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Disperzní a letová aktivita pakomárů stojatých vod
(Diptera: Chironomidae)

Diplomová práce

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Anotace

V této práci nejprve shrnuji význam disperze pro jedince a populace včetně nejvýznamnějších faktorů ovlivňujících jednotlivé fáze disperze, s důrazem na vodní hmyz obývající malé stojaté vody. Anglicky psaný rukopis vědeckého článku následně shrnuje kvantitativní studii krátkodobé a sezónní letové aktivity pakomárů (Chironomidae: Diptera) v pískovně Cep II v jižních Čechách. Práce přináší jeden z prvních ucelených pohledů na vliv počasí a sezonality na celkovou letovou aktivitu pakomárů, fenologii nejhojnějších druhů a rozdíly mezi suchozemskými a vodními druhy.

Annotation

I first review the significance of dispersal for individuals and populations including the role of the most significant environmental drivers on separate phases of dispersal. Emphasis is put on aquatic insects inhabiting small standing waters. The following manuscript summarizes a quantitative study of diel and seasonal flight patterns of adult Chironomidae in the Cep II sandpit in southern Bohemia. It provides one of the first comprehensive treatments of the effect of weather conditions and seasonality on the flight activity of adult Chironomidae, phenology of most common species and differences between terrestrial and aquatic species.

Prohlášení

Prohlašuji, že svoji diplomovou práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

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Podpis

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Cíle práce

- provést literární rešerši shrnující informace o sezónní a diurnální letové aktivitě vodního hmyzu se zaměřením na pakomáry stojatých vod
- v návaznosti na bakalářskou práci vyhodnotit pomocí moderních statistických metod sezónní a diurnální změny v letové aktivitě pakomárů v pískovně Cep II u Suchdola nad Lužnicí

Úvodní komentář

Sladkovodní habitaty stojatých vod jsou z geologického hlediska relativně mladé (Rundle, Bilton & Foggo, 2007) a pohybují se na gradientu velikostí od velkých trvalých jezer až po drobné vysychající tůně (Wellborn, Skelly & Werner, 1996). Přežití a zachování druhů obývajících tyto diskrétní habitaty obklopené terestrickým prostředím vyžaduje schopnost šířit se prostorem (Bilton, Freeland & Okamura, 2001). Navzdory zmíněné izolovanosti a malé propojenosti jednotlivých habitatů mají sladkovodní organismy poměrně široké geografické rozšíření, za které vděčí právě schopnosti dispergovat.

Společenstvům vodních nádrží bez ryb dominuje vodní hmyz, který dále můžeme rozlišit na dvě základní skupiny: hmyz s komplexním životním cyklem (téměř vždy vodní larvy a suchozemští dospělci) a na hmyz celým životním cyklem (někdy s výjimkou kukly) vázaným na vodní prostředí. Do první skupiny lze kromě vážek zařadit i řadu dvouřídých, např. pakomáry (Chironomidae) a komáry (Culicidae), kteří jsou na nestabilní prostředí drobných stojatých vod adaptováni rychlým larválním vývojem. Druhá skupina v čele s vodními brouky (Coleoptera) a plošticemi (Hemiptera) se naopak vyznačuje vysokou mírou disperze z vodních nádrží do okolí (Fernando & Galbraith, 1973).

Disperze

Disperze má zásadní vliv na fitness jedinců, populační dynamiku, abundanci a distribuci druhů (Nathan, 2001; Clobert *et al.*, 2012) i na strukturu společenstev (Bohonak, 1999; Clobert, Ims & Rousset, 2004). U prostorově strukturovaných populací zvyšuje jejich celkovou propojenost, kolonizaci nových lokalit nebo posiluje stávající populace a redukuje tak riziko náhodných extinkcí (Clobert *et al.*, 2001, 2004). Na míře disperze také závisí rychlost utváření společenstva (Bohonak & Jenkins, 2003).

Současný výklad chápe disperzi jako přenos jedinců především za účelem reprodukce napříč mozaikou různě velkých habitatových ostrůvků odlišné kvality (Bowler & Benton, 2005; Clobert *et al.*, 2012). Navzdory energetické náročnosti představuje disperze nedílnou součást životního cyklu většiny zástupců vodního hmyzu, bez které by se reprodukce v mnoha případech vůbec neuskutečnila (Danthanarayana, 1986).

Disperzi lze rozdělit do dvou základních kategorií: aktivní (bez cizího přičinění) a pasivní (pomocí přenosového vektoru, kterým může být vítr, proud vody či jiný organismus). Aktivní disperze plaváním, chůzí či letem umožňuje přenos na relativně krátké vzdálenosti, neboť je energeticky náročná. I přesto je však z velké části při opouštění

původní lokality upřednostňována (Shigesada & Kawasaki, 2002). Prostřednictvím pasivní disperze lze naopak dosáhnout větších disperzních vzdáleností, čímž se také zvyšuje pravděpodobnost kolonizace vzdálenějších lokalit (Ferriere *et al.*, 2000; o anemochorii viz níže), i když efektivita přenosu klesá s velikostí přenášeného organismu (De Bie *et al.*, 2012). Aktivně dispergující organismy jsou oproti pasivně přenášeným jedincům pokládány za úspěšnější především díky nenáhodnému výběru vhodného habitatu a nižší pravděpodobnosti vstoupení do habitatu nízké kvality (De Bie *et al.*, 2012).

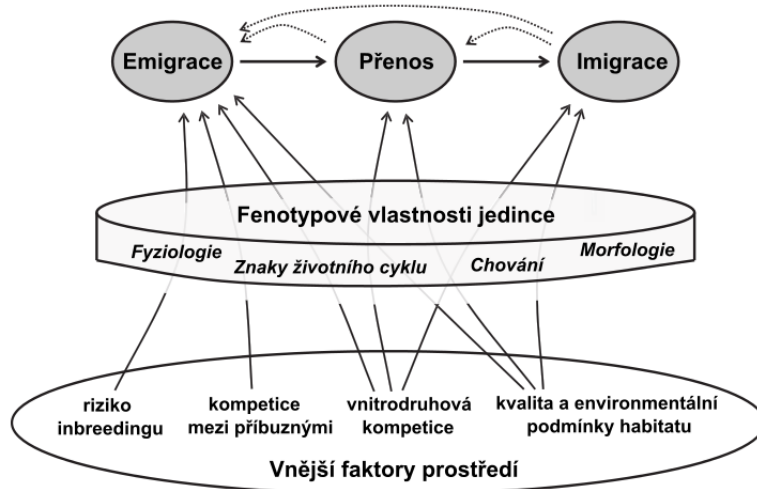
Proximální a ultimativní příčiny jednotlivých fází disperze

Proces disperze je tvořen třemi hlavními fázemi, které jsou ovlivněny různými faktory prostředí: opuštění stanoviště, fáze přenosu a příchod na nové stanoviště (Obr. 1; Ronce, 2007). Rozhodnutí jedince opustit původní stanoviště i osídlit nové závisí na podmínkách prostředí a fenotypu jedince (Clobert *et al.*, 2009). Disperze je spjata s variabilitou biotických a abiotických charakteristik habitatového ostrůvku (Bowler & Benton, 2005) včetně zvýšených populačních hustot a zvýšené kompetice o zdroje (potápníci: Yee, Taylor & Vamosi, 2009; plošnice: Pajunen & Pajunen, 2003; Baines, McCauley & Rowe, 2014), zvýšeného rizika predace (McCauley & Rowe, 2010) a fyziologického stavu jedince, např. vývoje ovaríí u samic (brouci z čeledi Helophoridae: Landin, 1980).

Vlastní přenos je na rozdíl od procesu rozhodování snadno pozorovatelný. Úspěšnost pohybu prostorem je ovlivněna fyziologickými aspekty jedince (např. plocha křídel, pohlaví, fyzická zdatnost) a strukturou či prostorovou heterogenitou habitatu (Bowler & Benton, 2005). Mnoho studií prokázalo působení vlivu počasí na disperzní let mnoha skupin vodního hmyzu (např. Landin & Stark, 1973; Waringer, 1991; Kovats *et al.*, 1996; Briers *et al.*, 2003; Boix *et al.*, 2011; Csabai *et al.*, 2012), zatímco informace o letové aktivitě pakomárů z velké části spíše chybí. Pozitivní korelace byla konkrétně pozorována mezi teplotou vzduchu a letovou aktivitou např. u pošvatek (Briers *et al.*, 2003), chrostíků (Waringer, 1991), zástupců vodních ploštic a brouků (Klečka, 2008; Boda & Csabai, 2012). Mortalita drobného hmyzu během letu je zvýšena rizikem dehydratace vlivem vysoké teploty a nízké vlhkosti vzduchu (Csabai *et al.* 2006). Nejpriznivější podmínky pro letovou aktivitu samičky komára tropického (*Aedes aegypti* L.) jsou při vlhkostním rozmezí 60–85 %; při nižších ale i vyšších hodnotách následoval pokles letové aktivity (Haufe, 1963). Dalším významným faktorem limitujícím letovou aktivitu je rychlost větru (Landin & Stark, 1973; Csabai & Boda, 2005).

Vhodnost nového stanoviště je posuzována pomocí druhově specifických mechanismů. U vodních brouků a ploštic byla například prokázána schopnost detekce vodních ploch na

základě vnímání polarizovaného světla (Horváth, 1995; Boda & Csabai, 2012), i když tento mechanismus vede ke vzniku *ekologických pastí*, kdy jedinci mylně vyhodnocují antropogenní objekty jako vodní hladinu (jepice a lesklý povrch asfaltových silnic: Kriska, Horváth & Andrikovics, 1998; vážky a ropné skvrny: Horváth & Zeil, 1996; vážky a leštěné náhrobní desky: Horváth *et al.*, 2007; lesklé povrchy tmavých aut: Wildermuth & Horváth, 2005). Dispergující plošnice jsou schopné rozlišit vodní nádrže o různých velikostech a hloubkách za účelem reprodukce na jaře a přezimování na podzim (Pajunen & Jansson, 1969). Se vzrůstající uraženou vzdáleností se ale požadavky na nové stanoviště snižují (Pajunen & Pajunen, 2003).



Obr.1 Schematické zobrazení individuální variability disperse a vzájemná propojenost a interakce mezi třemi úrovní procesu: vztahu mezi fázemi disperse (emigrace, přenosové fáze a imigrace), individuálním fenotypem jedince a vnějšími podmínky prostředí. Jedinci v závislosti na svém fenotypu mohou během jednotlivých fází disperse odlišně reagovat na okolní vlivy specifické pro konkrétní fázi disperse. Shromažďování a přenos informací druhově specifickými mechanismy může způsobit zpětné vazby mezi jednotlivými fázemi disperse (tečkované šipky). Upraveno dle Clobert *et al.* (2009).

Způsoby šíření vodního hmyzu

Dominantní část hmyzu s vodní larvou je v dospělosti schopna letu (více než 90 % všech druhů; Roff 1990). Variabilita v letových schopnostech souvisí především s velikostí organismů a objemem svalové hmoty (Rundle *et al.*, 2007). Mezi nejvýkonnější letce z řad vodního hmyzu patří zejména vážky a motýlice díky vysoce aerodynamickým křídům, efektivní svalové hmotě a vysokému poměru svalů v poměru k celkové hmotnosti těla (až 60 %; Rundle *et al.*, 2007). I mezi vážkami jsou přesto silně teritoriální druhy s omezenou

disperzí (Bohonak & Jenkins, 2003). Naopak drobný hmyz může uletět velké vzdálenosti; například chrostíci rodu *Hexagenia* byly nalezeny až 5 km od vodního zdroje (Kovats *et al.*, 1996). Přenosu pomocí vzdušných proudů (anemochorie) v různé výšce nad povrchem využívají nejen pasivně dispergující jedinci, ale i aktivní letci, nejčastěji drobný hmyz s délkou těla nepřesahující 4 mm (např. komáři, pakomáři, jepice, chrostíci a drobní brouci; Rundle *et al.*, 2007b). Mohou tak urazit až stovky kilometrů (např. muchnička *Simulium damnosum* v západní Africe: Magor & Rosenberg, 1980).

