Twenty years of ecological change in Arctic Muscidae (Diptera) at Zackenberg, Greenland

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‘The truth is that we need invertebrates but they don’t need us. If human beings were to disappear tomorrow, the world would go on with little change. (…) But if invertebrates were to disappear, I doubt that the human species could last more than a few months.’

Wilson (1987)

Dedication

This thesis is dedicated to Terry Wheeler, a passionate entomologist, outdoor enthusiast, talented speaker, and teacher. He was the person I enjoyed the most going to conferences with, chasing great talks. Without him, I would not have made the jump in the adventure of a Ph.D.

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Abstract

The depletion of insect populations in many parts of the world threatens the stability of ecosystem services and stability of ecosystems themselves. Understanding how climate change contributes to insect decline is a critical step to the development of conservation strategies. In the High-Arctic, while much of the terrestrial area is free from direct human disturbances, since 1950 warming has been twice as pronounced there in comparison to the rest of the planet. The main objective of this thesis is to better understand the effects of rapid warming on insect populations, using muscid flies (Diptera: Muscidae) as a focal taxon. These flies are common pollinators in the High-Arctic and have been in decline since 1996 at Zackenberg, Northern Greenland. I explored ecological changes in the muscids of Zackenberg, at multiple biological scales, from the assemblage to population level, between 1996 and 2014. In Chapter 1, I review climate change and its impacts on biodiversity, especially insects, globally and in the Arctic region. In Chapter 2, I examined how abundance, diversity, and composition of muscid fly assemblages have changed over the study period in three different habitats. Results show that the total abundance of muscid flies had declined by 80% between 1996 and 2014 across habitats. However, rates of abundance change over time varied by species and by habitat, resulting in reduced diversity and changes in composition over time, but not in biotic homogenization across habitats. Changes in species abundance, diversity and composition were more pronounced in the wet fen, implying that muscid species in wet habitats might be more at risk in the future because of drying soil. In Chapter 3, I analyzed how the timing and length of the flight period (i.e., phenology) of 12 species of muscid flies have changed between 1996 and 2014. I found that rapid warming has not only resulted in an earlier period of activity but also a shortened flight period for all muscid species studied, especially for those with smaller body size. Furthermore, the contraction of the flight period in a given year was related to a decline in abundance in the subsequent year(s),

indicating that the life cycle of muscid flies might have accelerated under rapid warming. In Chapter 4, I investigated how the population abundance of six muscid species was related to the length of the reproductive period by quantifying sex-specific changes in abundance and phenology as well as temperature sensitivity. The length of the reproductive period has shrunk over the study period, not because of a reproductive mismatch between the activity of males and females, but because the flight period of both sexes has shortened over time. The reduced reproductive period in a given year was associated with lower species abundance the following years, suggesting a potential Allee effect through mate limitation. My results showed that warmer temperatures were associated to insect decline directly, and also indirectly through impacts on insect phenology. Given future projections in temperature rise in Polar Regions, important changes in insect abundance, diversity, composition and phenology are expected and will continue to threaten ecosystem stability unless appropriate measures to mitigate climate change effects on biodiversity are taken.

Résumé

Le déclin des populations d'insectes dans de nombreuses régions du monde est devenu une préoccupation car cela menace la stabilité des écosystèmes et des services écosystémiques. Comprendre l’impact du réchauffement climatique sur les populations d’insectes est une étape cruciale dans l'élaboration des stratégies de conservation. Dans le haut-Arctique, la majeure partie de la zone terrestre est exempte de perturbations humaines directes, mais le réchauffement a été deux fois plus rapide que dans les autres régions du globe depuis 1950. L'objectif principal de cette thèse est donc de mieux comprendre les effets du réchauffement sur les populations d'insectes en me concentrant sur les mouches de la famille des Muscidés (Diptera, Muscidae). Ces mouches sont des pollinisateurs courants dans le haut-Arctique et sont en déclin depuis 1996 à Zackenberg. J’ai exploré les changements écologiques dans la famille des Muscidae et ce, à plusieurs échelles biologiques, de la communauté aux populations, entre 1996 et 2014. Dans le Chapitre 1, je décris le déclin des populations d’insectes, les changements climatiques dans la région Arctique ainsi que leurs impacts sur la biodiversité, en particulier celle des insectes. Dans le Chapitre 2, j'ai examiné comment l'abondance, la diversité et la composition des assemblages de mouches ont changé au cours de la période d'étude dans trois habitats différents. J'ai trouvé que l'abondance des mouches diminuait de 80% entre 1996 et 2014, entraînant une baisse de la diversité et un changement de composition au fil du temps, mais pas une homogénéisation biotique entre les habitats. Ces changements étaient plus prononcés dans le milieu humide, ce qui signifie que les espèces des habitats humides pourraient être plus vulnérables à l’avenir en raison du dessèchement du sol. Dans le Chapitre 3, j'ai analysé comment le moment et la durée de la période de vol (c.-à-d. la phénologie) de douze espèces de mouches ont changé entre 1996 et 2014. J'ai constaté que le réchauffement rapide a entraîné un devancement, mais aussi un

raccourcissement, de la période de vol des espèces de mouches, et en particulier pour celles dont la taille corporelle est plus petite. La contraction de la période de vol une année donnée était liée au déclin temporel de l'abondance des mouches les années suivantes, ce qui indique que la reproduction des mouches adultes pourrait être modifiée par un réchauffement rapide. Dans le Chapitre 4, j'ai donc étudié comment l'abondance de la population de six espèces était liée à la durée de la période de reproduction. De plus, j'ai évalué, pour chaque sexe, les changements phénologiques, les changements d’abondance et la sensibilité à la température. J'ai trouvé que la durée de la période de reproduction des mouches s'est raccourcie en raison du rétrécissement de la période de vol des mâles et des femelles. La période de reproduction réduite une année donnée a été associée à une plus faible abondance des espèces les années suivantes, suggérant une difficulté à trouver un partenaire sexuel durant cette courte période d’activité. En résumé, les résultats de cette étude démontrent que, en l’absence de perturbations humaines, le déclin des populations d’insectes est associé directement à une augmentation rapide de la température, et indirectement, suite à des modifications de la phénologie des insectes. Considérant l’augmentation prévue de température dans les régions polaires au cours des prochaines décennies, il est à prévoir que l’abondance, la diversité, la composition et la phénologie des insectes continueront à être modifié au détriment de la stabilité des écosystèmes.

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I would first like to thank my husband Mathieu for his endless love, support, and encouragement. With many obstacles and new adventurers at home, it would have been easy to fell slumped without his help and optimism. I would not have imagined a better partner to go through this mad project. Elliot, Cyril, Méli, and Zoé, thank you for bringing balance, laugh, wonder, and happiness to my life.

I want to thank my supervisors, Jade, Toke, and Chris, for their guidance, patience, and support. Despite the 600km that separate home and the Universities, 4 moves, 1 wedding, 2 thesis babies and 1 global pandemic (although I was not responsible for that), you never showed a sign of discouragement or disapproval. I was extremely fortunate to have not one but three amazing scientists with good comprehension. I have been happy to get to know Jade in recent years. In addition to having a good sense of humor and sensitivity, Jade is an outstanding scientist, with a talent for identification, editing, communication, and supervision. I generated a lot of results, always over-analyzing my data to make sure that my results would be similar with different methods. Jade was on the lookout for the story behind my numbers and figures. She never counted the hours when it came to supervising me and it was well appreciated. I would like to thank Jessica Gillung who, despite her new position at McGill, new life in Montreal and all the challenges that come along, took the time to read and comment this work, and send many warm encouragements. Jessica was a sunshine in these unexpected times. I would like to thank the defense committee including Kyle Elliott, Mark Vellend and Jim Fyles, for the constructive comments.

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I thank the team *Thèsez-vous?* who helped me find a method of working and writing to complete my thesis from home. I wish all graduate students to meet you! I take this opportunity to also thank the SEQ who granted me a scholarship the first year of my Ph.D, and the QCBS for their financial support to attend conferences and international courses, as well as all the R workshops, which have helped me to love statistical analysis and programming.

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Contribution of authors

All chapters were conceived and written by me and are the result of my research efforts. My supervisors, Christopher Buddle and Jade Savage, as well as Toke Høye from Aarhus University, Denmark, provided guidance, ideas, and comments on all my work. The sampling protocol used to collect insects at Zackenberg was developed in 1996 by Danish scientists and is used yearly since then (Meltofte & Thing, 1997).

Jade Savage trained me to identify muscid fly species from Greenland using morphological characters. I performed all species identifications, and Véronique Bellavance prepared the specimen for DNA barcoding. Identifications with morphological characters would have been difficult without the key developed by Jade and Véronique the year before I started my research. I conducted all statistical analyses and wrote all original manuscripts. Jade Savage also helped during the interpretation of results based on her knowledge of the ecological requirements of muscid species. Jade Savage, Christopher Buddle, and Toke Høye participated actively in the editing of the manuscripts. Niels Martin Schmidt provided access to the Zackenberg collection in Denmark and did some editing on Chapters 2 to 4. Finally, Sébastien Rivest helped to elaborate on the analysis of the temporal synchrony of males and females for Chapter 4. He edited the manuscript and gave critical comments for publication. Chapter 2 was published in *Ecography* (Loboda, Savage, Buddle, Schmidt, & Høye, 2018), Chapter 3 was prepared for publication in *Ecology and Evolution* and Chapter 4 for publication in *Polar Biology*.

Contributions to original knowledge

The overarching goal of my thesis was to better understand the ecological responses of muscid flies (Diptera: Muscidae) to rapid warming across biological scales, from the assemblage to populations, in the High-Arctic region between 1996 and 2014. This was done in the context of declines in insect abundance in a region where direct human pressures (pesticides, habitat fragmentation, and habitat loss) are less prevalent: the High-Arctic. My contributions to knowledge are as follows:

- I identified more than 18,000 specimens of muscid flies to species, which were collected between 1996 and 2014 with a standardized protocol over the years at Zackenberg, North-East Greenland. This collection represents the best curated detailed temporal series of insect species from the Arctic. It will facilitate future work on phenotypic or genetic changes over time, for example, or work on pollination networks since some muscid flies are important flower visitors in the Arctic region. The data will also be available through an online repository such as Dryad so it will continue to contribute to the assessment of insect population trends[1].

- I found that the decline in muscid abundance at Zackenberg documented at the family level in 2013, was widespread at the species level. Most species are declining in abundance since 1996, especially in the wet fen. Species diversity decreased over the study period, and the composition of the assemblage has changed with temperature increase in the summer. Few previous studies have been able to document such detailed changes at the assemblage level for an insect group in the Arctic. This study contributed to the climate change research area by

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[1] The data will be deposited on Dryad when the Chapter 3 will be published in a peer-reviewed journal.

showing that insect species with preferences for wet habitats in the Arctic are more at-risk to decline under rapid warming than species in arid or mesic habitats.

- My study represents the first analysis of phenological shifts in multiple species of Arctic insects of an abundant and common family of flies, over two decades of rapid warming. I documented this by testing how the timing of the flight period of muscid fly species has changed over time in relation to increased temperature. While phenological shifts of Arctic plant species and migratory birds in the spring and the fall are relatively well documented locally and globally, phenological shifts of Arctic insects at the species level have been documented only for few species of butterflies. My results draw attention to the great variety of phenological responses of species in one abundant family of insects.

- For the first time, I showed that the flight period has not only advanced but also shrunk with temperature increases for multiple species of muscids. My results showed how the timing of the flight period of muscid fly species has changed over the study period using multiple phenological events to describe the active period of adult flies in the summer. This information is novel, yet crucial to better predict potential mismatch between flowering plants and pollinators in the Arctic.

- Body size explained the magnitude of phenological shifts of muscid species observed at Zackenberg in recent decades. Smaller species had greater shifts in phenology than large ones, potentially because of their low thermoregulatory capacities. This result suggests that small changes in mean body size of insects could have a great impact on how species acclimate and adapt to climate change.

- To my knowledge, this study is the first to explore if differences in sex-specific responses could explain temporal changes in phenology and population sizes of insects in the context of climate change. I provide one of the first investigations of the relationship between insect demography and phenology in the context of climate change. Some shifts in phenology were associated with a decline in abundance. I also found that the reproductive period, defined as the period where both sexes are active in synchrony during the summer, has shortened. However, the reproductive period did not shrink because of a temporal mismatch between the activity of males and females. Instead, the flight period has shortened for both sexes. The contraction of the reproductive period was associated with a lower abundance of muscids in the following years.

Introduction and objectives

Introduction

Substantial declines in insect abundance and biomass around the globe over the last few decades have raised concerns about the stability and functions of terrestrial ecosystems (Cardoso et al., 2020; Shortall et al., 2009; Simmons et al., 2019; van Klink et al., 2020). Insects decline occurred even in protected areas where direct human pressures are absent (Hallmann et al., 2017), calling attention to global warming as a potentially critical factor affecting insect populations (Boggs, 2016; Forrest, 2016; Wagner, 2020). Through polar amplification, the Arctic is one of the regions on Earth most affected by rapid warming despite being less affected by direct human pressures (Arctic Monitoring and Assessment Programme, 2019), thus being a model region to explore the role of global warming on insect population changes over time.

The Arctic fauna is dominated by insects, particularly from the order Diptera (Gillespie, Alfredsson, Barrio, Bowden, Convey, Culler, et al., 2020). Flies are abundant, diverse, and, thus, play many roles in the ecological networks of northern systems, including decomposition and pollination (Marshall, 2006). Flies in the family Muscidae are known to be important flower visitors (Tiusanen, Hebert, Schmidt, & Roslin, 2016), and recently, their decline has been documented at Zackenberg (North-Eastern of Greenland), a site situated in the largest protected area in the world (Høye, Schmidt, Trøjelsgaard, & Forchhammer, 2013). Taking advantage of the unique long-term monitoring program established in 1996 at Zackenberg, I explored multiple patterns of ecological changes in the muscid fly family, between 1996 and 2014, to better understand how rapid warming could influence insect community structure, diversity, abundance, and phenology.

Research objectives and outline

The goal of my research is to highlight potential warming-induced processes altering insect abundance by documenting ecological responses of muscid fly species to rapid warming at Zackenberg, between 1996 and 2014, from the assemblage scale to populations.

