe, D., 1991. Brief-exposure of first and fourth instar *Chironomus riparius* larvae to equivalent assumed doses of cadmium: effects on adult emergence. *Water Air Soil Pollut.* 60, 395–403. <https://doi.org/10.1007/BF00282634>.
- Nakano, S., Murakami, M., 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. *Proc. Natl. Acad. Sci. USA* 98, 166–170. <https://doi.org/10.1073/pnas.98.1.166>.

- de la Noüe, J., Choubert, G., 1985. Apparent digestibility of invertebrate biomasses by rainbow trout. *Aquaculture* 50, 103–112. [https://doi.org/10.1016/0044-8486\(85\)90156-5](https://doi.org/10.1016/0044-8486(85)90156-5).
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2020. *vegan*: Community Ecology Package.
- Oliver, D.R., 1971. Life history of the chironomidae. *Annu. Rev. Entomol.* 16, 211–230.
- Paetzold, A., Schubert, C.J., Tockner, K., 2005. Aquatic terrestrial linkages along a braided-river: riparian arthropods feeding on aquatic insects. *Ecosystems* 8, 748–759. <https://doi.org/10.1007/s10021-005-0004-y>.
- Pedersen, T.L., 2020. *patchwork*: The Composer of Plots.
- Péry, A.R.R., Mons, R., Flammarion, P., Lagadic, L., Garric, J., 2002. A modeling approach to link food availability, growth, emergence, and reproduction for the midge *Chironomus riparius*. *Environ. Toxicol. Chem.* 21, 2507–2513. <https://doi.org/10.1002/etc.56202111133>.
- Pillot, H.K.M.M., 2014a. Chironomidae Larvae of the Netherlands and Adjacent Lowlands, Vol. 2: Biology and Ecology of the Chironomina. KNNV Publishing, Zeist.
- Pillot, H.K.M.M., 2014b. Chironomidae Larvae of the Netherlands and Adjacent lowlands, Vol. 3: Biology and Ecology of the Aquatic Orthocladinae. KNNV Publishing, Zeist.
- Pinder, L.C.V., 1986. Biology of freshwater chironomidae. *Annu. Rev. Entomol.* 31, 1–23. <https://doi.org/10.1146/annurev.en.31.010186.000245>.
- Ping, L., Wen-Ming, Z., Shui-Yun, Y., Jin-Song, Z., Li-Jun, L., 2005. Impact of environmental factors on the toxicity of *Bacillus thuringiensis* var. *israelensis* IPS82 to *Chironomus kiiensis*. *J. Am. Mosq. Control Assoc.* 21, 59–63. [https://doi.org/10.2987/8756-971X\(2005\)21\[59:IOEFOT\]2.0.CO;2](https://doi.org/10.2987/8756-971X(2005)21[59:IOEFOT]2.0.CO;2).
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Team, R.C., 2021. *nlme*: Linear and Nonlinear Mixed Effects Models.
- Polis, G.A., Anderson, W.B., Holt, R.D., 1997. Toward an Integration of Landscape and Food Web Ecology: The Dynamics of Spatially Subsidized Food Webs. *Annu. Rev. Ecol. Syst.* 28, 289–316. <https://doi.org/10.1146/annurev.ecolsys.28.1.289>.
- Poulin, B., Lefebvre, G., Paz, L., 2010. Red flag for green spray: adverse trophic effects of Bti on breeding birds. *J. Appl. Ecol.* 47, 884–889. <https://doi.org/10.1111/j.1365-2664.2010.01821.x>.
- Poulin, B., Lefebvre, G., Muranyi-Kovacs, C., Hilaire, S., 2017. Mosquito traps: an innovative, environmentally friendly technique to control mosquitoes. *Int. J. Environ. Res. Public Health* 14. <https://doi.org/10.3390/ijerph14030313>.
- R Core Team, 2021. *R: A Language and Environment for Statistical Computing*.
- Ristola, T., Pellinen, J., Ruokolainen, M., Kostamo, A., Kukkonen, J.V.K., 1999. Effect of sediment type, feeding level, and larval density on growth and development of a midge (*Chironomus riparius*). *Environ. Toxicol. Chem.* 18c, 756–764. <https://doi.org/10.1002/etc.5620180423>.
- Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-response analysis using R. *PLoS One* 10.
- Salvarina, I., Gravier, D., Rothhaupt, K.-O., 2018. Seasonal bat activity related to insect emergence at three temperate lakes. *Ecol. Evol.* 8, 3738–3750. <https://doi.org/10.1002/ece3.3943>.
- Saraiva, A.S., Sarmiento, R.A., Rodrigues, A.C., Campos, D., Fedorova, G., Zlábek, V., Gravato, C., Pestana, J.L., Soares, A.M., 2017. Assessment of thiamethoxam toxicity to *Chironomus riparius*. *Ecotoxicol. Environ. Saf.* 137, 240–246.
- Saraiva, A.S., Sarmiento, R.A., Gravato, C., Rodrigues, A.C.M., Campos, D., Simão, F.C.P., Soares, A.M.V.M., 2020. Strategies of cellular energy allocation to cope with paraquat-induced oxidative stress: chironomids vs Planarians and the importance of using different species. *Sci. Total Environ.* 741, 140443. <https://doi.org/10.1016/j.scitotenv.2020.140443>.
- SAS Institute Inc, 2012. LSMEANS statement.
- Schindler, D.E., Smits, A.P., 2017. Subsidies of aquatic resources in terrestrial ecosystems. *Ecosystems* 20, 78–93. <https://doi.org/10.1007/s10021-016-0050-7>.