Preference letové aktivity vodní hmyzu za určitých podmínek (daných především fotoperiodou a minimální, případně maximální teplotou) vede k vytvoření vzorců denního a sezónního disperzního chování (Csabai *et al.*, 2006; Boda & Csabai, 2012). Pro sezónní průběh disperze je rozhodující i načasování ukončení larválního vývoje, které závisí na teplotě a intenzitě slunečního světla v rámci sezóny (Johnson, 1960).

Shrnutí

Sladkovodní habitaty představují ostrovy izolované suchozemským prostředím. Přežití a zachování druhů obývajících tohoto heterogenního prostředí závisí na schopnosti přesunu mezi habitaty. Disperze je tak základním procesem populační a metapopulační ekologie umožňující propojení jednotlivých jinak izolovaných prostředí.

Disperzi ovlivňuje celá řada vzájemně interagujících faktorů, které se mění s ohledem na konkrétní fáze disperze. Mezi nejdůležitější faktory ovlivňující rozhodnutí dispergovat patří zejména stálost habitatu v prostoru a čase či populační hustota. Vlastní fáze přenosu závisí na konkrétních vlastnostech a zdatnosti jedince a je ovlivněna aktuální stavem podmínek (např. stavem počasí). Disperzní aktivita tak není konstantní v rámci dne ani sezóny. Fenologie jedince v podobě konkrétních disperzních vzorců je odpovědí především na změnu teploty a fotoperiody.

Vodní hmyz reprezentující důležitou složku sladkovodní bioty téměř vždy disperguje prostřednictvím okřídlených imag a disperzní let dospělců je klíčovým aspektem mnoha ekologických a evolučních procesů. Je tedy důležité porozumět tomu, jak podmínky prostředí ovlivňují letovou aktivitu vodního hmyzu. Přiložený manuskript se proto zabývá vlivem počasí na přenosovou fázi disperze jedné z dominantních složek společenstva stojatých vod – pakomárů.

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Seasonality and weather conditions jointly drive flight activity patterns of chironomids (Diptera: Chironomidae) in sandpit pools

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SUMMARY

1. Chironomids represent a major invertebrate taxon in many freshwater habitats including small standing waters. Their presence in such habitats depends on dispersal ability of adults to reach new habitats, yet the flight patterns of adult chironomids are not completely understood. In particular, little is known about the impact of environmental conditions on their flight activity.
2. To fill this gap, we investigated temporal flight activity patterns of adult Chironomidae in a reclaimed sandpit area using two sampling methods (Malaise traps and sweeping) and analyzed how weather conditions influenced total abundance and species composition of aquatic and terrestrial species.
3. Diel flight patterns (observed in August and September) were characterized by minimum flight activity of both aquatic and terrestrial species between ca. 12–15 hrs. and highest activity in the evening after accounting for weather conditions. Total abundance of aquatic species declined with increasing air temperature and cloud cover, whereas that of terrestrial species declined above ca. 50% relative humidity after accounting for temporal effects.
4. Total abundance and species composition of aquatic and terrestrial species varied substantially during the season (from March to September) and was influenced by both seasonality and weather conditions, although the latter had a weaker effect. Wind speed strongly affected abundance and community composition of both terrestrial and aquatic species. Beyond that, abundance of aquatic species was driven mainly by relative humidity, whereas that of terrestrial species varied mainly with changes in temperature. Terrestrial and aquatic species used partly alternating time windows for dispersal.
5. Our results suggest that aquatic and terrestrial chironomids use different dispersal strategies, which might be linked to the underlying differences in larval phenology or spatio-temporal availability of suitable oviposition sites. Our study also highlights the often overlooked difficulties that arise from inherent correlations of weather characteristics with time and among themselves. Additional long-time data and studies combining the larval and adult dynamics are needed to fully unravel the proximate environmental drivers of dispersal flight in chironomids and other aquatic insects.

Introduction

Long-term survival of species in changing environments depends on the ability of individuals to disperse to new habitats (Clobert et al., 2001, 2012). This is particularly true for the biota in small standing freshwaters, which represent highly dynamic environments (Bilton, Freeland & Okamura, 2001; Rundle, Bilton & Foggo, 2007). The main invertebrate groups occupying these waters utilize different dispersal strategies. While zooplankton disperse mostly as resting eggs, nearly all aquatic insects inhabiting standing waters disperse by flight (Rundle et al., 2007). Many aquatic insect species experience limited windows of dispersal opportunity due to relatively short-lived adults. Various biotic and abiotic factors further affect the decision making of individuals during the onset, duration and termination of the dispersal flight (Clobert et al., 2001; Bowler & Benton, 2005). Together with species phenologies, these constraints ultimately shape dispersal patterns of aquatic insects at diel and seasonal timescales (Csabai *et al.*, 2006, 2012; Boda & Csabai, 2012).

Flight activity of aquatic insects is strongly affected by weather conditions including air temperature (Waringer, 1991; Briers, Cariss & Gee, 2003; Csabai *et al.*, 2012), wind speed (Landin & Stark, 1973; Briers et al., 2003; Csabai & Boda, 2005), light intensity (Pajunen, 1962; Lewis & Taylor, 1964), and air humidity (Haufe, 1963). Thermal windows of flight activity are species specific and few individuals fly at temperatures outside the optimal window (Lewis & Taylor, 1964). The impact of environmental conditions on flight patterns of aquatic insects is best understood in the Coleoptera and Heteroptera. Their dispersal flight is rapidly inhibited by wind speeds above a certain threshold (Landin & Stark, 1973; Csabai & Boda, 2005) and modified by light intensity and solar elevation, which determines the amount of horizontally polarized light reflected by water surface (Csabai *et al.*, 2006) and thus enables individuals to locate suitable habitats and oviposition sites (Horváth, 1995; Bernáth, Gál & Horváth, 2004; Csabai *et al.*, 2012).

Dispersal patterns of Diptera and especially Chironomidae, one of the most successful and abundant macroinvertebrate groups in freshwater, are less well understood. Unlike Heteroptera and Coleoptera, flight performance of chironomids is generally weak (Vallenduuk & Moller Pillot, 2013) and they disperse by wind rather than through active dispersal (Armitage, Cranston & Pinder, 1995). Since adult chironomids are short lived and usually do not feed (Vallenduuk & Moller Pillot, 2013), their behaviour should primarily relate to reproduction and oviposition (Armitage *et al.*, 1995; Kovats, Ciborowski & Corkum, 1996). Small-bodied insects such as chironomids are also more vulnerable

to unfavourable weather conditions because they can cool down, overheat or desiccate rapidly (Willmer, 1982). For them, weather conditions represent a strong environmental filter (Ruhí *et al.*, 2014; Heino *et al.*, 2015) that ultimately affects individual fitness.

Dispersal flight of chironomids can involve several stages: initial movement from the site of emergence to the resting site, swarming flight, and oviposition flight of females (Oliver, 1971). Environmental conditions may affect these stages differently: wind and light conditions are key factors during the initial movement (Kokkinn & Williams, 1989; Wrubleski & Ross, 1989), while temperature and air humidity determine the selection of resting places and swarming activity (Armitage *et al.*, 1995). Moreover, strong wind force the flying adults to land (Syrjämäki, 1968; Soong & Leu, 2005). Beside studies focused on these stages of flight, works dealt with flight itself and factors influencing it are, however, rather sporadic (Delettre, Tréhen & Grootaert, 1992; Hodkinson *et al.*, 1996; Delettre & Morvan, 2000).

Differences in key characteristics related to dispersal suggest that environmental conditions may affect chironomids differently from other aquatic insect groups, with potentially important implications for the process of colonization of new habitats and local community assembly (Bohonak & Jenkins, 2003). Chironomids also include many species with semi-terrestrial or terrestrial larvae, mainly within the subfamily Orthocladiinae (Vallenduuk & Moller Pillot, 2013). Differences in flight patterns of aquatic and terrestrial chironomids could shed more light on the environmental drivers of dispersal activity of aquatic insects, but to our knowledge, few comparative studies are available and the existing ones focus primarily on differences in spatial distribution (Delettre *et al.*, 1992; Delettre & Morvan, 2000). Moreover, teasing apart the contribution of species phenology and environmental conditions on the observed flight patterns is inherently difficult due to the often strong correlations among environmental variables and their seasonality (Willmer, 1982).

The main purpose of this work was therefore to investigate and compare the diel and seasonal flight activity of aquatic and terrestrial chironomids, focusing on their assemblage in a reclaimed sandpit with an array of newly created pools as a case study. We also identified a suitable method to study flight activity of chironomids in sandpit, characterized their diel and seasonal flight patterns, and disentangled the effects of temporal and environmental factors on their flight activity.

Methods

Study site

The study was carried out in the Cep II sandpit near Suchdol nad Lužnicí, Czech Republic (GPS 48°91'85.51"N, 14°87'42.58"E). Most of the sandpit area is covered by a deep and turbid lake with ongoing sand extraction (Řehouňková *et al.*, 2016). The entire surface area in south-western part of the sandpit was remodelled and a cluster of ca. 40 small temporary to permanent pools (clayey-sandy bottom, surface area: mean \pm SD = 8.6 ± 3.5 m²; depth, 0.28 ± 0.09 m) created in October 2012 to conduct an experiment on community assembly of aquatic insects. The pools filled with water in the winter of 2012–2013 and their colonization by aquatic insects begun in early 2013 (D.S. Boukal *et al.*, unpublished data). The immediate vicinity of the pools consisted of bare clayey-sandy ground with very sparse cover of herbs and no shrubs. We sampled flying chironomids near the pools (Fig. 1) using two standard methods: sweeping with a hand-held aerial net and Malaise traps.

Diel flight activity in autumn 2013

To observe the diurnal flight patterns of chironomids and select the method for the subsequent long-term study, we sampled flying insects with a handnet and Malaise traps for 8 days in late summer (11–15 August and 23–25 September 2013). Sweeping was carried out with a handheld net with 55 cm diameter and a white mesh. One of us (LV) continuously swept the air ca. 1 m above the ground level while walking slowly (ca. 4 km.h⁻¹) for 15 minutes along one of two predetermined routes of approximately equal length – one on the shore of lake and close to the set of experimental ponds with sparse vegetation dominated by *Juncus effusus*, and another close to the largest pool (Fig. 1). This routine was performed 11 times every hour from 9 am to 7 pm each day with regular alternation between the two routes; we started each morning with a different route than the previous day. All captured insects were removed from the net with an aspirator and preserved in 80% ethanol. Samples were then evaluated separately for each hour and route (i.e., 11 samples per day; Table S1).