The specific objectives of the remaining chapters are:

Chapter 1: Describe the global decline in insect abundance and biomass observed recently, the environmental changes in the Arctic, the ecological responses of biodiversity and insects in particular, and the role of extended monitoring programs in detecting effects of climate change on biodiversity

Chapter 2: Examine temporal changes in diversity, abundance, and species composition of muscid flies sampled annually in three habitats at Zackenberg between 1996 and 2014

Chapter 3: Document species phenological shifts and explore the relationship between species abundance, phenology, and species' traits

Chapter 4: Determine intra-specific variations in responses to warming by examining sex-specific differences in the timing of activity and abundance of six species of muscid flies.

Chapter 1 Literature review

1.1 Insects and their recent decline

Insects have originated 400 million years ago and have been flying long before birds (Engel & Grimaldi, 2004) conquering all terrestrial and freshwater habitats on Earth. Invertebrates represent 80% of all known non-microbial species on the planet (Collen, Böhm, Kemp, & Baillie, 2012; Mora, Tittensor, Adl, Simpson, & Worm, 2011; Scheffers, Joppa, Pimm, & Laurance, 2012) and arthropods, which are invertebrates with segmented bodies such as insects, spiders, and crustaceans, form the most diverse taxon among invertebrates. There are ten times more beetle species on the planet than vertebrates (Marshall, 2006; Stork, McBroom, Gely, & Hamilton, 2015). Even more than the number of species, invertebrates dominate aquatic and terrestrial ecosystems by their abundance and biomass (Collen et al., 2012). Because of their dominance and diversity, insects are found in every trophic level, and they play major roles in ecosystem functions such as pollination, nutrient cycling, and seed dispersal (Cardoso et al., 2020; Noriega et al., 2018; Wilson, 1987).

Insects have survived several extinction events such as the Permian and the famous Cretaceous-Tertiary extinction event, but they are now threatened by human activities and climate change (Collen et al., 2012; Shortall et al., 2009; Simmons et al., 2019; van Klink et al., 2020; Warren, Price, Graham, Forstenhaeusler, & VanDerWal, 2018). Insects and other invertebrates are poikilothermic ectotherms, meaning that their body temperature depends on the ambient temperature. They are, therefore, readily responsive to small changes in temperature (Cohen, Lajeunesse, & Rohr, 2018). An estimated 12% of endangered terrestrial invertebrate species are threatened by climate change, and this number is expected to be strongly underestimated (Collen et al., 2012).

Some major groups of insects have been declining in abundance and/or biomass over the last few decades (Cardoso et al., 2020), including domesticated and wild pollinators such as bees (Bartomeus et al., 2013; Potts et al., 2010; Soroye, Newbold, & Kerr, 2020), fireflies (Lewis et al., 2020), butterflies and moths (Bell et al., 2020; Fox et al., 2019; J. A. Thomas et al., 2004), some aphids (Bell, Blumgart, & Shortall, 2020; Bell et al., 2015), beetles (D. R. Brooks et al., 2012), flies (Grabener, Oldeland, Shortall, & Harrington, 2020) and dragonflies (Clausnitzer et al., 2009). Human activities and disturbances such as pesticide use, fragmentation, and loss of habitat are the main drivers behind these declines, followed by climate change (Cardoso et al., 2020; Ewald et al., 2015; Habel, Samways, & Schmitt, 2019; Maxwell, Fuller, Brooks, & Watson, 2016). Even though biodiversity decline has been of concern among scientists for a while (Union of Concerned Scientists, 1992), the documentation of the modern extinction is focused almost exclusively on mammals and birds, for which robust data is available (Horwitz, Recher, & Majer, 1999). Estimates of extinction rates for the contemporary "sixth mass extinction" are unsurprisingly expressed as 200 mammals per 10,000 species per 100 years (Ceballos et al., 2015).

The spotlight was shed on the ‘little things that run the world’ when a drop in biomass of flying insects by 75% in protected areas of Germany between 1989 and 2016 was revealed (Hallmann et al., 2017). Sánchez-Bayo and Wyckhuys (2019), who first claimed the "Insectageddon" or "Insect Armageddon", reviewed the global state of insect populations and suggested that ‘the collapse of nature’ would occur within decades with the extinction of 40% of the world’s insect species. This study has been heavily criticized by scientists, who do not deny the potential threat of insect extinctions but are more cautious on the generality of insect declines (Didham et al., 2020; Komonen, Halme, & Kotiaho, 2019; Montgomery et al., 2020; Mupepele et al., 2019; Saunders, Janes, & O'Hanlon, 2019; Simmons et al., 2019; Thomas, Jones, & Hartley, 2019; Wagner, 2019). Most studies with long time series of insect data are geographically and-or taxonomically restricted, thereby making generalizations about the global "Insectageddon" unreliable (Montgomery et al., 2020; Simmons et al., 2019). The most recent meta-analysis on insect abundance trends, using 166 long-term surveys of insects and arachnids across 1676 sites, found a significant decline in terrestrial insects over time of 9% per decade but a significant increase in aquatic insects by 11% (van Klink et al., 2020), highlighting the high variability in temporal trends of insect biomass and abundance globally (Dornelas & Daskalova, 2020). Moreover, they tested if temporal trends in abundance and biomass changed over time using different time windows (since 1960, 1970, 1980). They found that the decline in terrestrial insects in Europe was more pronounced in recent decades, while the opposite was observed in North-America (van Klink et al., 2020), suggesting that temporal changes in insect abundance are far from being linear and constant over time and space.

1.2 The Arctic region under climate change

In recent decades, human activities have caused substantial changes to the climate around the Earth (IPCC, 2019). Several of the warmest years since 1880 have been occurring in the last decade (Arguez et al., 2020). Despite being among the least inhabited part of the world, the Arctic region, defined as the land above the treeline (Callaghan et al., 2005), has experienced severe changes in climate because of ‘polar amplification’ (Arctic Climate Impact Assessment, 2005; Arctic Monitoring and Assessment Programme, 2017; CAFF, 2013; Post et al., 2019; Serreze & Francis, 2006). Polar amplification results from the effects of feedback processes, including snow and ice-albedo feedback or reduced albedo, from the increased vegetation growth promoted by increased carbon dioxide in the atmosphere (Arctic Climate Impact Assessment, 2005; Post et al., 2019). Over the last 40 years, the atmosphere in the Arctic has warmed twice as much as the rest of the Earth (IPCC, 2019), and in the future, the Arctic is predicted to experience the highest temperature increase, with pronounced rises in the fall and the winter (Arctic Climate Impact Assessment, 2005; Cook, Smerdon, Seager, & Coats, 2014). If the 2°C upper limit of temperature increase (since pre-industrial levels) chosen by developing countries during the Paris agreement is reached by 2050, this will result in a temperature rise estimated between 2.8 and 7.8°C in the Arctic (CAFF, 2013; Post et al., 2019).

Climate change in the Arctic is projected to affect many aspects of northern ecosystems, including vegetation zones (Myers-Smith et al., 2015; Post et al., 2019), as increased air temperatures promote the growth of shrubs and trees into the tundra (Arctic Monitoring and Assessment Programme, 2019; Elmendorf et al., 2012; Martin, Jeffers, Petrokofsky, Myers-Smith, & Macias-Fauria, 2017). Analyses of satellite images have shown that the tundra is ‘greening’ due to the growth and northward expansion of shrubs (Arctic Climate Impact Assessment, 2005; Daniëls et al., 2013; Forbes, Fauria, & Zetterberg, 2010; Jia, Epstein, & Walker, 2003; Jia, Epstein, & Walker, 2009; Myers-Smith et al., 2011) and changes in vegetation type have been more pronounced in moist than dry habitats (Daniëls et al., 2013; Jia et al., 2009; Myers-Smith et al., 2015). Even if some warming experiments suggest that plant communities might be resilient to moderate temperature increase over time in Canada (Hudson & Henry, 2010), it is likely that once the expansion of the boreal forest becomes substantial, it will accelerate both warming and the replacement of the tundra because of higher absorption of solar radiation by the vegetation (Myers-Smith et al., 2020).

The increase in air temperature has accelerated the melting of the ice and snow covers of the Arctic region. For instance, the winter sea ice maximum was the lowest recorded in 2015, 2016, 2017, and 2018 since 1979 (Arctic Monitoring and Assessment Programme, 2019). Many projections suggest that the Arctic Ocean will be ice-free during the summer within 30 to 40 years (Arctic Monitoring and Assessment Programme, 2017, 2019; IPCC, 2019). Thawing permafrost is another consequence of rapid warming in the Arctic. Permafrost, defined as the permanently frozen layer of soil in the Arctic, is a reservoir of methane and carbon dioxide (McGuire et al., 2009), and the release of gas by the thawing permafrost will initiate positive feedback on temperature (Arctic Climate Impact Assessment, 2005; McGuire et al., 2009). The presence of permafrost also limits plant growth by lowering the soil temperature and limiting the active layer available for roots to grow. Thawing of permafrost may thus contribute to the "greening" of the tundra. It is also likely to lead to a drier tundra due to drainage modifications even if precipitations are expected to increase in the future (Arctic Climate Impact Assessment, 2005).

Considering the speed at which the distribution of boreal vascular plants expands into the tundra, recent projections suggest that half of the actual tundra will be replaced by shrubs and trees from the south by the end of our century (Myers-Smith et al., 2020; Post et al., 2019). However, pest insects, grazing by herbivores, increasing periods of summer drought and increasing frequency of forest fires might slow the establishment of woody plants in the Arctic (Barrio et al., 2017;

Hagen, Jepsen, Ims, & Yoccoz, 2007; Lund et al., 2017; Myers-Smith et al., 2020). In the future, the tundra is projected to cover smaller areas, be drier, and be covered by a greater diversity of plants and shrubs (Post et al., 2019).

1.3 Impacts of climate change on global and Arctic biodiversity

1.3.1 Diversity and composition

Globally, climate change has already had profound effects on biodiversity (Butchart et al., 2010; Dornelas et al., 2014) and, due to its pervasive and complex impacts on ecosystems, it is regarded as the most severe threat to biodiversity in the Arctic (CAFF, 2013). However, all species are not equally affected by those rapid environmental changes (Davey, Devictor, Jonzén, Lindström, & Smith, 2013; Moritz & Agudo, 2013). Rare species may go locally extinct, while common ones often take advantage of warmer conditions by expanding their distributional ranges (Davey et al., 2013; Dornelas et al., 2014; Parmesan, 2006). Even though 18-37% of terrestrial species are expected to go extinct by 2050 (C. D. Thomas et al., 2004), several meta-analyses have demonstrated that local diversity, calculated as a number of species per unit area, is rarely altered by significant and rapid environmental changes (Dornelas et al., 2014; Vellend et al., 2013; Vellend et al., 2017). Species composition, however, has been changing through time as a result of species-specific responses to climate change (Davey et al., 2013; Dornelas et al., 2014) because the distributional expansion of widespread species can lead to biotic homogenization of communities at large spatial scales (Davey et al., 2013; Eskildsen et al., 2015; Jurasinski & Kreyling, 2007; Savage & Vellend, 2015).

In the Arctic, the environment is so extreme that abiotic constraints are the major drivers of biodiversity patterns (Culp, Lento, Curry, Luiker, & Halliwell, 2019; Danks, 1981). The region is home to more than 21,000 cold-adapted species and is expected to be colonized by competing species from southern areas in a warming future (Post et al., 2019). This movement of species may not change richness that much if northern species go locally extinct but it would have an impact on species composition. Recent research at Zackenberg, a High-Arctic site in Greenland, found that the community composition of arthropods has changed between 1996 and 2014. This change in composition did not result from the addition of southern or invasive species but from changes in the relative abundance of taxa, with herbivores and parasitoids being more abundant with warmer summers and detritivores being less numerous over time (Koltz, Schmidt, & Høye, 2018).

1.3.2 Phenology

One of the most documented ecological consequences of climate change is the modification of phenology, i.e., the timing of biological events (Brown et al., 2016; Høye, Post, Meltofte, Schmidt, & Forchhammer, 2007; Ovaskainen et al., 2013; Parmesan, 2006, 2007; Root et al., 2003; Walther et al., 2002). In the global meta-analysis realized by Parmesan and Yohe (2003), change in spring phenology was quantitatively assessed for more than 677 species of trees, birds, frogs, and butterflies for which data was available over a time period range of 16-132 years. They found that spring phenology has advanced by 3.7 days per decade for butterflies and birds, 7.6 days per decade for amphibians, and 3.3 days per decade for trees (Parmesan, 2007; Parmesan & Yohe, 2003). However, more recent meta-analyses demonstrated that insects were more sensitive to changes in temperature than birds, amphibians or mammals, and had greater phenological shifts (Cohen et al., 2018; Thackeray et al., 2016). In the highly seasonal environments of the

Arctic, the active season is short, and the timing of ecological life cycle events such as flowering or insect activity with appropriate environmental conditions is critical for population persistence. Climate warming has induced earlier spring phenology in Arctic plants, invertebrates, birds, and mammals (CAFF, 2013; Høye et al., 2007; Post & Forchhammer, 2008). Høye et al. (2007) have documented an average advancement of spring phenology of Arctic species (across bird, plant and arthropod taxa) of 14.5 days between 1996 and 2005, which was much higher than phenological shifts observed at lower latitudes. In Finland, phenological shifts of northern species of butterflies were greater than those of species and populations at southern latitudes because changes in climate were more pronounced at high latitudes (Valtonen et al., 2014).

The timing of fall biological events such as leaf senescence and diapause induction has been delayed in many groups such as deciduous trees (Gill et al., 2015) and pest insects (Stoeckli et al., 2012). However, some taxa have seen advancements such as long-distance migrant birds in Western-Europe migrating earlier in the fall in recent years, probably because of the challenging cross of the desert during the dry period (Gallinat, Primack, & Wagner, 2015; Jenni & Kéry, 2003). Because of earlier spring and delayed fall phenology, the growing season of plants in Europe and North-America has substantially extended over time (Chmielewski & Rötzer, 2001; Linderholm, 2006; Menzel et al., 2006). In the Arctic, the analysis of satellite images has confirmed that the growing season of plants across the whole Arctic region has extended too (Zeng, Jia, & Epstein, 2011). Nevertheless, the flowering season of Arctic plants has shortened (Prevéy et al., 2019), limiting the temporal window during which pollinators have access to their flower resources. As for plants, insects in temperate regions are taking advantage of the increased ambient temperature by expanding their active season with an earlier emergence at the beginning of the season and an extended activity late in the fall, with the addition of generations in some cases (Bell et al., 2015; Gallinat et al., 2015; Hassall, Thompson, French, & Harvey, 2007; Stoeckli et al., 2012). However, some exceptions exist such as some butterfly species from Massachusetts have reduced their period of activity in the fall between 1993 and 2014 (Zipf, Williams, Primack, & Stichter, 2017), or in Manitoba, where most butterfly species have not advanced their spring activity but have prolonged their flight period in the fall (Westwood & Blair, 2010).