- Schmidt, T.S., Kraus, J.M., Walters, D.M., Wanty, R.B., 2015. Correction to emergence flux declines disproportionately to larval density along a stream metal gradient. *Environ. Sci. Technol.* 49, 13079. <https://doi.org/10.1021/acs.est.5b03539>.
- Schulz, R., Liess, M., 2000. Toxicity of fenvalerate to caddisfly larvae: chronic effects of 1- vs 10-h pulse-exposure with constant doses. *Chemosphere* 41, 1511–1517. [https://doi.org/10.1016/S0045-6535\(00\)00107-7](https://doi.org/10.1016/S0045-6535(00)00107-7).
- Schulz, R., Liess, M., 2001. Toxicity of aqueous-phase and suspended particle-associated fenvalerate: chronic effects after pulse-dosed exposure of *Limnephilus lunatus* (Trichoptera). *Environ. Toxicol. Chem.* 20, 185–190. <https://doi.org/10.1002/etc.5620200121>.
- Schulz, R., Bundschuh, M., Gergs, R., Brühl, C.A., Diehl, D., Entling, M.H., Fahse, L., Frör, O., Jungkunst, H.F., Lorke, A., Schäfer, R.B., Schaumann, G.E., Schwenk, K., 2015. Review on environmental alterations propagating from aquatic to terrestrial ecosystems. *Sci. Total Environ.* 538, 246–261. <https://doi.org/10.1016/j.scitotenv.2015.08.038>.
- Shipley, J.R., Twining, C.W., Mathieu-Resuge, M., Parmar, T.P., Kainz, M., Martin-Creuzburg, D., Weber, C., Winkler, D.W., Graham, C.H., Matthews, B., 2022. Climate change shifts the timing of nutritional flux from aquatic insects. *Curr. Biol.* 32, 1342–13493. <https://doi.org/10.1016/j.cub.2022.01.057>.
- Silvestre Pereira Dornelas, A., A Sarmiento, R., C Rezende Silva, L., de Souza Saraiva, A., J de Souza, D., D Bordalo, M., MVM Soares, A., LT Pestana, J., 2020. Toxicity of microbial insecticides toward the non-target freshwater insect *Chironomus xanthus*. *Pest Manag. Sci.* 76, 1164–1172. <https://doi.org/10.1002/ps.5629>.
- Soininen, J., Bartels, P., Heino, J., Luoto, M., Hillebrand, H., 2015. Toward more integrated ecosystem research in aquatic and terrestrial environments. *BioScience* 65, 174–182. <https://doi.org/10.1093/biosci/biu216>.
- Sokolova, I.M., Frederich, M., Bagwe, R., Lannig, G., Sukhotin, A.A., 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Mar. Environ. Res.* 79, 1–15. <https://doi.org/10.1016/j.marenvres.2012.04.003>.
- Stehle, S., Manfrin, A., Feckler, A., Graf, T., Joschko, T.J., Jupke, J., Noss, C., Rösch, V., Schirmel, J., Schmidt, T., Zubrod, J.P., Schulz, R., 2022. Structural and functional development of twelve newly established floodplain pond mesocosms. *Ecol. Evol.* 12, 8674. <https://doi.org/10.1002/ece3.8674>.
- Theisinger, K., Röder, N., Allgeier, S., Beermann, A.J., Brühl, C.A., Friedrich, A., Michiels, S., Schwenk, K., 2019. Mosquito control actions affect chironomid diversity in temporary wetlands of the Upper Rhine Valley. *Mol. Ecol.* 28, 4300–4316. <https://doi.org/10.1111/mec.15214>.
- Timmermann, U., Becker, N., 2017. Impact of routine *Bacillus thuringiensis israelensis* (Bti) treatment on the availability of flying insects as prey for aerial feeding predators. *Bull. Entomol. Res.* 107, 705–714. <https://doi.org/10.1017/S0007485317000141>.
- Twining, C.W., Shipley, J.R., Winkler, D.W., 2018. Aquatic insects rich in omega-3 fatty acids drive breeding success in a widespread bird. *Ecol. Lett.* 21, 1812–1820. <https://doi.org/10.1111/ele.13156>.
- Vaughan, N., 1997. The diets of British bats (Chiroptera). *Mammal. Rev.* 27, 77–94. <https://doi.org/10.1111/j.1365-2907.1997.tb00373.x>.
- Wickham, H., 2011. The Split-Apply-Combine Strategy for Data Analysis 40, 129.
- Wickham, H., 2016. *ggplot2: Elegant Graphics for Data Analysis*.
- Wickham, H., 2021. *tidyr: Tidy Messy Data*.
- Wickham, H., Seidel, D., 2020. *scales: Scale Functions for Visualization*.
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller, E., Bache, S.M., Müller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H., 2019. Welcome to the tidyverse. *J. Open Source Softw.* 4, 1686. <https://doi.org/10.21105/joss.01686>.
- Wickham, H., François, R., Henry, L., Müller, K., 2021. *dplyr: A Grammar of Data Manipulation*.
- Wolfram, G., Wenzl, P., Jerrentrup, H., 2018. A multi-year study following BACI design reveals no short-term impact of Bti on chironomids (Diptera) in a floodplain in Eastern Austria. *Environ. Monit. Assess.* 190, 709. <https://doi.org/10.1007/s10661-018-7084-6>.
- Zahn, A., Rodrigues, L., Rainho, A., Palmeirim, J.M., 2007. Critical times of the year for *Myotis myotis*, a temperate zone bat: roles of climate and food resources. *Acta* 9, 115–125. [https://doi.org/10.3161/1733-5329\(2007\)9\[115:CTOTYF\]2.0.CO;2](https://doi.org/10.3161/1733-5329(2007)9[115:CTOTYF]2.0.CO;2).