Four Malaise traps (Fig. S1; effective intercept area of each trap 8.7 m²) were placed across the study area on the same dates, two near the lake shore and two near the largest pool (distance within each group: ca. 12 m, distances between groups: ca. 25 m). Intercepted flying insects that flew upwards were accumulated in a 0.5-L bottle filled with glycerated

80% ethanol. Traps were exposed continuously between 8 am and 8 pm and the entire sample was collected afterwards (i.e., four samples per day). Temperature and humidity in the area was continuously monitored every 15 minutes with two data loggers (Ebro EBI 20-TH) placed 1 m above the ground in a shaded ventilated space, one near the lake and another near the experimental pools. We also recorded cloud cover (4 categories: clear sky, mostly sunny, mostly cloudy, and cloudy) and wind speed on the Beaufort scale (Table S2).

Seasonal flight activity in 2014–2015

We deployed the same four Malaise traps for four days every month between May and September 2014 and in March and April 2015 to study the seasonal flight patterns. The dates were chosen a priori and changed only to avoid rainy days, when sampling was not possible. The traps were placed as in 2013 but the samples were collected 5 times a day every 4 hours (first at 6:30 am and the last at 10:30 pm). Based on the results from 2013 along with preliminary inspection of the 2014 samples, we analysed only data on chironomids captured in the afternoon and evening (from 2:30 pm to 10:30 pm). We pooled data from each trap on each day as one sample and used only average values of environmental characteristics during this period (Table S1). Our results thus convey the daily average response of chironomids, but the weather on the sampling dates was relatively stable and the average environmental characteristics were strongly correlated with minima and maxima during the sampling period (not shown). We are thus confident that the results are robust.

Air temperature and humidity on the site were recorded as in 2013. In addition, hourly data on average air pressure and wind speed and point data on cloud cover (recorded at 7 am, 2 pm, and 10 pm; Table S2) were obtained from the field site of the Czech Hydrometeorological Institute in Třeboň 12 km away from the study site. Humidity and temperature data measured at our experimental site were almost always very close to the data from Třeboň (Fig. S2) and we thus used data from the site. We recorded no rain during the sampling dates.

Adult males were identified under Olympus SZX9 and Olympus BH microscopes using keys and descriptions by Langton and Pinder (2007), Moller Pillot (2008), and Oyewo and Saether (2008) to the species or genus level. Females were excluded from the analyses

because their identification except a few species is difficult or impossible (Ekrem, Stur & Hebert, 2010).

Data analyses

We carried out five analyses: (1) calculation of species rarefaction curves (Chao *et al.*, 2014) to compare the two sampling techniques used in 2013, (2) univariate analysis of time- and weather-dependent changes in total abundance of adult chironomids in 2013 and in 2014–2015, (3) multivariate analysis of seasonal and weather-dependent changes in the composition of chironomid assemblages in 2014–2015 including variation partitioning to detect pure effect of season and environmental factors, (4) analysis of seasonal flight phenology of common species in 2014–2015 using species response curves, and (5) multivariate analysis of seasonal flight patterns in 2014–2015 including the larval habitat as species trait. In order to detect possible differences in flight patterns explained by larval habitat, we performed the second, third and fourth analysis separately for aquatic and terrestrial species; the few rare semi-terrestrial species were treated as terrestrial. Univariate analyses and calculations of rarefaction curves were done in R version 3.1.2 (R Core Team, 2014). Multivariate analyses and species response curves were calculated in CANOCO 5 (ter Braak & Šmilauer, 2012).

Rarefaction analysis was implemented in the *iNEXT* package version 2.0.8 (Hsieh, Ma & Chao, 2016) with the number of individuals as the rarefaction unit. All handnet and Malaise trap samples from each part of the day were respectively pooled into one aggregated sample regardless of locality, and we also ran a supplementary analysis in which we pooled the data by locality (Table S1). We calculated rarefaction curves for the whole chironomid assemblage and for aquatic species only. The results were used to select the sampling method for the survey of seasonal patterns in 2014–2015.

Generalized linear models (GLMs) were used to analyse the effects of air temperature T , relative humidity H , wind speed W , cloud cover C , and air pressure P (the latter only in 2014–2015) on the total abundance of chironomids in the handnet samples in 2013 and in the Malaise trap samples in 2014–2015. All five environmental variables (T , H , C , P , and W) were standardized and included as second-order orthogonal polynomials to detect nonlinear responses except a few cases outlined below. Some of the environmental variables were strongly correlated. Moreover, we detected two distinct weather regimes with highly correlated temperature and humidity (one in May–September 2014 and another in March

and April 2015; Fig. S3). We thus considered a weather regime (categorical variable TH , set to 0 for the 2014 data and to 1 for the 2015 data) instead of humidity or temperature in some models (see below). Due to the strong correlation between temperature and humidity, we used these explanatory variables separately for both datasets. We included the respective effect of daytime ($time$, continuous) and season (either as continuous $season$, scaled linearly between -0.5 and 0.5 with the minimum corresponding to 1 January and maximum to 31 December, or discrete $month$) for the 2013 and 2014–2015 data.

We created respectively two and four saturated models D1–D2 and S1–S4 of diel and seasonal flight patterns and applied each of them separately to aquatic and terrestrial species data. For diel flight patterns, we evaluated the effect of weather conditions changing through time of day on total abundance N of adults captured in the handnet in a given month with temperature (D1) and humidity (D2). Nonlinear responses were considered except cloud cover, which was treated as a factor (C_F) in 2013.

$$N \sim month + Q(time) + Q(T) + C_F + Q(W) + place \quad (D1)$$

$$N \sim month + Q(time) + Q(H) + C_F + Q(W) + place \quad (D2)$$

where $Q(x)$ stands for a second-order orthogonal polynomial of the variable x . Models for seasonal flight activity in 2014–2015 were based on similar reasoning:

$$N \sim month + Q(T) + Q(C) + Q(W) + Q(P) + place \quad (S1)$$

$$N \sim month + Q(H) + Q(C) + Q(W) + Q(P) + place \quad (S2)$$

$$N \sim season + Q(T) + TH + Q(C) + Q(W) + Q(P) + place \quad (S3)$$

$$N \sim season + Q(H) + TH + Q(C) + Q(W) + Q(P) + place \quad (S4)$$

The data were overdispersed and we thus used quasi-Poisson distribution. For each of the full models D1–D2 and S1–S4, we performed manual stepwise selection based on quasi-AICc criterion corrected for small sample size (qAICc, Bartoň, 2012) with repeatedly extracted overdispersion parameter to select the most parsimonious model. To identify the overall best model describing the daily and seasonal pattern, we compared the resulting most parsimonious models based respectively on D1–D2 and on S1–S4 using qAICc with the dispersion parameter calculated from a new saturated model containing all explanatory variables included respectively in D1–D2 and in S1–S4 ($season$ and TH were left out from the saturated model based on S1–S4 because they were determined by $month$). We calculated McFadden's pseudo- R^2 for each model (D1–D2 and S1–S4) as the difference between null and residual deviance divided by null deviance of the model (Faraway, 2016). We verified

that the residuals of the final models were approximately homoscedastic and did not show trends when plotted against the explanatory variables. Significance of all explanatory variables in the final models was assessed by *drop1* function and model fits illustrated using the *effects* package version 3.0-6 (Fox, 2003).

We further assessed the seasonal flight patterns and the effect of environmental parameters on species composition of chironomid assemblages using redundancy analysis (RDA) for the 2014–2015 Malaise trap data. Species abundances n were transformed as $\log_{10}(n + 1)$ and centred prior to the analyses. Single-term ordinations were separately computed for the season and environmental parameters. To obtain the pure effect of each weather characteristics, we applied partial RDA with season as a covariate. In order to explain any differences between the flight activity of aquatic and terrestrial chironomid species, we used the same RDA analysis as above on the pooled aquatic and terrestrial species data with larval habitat as a species trait. To minimize the influence of rare or randomly recorded species in all multivariate analyses, we used species with at least 5 occurrences in the data for both aquatic and terrestrial species. We also computed variation explained only by season, only by environmental factors and shared variation by variation partitioning.

Seasonal changes in the flight activity of the most frequently found species were illustrated by species response curves implemented as generalized additive models (GAMs) with quasi-Poisson distribution. We used five degrees of freedom as the highest resolution that was then modified for each species based on stepwise selection using AIC. We used total abundance pooled across all four traps per day as the response variable and month as the time variable for 2014–2015 data (Table S1). All models were based on Monte Carlo tests with 9999 unrestricted random permutations.

Results

We collected 2590 male chironomids representing at least 37 aquatic, 1 semi-terrestrial, and 6 terrestrial species in August and September 2013. The 2014–2015 Malaise traps samples included 2356 males representing at least 90 aquatic, 1 semi-terrestrial, and 18 terrestrial species (Table 1). Abundances were highly skewed in both datasets, with 33 species in 2013 and 89 species in 2014–2015 represented by less than 10 individuals. On the contrary, only 7 and 6 species were common (>100 males) in 2013 and 2014–2015,

respectively. Interannual species turnover was substantial, with only 37% of the species shared between both datasets; these species were common throughout the study.

Handnet and Malaise traps differed significantly in their ability to cover the 2013 chironomid assemblage (Fig. 2 and S4). Although we caught more adults with handnet ($N = 1566$ males) than with the Malaise trap ($N = 1024$ males), the latter yielded more species (Malaise trap: 41 species; handnet: 16 species) and contained all species captured by handnet. Aquatic species were more diverse but less abundant than terrestrial ones in samples collected by each method (Fig. 2). Rarefaction analysis revealed that the Malaise trap still missed some additional species while sweeping provided a nearly complete inventory of the part of the assemblage available through the method (mean predicted species diversity at 3000 individuals, 95% CI in parentheses: handnet, 18.2 (13.8–23.5) species; Malaise trap, 62.6 (47.3–77.8) species; Fig. 2). Differences between both sampling sites were minor, especially for the Malaise trap (Fig. S4). We thus chose Malaise traps as the appropriate method to follow species community composition in the 2014–2015 survey.

Diel flight activity in autumn 2013

Proportions of explained variability of the final models were high and almost identical for both groups (aquatic: $D1 = 46.3\%$ and $D2 = 45.6\%$; terrestrial: $D1 = 44.8\%$ and $D2 = 45.8\%$). The most parsimonious models (Table 2 and Fig. 3) showed that the total flight activity of terrestrial and aquatic species changed significantly during the day (aquatic: $F = 25.5$, d. f. = 1, $P < 10^{-4}$; terrestrial: $F = 17.8$, d. f. = 1, $P < 10^{-4}$) as they flew preferentially in the morning and especially in the evening (aquatic: 58 %, terrestrial: 50 % of individuals). Flight activity of aquatic species further decreased with temperature (linear term, $F = 7.1$, d. f. = 1, $P = 0.009$) and depended on cloud cover ($F = 5.5$, d. f. = 3, $P = 0.002$) with higher activity during sunny weather. Marginally fewer individuals of aquatic species were collected near the experimental pools ($F = 2.8$, d. f. = 1, $P = 0.09$). Flight activity of terrestrial species varied between months ($F = 18.7$, d. f. = 1, $P < 10^{-4}$), depended on humidity ($F = 14.8$, d. f. = 1, $P < 10^{-4}$) with a maximum near 40–50 % relative humidity, and was marginally higher near the experimental pools ($F = 3.5$, d. f. = 1, $P = 0.06$). We found no significant effect of wind speed on either group.

Seasonal flight activity in 2014–2015

Flight activity of both terrestrial and aquatic species varied markedly during the season and with weather conditions. We recorded the highest flight activity in June 2014 (32 % of all males), July (20 %) and April 2015 (19 %), which contrasted with very few individuals collected in March 2015 (0.2 %). The available environmental gradients were strong (Table S2) and included extreme values beyond which flight activity completely ceased. For example, the minimum average temperature at which any adults were caught was 5.2 °C on 15 March 2015 (aquatic *Tanytarsus sylvaticus* and terrestrial *Bryophaenocladus* cf. *illimbatus*).