Regardless of general trends, the phenological responses of different species remain far from uniform as the direction and magnitude of phenological changes over time vary greatly by traits such as dispersal ability and body size (Altermatt, 2010; S. J. Brooks et al., 2017; Gillespie, Birkemoe, & Sverdrup-Thygeson, 2017; Kharouba, Paquette, Kerr, & Vellend, 2014), trophic level (Thackeray et al., 2016), and by populations within species (Høye et al., 2007; Ovaskainen et al., 2013; Thackeray et al., 2016). For instance, in Norway, species of beetles with small body sizes have extended their period of activity in warmer years between 2001 and 2013 while the opposite was observed for large species (Gillespie et al., 2017). Differential responses between species can also be explained by species-specific sensitivities to temperature (Kharouba et al., 2014; Thackeray et al., 2016). Important spring phenological shifts have been recorded for butterfly and bee species with early emergence in the season compared to species emerging later (Bartomeus et al., 2011; S. J. Brooks et al., 2017; Kharouba et al., 2014). This pattern has also been reported for plants (Miller-Rushing & Primack, 2008), even at high latitudes (Lessard-Therrien, Davies, & Bolmgren, 2014), suggesting that early-active species are more tightly related to the same environmental cues than late-active species, and thus, respond more readily to spring climate change (Lessard-Therrien et al., 2014). Ecological (i.e., temperature sensitivity, habitat and trophic specialization) and life-history traits (i.e., body size) are therefore generally good predictors of species responses to climate change.

Differential phenological shifts can lead to a temporal mismatch between interacting species including the disruption of predator-prey systems (Plard et al., 2014; Post & Forchhammer, 2008; Renner & Zohner, 2018) or the mismatch of mutualist species in a plant-pollinator network (Forrest, 2016; Forrest & Thomson, 2011; Hegland, Nielsen, Lázaro, Bjerknes, & Totland, 2009; Memmott, Craze, Waser, & Price, 2007). For example, in France, the timing of roe deer births did not track changes in the timing of high-quality spring vegetation, which caused the decline in population fitness over time (Plard et al., 2014). In the Arctic, when the phenological mismatch between plants and the hatching date of goslings was high (>9 days), goslings had lower body mass (Doiron, Gauthier, & Lévesque, 2015). Phenological shifts of insects have the potential to influence many other organisms, including mammals, birds, and other insects, since they play several important roles in ecological networks (Franke, Lamarre, & Hedlin, 2016; Gaston & Elliott, 2013). For example, the earlier emergence of endoparasitic oestrid flies in the Arctic could affect caribou feeding behavior by increasing harassment (Witter, Johnson, Croft, Gunn, & Gillingham, 2012). Furthermore, Arctic mosquitoes in ponds could increase their population size when the temperature is higher because aquatic larvae develop faster, and adults emerge before the active period of their beetle predators (Culler, Ayres, & Virginia, 2015). Despite many reports of earlier spring activity of insects in the Arctic (Høye et al., 2007; Ovaskainen et al., 2013; Tulp & Schekkerman, 2008; Valtonen et al., 2014), studies on insect phenological changes over time have neglected to document species level phenological shifts, and how the end of the active period in the fall has been affected (but see Høye et al. (2015)) which prevents the documentation and prediction of potential phenological mismatches between interacting species in the Arctic.

1.3.3 Development and life cycle

Recent climate warming has affected not only the diversity, community composition and phenology, but also the growth and development of organisms (Sheridan & Bickford, 2011; Van Dyck, Bonte, Puls, Gotthard, & Maes, 2015). On one hand, warmer temperatures accelerate the development of organisms, especially insects and other ectotherms, leading to smaller individuals (Bestion, Teyssier, Richard, Clobert, & Cote, 2015; Buckley, Arakaki, Cannistra, Kharouba, & Kingsolver, 2017; Kingsolver et al., 2011). Body size, one of the most studied life-history traits, is related to fitness, survival, dispersal, geographic range size, and thermoregulatory capacities of species (Bishop & Armbruster, 1999; Gardner, Peters, Kearney, Joseph, & Heinsohn, 2011; Scheffers et al., 2016; Zeuss, Brunzel, & Brandl, 2017). A reduction of body size over time is expected to result in lower individual fitness (Bennett, Severns, Parmesan, & Singer, 2015) and reduced ability of dispersal, even at high latitudes (Sheridan & Bickford, 2011). At Zackenberg, Greenland, wing length (a proxy of body size) of two species of butterflies got smaller between 1996 and 2013 in response to warmer summer during the larval stages (Bowden et al., 2015). Increased temperature has also influenced the body size of Arctic wolf spiders under experimental conditions. However, the rate of changes in body size was different for males and females, altering the sexual dimorphism, which could have consequences on reproduction and demography (Høye, Hammel, Fuchs, & Toft, 2009). Nonetheless, accelerated development of ectotherms under warmer conditions affects the life cycle and longevity of species. For some multivoltine insect species in temperate regions, the accelerated development of the different life stages has allowed a new generation to be added during the active season (Altermatt, 2009; Roy & Sparks, 2000), thereby affecting the voltinism of these species (Boggs, 2016).

1.3.4 Phenology and population dynamics

While abundance and phenological changes over time are prevalent in the Anthropocene, these responses are rarely considered together, even though one can influence the other. As mentioned previously, temporal mismatches induced by differential phenological shifts in interacting species have the potential to reduce fitness and affect population size (reviewed by Kharouba et al. (2018)). Phenological shifts can also directly affect abundance by altering population rates such as survival and reproduction (Bestion et al., 2015; CaraDonna, Cunningham, & Iler, 2018; Lane, Kruuk, Charmantier, Murie, & Dobson, 2012). For example, demographic models of the mustard white butterfly (*Pieris oleracea* Harris), which is common and widespread in North-America, spanning from New England up to the limit of the Canadian Arctic region, showed that earlier spring activity would lead to higher population growth rate in the southern range of the species but not in the northern populations (Kerr et al., 2020). This difference could be explained by the "developmental trap hypothesis", which suggests that the addition of a generation induced by warmer temperature and a faster life-cycle in ectotherms could lead to a local population decline if individuals of this extra generation fail to reach the proper life stage for the winter and die (Van Dyck et al., 2015). Although the influence of phenology on demography has been documented in a number of species (Nielsen, Chen, & Fleischer, 2016; Steinbauer, Kriticos, Lukacs, & Clarke, 2004), there is still much to learn about how both could be tied (Kerr et al., 2020; Miller-Rushing, Høye, Inouye, & Post, 2010; Van Dyck et al., 2015).

1.4 Long term monitoring and the special case of Zackenberg

The effects of climate change on Arctic species will generally take place over multiple generations. Ecological responses of species to climate change will then occur along temporal scales greater than a classic 3-year graduate research project (see Savage and Vellend (2015)). It is thus necessary to investigate ecological dynamics at an appropriate temporal scale to avoid over- or under-estimating the impacts of rapid climate change on ecosystems. To study the effects of climate change on biodiversity, "space for time" analyses have proven to be of limited value due to the interplay of multiple environmental factors (Krebs & Berteaux, 2006). Moreover, there can be a delay between environmental changes and biotic responses (Savage & Vellend, 2015). In this context, long-term environmental monitoring, defined as the process of gathering information about state variables (e.g., animal abundance, forest cover, water pH), play a critical role in understanding ecological responses of biodiversity to climate change (Arctic Climate Impact Assessment, 2005; Gillespie, Alfredsson, Barrio, Bowden, Convey, Coulson, et al., 2020). Datasets from long-term environmental monitoring allow for teasing apart the "baseline" variability of ecological and environmental dynamics from the "unusual" variability due to recent climate change. Insects are not considered in most biodiversity monitoring programs, even though they are easily sampled in space and time (Collen et al., 2012). Long-term, robust, and repeatable data about their diversity and community structure are rare (Sikes et al. (2017) but see Ives, Einarsson, Jansen, and Gardarsson (2008) or Hodkinson et al. (1998) for exceptions). More than two hundred programs of biodiversity monitoring are active in the Arctic, and an international effort toward standardized monitoring across all sites that include monitoring of invertebrates has been proposed to produce coherent and comparable datasets across the Arctic (Petersen, Zockler, & Gunnarsdottir, 2004). The proposed design and protocol for invertebrates has been greatly inspired by the work accomplished at Zackenberg in Greenland since 1996.

The ZERO (Zackenberg Ecological Research Operations) program was initiated in 1995 by the Danish Polar Center to provide long-term data for a whole High Arctic ecosystem. The Zackenberg study area (74°28’12’’N, 20°34’23’’ W) in North-east Greenland, was selected because it is situated at the transition between the relatively rich southern area of the High Arctic and the arid northern part, making species diversity relatively high for this latitude (Meltofte & Thing, 1997). The ZERO program is unique as it is the most extensive and comprehensive monitoring program across the whole Arctic region with several complementary monitoring programs to record climatic (ClimateBasis and GlacioBasis), abiotic (GeoBasis) and biotic parameters (BioBasis). As part of the BioBasis, insects and other terrestrial invertebrates are collected weekly during the summer, from snowmelt to snowfall, in multiple habitats using several types of traps, since 1996 (Schmidt, Hansen, Hansen, Berg, & Meltofte, 2016). Invertebrates are kept in ethanol and identified to the family level, providing valuable data on temporal variation of abundance of more than 15 families of invertebrates in three habitat types since 1996 (Gillespie, Alfredsson, Barrio, Bowden, Convey, Culler, et al., 2020). Since the establishment of the program in 1995, the Zackenberg area underwent important climatic changes including a significant increase in mean spring, summer and fall temperatures (Koltz et al., 2018; Loboda et al., 2018), a reduction in the number of freeze-thaw events during winter (Koltz et al., 2018), and the advancement of the timing of snowmelt (Høye et al., 2013).

1.5 Diptera and Muscidae

Research on the effects of climate change on Arctic insects would benefit from an extensive and comprehensive exploration of the ecological responses of an abundant and diverse taxon to recent environmental changes. Diptera (true flies) is one of the four megadiverse orders of insects, representing 15-20% of all animal species (Marshall, 2006), and their representation and dominance increase at high latitudes with 55% of Arctic animal species belonging to the group (Danks, 1981). Abundant families of flies represented in the Arctic include the non-biting midges (Chironomidae), Culicidae (mosquitoes), and Tipulidae (cranes flies), three families with mostly aquatic and semi-aquatic larval stages, as well as dance flies and relatives (superfamily Empidoidea) and finally, the Muscidae (houseflies and relatives) and Anthomyiidae (root-maggot flies and relatives). Muscidae is a diverse cosmopolitan fly family with more than 140 species recorded in the Arctic ecosystems of Canada and Alaska, accounting for 10% of the Arctic insect diversity (Danks, 1981). Muscids are mostly saprophagous, predaceous, coprophagous, or anthophilous, depending on life stage, and can be found in a wide variety of habitats in the Arctic (Michelsen, 2015; Renaud, Savage, & Roughley, 2012). In Arctic ecosystems, the high abundance of muscid flies and their relatively big body size compared to aquatic flies make them one of the top families of terrestrial invertebrates in terms of biomass (Bolduc, Casajus, Legagneux, McKinnon, Gilchrist, Leung, et al., 2013). Therefore changes in muscid abundance and phenology could have a great impact on prey availability for Arctic birds and other predators. Early work on muscid flies had already established that they are important flower visitors in the Arctic as most adults will feed on nectar and occasionally pollen as well (Elberling & Olesen, 1999; Kevan, 1972; Pont, 1993).

The potential effects of climate change on species interaction has resulted in many studies on pollination and thus, on muscid flies (Høye et al., 2013; Larson, Kevan, & Inouye, 2001; Olesen, Bascompte, Elberling, & Jordano, 2008; Rasmussen, Dupont, Mosbacher, Trøjelsgaard, &

Olesen, 2013; Schmidt, Mosbacher, et al., 2016; Tiusanen et al., 2016). At Zackenberg, the decline in flower visitor abundance, including Muscidae, between 1996 and 2009, has been found in parallel to a temporal mismatches between their activity and flowering (Høye et al., 2013; Schmidt, Mosbacher, et al., 2016). The authors have suggested that decline of pollinators might be a consequence of food limitation through phenological mismatch (Høye et al., 2013; Schmidt, Mosbacher, et al., 2016). Yet, the decline in muscid flies could affect pollination as seed production of flowering plants has been linked to the presence of these flies at Zackenberg, and of one species in particular, *Spilogona sanctipauli* (Tiusanen et al., 2016). Not all muscid species visit flowers regularly so the impact of muscid decline of pollination requires a study of abundance and phenological change at the species-level.

Advanced spring activity of muscid flies, documented at Zackenberg between 1996 and 2005 at the family level (Høye et al., 2007), varied by habitat type, with values between -11.36 and -35.05 days per decade (Høye et al., 2007). This result highlights the importance of documenting ecological responses in multiple habitats when possible. This type of data offers an exceptional opportunity to investigate climate factors and traits influencing insect population sizes over time, species phenological shifts, and the potential relationship between these ecological responses.

Linking statement

The literature review in Chapter 1 has provided the context of this thesis and the specific research objectives that will be described in the next three chapters. As mentioned in Chapter 1, a decline in muscid abundance at Zackenberg has been previously documented at the family level, but it was unknown whether this decline reflected or hid patterns at the species-level. In Chapter 2, I thus estimated temporal changes in diversity, species relative abundance, and composition in three assemblages of muscid flies from different habitats in the Zackenberg Valley, between 1996 and 2014.

Chapter 2 Declining diversity and abundance of High Arctic fly assemblages over two decades of rapid climate warming

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2.1 Abstract

Insects are particularly vulnerable to rapid environmental changes, which are disproportionally affecting high latitudes. Increased temperature could influence insect species differentially and reshape assemblages over time. We quantified temporal assemblage turnover of Arctic Diptera (flies) in the Muscidae, one of the most diverse and abundant families of Arctic insects, using time series data from Zackenberg, North-East Greenland. We measured temporal patterns of abundance, diversity, and composition of muscid assemblages in wet fen, mesic and arid heath habitats from yearly collections spanning 1996-2014 and tested their relationship to climate. A total of 18,385 individuals representing 16 species of muscid flies were identified. A significant decrease of 80% of total muscid abundance was observed during the study period. Species richness declined in each habitat type but this trend was not significant across habitats. The number of common and abundant species also decreased significantly over time across habitats revealing a temporal modification of species evenness. Significant temporal changes in composition observed in the wet fen and across habitats were mainly driven by a change in relative abundance of certain species rather than by species replacement. Shift in composition in each habitat and decline in muscid abundance across habitats were associated with summer temperature, which has significantly increased over the study period. However, relationships between temperature and muscid abundance at the species level were noticeable for a few species only. Significant directional change in composition was documented in the wet fen but no biotic

homogenization across habitats was observed. As one of the few studies of species-level changes in abundance, diversity and composition of an insect taxon in the Arctic over the past two decades, our study shows that habitat types may modulate insect species responses to recent climate change and that contrasting species responses can alter species assemblages within a few decades.

2.2 Introduction

As atmospheric and oceanic temperatures increase, effects of climate change on natural systems are pronounced and important (Butchart et al., 2010; Dornelas et al., 2014; IPCC, 2014). However, species are not equally affected by those rapid and intense environmental changes (Davey, Devictor, Jonzén, Lindström, & Smith, 2013; Moritz & Agudo, 2013). Rare species may go locally extinct while common ones often expand their ranges (Davey et al., 2013; Parmesan & Yohe, 2003). Species-specific responses to environmental change have led to contrasting responses at the community level, and ecological traits could influence how species will respond to climate change. For example, differential habitat specialization of species at local scale can lead to a biotic homogenization of communities at large spatial scales due to extended distribution of widespread species (Davey et al., 2013; Eskildsen et al., 2015; Jurasinski & Kreyling, 2007; Savage & Vellend, 2015). Other ecological traits influencing the sensitivity of species under important environmental changes include life-history, functional and dispersal traits (Lavergne, Mouquet, Thuiller, & Ronce, 2010).

Despite increased research efforts dedicated to studying the effects of climate change on biodiversity (Bellard, Bertelsmeier, Leadley, Thuiller, & Courchamp, 2012; Chen, Hill, Ohlemüller, Roy, & Thomas, 2011; Lurgi, López, & Montoya, 2012; Norberg, Urban, Vellend, Klausmeier, & Loeuille, 2012; Parmesan, 2006; Walther et al., 2002), there is still a lack of knowledge about how entire communities may be influenced by climate change. Arthropods (insects and their relatives) offer a unique opportunity to study community structure across different environmental, spatial and temporal gradients since they are abundant in all ecosystems and highly diverse. Arthropods also have short generation time and respond readily to small environmental change, which makes them an ideal group to study biological responses to rapid environmental change at the species-level (Bowden, Eskildsen, et al., 2015; Culler, Ayres, & Virginia, 2015; Høye et al., 2015). However, even if arthropods are easily sampled in space and time, long-term, robust and repeatable data about their diversity and community structure are rare in part due to taxonomic and logistical challenges. In the Arctic, where the impacts of climate changes are especially pronounced (Arctic Climate Impact Assessment, 2005; IPCC, 2014; Post & Forchhammer, 2008) insects represent more than 60% of the described terrestrial animal diversity and up to 80 % in some part of the High Arctic (Hodkinson et al., 2013; Tiusanen, Hebert, Schmidt, & Roslin, 2016; Wirta et al., 2016). Overall species richness is, however, generally reduced compared to lower latitudes, thereby diminishing the taxonomic impediment often encountered in other systems.

Temporal changes in phenology and body size due to climate change have been already observed in Arctic insects and related invertebrates (Bowden, Eskildsen, et al., 2015; Bowden, Hansen, Olsen, & Høye, 2015; Høye et al., 2015; Høye, Hammel, Fuchs, & Toft, 2009). One worrying temporal trend documented at the community-level is a decline in flower visitor abundance in the tundra ecosystem of Zackenberg, in North-East Greenland (Høye et al., 2015; Høye & Forchhammer, 2008; Høye, Schmidt, Trøjelsgaard, & Forchhammer, 2013). Since abundance of common species might be more important than the diversity of species in the community per se in providing ecosystem functions (Winfree, Fox, Williams, Reilly, & Cariveau, 2015), it is essential to investigate abundance trends in relation to climate at the species-level. Specifically, it remains unknown whether the community trend masks contrasting temporal dynamics in the abundance of common and rare species (Supp & Ernest, 2014).

In Arctic environments, true flies (Order Diptera) are the most abundant and diverse group of insects (S. J. Coulson et al., 2014; Hodkinson et al., 2013; Marshall, 2006). The Muscidae (house flies and relatives) are one of the most diverse families of Arctic insects with over 220 described species north of the Arctic Circle in the Nearctic realm (Hodkinson et al., 2013) and 37 species known from Greenland (Michelsen, 2015). Northern muscids are generally predators of other flies and the adults of many species visit flowers to obtain nectar and/or pollen (Elberling & Olesen, 1999; Hocking, 1968; Kevan, 1972; Michelsen, 2015; Philipp, 1990; Rasmussen, Dupont, Mosbacher, Trøjelsgaard, & Olesen, 2013; Tiusanen et al., 2016). Using one of the longest time series data sets on Arctic insect biodiversity, the Greenland Ecosystem Monitoring programme initiated in 1996 at Zackenberg in North-East Greenland, we documented interannual patterns and long-term trends of abundance, diversity and composition of muscid fly assemblages at the species-level, in three habitats, between 1996 and 2014. Furthermore, we examined whether assemblage similarity between habitats changed over time and whether it resulted in biotic homogenization of the muscid assemblage across habitats. Finally, we assessed the relationship between assemblage changes and two abiotic variables: air temperature and timing of snowmelt.

Given the decline in overall abundance of the Muscidae already documented at Zackenberg (Høye et al., 2013), we predicted that diversity (richness and evenness) and composition of the species assemblages of muscid flies in Zackenberg changed through time. However, since the tundra is highly heterogeneous and some habitats are affected differently by environmental changes in the Zackenberg valley (Koltz, 2015; Schmidt, Kristensen, Michelsen, & Bay, 2012), we hypothesized that temporal changes in diversity and composition of muscid flies would differ among habitats and that the greatest temporal changes in diversity, abundance and composition would occur in the wet fen, where significant changes in vegetation composition have been observed during the study period (Schmidt et al., 2012).

2.3 Materials and methods

2.3.1 Study area and data collection

As part of the Greenland Ecosystem Monitoring BioBasis programme (Schmidt, Hansen, Hansen, Berg, & Meltofte, 2016), arthropods and climate data were collected at Zackenberg, North-East Greenland (74°28’N, 20°34’W), since 1996. Each summer, muscid flies were sampled in three different habitats; an arid heath, a mesic heath, and a wet fen (Figure 2-1), and collected using two yellow pitfall traps per habitat. Each trap measured 10 cm in diameter and was colored bright yellow to attract flower visitors. Because of the low vegetation at the sample sites (typical height of 5-10cm), previous work (Bowden, Eskildsen, et al., 2015; Høye et al., 2015; Meltofte & Thing, 1997) has demonstrated that yellow pitfall traps function like pan traps and catch large numbers of flying insects. Traps were emptied every week during the growing season. The sampling covers the entire active season of muscid flies at Zackenberg which generally extends between early June (day of year 161) and end of August (day of year 244) for a total of 12 weeks of activity. Unfortunately, specimens collected in 2010 were lost during transport before sorting.

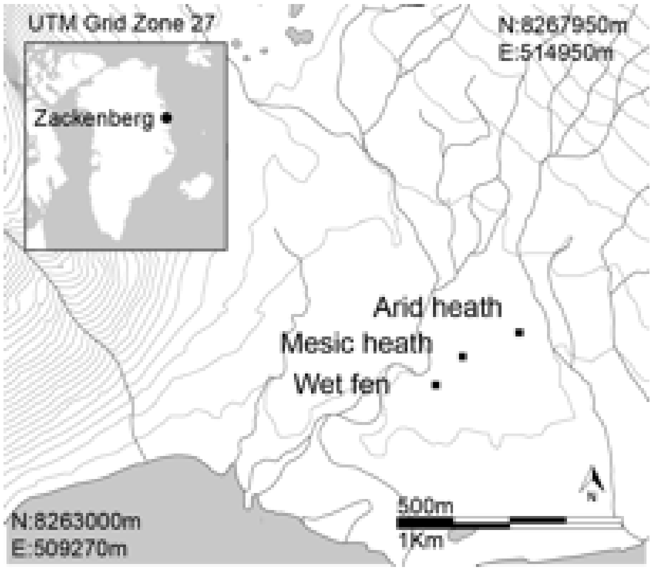

Figure 2-1 Map of the Zackenberg valley with location of the three habitats (wet fen, mesic heath, and arid heath).

Morphological identification of adult muscid flies was done using Collin (1930), Huckett (1965), and Michelsen (2006, 2015) and the identity of all species was corroborated with DNA barcoding (Folmer region of COI). DNA was extracted from 61 specimens and successfully sequenced for 58 of these (most species were represented by at least two specimens and both sexes). One representative sequence for each species encountered in this study can be retrieved from BOLD (www.barcodinglife.org) in the public dataset: Muscidae of Zackenberg (dx.doi.org/10.5883/DS-MOZK).

Soil moisture is an important environmental factor for muscid development, especially in the larval stages (Michelsen, 2015; Skidmore, 1985), therefore, the three habitats were selected along a soil moisture gradient to study different muscid assemblages (Figure 2-1). In addition to variation in soil moisture, these sites have different snowmelt timing and vegetation composition (Høye & Forchhammer, 2008). The wet fen is dominated by mosses and grasses. Both heath habitats have a well-developed layer of lichens and differ in their flowering plants; *Cassiope tetragona* is widespread in the mesic heath while *Dryas* spp. is dominant in the arid heath.

2.3.2 Data analyses

Analyses described below were conducted separately for habitat-specific ensembles and for the whole assemblage pooling all habitats (defined hereafter as “across habitats”). All statistical analyses were carried out in the R 3.2.0 platform (R Development Core Team, 2016).

2.3.2.1 Climate

Temperature and timing of snowmelt determine the phenology of many terrestrial arthropods in the Arctic (Høye & Forchhammer, 2008; Tulp & Schekkerman, 2008). We used simple linear regression to examine trends in habitat-specific timing of snowmelt which influence season length, as well as trends in average summer, spring, winter and fall temperatures over the study period (respectively average in July-August, May-June, January-February temperature and average temperature of October-November of the previous year).

2.3.2.2 Abundance

To assess whether the decrease in muscid abundance previously observed at Zackenberg over time (Høye et al., 2013) was habitat-specific and/or driven by one or a subset of species across habitats, we explored long-term trends in abundance for all species pooled and separately for dominant species (species making up at least 15% of the total abundance). Temporal trends in muscid abundance were assessed with simple linear models for each assemblage with year as independent variable. When first order temporal autocorrelation was significant according to the Ljung-Box test, temporal trends in muscid abundance were adjusted with the Cochrane-Orcutt estimation (Spada, Quartagno, Tamburini, & Robinson, 2018). In 1996, arthropods were sampled with four yellow pitfall traps in each habitat and samples from the four traps were pooled for each sampling period (each week). Because of the different sampling methods used in 1996, we divided abundance of muscids in 1996 by two to compare with annual abundances from other years based on two pitfall traps.

2.3.2.3 Diversity

In order to compare muscid diversity between years with different abundance, diversity metrics were calculated using individual-based rarefaction and extrapolation curves for each year. Diversity metrics were plotted against annual number of individuals and extrapolated to one common sample size (highest annual abundance of muscid flies of each assemblage) using the iNEXT package (Chao et al., 2014; Hsieh, Ma, & Chao, 2014). Species diversity changes between 1996 and 2014 were assessed using temporal variations of three diversity metrics of the Hill family that incorporate both relative abundance and species richness: ${}^{0}D$, ${}^{1}D$ and ${}^{2}D$ (Chao et al., 2014; Gotelli & Chao, 2013). One advantage of these diversity metrics is the unit of measurement. Each Hill number ${}^{q}D$ calculates diversity in units of effective number of species and differ from others only by an exponent q, which controls for the sensitivity of the index to species relative abundance (Hill 1973). When q=0, relative abundance is not taken into account and ${}^{0}D$ equals species richness of the assemblage. The Hill number ${}^{1}D$ corresponds to the number of “typical” or “common” species in the assemblage while ${}^{2}D$ represents the number of abundant species in the assemblage (Chao et al., 2014; Gotelli & Chao, 2013). Linear models were used to identify significant trends in diversity change (${}^{0}D$, ${}^{1}D$ and ${}^{2}D$) between 1996 and 2014 and the Cochrane-Orcutt estimation was used to correct for autocorrelation when detected by the Ljung-Box test.

2.3.2.4 Composition

To explore patterns and shifts in community composition over time, the similarity of muscid assemblages across years was assessed with non-metric multidimensional scaling ordinations (NMDS) in two dimensions based on Bray-Curtis dissimilarities, using the function *metaMDS* in the vegan package with default settings (Oksanen et al., 2015). Bray-Curtis dissimilarity distances were calculated using standardized number of individuals per species caught per trap per day. To test for significant shift in species composition similarity over time, we used a permutational MANOVA or PERMANOVA (9999 permutations using the *adonis* function in the vegan package), implemented with permutations of samples that consider the response variables over time as repeated measures (M. Anderson et al., 2008; Oksanen et al., 2015).

To further explore shifts in compositional similarity in each assemblage, a temporal turnover measure named “D”, developed by Shimadzu, Dornelas, and Magurran (2015), was calculated to compare each annual assemblage to the assemblage of 1996. One advantage of this abundance-based measure of temporal turnover is that it teases apart two processes driving compositional turnover by deriving two additive components: the first component D1 is the fraction of the turnover that is due to compositional change, or in other words change in relative abundance distribution within the assemblage, while the second component D2 is the fraction of the turnover due to change in the total size of the assemblage. This measure is intuitive as a decrease in D2, for example, indicates a decrease of the size of the community over the time period. Raw species abundance (standardized annual number of individuals caught in two pitfall traps for each species) was used to calculate the turnover index D and its components D1 and D2 (see Shimadzu et al. (2015) for more details).

Since we investigated changes in compositional similarity over time within habitats, we assessed potential homogenization of the muscid assemblage across habitats by testing how similarity of composition between habitats varied over time. If assemblages of muscid flies in each habitat become increasingly similar over the study period, we can conclude that temporal homogenization has occurred. We performed this analysis with a test of multivariate homogeneity of group dispersions (M. Anderson et al., 2008; M. J. Anderson, Ellingsen, & McArdle, 2006), an analysis performed by the function *betadisper* in the vegan package which is based on Bray-Curtis dissimilarities (Oksanen et al., 2015).