Flight activity patterns, expressed as the total number of individuals, were similar but not identical in aquatic and terrestrial species (Table 2 and Fig. 4). Proportions of explained variability of the final models were even higher than for the diel patterns (aquatic: S1 = 70.4 %, S2 = 70.9 %, S3 = 67.9 %, and S4 = 64.4 %; terrestrial: S1 = 64.4 %, S2 = 4.5 %, S3 = 43.9 %, and S4 = 36.3 %). Most parsimonious models for both aquatic and terrestrial species showed that their flight activity varied strongly between months (aquatic: $F = 20.1$, d. f. = 6, $P < 10^{-4}$; terrestrial: $F = 19.8$, d. f. = 6, $P < 10^{-4}$) and decreased with higher wind speed (aquatic: $F = 11.6$, d. f. = 1, $P < 10^{-4}$; terrestrial: $F = 43.5$, d. f. = 1, $P < 10^{-4}$), lower air pressure (aquatic: $F = 5.2$, d. f. = 1, $P = 0.02$; terrestrial: $F = 3.6$, d. f. = 1, $P = 0.06$), and distance from the experimental pools (aquatic: $F = 14.7$, d. f. = 1, $P = 0.0002$; terrestrial: $F = 2.9$, d. f. = 1, $P = 0.09$). The models predicted the highest abundance of aquatic adults under mean weather conditions (i.e., with weather conditions averaged across the whole dataset) in July followed by September, which contrasts with the predicted April and June maxima for the terrestrial species. That is, our data are consistent with fluctuations in total abundance of either group that cannot be described by a simple continuous change during the season. Moreover, we found no significant effect of cloud cover on either group.

Aside from steeper dependence on wind speed and weaker dependence on air pressure and distance from experimental pools in the terrestrial species, total flight activity of both groups differed primarily in their responses to the (correlated) air temperature and relative humidity. While flight activity of aquatic species decreased significantly with relative humidity ($F = 4.8$, d. f. = 1, $P = 0.02$), terrestrial species appeared to have a unimodal response to air temperature with a maximum around 18 °C ($F = 9.9$, d. f. = 1, $P = 0.002$).

The dependence of total flight activity on season and weather conditions was largely but not fully reflected on the species level. Community composition of both aquatic and terrestrial assemblage changed markedly between months (Table 3 and Fig. 5). Most aquatic species flew mainly under conditions characterized by light wind, higher temperatures and low humidity (Fig. 5a). Composition of terrestrial assemblage changed with humidity, wind speed and cloud cover; unlike the aquatic assemblage, some terrestrial species flew preferably under higher humidity (Fig. 5b). When analyzed together, the flight patterns of aquatic and terrestrial species differed only in their seasonality and were indicative of alternating main periods of emergence of both groups (Fig. 6c; RDA: 53.0 % of adjusted variance, $F = 5.9$, $P = 0.0015$).

Flight activity of the most common species was characterized by more or less narrow, single peaks of emergence (Fig. 6; aquatic species: *Tanytarsus volgensis* and *Microchironomus tener*; terrestrial: *B. cf. illimbatus* and *Smittia edwardsi*), while other taxa had more protracted periods of flight activity (aquatic: *Psectrocladius* gr. *limbatellus*; terrestrial: *Hydrosmittia oxoniana*) or were indicative of two generations per year (terrestrial: *Smittia* sp.; aquatic: *P. choreus*, *H. curtilamellata*, and *P. bathophila*).

Since numerous other studies attempted to explain changes in flight activity and species composition by temperature or other weather conditions alone, we also used constrained partial ordination test to detect the marginal effects of the significant environmental variables ($P < 0.05$) after accounting for the seasonality as covariate. Surprisingly, we found no marginal effect of weather conditions on the aquatic species, which contrasted with a significant marginal effect of humidity, wind speed and cloud cover on terrestrial species (Table 3). In particular, the marginal effect of temperature on the flight activity of either group was insignificant (aquatic species: $P = 0.65$) after the main effect of seasonality was removed. Moreover, variation partitioning confirmed a strong effect of seasonality which dominated over the joint effect of temperature, humidity, wind speed and air pressure for both aquatic (only season: 13.2 %; only environmental factors: 2.9 %; shared: 4.9 % from 21 % of total explained variation) and terrestrial species (only season: 24.6 %; only environmental factors: 10.0 %; shared: 24.0 % from 58.6 % of total explained variation).

Discussion

Our study provides the first detailed quantitative analysis of the short- and long-term temporal patterns of flight activity of adult chironomids and its dependence on weather conditions in a temperate zone that is not based on emergence traps. Moreover, it directly compares the activity of terrestrial and aquatic species.

Diel flight activity

Flight activity of all insects is restricted to some periods during a day (Lewis & Taylor, 1964). We found that most adult chironomids flew during evening and afternoon, with another lower morning maximum in the terrestrial species. This differs from reported diel flight activity patterns in aquatic beetles and bugs, which fly predominantly in mid-morning, at noon and at dusk (Csabai *et al.*, 2006; Boda & Csabai, 2012). Flight during the day has been associated with higher probability of being carried over long distances by air turbulences and convection, whereas flight during the evening and around dusk upon more stable air conditions is supposedly optimal for local flight (Lewis & Taylor, 1964). Furthermore, flights during the evenings under higher relative humidity should reduce the risk of dehydration (Oliver, 1971; Csabai *et al.*, 2006). Dusk is also the preferred period for oviposition flight of chironomid females (Armitage *et al.*, 1995).

Aquatic and terrestrial chironomids in our study differed in diel flight activity patterns and their dependence on weather conditions, although the overall differences were relatively minor. The observed unimodal response of terrestrial species to relative humidity was in line with earlier studies of the total activity of chironomids, expressed as biomass captured by Malaise traps, in the Arctic (Hodkinson *et al.*, 1996) although the predicted optima of ca. 40–50% were below the values found in other studies, e.g., for adult *Aedes aegypti* mosquitoes, which flew predominately under more humid conditions of ca. 65–80 % relative humidity (Haufe, 1963). This discrepancy could be due to our sampling period in late summer and early autumn, during which higher humidity also entails low temperatures that inhibit flight. It could have also arisen from our statistical approach, in which we derived the response to a particular driver from the marginal effects that assume mean values of all other explanatory variables, while the raw data suggest that flight activity is unrelated to relative humidity (Fig. 3). Particularly for small insects, air humidity provides an important constraint related to internal water balance (Csabai *et al.*, 2006) and flight during

inappropriate conditions may increase the risk of dehydration and mortality (Armitage *et al.*, 1995).

Surprisingly, we found no significant effect of wind speed on diel flight activity patterns, which could be caused by the relatively limited underlying dataset rather than lack of contrast in the data. Other studies considered wind as a key factor determining the flight activity of aquatic insects. Strong declines in flight activity with rising wind speeds or a complete cessation of flight above a certain wind threshold were observed in stoneflies (Briers *et al.*, 2003), water beetles and bugs (Popham, 1964; Landin & Stark, 1973; Weigelhofer, Weissmair & Waringer, 1992; Csabai & Boda, 2005; Boix *et al.*, 2011) as well as swarming chironomids (Syrjämäki, 1964, 1968; Otto & Schiegg, 1999).

Seasonal flight activity

Effects of environmental parameters on seasonal flight activity of aquatic and terrestrial species were similar but not identical. Changes in flight activity and species composition of both aquatic and terrestrial chironomids were driven mainly by seasonality, although the role of weather conditions was also substantial.

Rising wind speeds strongly inhibited flight activity in line with the majority of previous studies, although few species showed some tendency to fly even upon stronger winds (e.g., *C. sylvestris*). Moreover, raw data were indicative of increased flight activity under light wind conditions (ca. 1 m.s⁻¹) that likely facilitated long-distance dispersal beyond the experimental site. Despite the strong overall effect of wind on insect flight performance, fluctuating and rapidly changing wind speeds lead to irregular influence on daily timescales and therefore play only a minor role in general dispersal flight pattern (Boda & Csabai, 2009). Positive response to air pressure was presumably due to the association with good, stable weather as in the study of the Arctic chironomid assemblage (Hodkinson *et al.*, 1996).

Flight activity of aquatic species further declined with relative humidity and species composition also changed with temperature, while total activity of terrestrial species seemed to vary with temperature and species composition with humidity and cloud cover. Humidity and temperature are often highly correlated and their effects difficult to separate (Willmer, 1982). Nevertheless, water and thermal balance of individuals is supposed to influence activity patterns through physiological mechanisms (Willmer, 1982) and the importance of the joint effect of temperature and humidity on flight activity was recognized for other groups (e.g., Shipp, Grace & Schaalje, 1987; Peng, Fletcher & Sutton, 1992; Csabai *et al.*,

2006) as well as chironomids in the high Arctic (Hodkinson *et al.*, 1996). The differential responses of individual species observed in our study could be caused by their adaptation to environmental conditions that prevail during the main period of emergence. For example, species emerging on average later in the season (such as *H. curtilamellata*, *M. tener* and *P. choreus*) flew during periods characterized by relatively higher humidity in comparison to other species emerging in spring and early summer.

Many studies highlighted the crucial role of temperature in dispersal activity patterns of various taxa (aquatic Heteroptera and Coleoptera: Landin & Stark, 1973; Zalom *et al.*, 1980; Weigelhofer *et al.*, 1992; Csabai *et al.*, 2012; Plecoptera: Briers *et al.*, 2003; Trichoptera: Waringer, 1991; Diptera: Platt *et al.*, 1957; Haufe, 1964; Peng *et al.*, 1992). Surprisingly, our results suggest that seasonality is the main driver of variation in species composition of flying adult chironomids, especially in the aquatic species.

Adult chironomid lifespan is very short, often less than one day and rarely up to 1–2 weeks (Vallenduuk & Moller Pillot, 2013). Their flight activity is thus mostly determined by the timing of emergence (Johnson, 1960; Danks & Oliver, 1972) and detailed data on phenology may require long-term use of emergence traps (Vallenduuk & Moller Pillot, 2013), which we could not deploy at our freely accessible site. However, observed flight patterns in our study indicate that we did not miss important events, at least not for the commonest aquatic species. Our data are consistent with a univoltine life cycle and more or less narrow summer emergence period in *T. volgensis* and *M. tener*, bivoltine life cycle with two emergence periods in *P. choreus*, *H. curtilamellata* (both in summer and autumn) and especially *P. bathophila* (spring and summer), and protracted emergence with increased abundance in summer in *P. gr. limbatellus*. These patterns including the peak emergence periods are consistent with data from other regions (Moller Pillot, 2009; Mundie, 1957), although they may not hold across the whole distribution area (e.g., *P. bathophila* was reported to have up to 3 or 4 generations in Bavarian lakes; Moller Pillot, 2013).