2.3.2.5 Relationship between species responses and environmental changes

To investigate the relationship between muscid assemblage composition and climate (habitat-specific timing of snowmelt and average summer, spring, previous winter and previous fall temperatures), we fitted smoothed surfaces of environmental variables on the NMDS ordinations previously obtained with the *ordisurf* function of the vegan package, which uses generalized additive models. We also examined whether changes in total muscid abundance and species abundance (for the dominant species in each assemblage) are related to variation in temperature or timing of snowmelt. Since year can have a confounding effect when climate and abundance vary through time, we first removed the potential effect of year on climate-abundance correlations by using residuals of climate and abundance variables from simple linear regression with year as the predictor (Iler, Inouye, Schmidt, & Høye, 2017). Abundance residuals were then regressed against climate residuals to determine relationship between climate and total muscid abundance in each assemblage as well as species abundance of the dominant species in each assemblage (species making up at least 15% of the total abundance).

2.4 Results

2.4.1 Climate change at Zackenberg

The date of snowmelt in the wet fen significantly advanced by 6.5 ± 2.9 days per decade between 1996 and 2014 at Zackenberg. A similar, but non-significant, trend was observed in the mesic and arid heath (Figure 2-2a). Average spring and summer temperature have increased significantly by 1.1 ± 0.5 and 1.3 ± 0.6°C per decade during the study period, respectively, while fall and winter temperatures have shown no significant change (Figure 2-2b). Timing of snowmelt were correlated across the three habitats (arid-mesic: r=0.68, $p<0.01$; arid-wet: r=0.60, $p<0.01$; mesic-wet: r=0.77, $p<0.01$, Pearson). Snowmelt was also negatively correlated to spring temperature (r=-0.50, $p=0.03$) and positively correlated to winter temperature (r=0.50, $p=0.04$).

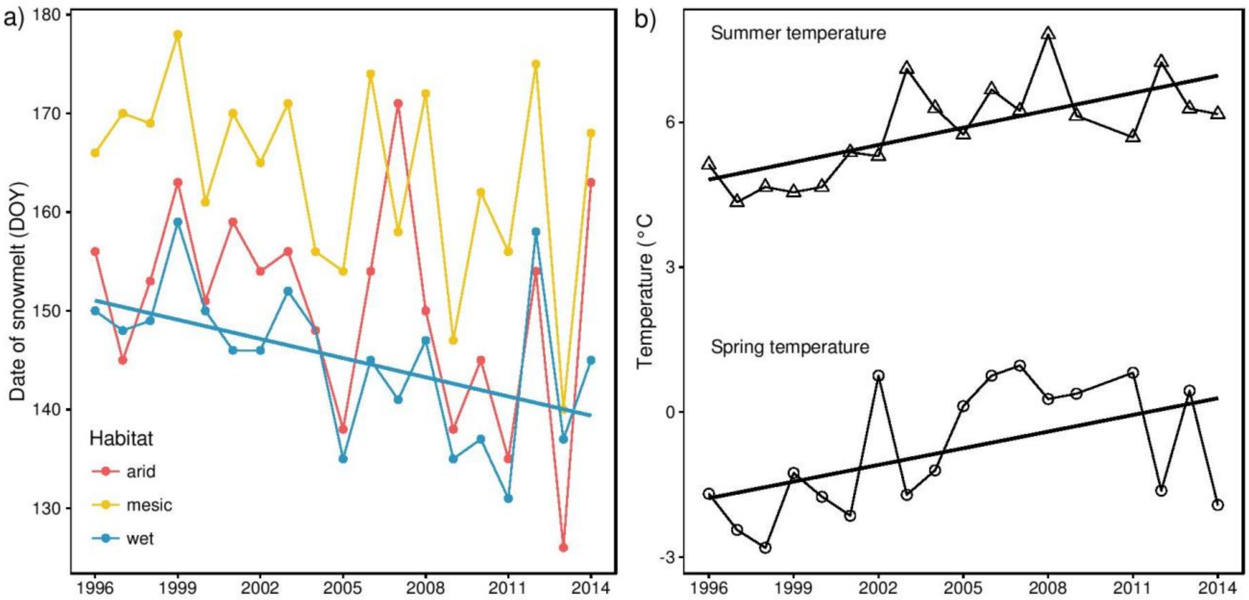

Figure 2-2 Trends in a) timing of snowmelt in the wet (F1,18 =5.05, estimate=-0.65, R^2_{adj}=0.18, p=0.04), the mesic (F1,18 =2.91, estimate=-0.68, R^2_{adj}=0.10, p=0.11) and arid heath (F1,18 =1.79, estimate=-0.60, R^2_{adj} =0.04, p=0.20) and b) average summer temperature (July-August; $F_{1,18}$ =14.0, estimate=0.12, R^2_{adj}=0.43, p=0.002) and spring temperature (May-June; $F_{1,18}$ =5.31, estimate=0.12, R^2_{adj}=0.20, p=0.04) at Zackenberg between 1996 and 2014. Non-significant trends in fall (October-November; $F_{1,17}$ =0.70, estimate=-0.10, R^2_{adj}=-0.02, p=0.42) and winter temperature (January-February; $F_{1,16}$ =0.75, estimate=0.10, R^2_{adj}=-0.02, p=0.40) are not displayed.

2.4.2 General results on muscid assemblages

A total of 18,385 muscid specimens collected between 1996 and 2014 in the three habitats at Zackenberg were identified to the species level. These represented 16 species in 4 genera (*Spilogona* Schnabl, *Drymeia* Meigen, *Limnophora* Robineau-Desvoidy, *Phaonia* Robineau-Desvoidy). Details on the number of individuals caught in each habitat per species are given in Supplementary Table 2-1. Twelve species belonged to *Spilogona*, which was also the most abundant genus, representing 74% of all individuals caught across habitats. *Spilogona sanctipauli* (Malloch) and *Drymeia segnis* (Holmgren) were the two dominant species, representing 33% and 25% of the total number of individuals caught across habitats, respectively (Figure 2-3a); both species were mainly present in the arid and mesic heaths (Figure 2-3b and 2-3c). Overall, most muscid individuals were collected in the wet fen (8083 specimens, 1137 trap days), which also contained the most species (15), compared to 13 in both the mesic (5485 specimens, 1021 trap days) and arid heaths (4817 specimens, 1166 trap days).

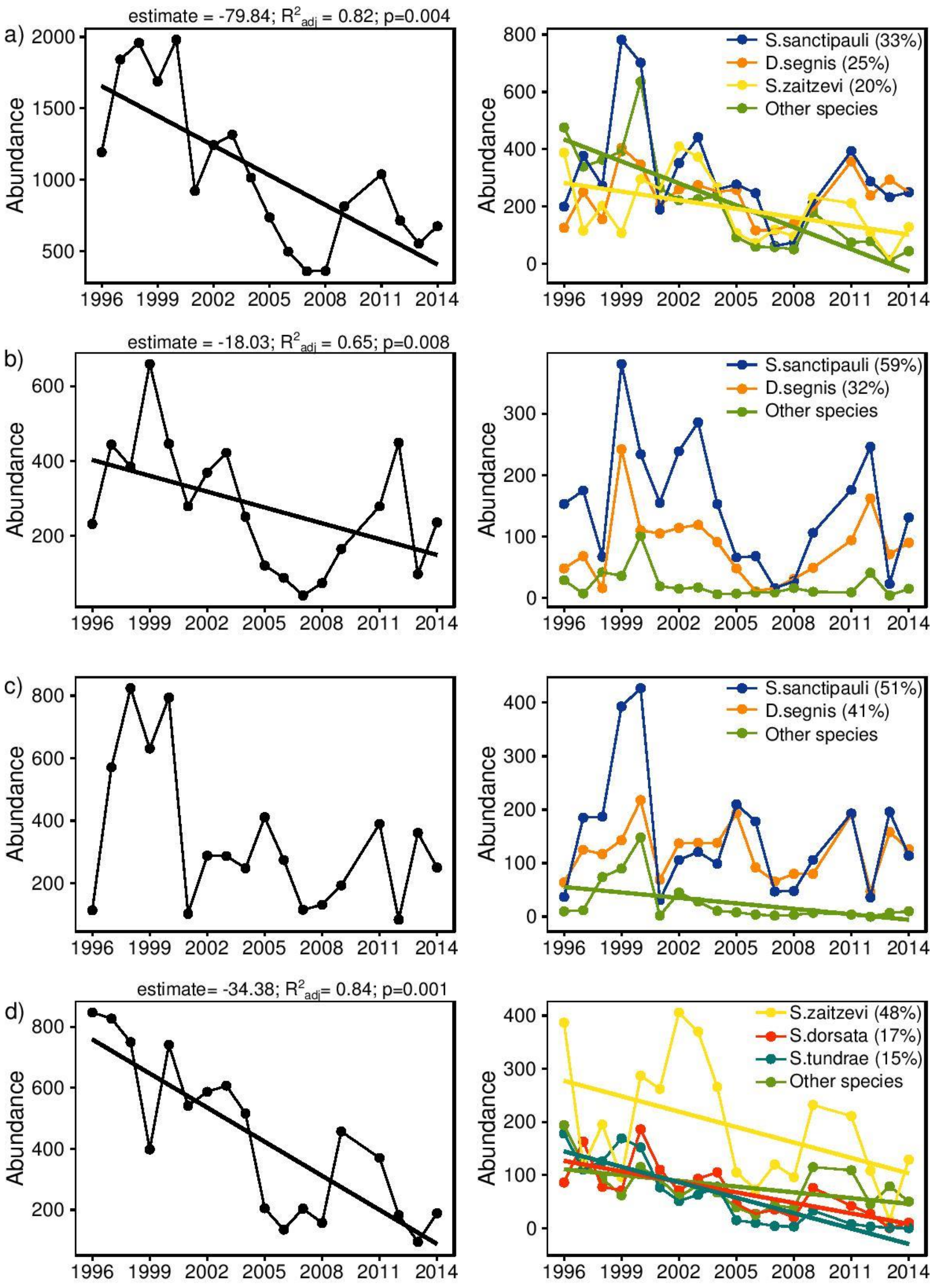

Figure 2-3 Temporal variations of annual muscid abundance (left column) and species-level abundance of common species (right column) at Zackenberg, NE Greenland, between 1996 and 2014 (a) across habitats and within each habitat; arid heath (b), mesic heath (c) and wet fen (d). Percentages in parentheses represent the proportion of individuals of a particular species in the assemblage, from 1996 to 2014. Regression lines based on simple linear regression analysis are given when the temporal trend in abundance in each assemblage is significantly different from

zero. Regressions were corrected for first-order temporal autocorrelation when needed (see Supplementary Table 2-2). Estimates, standard errors and p-values for species-specific trends in abundance are presented for each assemblage in Supplementary Table 2-2.

2.4.3 Temporal patterns of muscid abundance

Significant declines in muscid abundance were found across habitats (Figure 2-3a), in the arid heath (Figure 2-3b) and in the wet fen (Figure 2-3d). The declines in abundance correspond to a loss of 80% of individuals between 1996 and 2014 across habitats, 85% in the wet fen and 71% in the arid heath. Despite some annual variations, the abundance of the most abundant *Spilogona* species in the wet fen (*S.dorsata*, (Zetterstedt), *S.tundrae* (Schnabl) and *S.zaitzevi* (Schnabl)) decreased between 1996 and 2014 (Figure 2-3d), while the abundance of *S. sanctipauli* and *D. segnis* did not show any trend over the study period in the arid heath (Figure 2-3b), mesic heath (Figure 2-3c) or across habitats (Figure 2-3a).

2.4.4 Temporal patterns of muscid diversity

Species richness (Hill number ^{0}D) significantly decreased over time within each habitat (Figure 2-4b, 2-4c, 2-4d) but not across habitats (Figure 2-4a). Numbers of common (Hill number ^{1}D) and abundant species (Hill number ^{2}D) decreased significantly between 1996 and 2014 across habitats (Figure 2-4a). However, changes in diversity ^{1}D and ^{2}D were habitat-specific. Significant decreases in the number of common (Hill number ^{1}D) and abundant (Hill number ^{2}D) species were observed in the wet fen (Figure 2-4d) but not in the arid heath (Figure 2-4b). In the mesic heath, decline in the number of common species (Hill number ^{1}D) was significant (Figure 2-4c).

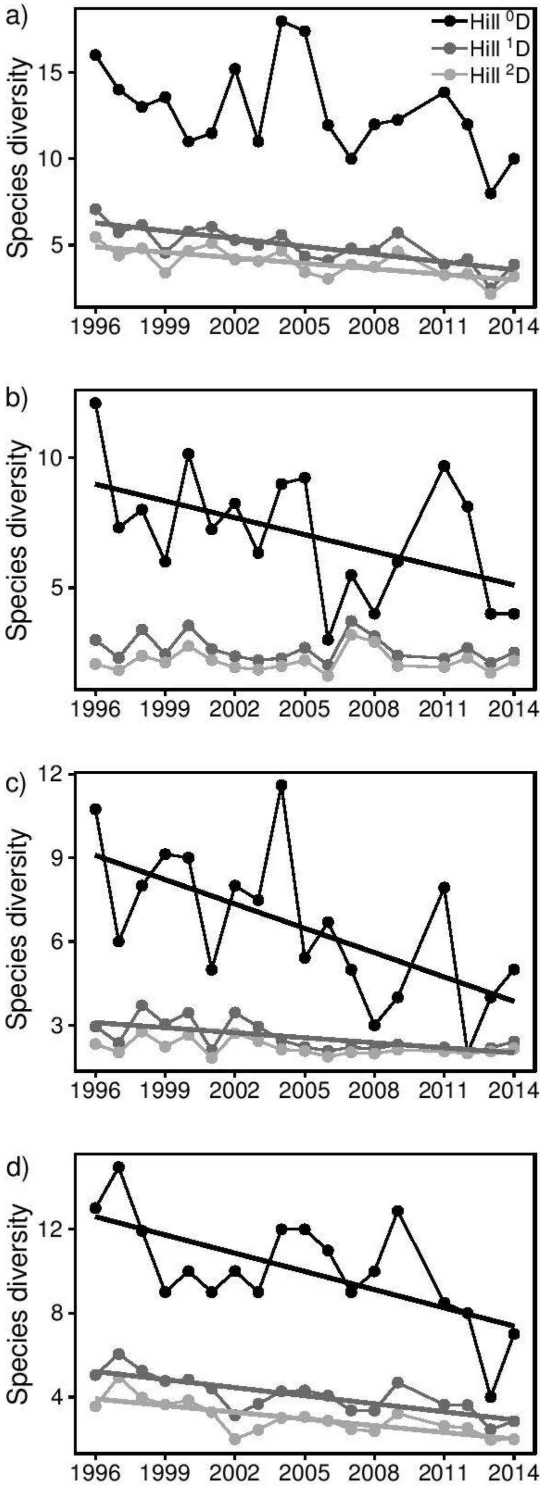

Figure 2-4 Temporal variations of muscid fly diversity at Zackenberg, NE Greenland, between 1996 and 2014. Analyses were conducted separately for each assemblage collected across habitats (a) and in distinct habitat; arid heath (b), mesic heath (c) and wet fen (d). Diversity of each assemblage is described by Hill numbers ^{0}D, ^{1}D and ^{2}D. Linear trends in diversity changes

through time at Zackenberg were assessed with simple linear regression analysis. Slopes, adjusted R^2 values and p-values for diversity trends in each assemblage are presented in Supplementary Table 2-3.

2.4.5 Temporal patterns of muscid assemblage composition

According to the PERMANOVA, changes in composition similarity over time were significant across habitats (R^2=0.37, *p*=0.001, Figure 2-5a) and in the wet fen (R^2=0.42, *p*=0.001, Figure 2-5d), but not in the arid (R^2=0.15, *p*=0.06, Figure 2-5b) or mesic heath (R^2=0.11, *p*=0.17, Figure 2-5c). To understand the respective contribution of change in species composition and change in the size of the assemblage, temporal shifts in composition were assessed using a temporal turnover measure D, additively decomposed in two components, D1 and D2, representing the contribution of each process. Temporal turnover D across habitats (Figure 2-5e) and in the wet fen (Figure 2-5h) decreased over time, indicating a directional change of composition over the study period where the compositions in the recent assemblages were different from those in 1996. Changes in the turnover D are primarily due to change in the total size of the assemblage measured by D2 (Figure 2-5e to 2-5h). In the arid (Figure 2-5f) and mesic heath (Figure 2-5g) habitats, no significant change in composition over time was observed (according to the PERMANOVA tests) and no important cumulative or directional change in composition over time was measured by the turnover D.

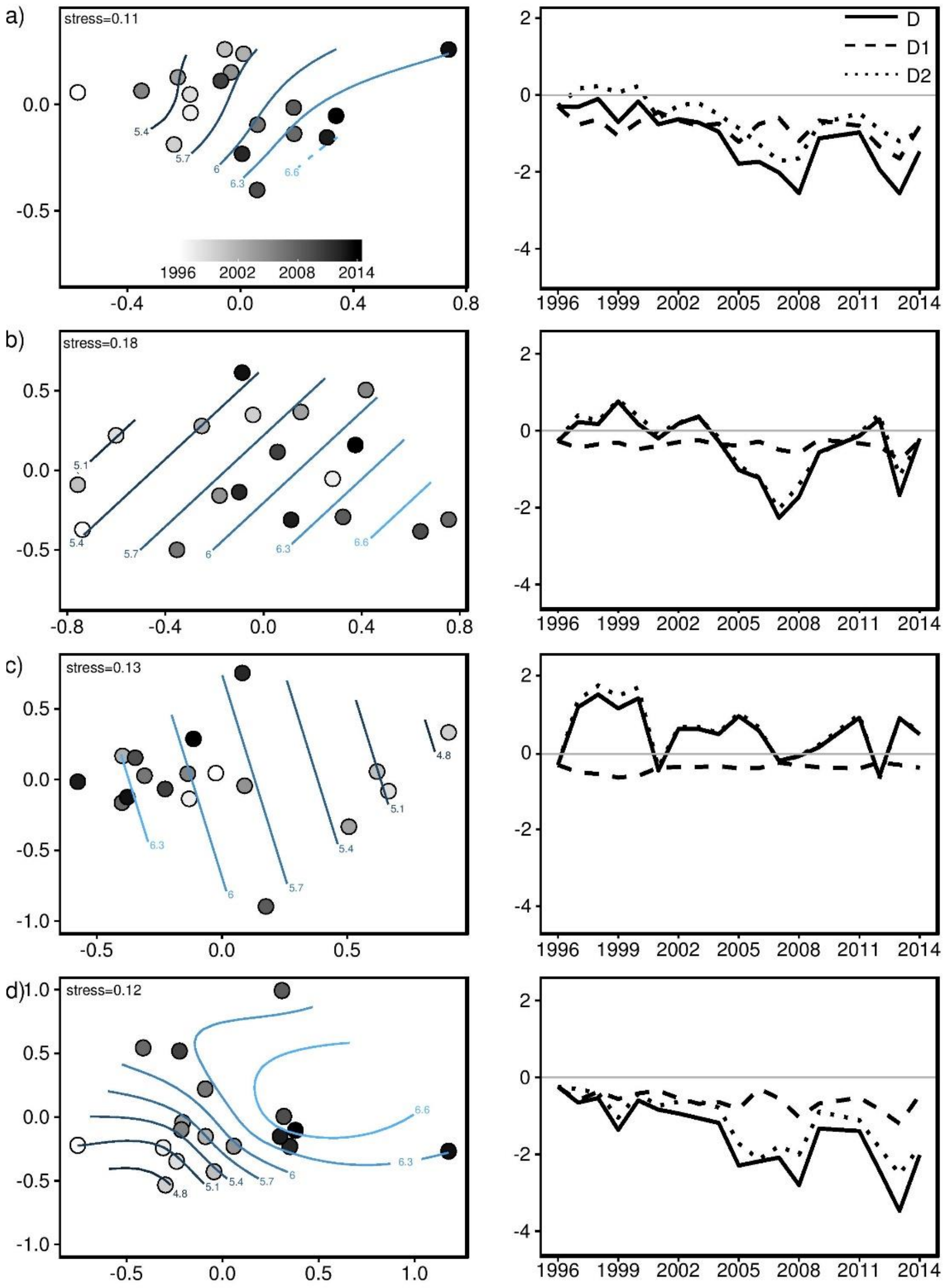

Figure 2-5 Temporal variations of muscid assemblage similarity assessed by 1) non-metric multidimentional scaling (NMDS) ordinations in two dimensions (first column) of the muscid assemblage sampled at Zackenberg, NE Greenland, each year between 1996 and 2014 across habitats (a) and for the arid heath (b), the mesic heath (c) and the wet fen (d) habitats separately and 2) temporal turnover D and its component D1, change in composition and D2, change in assemblage size (second column) across habitats (e), in the arid heath (f), the mesic heath (g) and the wet fen (h). When D, D1 or D2 measure stays around zero over time, this indicates low temporal change in similarity. In the four ordinations, similarity was calculated with the Bray-Curtis dissimilarity measure; each data point is muscid composition at a given year. Habitat-

specific ordinations are overlaid on summer temperature (July-August average) contour lines (value of each contour line is given in °C). In the wet habitat, summer (black contour lines) and spring (grey contour lines) temperature are overlaid as both were significantly related to compositional change. Temperatures were fitted on the ordinations using generalized additive models. Stress values between 0.1 and 0.2 indicate that the structure of the data is well represented in two dimensions.

Temporal changes in composition similarity were habitat-specific, but similarity between habitats did not change over time ($F_{2,51}$= 0.82; p=0.45), indicating that there was no biotic homogenization across habitats of the muscid assemblage during the study period at Zackenberg.

2.4.6 Relationship between species responses and environmental changes

Summer temperature was significantly related to assemblage composition in the arid heath (R^2_{adj} =0.32, p=0.02, Figure 2-5b), in the mesic heath (R^2_{adj} =0.33, p=0.02, Figure 2-5c), and in the wet fen (R^2_{adj} =0.50, p=0.01, Figure 2-5d). Recent assemblages are associated to warmer temperatures. In the wet fen, spring temperature was also related to compositional change over time (R^2_{adj} =0.42, p<0.01). Timing of snowmelt was not related to compositional change over time (across habitats: R^2_{adj} =0.03, p=0.30; arid: R^2_{adj} <0.01, p=0.75; mesic: R^2_{adj} <0.01, p=0.74 ; wet: R^2_{adj} =0.23, p=0.06) neither was winter (across habitats: R^2_{adj} <0.01, p=0.56; arid: R^2_{adj} =0.03, p=0.39 ; mesic: R^2_{adj} <0.01, p=0.71; wet: R^2_{adj} <0.01, p=0.45), fall (across habitats: R^2_{adj} =0.15, p=0.11; arid: R^2_{adj} =0.13, p=0.13 ; mesic: R^2_{adj} <0.01, p=0.53; wet: R^2_{adj} =0.15, p=0.11) and spring temperature (across habitats: R^2_{adj} =0.06, p=0.25; arid: R^2_{adj} =0.09, p=0.18; mesic: R^2_{adj} =0.25, p=0.14).

According to our regressions with detrended variables, variation in muscid fly abundance at Zackenberg was significantly related to temperature in a few cases, but not to timing of snowmelt

(Table 2-1). Total muscid abundance decreased significantly as summer temperature increased across habitats and in the mesic heath. In the arid heath, total muscid abundance was negatively related to spring temperature. However, at the species level, most relationships between abundance variation and climate predictors were not significant (Table 2-1). The number of individuals of *D. segnis* across habitats and *S. sanctipauli* in the mesic heath were negatively related to summer temperature. Furthermore, the abundance of *S. tundrae* in the wet fen was positively related to fall temperature of the previous year.

Table 2-1 Summary of simple linear regression results of muscid abundance (total abundance of the assemblage or species specific abundance) detrended by year (residuals) and detrended climatic variables as predictor to identify drivers of muscid fly assemblage changes at Zackenberg between 1996 and 2014. Summer $temp_t$ = mean July-August temperature, Spring $temp_t$ = mean May-June temperature, Winter $temp_t$ = mean January-February temperature, Fall $temp_{t-1}$ = mean October-November temperature of the previous year. Timing of snowmelt was measured in each habitat and the average timing was used to test for relationship between snowmelt and muscid abundance variations across habitats.

Assemblage	Abundance	Climate variable	Estimate ± Std error	*P* value
Across habitats	all species	Summer $temp_t$	-277.80 ± 103.91	**0.02**
		Spring $temp_t$	-146.08 ± 71.27	0.06
		Winter $temp_t$	-16.90 ± 36.98	0.65
		Fall $temp_{t-1}$	32.74 ± 33.64	0.35
		Snowmelt	-3.53 ± 11.51	0.76
	S.sanctipauli	Summer $temp_t$	-95.33 ± 54.48	0.10
		Spring $temp_t$	-21.82 ± 37.70	0.57
		Winter $temp_t$	-6.55 ± 17.63	0.72
		Fall $temp_{t-1}$	3.68 ± 16.44	0.83
		Snowmelt	2.15 ± 5.47	0.70
	D.segnis	Summer $temp_t$	-58.08 ± 25.33	**0.04**
		Spring $temp_t$	-10.66 ± 18.51	0.57
		Winter $temp_t$	-4.48 ± 8.62	0.61
		Fall $temp_{t-1}$	-0.88 ± 8.08	0.92
		Snowmelt	-1.40 ± 2.67	0.61
	S.zaitzevi	Summer $temp_t$	21.40 ± 34.98	0.55
		Spring $temp_t$	2.19 ± 22.67	0.92
		Winter $temp_t$	11.92 ± 10.11	0.26

		Fall temp$_{t-1}$	13.49 ± 9.20	0.16
		Snowmelt	-0.63 ± 3.27	0.85
Arid	all species	Summer temp$_t$	-60.20 ± 49.39	0.24
		Spring temp$_t$	-64.98 ± 28.82	**0.04**
		Winter temp$_t$	11.11 ± 15.12	0.47
		Fall temp$_{t-1}$	22.94 ± 13.10	0.10
		Snowmelt	1.68 ± 3.49	0.64
	S.sanctipauli	Summer temp$_t$	-14.34 ± 32.26	0.66
		Spring temp$_t$	-22.16 ± 20.05	0.29
		Winter temp$_t$	9.44 ± 9.37	0.33
		Fall temp$_{t-1}$	13.08 ± 8.38	0.14
		Snowmelt	1.70 ± 2.17	0.45
	D.segnis	Summer temp$_t$	-15.23 ± 19.44	0.45
		Spring temp$_t$	-14.45 ± 12.17	0.25
		Winter temp$_t$	5.68 ± 5.73	0.34
		Fall temp$_{t-1}$	7.42 ± 5.16	0.17
		Snowmelt	0.91 ± 1.33	0.50
Mesic	all species	Summer temp$_t$	-164.02 ± 58.52	**0.01**
		Spring temp$_t$	-31.14 ± 45.11	0.51
		Winter temp$_t$	-26.50 ± 20.22	0.21
		Fall temp$_{t-1}$	-13.61 ± 19.50	0.50
		Snowmelt	-3.36 ± 5.39	0.54
	S.sanctipauli	Summer temp$_t$	-78.35 ± 32.17	0.03
		Spring temp$_t$	3.26 ± 24.12	0.89
		Winter temp$_t$	-14.02 ± 10.65	0.21
		Fall temp$_{t-1}$	-9.37 ± 10.17	0.37
		Snowmelt	-1.55 ± 2.85	0.59
	D.segnis	Summer temp$_t$	-31.40 ± 15.01	0.05
		Spring temp$_t$	4.12 ± 10.80	0.71
		Winter temp$_t$	-7.42 ± 4.69	0.13
		Fall temp$_{t-1}$	-8.27 ± 4.21	0.07
		Snowmelt	-2.02 ± 1.19	0.11
Wet	all species	Summer temp$_t$	-50.75 ± 48.32	0.31
		Spring temp$_t$	-49.97 ± 29.47	0.11
		Winter temp$_t$	-1.27 ± 14.88	0.93
		Fall temp$_{t-1}$	23.39 ± 12.54	0.08
		Snowmelt	-2.81 ± 5.30	0.60
	S.zaitzevi	Summer temp$_t$	22.84 ± 35.04	0.52
		Spring temp$_t$	2.42 ± 22.74	0.92
		Winter temp$_t$	11.67 ± 10.16	0.27
		Fall temp$_{t-1}$	13.33 ± 9.25	0.17
		Snowmelt	-1.16 ± 3.79	0.76
	S.dorsata	Summer temp$_t$	-10.84 ± 11.45	0.36
		Spring temp$_t$	-9.00 ± 7.20	0.23
		Winter temp$_t$	-2.86 ± 3.43	0.42
		Fall temp$_{t-1}$	0.62 ± 3.26	0.85

	Snowmelt	-0.28 ± 1.26	0.83
S.tundrae	Summer temp$_t$	-15.94 ± 10.10	0.13
	Spring temp$_t$	-11.66 ± 6.32	0.08
	Winter temp$_t$	0.34 ± 3.23	0.91
	Fall temp$_{t-1}$	6.14 ± 2.59	**0.03**
	Snowmelt	1.50 ± 1.10	0.19

2.5 Discussion

Our study is among the first long-term species-level assessments of interannual variation in Arctic insect communities in the context of climate change. We present strong evidence of temporal shifts in abundance, diversity and composition in muscid fly assemblages between 1996 and 2014 at Zackenberg, North-East Greenland. Our results suggest that change in species composition was mainly attributable to significant decreases in species abundance, with a loss of 80% of muscid individuals across habitats documented between 1996 and 2014 at Zackenberg. The decline in the number of common species across habitats and in the wet fen is a result of the decrease in abundance of some dominant *Spilogona* species. No biotic homogenization across habitats was found during the study period because most species decreased in abundance, especially in the wet fen, and limited replacement or turnover in species composition was recorded. Temporal shifts in composition in each habitat assemblage and declining abundance of some individual species of muscid flies were related to increasing summer temperature.