Differences between sites and methods

Differences in flight activity of individual chironomid species could be explained by different dispersal abilities. Even species with identical wing morphology and equal body size can disperse differently including largely passive or active model of dispersal (Delettre, 1988; Delettre *et al.*, 1992). Flight activity of chironomids is also influenced by the surrounding environment and should generally decline with distance from water due to

spatial dilution of the dispersing individuals as in Delettre and Morvan (2000). We found more individuals of the aquatic species near the experimental pools in 2014–2015, which is in line with the spatial dilution effect but could also be caused by environmental heterogeneity at fine spatial scales: this locality was first shaded during the afternoon, which could be beneficial in the overall dry conditions of the sandpit habitat. Moreover, we were not able to determine if the individuals performed very short local flight or if they underwent flight from more remote places (Hodkinson *et al.*, 1996). Nevertheless, females were 2.3 times as abundant as males in our data. Mating swarms typically consist of males and only few females (Vallenduuk & Moller Pillot, 2013) and we are thus confident that our data are based on dispersing individuals rather than swarming activity.

Last but not least, our results contribute to the discussion of suitable sampling methods and protocols (Klečka & Boukal, 2011 and references therein, Briers *et al.*, 2003). We found Malaise traps to be more appropriate than handnet for studies of adult chironomids, which have been used in other quantitative studies of adult chironomid assemblages (Hodkinson *et al.*, 1996). Efficiency of Malaise traps might be compromised by strong wind that would deform the traps. However, Briers *et al.* (2003) argued that periods of strong wind conditions are unlikely to significantly affect the overall results.

Conclusion

Overall, our results imply that species phenologies and the conditions experienced by the larvae have the upper hand in determining adult flight patterns of chironomids and probably also other short-lived aquatic insects. This may provide a crucial driving mechanism for the evolution of diversification of life history strategies such as cohort splitting, which ensure that at least part of the emerging adults experience favourable conditions for dispersal (Soong & Leu, 2005).

Seasonal changes in adult abundance necessarily reflect the fates of their larvae. Local emergence patterns as well as the voltinism of chironomids may be determined by latitude and thermal regime in the larval habitat (Armitage *et al.*, 1995). Beyond the effects of temperature and photoperiod, species-dependent phenologies also result from other biotic factors such as resource dynamics, predation risk perceived by the larvae and the degree of larval competition for food (Welch, Jorgenson & Curtis, 1988; Armitage *et al.*, 1995). Long-term studies coupling detailed observations of environmental conditions both in and out of water, larval dynamics and adult emergence and flight activity at the given site would be

thus particularly useful to disentangle the effects of different biotic and abiotic drivers on the life histories and population dynamics of aquatic chironomids and other aquatic insect groups.

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Tables

Table 1 Total abundances and habitat associations of chironomid taxa captured by the two methods in 2013 and in 2014–2015.

Taxon	Habitat	Species code	Handnet 2013	Malaise trap 2013	Malaise trap 2014–15
Chironominae					
<i>Cladopelma edwardsi</i> (Kruseman, 1933)	aqua		0	1	2
<i>Cladopelma virescens</i> (Kruseman, 1933)	aqua		0	1	1
<i>Cladotanytarsus atridorsum/difficilis</i>	aqua		0	0	1
<i>Cladotanytarsus bicornutus</i> Kieffer, 1922	aqua		0	0	1
<i>Cladotanytarsus mancus</i> (Walker, 1856)	aqua	<i>CladManc</i>	21	92	38
<i>Cladotanytarsus</i> sp. A	(aqua)		0	0	5
<i>Cladotanytarsus</i> sp. B	(aqua)		0	0	1
<i>Cladotanytarsus vanderwulpi</i> (Edwards, 1929)	aqua		0	0	2
<i>Cryptotendipes usmaensis</i> (Pagast, 1931)	aqua		0	0	7
<i>Cryptotendipes usmaensis/holsatus</i>	aqua		1	2	0
<i>Demicryptochironomus vulneratus</i> (Zetterstedt, 1838)	aqua		0	0	2
<i>Dicrotendipes pulsus</i> (Walker, 1856)	aqua		0	1	1
<i>Endochironomus tendens</i> (Fabricius, 1775)	aqua		0	1	1
<i>Glyptotendipes</i> cf. <i>paripes</i> (Edwards, 1929)	aqua		0	0	2
<i>Glyptotendipes pallens</i> (Meigen, 1804)	aqua		0	1	1
<i>Glyptotendipes signatus</i> (Kieffer, 1909)	aqua		0	0	1
<i>Glyptotendipes</i> sp.	(aqua)		0	0	1
<i>Chironomus</i> cf. <i>venustus</i> Pinder, 1978	aqua		0	0	4
<i>Chironomus obtusidens</i> Goetghebuer, 1921	aqua		0	1	6
<i>Chironomus plumosus</i> (Linnaeus, 1758)	aqua		0	0	5
<i>Chironomus riparius</i> agg. Meigen, 1804	aqua		0	0	4
<i>Chironomus</i> sp.	(aqua)		0	0	4
<i>Microchironomus tener</i> (Kieffer, 1918)	aqua	<i>MicrTenr</i>	5	15	78
<i>Micropsectra atrofasciata</i> (Kieffer, 1911)	aqua		0	0	2
<i>Micropsectra lindrothi</i> Goetghebuer, 1931	aqua		0	0	2
<i>Microtendipes pedellus</i> (De Geer, 1776)	aqua		0	0	6
<i>Paracladopelma laminatum</i> (Kieffer, 1921)	aqua		0	0	4
<i>Parachironomus</i> sp.	(aqua)		0	1	1
<i>Polypedilum bicrenatum</i> Kieffer, 1921	aqua		0	1	1
<i>Polypedilum</i> cf. <i>uncinatum</i> (Goetghebuer, 1921)	aqua		0	0	1
<i>Polypedilum nubeculosum</i> (Meigen, 1804)	aqua		0	1	1
<i>Polypedilum nubifer</i> (Skuse, 1889)	terr		793	107	0
<i>Polypedilum sordens</i> (van der Wulp, 1875)	aqua		0	0	1
<i>Polypedilum</i> sp. A	(aqua)		0	1	0
<i>Polypedilum</i> sp. B	(aqua)		0	2	1
<i>Polypedilum tritum</i> (Walker, 1856)	aqua		0	0	4
<i>Rheotanytarsus muscicola</i> (Thienemann, 1929)	aqua		0	1	2

<i>Saetheria reissi</i> Jackson, 1977	aqua		0	0	1
<i>Stempellinella edwardsi</i> Spies & Saether, 2004	aqua	<i>StemEdwr</i>	2	2	29
<i>Stictochironomus pictulus</i> (Meigen, 1830)	aqua		0	0	2
<i>Stictochironomus</i> sp.	(aqua)		0	3	8
<i>Tanytarsini</i> gen. sp.	(aqua)		0	0	2
<i>Tanytarsus bathophilus</i> Kieffer, 1911	aqua		0	1	12
<i>Tanytarsus brundini</i> Lindeberg, 1963	aqua		0	0	7
<i>Tanytarsus brundini/curticornis</i>	(aqua)		0	0	2
<i>Tanytarsus buchonius</i> Reiss & Fittkau, 1971	aqua	<i>TantBuch</i>	0	0	31
<i>Tanytarsus eminulus</i> (Walker, 1856)	aqua		0	0	2
<i>Tanytarsus heusdensis</i> Goetghebuer, 1923	aqua		0	0	1
<i>Tanytarsus chinyensis</i> Goetghebuer, 1934	aqua		0	1	10
<i>Tanytarsus lestagei</i> agg. Goetghebuer, 1922	aqua		0	0	1
<i>Tanytarsus lestagei/longitarsus</i>	aqua		0	0	1
<i>Tanytarsus lugens/gregarius</i>	aqua		0	0	1
<i>Tanytarsus mendax</i> Kieffer, 1925	aqua		0	0	2
<i>Tanytarsus palettaris</i> Verneaux, 1969	aqua		0	0	1
<i>Tanytarsus pallidicornis</i> (Walker, 1856)	aqua		0	0	4
<i>Tanytarsus signatus</i> (van der Wulp, 1859)	aqua		0	0	1
<i>Tanytarsus striatulus</i> Lindeberg, 1976	aqua		0	1	0
<i>Tanytarsus sylvaticus</i> (van der Wulp, 1859)	aqua		0	0	8
<i>Tanytarsus volgensis</i> Miseiko, 1967	aqua	<i>TantVolg</i>	11	101	160
<i>Virgatanytarsus</i> sp.	aqua		0	1	2
Tanypodinae					
<i>Ablabesmyia longistyla</i> Fittkau, 1962	aqua		2	4	11
<i>Ablabesmyia monilis</i> (Linnaeus, 1758)	aqua		2	1	21
<i>Ablabesmyia</i> sp.	(aqua)		0	0	2
<i>Conchapelopia melanops</i> (Meigen, 1818)	aqua		0	0	1
<i>Conchapelopia</i> sp.	(aqua)		0	0	1
<i>Conchapelopia viator</i> (Kieffer, 1911)	(aqua)		0	0	3
<i>Macropelopia adauca</i> Kieffer, 1916	aqua		0	0	4
<i>Procladius</i> cf. <i>flavifrons</i> Edwards, 1929	aqua		0	0	2
<i>Procladius choreus</i> (Meigen, 1804)	aqua	<i>ProcChor</i>	2	83	148
<i>Procladius sagittalis</i> (Kieffer, 1909)	aqua		0	3	4
<i>Procladius</i> sp.	(aqua)		0	0	1
Orthoclaadiinae					
<i>Acricotopus lucens</i> (Zetterstedt, 1850)	aqua		0	6	0
<i>Allocladius bothnicus</i> (Tuiskunen, 1984)	terr		0	0	3
<i>Bryophaenocladus</i> cf. <i>illimbatus</i> (Edwards, 1929)	terr	<i>BryoCfl</i>	0	0	206
<i>Bryophaenocladus ictericus</i> (Meigen, 1830)	terr		0	0	4
<i>Bryophaenocladus nidorum/tuberculatum</i>	terr		0	0	4
<i>Bryophaenocladus nitidicollis</i> (Goetghebuer, 1913)	terr		0	0	2
<i>Bryophaenocladus</i> sp.	(terr)	<i>BryopSp</i>	1	3	11
<i>Camptocladus stercorarius</i> (De Geer, 1776)	terr	<i>CampSter</i>	0	0	17
<i>Corynoneura</i> sp.	(aqua)		0	1	0
<i>Cricotopus albiforceps</i> (Kieffer, 1916)	aqua		0	0	1