Declining abundance was a key species-level response in this study as was previously reported for Muscidae (Høye et al., 2013). A major decrease in the number of muscid flies caught annually across all habitats and in the wet fen in particular led to significant shifts in assemblage composition over the study period at Zackenberg, a result that could be detected mainly because of the repeated and standardized collection protocol. Some studies in which species abundance could not be considered due to differences in sampling protocols have failed to find any temporal shifts in Arctic insect biodiversity by comparing contemporary and historical data with presence-absence data (Renaud, Savage, & Roughley, 2012; Timms, Bennett, Buddle, & Wheeler, 2013). Our results show that abundance is a key aspect of biodiversity that needs to be measured and monitored in order to better document, understand and predict the effects of environmental changes on biodiversity in the Arctic. Furthermore, because ecosystem functions and services, especially pollination, may depend strongly on the abundance of common species and not necessarily on the number of species delivering the service (Kleijn et al., 2015; Winfree et al., 2015), how ecosystem services involving muscid flies and other arthropods are affected by temporal changes in species-level abundance should be further investigated.

Temporal patterns of abundance at the family level did not reflect patterns at the species level in our study. For example, the abundance of *S. sanctipauli*, the most abundant species in our data set and the main pollinator in the Zackenberg Valley (Tiusanen et al., 2016), does not show significant decrease over the study period even if total muscid abundance across habitats declined by 80%. This result may be explained by the fact that *S. sanctipauli*, like *D. segnis*, is a muscid species caught mainly in the arid and mesic heath, both dry habitats in which muscid assemblages showed low changes compared to the assemblage in the wet fen. At Zackenberg, Schmidt et al. (2012) have documented larger temporal changes in Arctic plant assemblage diversity and composition in wet habitats compared to dry habitats and suggested that wet habitats are drying over time as a result of altered snow precipitation patterns. Because vegetation types are differentially affected by climate change, habitat types may modulate effects of rapid environmental changes on insect species (Bowden, Hansen, Olsen, & Høye, 2015).

Species-specific patterns of abundance may be due to ecological differences among species. In a recent study on temporal changes in muscid communities in multiple habitats around Churchill (Manitoba), a Canadian sub-Arctic locality, limited changes in species composition were observed overall but a decrease in *Spilogona* species richness was measured between 1965 and 2010 (Renaud, Savage, & Roughley, 2012). The larvae of some Arctic *Spilogona* species are known to be aquatic or semi-aquatic predators of other Diptera larvae with adults breeding in damp wet surfaces or in algae along water bodies (Michelsen, 2015). As suggested by Renaud et al. (2012), many *Spilogona* species may be directly affected by changes in soil moisture recorded in Churchill (Ballantyne, 2009) or Zackenberg (Schmidt et al., 2012). This may explain why, in our study, the most pronounced decreases in abundance over the study period were recorded in three *Spilogona* species. Unfortunately, detailed breeding habits are still undescribed for most arctic insect species, including Muscidae, and therefore correlations between species responses and ecological traits could not be evaluated in the present work. The deficient state of knowledge regarding Arctic insect species ecology is an impediment to understanding and predicting how future climate change will influence species interactions and ecosystem functions (Hodkinson et al., 2013).

Increased summer temperature was the only climatic variable correlating with a change (decrease) in total Muscidae abundance across habitats. However, at the habitat and species levels, contrasting patterns were observed (Table 2-1). The abundance of most species did not correlate with temperature but some of the most abundant taxa showed unique patterns; the abundance of *D. segnis* and *S. sanctipauli* both decreased with increasing summer temperatures but the trend was only detectable across habitats for *D. segnis* and in the mesic heath for *S. sanctipauli*. These results suggest that muscid species are not equally sensitive to temperature and that habitat characteristics might modify their temperature response. Controlled experiments investigating the influence of temperature on population dynamics of muscid flies could allow us to better understand their resilience towards climate change in a context where summer temperatures have increased markedly in the Arctic region, and are expected to continue to increase in the coming century (IPCC, 2014).

Significant declines in muscid species richness were observed between 1996 and 2014 in each habitat-specific assemblage but no significant change in richness was recorded across habitats at Zackenberg. This result differs from previous reviews of temporal patterns of local richness across taxa and biomes which have found that local species richness tend to stay constant over time even after major anthropogenic and natural disturbances (Dornelas et al., 2014; McGill, Dornelas, Gotelli, & Magurran, 2015; Supp & Ernest, 2014). However, this discrepancy could be due to differences in the definition of “local” scale, usually defined as a scale dominated by species interactions and environmental constraints (McGill et al., 2015). By looking at habitat-specific patterns of biodiversity, our study focused on smaller local scale than ecological research and indicates that the ‘habitat’ scale is appropriate to study biodiversity patterns of Arctic insects.

Rapid environmental changes often lead to biotic homogenization through the establishment of generalist and potentially invasive species and the expense of rare specialist species locally (Davey et al., 2013; Eskildsen et al., 2015; Parmesan & Yohe, 2003; Vellend et al., 2013). Although habitat-specific changes in muscid assemblages were observed in the present work, they did not lead to an increased similarity of assemblages across habitats. This result may in part be explained by the fact that no new muscid species have appeared at Zackenberg between 1996 and 2014. The geographic location and relative isolation of the Zackenberg valley probably limit species introduction in this part of Greenland, even if exceptional migration via wind currents are still possible (Coulson et al. 2002). We also did not document any apparent extirpation, but considering that most muscid species are decreasing in abundance over time in the Zackenberg valley, it is possible that some taxa, and especially *Spilogona* species with wet habitat preferences, will eventually go extinct locally, since the fragmented wetlands of North-Eastern Greenland are strongly affected by climate change (Juncher Jørgensen, Lund Johansen, Westergaard-Nielsen, & Elberling, 2015).

Our study has revealed substantial shifts in abundance, diversity and composition of muscid flies, a diverse and ecologically important group of flying insects in Arctic ecosystems between 1996 and 2014 at Zackenberg, a period of rapid warming. Our results demonstrate why some muscid flies should be classified as “losers” (declining species abundance over time) in the context of rapid environmental change in the Arctic (sensu McGill et al. (2015). Further studies into the ecological function and demographic sensitivity to climate change of individual species of muscids should be prioritized to better understand why some species tend to decline while others appear to be more resilient. Finally, the development of standardized biodiversity monitoring programs in a wide range of habitats and locations should be a high priority, especially in the Arctic region where climate change is expected to escalate in the next century (CAFF, 2013; Heller & Zavaleta, 2009).

2.6 Acknowledgements

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Linking statement

In Chapter 2, I demonstrated that the total abundance of muscid flies at Zackenberg had declined drastically as a result of increased summer temperature between 1996 and 2014, affecting the diversity and composition of muscid assemblages over time in the wet fen mainly. In Chapter 3, I investigated how decline in abundances are related to species timing and duration of the flight period (i.e. phenology). This chapter summarizes the first analyses of phenological shifts at the species level for some Arctic insects other than butterflies. I used a species’ traits approach to see if a common ecological trait (phenological niche, flower visitation habits and temperature sensitivity) or life history trait (body size) could explain variation in species phenological shifts over time.

Chapter 3 Earlier and shorter flight periods of small-bodied Arctic fly species linked to declining abundances

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3.1 Abstract

Climate change affects population abundance over time, and in northern systems, it advances species activity in the spring. These two types of ecological responses are rarely explored simultaneously to investigate their potential relationship. Our three objectives were to examine: 1) phenological shifts in 12 muscid fly species from Zackenberg, Greenland across two decades, 2) differences in species' traits in relation to these trends, 3) whether phenological shifts were associated with changes in species' relative abundance for the six most common species. Generalized additive models and regressions tested the temporal trends in species phenology of muscid flies collected between 1996 and 2014. With mixed models, we analyzed whether species traits (body size, phenological niche, anthophilous habits, and temperature sensitivity) could explain phenological shifts, and if phenological shifts were related to species abundance. Muscid species were active earlier in the season as a result of temperature increase, with the most significant advancements in the first decade of the study period. The advancement was greater for the end than for the onset of the active season, thereby reducing the length of the flight period by 5.7 days per decade. The degree of phenological shifts varied by species, and more pronounced shifts were observed for small-bodied species. As the onset and end of emergence of muscid species advanced in the season, the annual abundance decreased significantly the following summer. However, this relationship was not apparent at the species level. Despite increasingly

warmer and prolonged summer seasons in the Arctic, the activity of some valuable pollinator species has advanced, and their flight period has been reduced, especially for small species. Even if a relationship between abundance and phenology was found across species, further studies are needed to identify the mechanism that links phenology and demography through the species life cycle.

3.2 Introduction

Since 1950, anthropological disturbances and human-mediated climate change have impacted more than 4,000 wild species of mammals, birds, reptiles, and amphibians (Martay et al., 2017; WWF, 2018). Invertebrates are also declining worldwide (Dirzo et al., 2014), which has cascading effects on higher trophic levels, leading to declines in bird, frog, and lizard populations (Lister & Garcia, 2018; Nebel, Mills, McCracken, & Taylor, 2010). In parallel, the timing of biological activities (phenology) is also changing (Cohen, Lajeunesse, & Rohr, 2018; Kharouba, Paquette, Kerr, & Vellend, 2014; Ovaskainen et al., 2013; Thackeray et al., 2016), and the most recent global meta-analysis on animal phenological shifts has documented a spring advancement of 4.15 days per decade since 1950 for small ectotherms like insects (Cohen et al., 2018). While impacts of climate change on abundance and phenology are common, these responses are rarely considered together, even though one can influence the other. For example, phenological shifts can directly affect abundance by altering population rates such as survival and reproduction (Bestion, Teyssier, Richard, Clobert, & Cote, 2015; CaraDonna, Cunningham, & Iler, 2018; Lane, Kruuk, Charmantier, Murie, & Dobson, 2012). As an example, the earlier emergence of some North American butterfly species under warmer temperatures can lead to smaller adults and less fecund females due to accelerated development (Bennett, Severns, Parmesan, & Singer, 2015).

Changes in phenology have the potential to reduce fitness and affect population size indirectly by disrupting species interactions through temporal mismatch (reviewed by Kharouba et al. (2018)) or directly by altering climatic conditions experienced by individuals. Under warmer conditions, insects have a shorter life cycle because higher temperatures accelerate the developmental rate of ectotherms. For some insect species in temperate regions, a supplementary generation can even be added on warmer years (Altermatt, 2009; Roy & Sparks, 2000). However, if the extra generation fails to reach the proper life stage for the winter, individuals from this generation can die and cause a local population decline (Van Dyck, Bonte, Puls, Gotthard, & Maes, 2015), as seen in the northern populations of the mustard white (*Pieris oleracea* Harris) in North-America (Kerr et al., 2020) or some populations of wall brown (*Lasionnata megera* Linnaeus) in Europe (Van Dyck et al., 2015). Phenology and abundance are interconnected, and climate change may affect these biological elements directly, and indirectly through impacts on their relationships.

Using a trait-based approach can help understand interspecific differences in phenological shifts (Bartomeus et al., 2013; Clements & Ozgul, 2016; Pacifici et al., 2017). Species-specific phenological responses to climatic changes vary greatly, usually according to sensitivities to temperature and ecological traits such as phenological niche (early-active vs. late-active species) (Brooks et al., 2017; Kharouba et al., 2014; Maurer et al., 2018). Early-active species of butterflies, bees, and early-flowering plants usually display more pronounced spring advancement in emergence than late-active species (Bartomeus et al., 2011; Kharouba et al., 2014), probably because of differences in species sensitivities to temperature variation (Kharouba et al., 2014; Miller-Rushing & Primack, 2008). Other life-history traits can modulate species responses over time, such as voltinism or body size (Diamond, Frame, Martin, & Buckley, 2011; Dunn & Møller, 2014; Thomsen et al., 2016). Smaller species of animals have demonstrated more pronounced phenological shifts over the last few decades of rapid warming because they acclimate faster to changes in temperature than large ones (Cohen et al., 2018, Rohr et al., 2018).

At Zackenberg, North-Eastern Greenland, the abundance of flies in the Muscidae family has declined significantly between 1996 and 2014 (Loboda, Savage, Buddle, Schmidt, & Høye, 2018). In this study, we measured phenological shifts and analyzed the relationships between temporal changes in abundance, life-history traits and phenological shifts for 12 muscid fly species using data from Zackenberg collected between 1996 and 2014, a period during which mean summer and spring temperatures have increased (Loboda et al., 2018). Our objectives were to (i) Determine if the onset, peak, end, and duration of the flight period of muscid species have changed at Zackenberg between 1996 and 2014; (ii) Investigate the role of some species functional traits such as phenological niche, body size, anthophilous habits (frequent vs. irregular flower visitors), and species temperature sensitivity in explaining phenological trends; and finally (iii) Test how abundance variation over time relates to phenological variations.