<i>Cricotopus brevipalpis</i> Kieffer, 1909	aqua		3	8	2
<i>Cricotopus cf. trifasciatus</i> (Meigen, 1810)	aqua		0	5	0
<i>Cricotopus festivellus</i> (Kieffer, 1906)	aqua		0	0	5
<i>Cricotopus intersectus</i> (Staeger, 1839)	aqua		0	0	7
<i>Cricotopus</i> sp. A	(aqua)		0	2	0
<i>Cricotopus</i> sp. B	(aqua)		0	0	1
<i>Cricotopus sylvestris</i> (Fabricius, 1794)	aqua	<i>CricSylv</i>	0	0	26
<i>Cricotopus vierrensis</i> Goetghebuer, 1935	aqua		2	2	0
<i>Eukiefferiella</i> sp.	(aqua)		0	0	1
<i>Harnischia curtilamellata</i> (Malloch, 1915)	aqua	<i>HarnCurt</i>	2	17	70
<i>Heterotrissocladius marcidus</i> (Walker, 1856)	aqua		0	0	1
<i>Hydrosmittia oxoniana</i> (Edwards, 1922)	terr	<i>HydrOxon</i>	0	0	79
<i>Hydrosmittia ruttneri</i> Strenzke & Thienemann, 1942	terr		0	0	4
<i>Limnophyes pumilio</i> (Holmgren, 1869)	aqua	<i>LimnPuml</i>	2	6	23
<i>Limnophyes</i> sp.	ND		0	2	17
<i>Metriocemus cf. ursinus</i> (Holmgren, 1869)	aqua		0	1	1
<i>Metriocnemus cf. beringensis</i> (Cranston & Oliver, 1988)	semi		0	0	4
<i>Metriocnemus picipes</i> (Meigen, 1818)	terr		0	0	1
<i>Metriocnemus</i> sp.	ND		0	0	3
<i>Metriocnemus tristellus</i> Edwards, 1929	aqua		0	0	11
<i>Orthocladius cf. rubicundus</i> (Meigen, 1818)	aqua		0	0	1
<i>Orthocladius</i> sp.	(aqua)		0	0	1
<i>Parakiefferiella bathophila</i> (Kieffer, 1912)	aqua	<i>ParkBath</i>	44	34	118
<i>Paraphaenocladius impensus</i> (Walker, 1856)	semi		1	2	0
<i>Psectrocladius bisetus</i> Goetghebuer, 1942	aqua		0	0	2
<i>Psectrocladius gr. limbatellus</i> (Holmgren, 1869)	aqua	<i>PsecGrLm</i>	0	14	64
<i>Psectrocladius platypus</i> (Edwards, 1929)	aqua		0	0	1
<i>Pseudorthocladius</i> sp.	(terr)		0	0	1
<i>Pseudorthocladius curtistylus</i> (Goetghebuer, 1921)	terr		0	0	2
<i>Pseudosmittia holsata</i> Thienemann & Strenzke, 1940	terr		0	0	4
<i>Pseudosmittia mathildae</i> Albu, 1968	ND		0	0	2
<i>Pseudosmittia</i> sp.	(terr)		0	0	1
<i>Rheocricotopus fuscipes</i> (Kieffer, 1909)	aqua		0	0	1
<i>Rheocricotopus</i> sp.	(aqua)		0	0	1
<i>Rheosmittia spinicornis</i> (Brundin, 1956)	aqua		0	0	1
<i>Smittia aterrima</i> (Meigen, 1818)	terr		0	0	2
<i>Smittia edwardsi</i> Goetghebuer, 1932	terr	<i>SmitEdwr</i>	0	0	28
<i>Smittia leucopogon</i> (Meigen, 1804)	terr	<i>SmitLeuc</i>	0	0	16
<i>Smittia</i> sp.	(terr)	<i>SmitSp</i>	0	0	908
terrestrial <i>Orthoclaadiinae</i> *	terr		673	490	0
<i>Tvetenia cf. discoloripes</i> (Goetghebuer & Thienemann, 1936)	aqua		0	0	1
<i>Tvetenia cf. verralli</i> (Edwards, 1929)	aqua		0	0	13
<i>Tvetenia</i> sp.	(aqua)		0	0	4

Total number of aquatic males	98	419	1037
Total number of aquatic species	13	37	90
Total number of semi- and terrestrial males	1468	602	1300
Total number of semi- and terrestrial species	7	7	19
Ratio aquatic males : total males (%)	6.3	40.9	44.0
Total number of females ¶	ND	ND	5286

* 2 species of *Smittia* + 2 species of *Hydrosmittia*.

¶ Females were not considered in analyses.

Abbreviations: aqua = aquatic larvae, semi = semi-terrestrial larvae living mostly outside or near water but requiring high humidity, terr = terrestrial larvae, ND = no data; habitat data in parentheses = dominant habitat preference of other members of the genus. Habitat preferences based on Fittkau and Reiss (1978), Otto and Schiegg (1999), Moller Pillot (2009, 2013), and Vallenduuk and Moller Pillot (2013).

Table 2 Summary of the most parsimonious models of total diel and seasonal flight activity of adult chironomids. Corresponding initial saturated model given in front of each final model. $\Delta qAICc$ = $qAICc$ difference from the most parsimonious model, d. f. = degrees of freedom, w = $qAICc$ weight, R^2 = McFadden's pseudo- R^2 . See Methods for abbreviations of variables; H^2 and W^2 = linear term not retained in the final model. Terrestrial species also include semi-terrestrial taxa.

Model	$\Delta qAICc$	d. f.	w	R^2
Diel pattern: aquatic species, 2013 data				
D1: $N \sim Q(time) + T + C_F + place$	0.0	8	0.62	0.463
D2: $N \sim month + Q(time) + C_F + place$	1.0	8	0.38	0.456
Diel pattern: terrestrial species, 2013 data				
D2: $N \sim month + Q(time) + Q(H) + place$	0.0	7	0.64	0.448
D1: $N \sim Q(time) + Q(T) + W^2 + place$	1.2	7	0.36	0.458
Seasonal patterns: aquatic species, 2014–2015 data				
S2: $N \sim month + H + W + P + place$	0.0	11	0.58	0.704
S1: $N \sim month + T + W + P + place$	1.6	11	0.25	0.709
S3: $N \sim season + Q(T) + TH + W + P + place$	2.5	8	0.17	0.679
S4: $N \sim season + Q(H) + C + W + P + place$	13.9	8	<0.001	0.644
Seasonal patterns: terrestrial species, 2014–2015 data				
S1: $N \sim month + Q(T) + W + P + place$	0.0	11	0.76	0.644
S2: $N \sim month + H^2 + C + W + P + place$	2.3	12	0.24	0.645
S3: $N \sim Q(T) + TH + C + Q(W)$	48.6	7	<0.001	0.439
S4: $N \sim H + Q(W)$	63.6	4	<0.001	0.363

Table 3 Summary of multivariate analyses (RDA and partial RDA) of seasonal and weather-dependent changes in the composition of male chironomid assemblages in 2014–2015. Only species with at least 5 occurrences included. Significant results ($P < 0.05$) in bold. Covariates used in partial RDA given in parentheses. AEV = adjusted explained variation of the model.

Model	Aquatic species			Terrestrial species		
	AEV	Pseudo-F	<i>P</i>	AEV	Pseudo-F	<i>P</i>
Month	67.9 %	10.2	0.0001	52.7 %	5.5	0.0001
Humidity	16.0 %	6.0	0.0005	16.5 %	5.7	0.0008
Temperature	22.8 %	8.7	0.0001	4.1 %	2.0	0.10
Wind	7.5 %	3.1	0.013	12.8 %	4.5	0.003
Cloud cover	3.2 %	1.9	0.10	11.2 %	4.0	0.007
Air pressure	1.1 %	1.3	0.23	0.7 %	1.2	0.31
Temperature + humidity	35.9 %	8.3	0.0001	22.4 %	4.5	0.0002
Temperature + weather regime	36.1 %	8.3	0.0001	-	-	-
Humidity + weather regime	33.2 %	7.5	0.0001	22.4 %	4.5	0.0003
Humidity + temperature + wind *	38.9 %	6.5	0.0001	-	-	-
Humidity + temperature + wind (month)	0 %	0.9	0.65	-	-	-
Humidity + wind + cloud cover ¶	-	-	-	32.0 %	4.8	0.0002
Humidity + wind + cloud cover (month)	-	-	-	18.6 %	2.4	0.017

* and ¶ visualised in Fig. 5

Figure legends

Fig. 1 Schematic map of the study area showing experimental pools (grey circles), lake (grey area), vegetation (dotted area), steep slopes (checkerboard) and drains (parallel lines), position of Malaise traps (x) and sweeping route (dashed line) within locations (A and B) in the sandpit.

Fig. 2 Rarefaction curves (data: solid lines, extrapolation: dashed lines) of the net (line with triangle), Malaise trap (line with dot) for (a) whole chironomid assemblage and (b) only for aquatic species. Shaded areas indicate 95 % confidence intervals.

Fig. 3 Drivers of diel patterns of total flight activity of (a–d) aquatic and (e–h) terrestrial species. Routes of sweeping: A = on the shore of lake and B = close to the set of experimental ponds, categories for cloud cover: 1 = clear sky, 2 = mostly sunny, 3 = mostly cloudy, and 4 = cloudy. Solid black lines and black points = model fit; dashed lines and grey error bars = 95 % confidence intervals; grey points = overlaid raw data with small amount of jitter added. Y-axis on \log_{10} scale with zeroes placed at 0.1.

Fig. 4 Drivers of seasonal flight activity of total flight activity of (a–e) aquatic and (f–j) terrestrial chironomids. Symbols and axes as in Fig. 3.

Fig. 5 Response to environmental parameters of (a) aquatic chironomid assemblage (RDA, axis 1 = 29.7 %, axis 2 = 15.1 % of total explained variance), and (b) terrestrial species (RDA, axis 1 = 20.7 %, axis 2 = 19.4 % of total explained variance). See Table 1 for species abbreviations.

Fig. 6 Seasonal flight patterns of chironomids. (a and b) Species response curves for most common taxa with >5 occurrences for (a) aquatic species (RDA, axis 1 = 40.0 %, axis 2 = 17.2 % of total explained variance), and (b) terrestrial species (RDA, axis 1 = 36.9 %, axis 2 = 19.7 % of total explained variance). Degrees of freedom (see Methods for details): *HarnCurt* = 4, *MicrTenr* = 3, *ParkBath* = 5, *ProcChor* = 5, *PsecGrLm* = 5, *TantVolg* = 3, *BryoCfll* = 4, *HydrOxon* = 3, *SmitEdwr* = 4, *SmitSp* = 5. (c) Differences between aquatic and terrestrial species (RDA, axis 1 = 63.9 % of total explained variance). See Table 1 for species abbreviations.

Figures

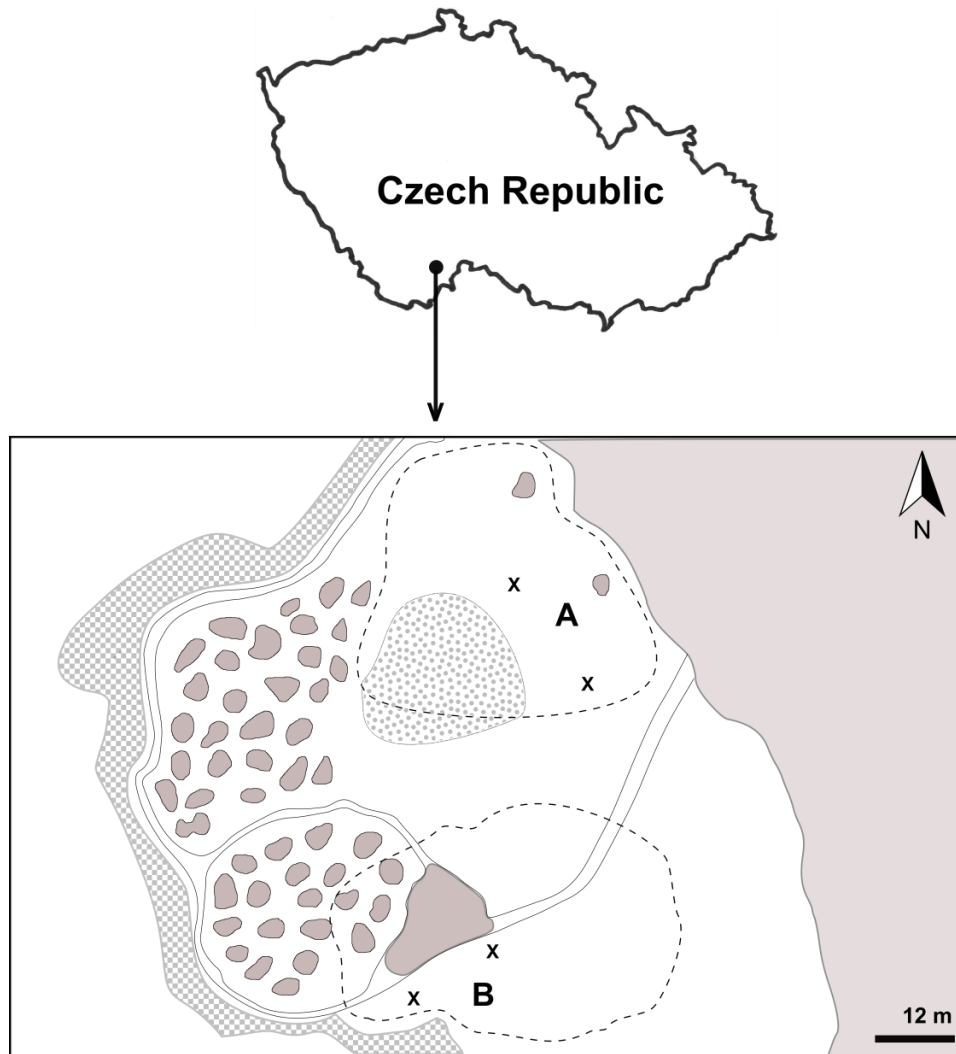


Fig. 1

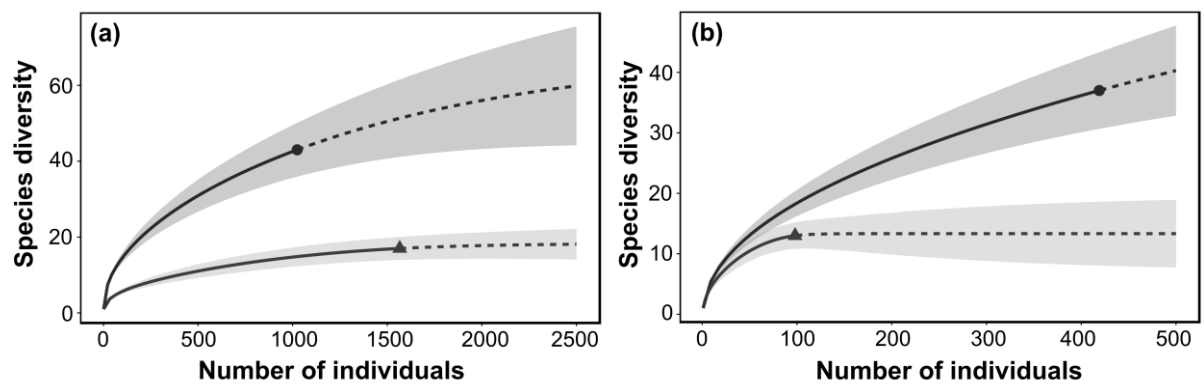


Fig. 2

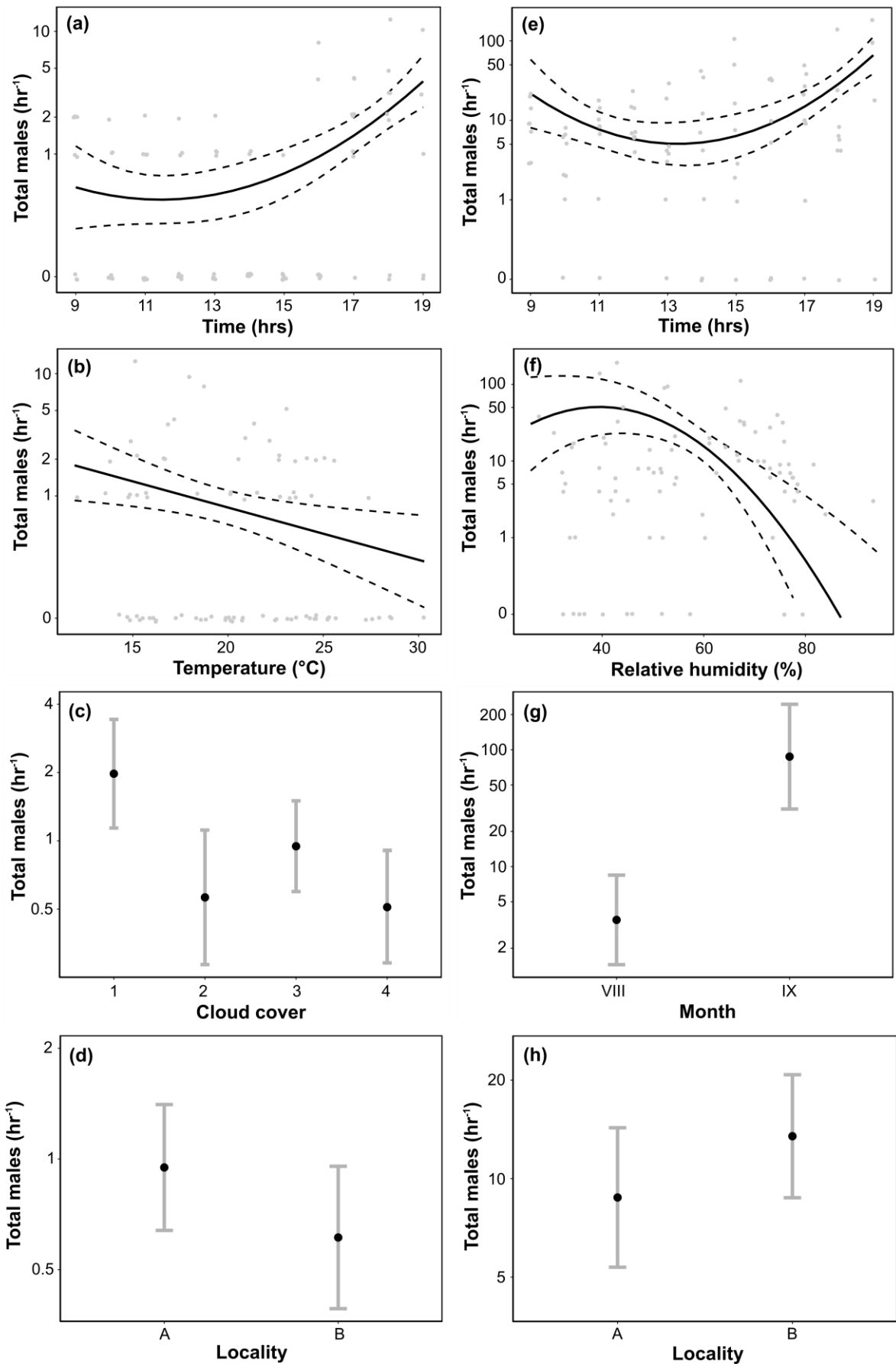


Fig. 3

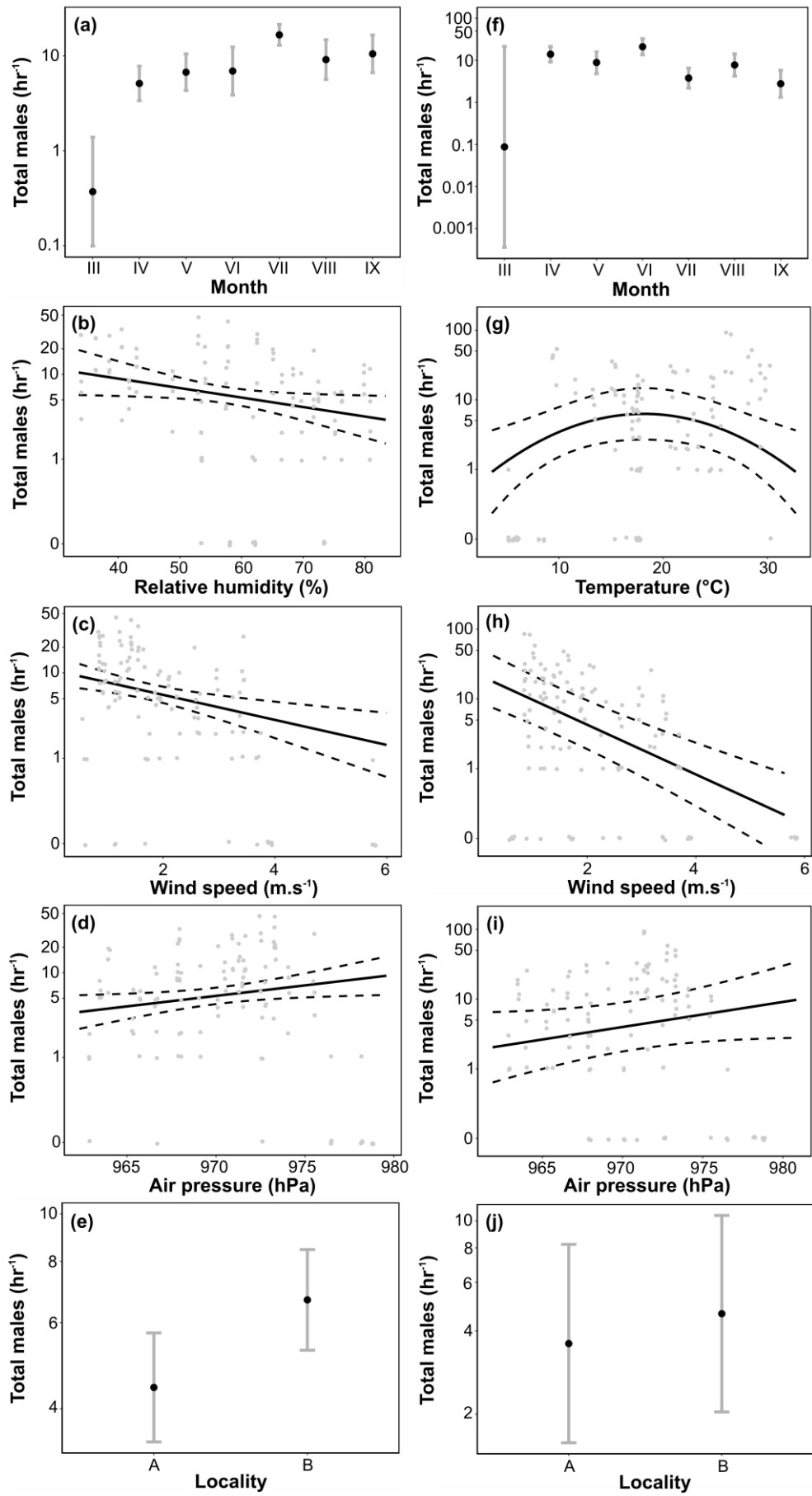


Fig. 4

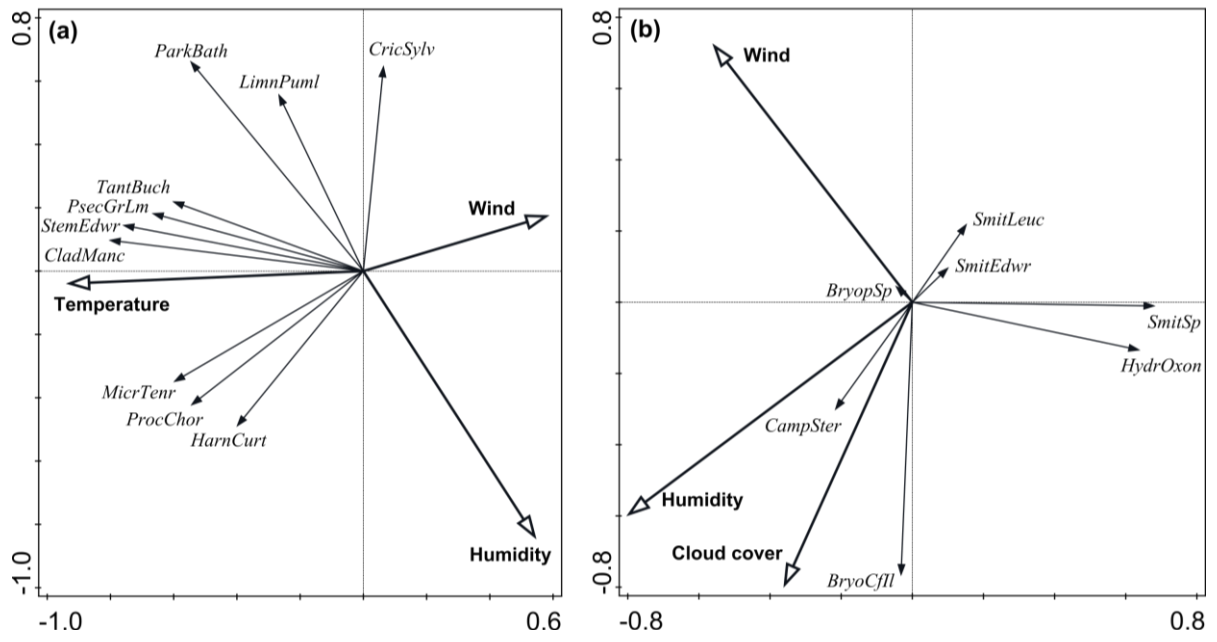


Fig. 5

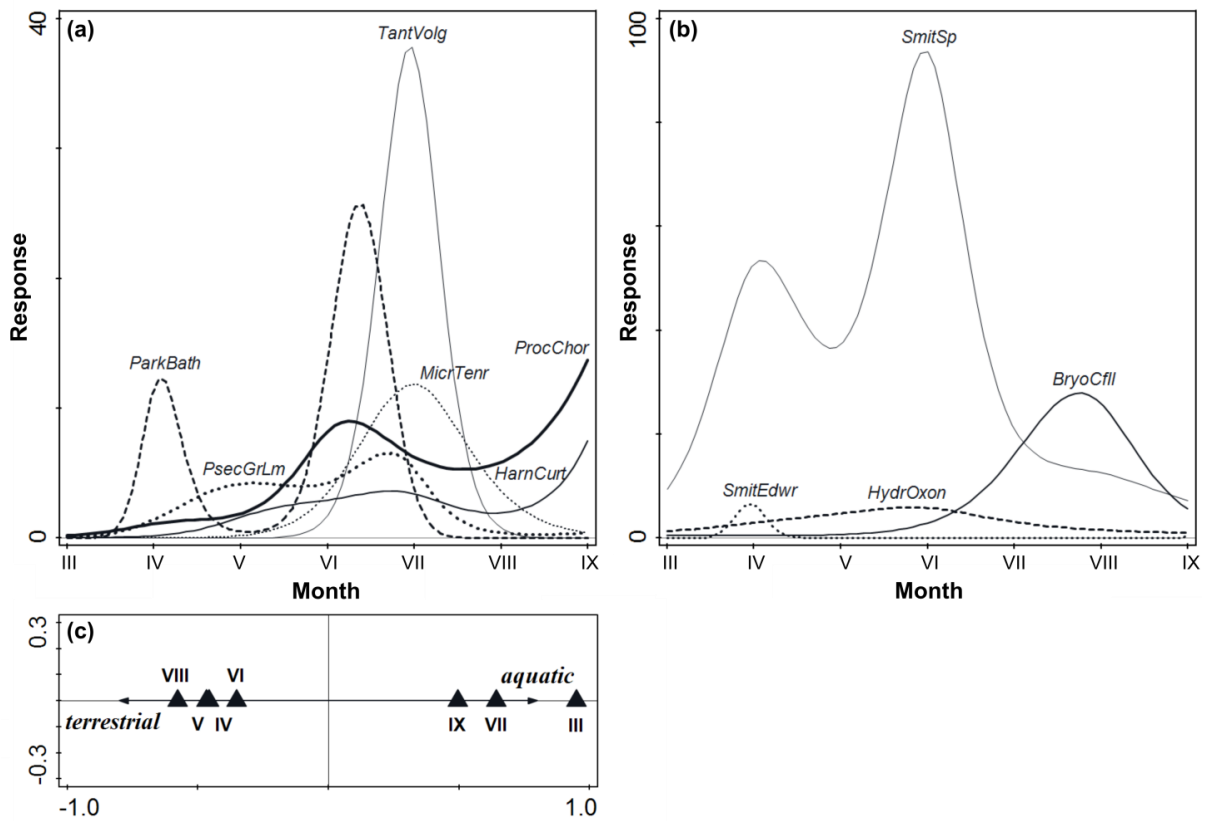


Fig. 6

Supporting Information

Table S1 Overview of spatiotemporal resolution and pooling of samples in the analyses of the 2013 and 2014–2015 datasets. For more information see Methods.

Dataset	Method	Temporal resolution	Spatial resolution
Handnet 2013	univariate	1 hour (11 per day)	separate routes (1 per each hour)
Handnet vs. Malaise traps 2013	rarefaction	pooled daily data	(a) all sites across each method pooled (b) separate sites for both methods
Malaise traps 2014–2015	univariate	pooled afternoon and evening data (1 per day)	separate traps (4 per day)
	multivariate, species response curves, traits	pooled afternoon and evening data (1 per day)	all traps pooled (1 per day)

Fig. S1 Malaise trap used in the study. The trap consisted of the main trapezoid vertical panel (black fine mesh, heights 1.85 m and 1 m, upper edge 2.07 m long, lower edge 1.80 m long) bordered by two larger (1.06 x 0.68 m) and two smaller (0.92 x 0.6 m) perpendicular panels (black fine mesh) at each side of the panel and a sloping roof (white fine mesh). Experimental pools and the steep slope shown in Fig. 1 visible in background.



Table S2 Weather conditions during the sampling dates in 2013 and 2014–2015. Summary based on point data measured every 15 minutes on the site except the wind, cloud cover and air pressure data from 2014–2015 that were provided by the Czech Hydrometeorological Institute weather station in Třeboň. Asterisk: cloud cover data from 2013 and 2014–2015 are on different scale (see Methods for details). Categories for cloud cover: 1 = clear sky, 2 = mostly sunny, 3 = mostly cloudy, and 4 = cloudy.

Variable	2013			2014–2015		
	Mean \pm SD	Min	Max	Mean \pm SD	Min	Max
Temperature ($^{\circ}$ C)	19.9 \pm 4.4	9.5	30.5	17.6 \pm 7.9	-1.7	36.8
Relative humidity (%)	58.8 \pm 17.7	25.8	97.3	59.4 \pm 18.3	19.8	96.5
Wind ($\text{m}\cdot\text{s}^{-1}$)	3.5 \pm 2.4	0.1	9.4	2.2 \pm 1.7	0	8.4
Cloud cover *	3	1	4	5 \pm 3.2	0	10
Air pressure (hPa)	-	-	-	970.8 \pm 4.5	962.5	979.9

Fig. S2 Relationship between values recorded in 2014–2015 in sandpit (y) and Třeboň (x) for (a) temperature: $y = 1.11x - 0.42$ ($R^2 = 0.88$), and (b) relative humidity: $y = 0.74x + 14.37$ ($R^2 = 0.65$). Months: March (brown), April (pink), May (red), June (gold), July (violet), August (green) and September (blue).

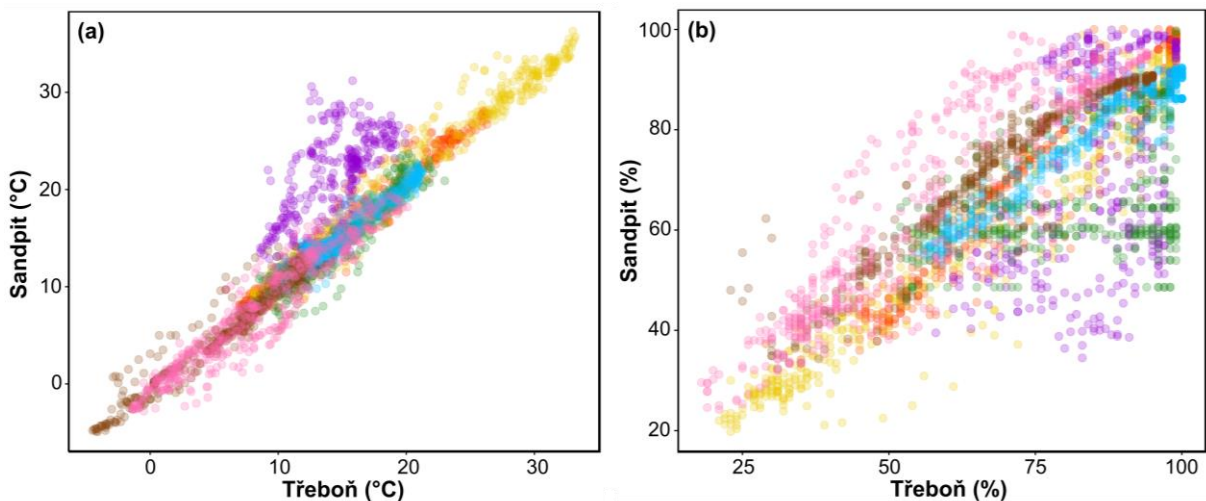


Fig. S3 Two different temperature-humidity regimes in sandpit during the 2014–2015 sampling dates. Linear regression of temperature T against relative humidity H based on 1-hour average data measured directly at the site: $T = -0.29 H + 39.1$ ($R^2 = 0.91$) in May–September 2014 (upper) and $T = -0.27 H + 24.6$ ($R^2 = 0.87$) in March and April 2015 (lower). Symbols for months as in Fig. S2.

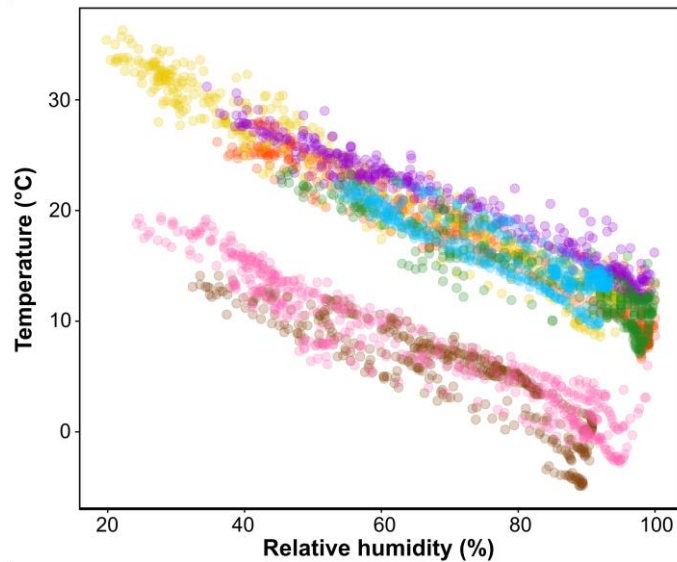


Fig. S4 Rarefaction curves (data: solid lines, extrapolation: dashed lines) of the net (blue and violet lines), Malaise trap (red and green lines) for (a) whole chironomid assemblage and (b) only for aquatic species with data disaggregated between the sites near the lake shore (red and blue lines) and close to the southern edge of the experimental pools (green and violet lines). Shaded areas indicate 95 % confidence intervals.