3.3 Materials and methods

3.3.1 Study site and data collection

Muscid flies were collected at Zackenberg in High-Arctic Northeast Greenland (74°28'N, 20°34'W) as part of the Zackenberg Ecological Research Operations (see Schmidt, Hansen, Hansen, Berg, and Meltofte (2016) for a complete collection protocol). Insects were monitored in three habitats (arid heath, mesic heath, wet fen) throughout the active season from snowmelt to snowfall (May-September) from 1996 to 2014. Specimens were collected using four yellow pitfall traps per habitat, serviced every week, and stored in ethanol after being identified to family. Flies of the family Muscidae collected from two pitfall traps in each habitat were identified to species using morphological characters (Collin, 1930; Huckett, 1965; Michelsen, 2006, 2015) and DNA barcoding (see Loboda et al., 2018). A total of 18,385 individuals were identified, representing 16 species. The species list and details on the number of individuals per species are available in Loboda et al. (2018).

3.3.2 Analyses

3.3.2.1 Phenology of the flight season

Annual onset, peak, and end of the flight season of muscid fly species across habitats were calculated using a generalized additive model (GAM) smoothing method as it provides the most accurate estimations of species phenology even with bimodal distributions, and varying sample size and sampling efforts (gaps in the distribution) (Moussus, Julliard, & Jiguet, 2010). We first fitted curves of muscid abundance over the season using GAMs, assuming a Poisson error, for each species across habitats within each year (Figure 3-1). We modeled species abundance if: 1) the species was present in at least two weeks of the same season; 2) the species was represented by at least three individuals over the season; and 3) the GAM could generate a satisfying curve over the season (for example, in 1998 and 1999, sampling stopped during the peak of abundance of some late-emerging species so the activity phenology could not be calculated accurately for some species these years). The average number of data points to fit a model for each year for each species was 16. Annual onset, peak, and end of the flight period were then calculated as the day of the year, at which 10%, 50%, and 90% of the area under the curve were reached respectively (Figure 3-1). The duration of the flight period was calculated as the number of days between the onset and end of the season for each species (Figure 3-1). Each GAM was inspected visually to see if it fits the data well, normality of model residuals was assessed with QQ plots, and the k-index was used to evaluate how adequate the basis dimension was for each GAM (*gam.check* function from the mgcv package (Wood, 2011))

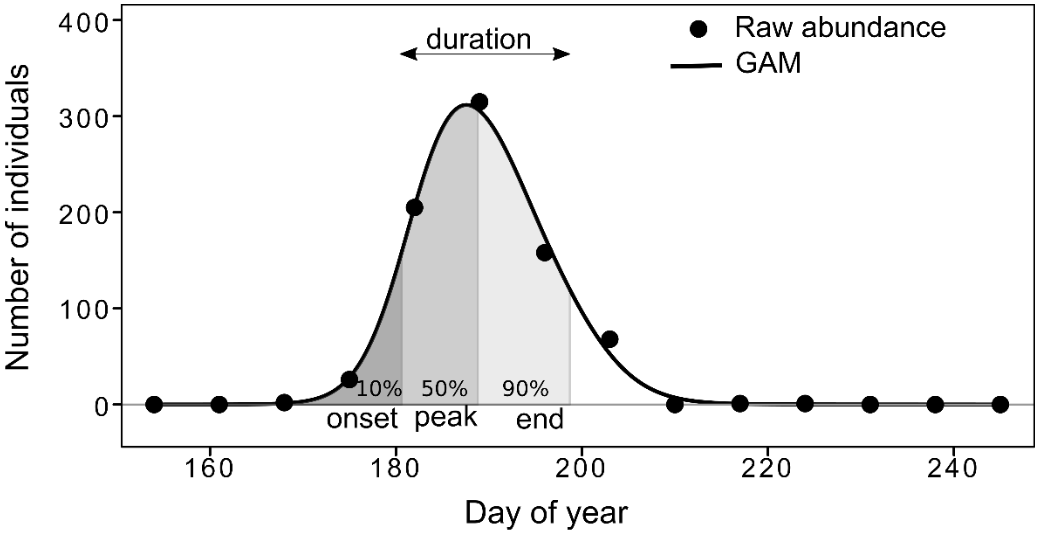

Figure 3-1 Example of curve fitting using GAM (Poisson family) for abundance data of muscid species to calculate phenology. This example is based on raw abundance data for *Spilogona. zaitzevi* (Schnabl) in 1996.

Once annual onset, peak, and end of the flight season were estimated, temporal trends in the timing of these events were measured as the slope of the regression against year, corrected for one year temporal autocorrelation when detected by a Ljung-Box test to meet the assumption of the non-autocorrelated errors for the regression. Temporal trends in phenological events were calculated for each of the species for which we were able to estimate phenology of the flight period for at least six years across the study period (minimum of six data points to estimate regression of phenology against time). Data were satisfactory for 12 out of the 16 species present in our samples (Loboda et al., 2018). Average temporal trends in the timing of onset, peak, and end of the flight period for muscid flies were calculated using linear mixed models (LMMs) with each phenological event as the dependent variable, time as a fixed effect, and species as a random effect. Diagnostic plots for normality of residuals and variance homogeneity were satisfactory.

To test if the temporal trends in flight season phenology of species of Muscidae at Zackenberg have changed in recent years, we fragmented the study period in 10 decade time-windows and estimated the temporal trends in each segments (see Iler, Høye, Inouye, & Schmidt, 2013). For each decade, we calculated the average temporal trends of phenology (onset, peak, and end of the flight period) of the 12 species with satisfactory data using LMMs with species as a random effect.

Correlations between phenological events were estimated with the Pearson correlation test (see Supplementary Figure 3-1). The timing of the onset, peak, and end of flight period of each species were significantly correlated. Since the duration of the flight period was not correlated to the onset or peak of the season for most species, we decided to perform the following analyses with only the timing of the peak and the duration of the flight period as the two response variables describing the phenology of each species: trait analyses, climate analyses, and relationship between phenology and abundance.

3.3.2.2 Species' traits and species phenology

To investigate how species' traits could influence phenological shifts, we used information on four selected traits: the phenological niche, the body size, anthophilous habits and temperature sensitivity. The mean date of activity peak across the study period was used as the metric of the phenological niche. Average wing length, used as a proxy of body size, was found in Michelsen (2015) for species of *Spilogona* and extracted from images published on boldsystem.org for the remaining species (*Drymeia groenlandica* (Lundbeck), *Drymeia segnis* (Holmgren) and *Phaonia bidentata* (Ringdahl). The rate of flower visitation and the number of plant species visited published in Olesen, Bascompte, Elberling, and Jordano (2008) were used to categorize species as 'frequent' or 'irregular' flower visitors (see Supplementary Table 3-1 for details on each categorical variables). Species temperature sensitivity was determined as the slope of the linear regression of the activity peak as a function of temperature (mean temperature within predefined time window).We identified the time-window of temperature that most affects activity for each species using a sliding window analysis with the climwin R package (Bailey & Van De Pol, 2016; van de Pol et al., 2016). Using daily mean soil temperature recorded at ground level (0 cm depth) at the Zackenberg weather station (Schmidt, Hansen, et al., 2016), models on the influence of temperature on peak timing of activity were performed with each possible time window from a reference date. We set the reference date as the mean date of the activity peak per species so all possible windows (different durations in number of days and starting days) before that reference date were compared based on their AICc values. This procedure compares many climate models which increase the risk of false positives. To evaluate the reliability of the detected temperature windows, we carried out randomizations by reordering the response variable (phenology) and calculated a Pc probability statistic, which compares the number of models in the 95% confidence set in observed vs. randomized data (van de Pol et al., 2016). The Pc value indicated if the relation was likely due to chance (Pc close to 1) or not (Pc close to 0). The Pc requires only five randomizations to be reliable (van de Pol et al., 2016). A similar analysis using air temperature recorded at 200 cm above the ground did not identify any time-window of temperature for muscid species. Temperature-windows identified for each species with the sliding window analysis are presented in Supplementary Figure 3-2.

To evaluate the effects of phenological niche, body size, anthophilous habits, and temperature sensitivity on temporal trends in the species flight season, we modeled phenological shifts in the peak of activity and duration of the flight period as a function of each trait variable separately, with their interactions and species as a random effect.

3.3.2.3 Species phenology and abundance

To test the hypothesis that changes in species abundance are related to species phenology, we used three linear mixed models of species abundance against each phenological event (onset, peak, end of season or duration of the active season) with ‘species’ as a random factor: the first one to test the relationship between abundance and phenology of the same year (Abundance_t), the second to test the relationship between abundance and phenology the previous year (Abundance_{t-1}) and the last one to test the relationship between abundance and phenology two years earlier (Abundance_{t-2}). To investigate the correlation between species abundance and phenology with a lagged effect in time, we needed long time series with consecutive annual values of phenology and abundance as any missing annual data point would lower the statistical power of the analysis. Therefore, we determined relationship between variations in abundance and timing of emergence for each species individually for the six most abundant species: *Drymeia segnis, Spilogona almqvistii* (Holmgren), *Spilogona dorsata* (Zetterstedt), *Spilogona sanctipauli* (Malloch), *Spilogona tundrae* (Schnabl), and *Spilogona zaitzevi* (See Supplementary Table 3-1). Since decline in abundance of muscid species over time has been documented at Zackenberg (Loboda et al., 2018), species standardized annual abundance was detrended before analyses following the same procedure as for phenological or climatic variables.

All analyses were performed using R 3.6.1 (R Core Team, 2019). Generalized additive models were constructed with the mgcv package , mixed models were run with the lme4 package (Bates, Maechler, Bolker, & Walker, 2015), and the figures were created with the package ggplot2 (Wickham, 2016)..

3.4 Results

3.4.1 Phenology of the flight season

On average over the study period, muscid species have advanced their activity onset by 2.9 ± 0.7 ($F_{1,464}$= 15.56, p<0.001), the peak by 6.3 ± 0.8 ($F_{1,462}$= 61.06, p<0.001) and the end by 8.6 ± 1.0 days per decade ($F_{1,462}$=71.79, p<0.001) (left column in Figure 3-2a, 3-2b, and 3-2c). Even though temporal trends in the timing of activity were mainly negative, indicating that most species advanced their activity over time (Figure 3-2), *Phaonia bidentata* and *Spilogona almqvistii* showed the opposite trends for all phenological events (Figure 3-2). The advancement in timing for all phenological events over the study period was most pronounced in *Spilogona deflorata* (Holmgren) (onset: 22.2 ± 12.0, *P*=0.29; peak: 20.0 ± 10.0, *P*=0.34; end: 26.1 ± 6.7, *p*= 0.01) and *Spilogona megastoma* (Boheman) (onset: 13.2 ± 7.9, *p*=0.15; peak: 20.6 ± 4.9, *p*<0.001; end: 27.4 ± 3.4, *p*= 0.01) but these results may have been affected by low sample sizes for these species (see Supplementary Table 3-1). Ten species advanced their end of the season (Figure 3-2c), but the duration of the flight season was reduced for all twelve species over the study period, with a significant mean reduction of 5.7 ± 0.8 days per decade across species ($F_{1,462}$=47.27, *p*<0.001; left column in Figure 3-2d). More pronounced advancement for the end than for the onset of the flight season has resulted in a shorter active season (Figure 3-2d). Even in *P. bidentata* and *S. almqvistii*, the two species for which the onset was delayed over time, the flight period was reduced too because the delay of the onset was greater than the delay of the end of the season.

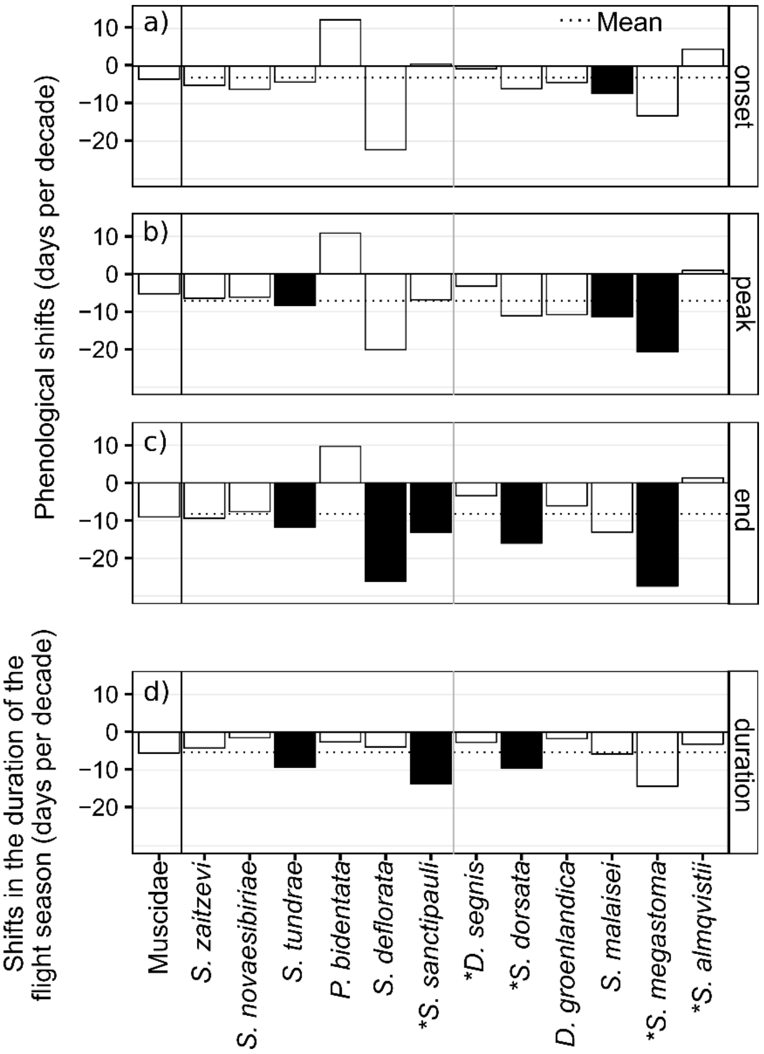

Figure 3-2 Phenological shifts in the timing of onset (a), peak (b) and, end of flight season (c) and resulting changes in the duration of the flight season over time (d) for muscid flies at Zackenberg between 1996 and 2014. Black bars represent significant shifts over time (*p*<0.05). Asterisks in front of species' names indicate common flower visitors.

Once the study period was fragmented in 10-years segments, phenological shifts of the onset and the peak in the first half of the study period (1996-2005) were significantly different from shifts recorded over the second decade of the study period (2005-2014) (Figure 3-3a and b). However, the largest shifts in the duration of the flight season occurred between 2001 and 2010 (Figure 3-3d). The advancement of the activity period was much more pronounced for all phenological events in the ten-year windows between 1998 and 2007 than between 2005 and 2014, resulting in a relatively modest advancement of activity phenology over the entire study period.

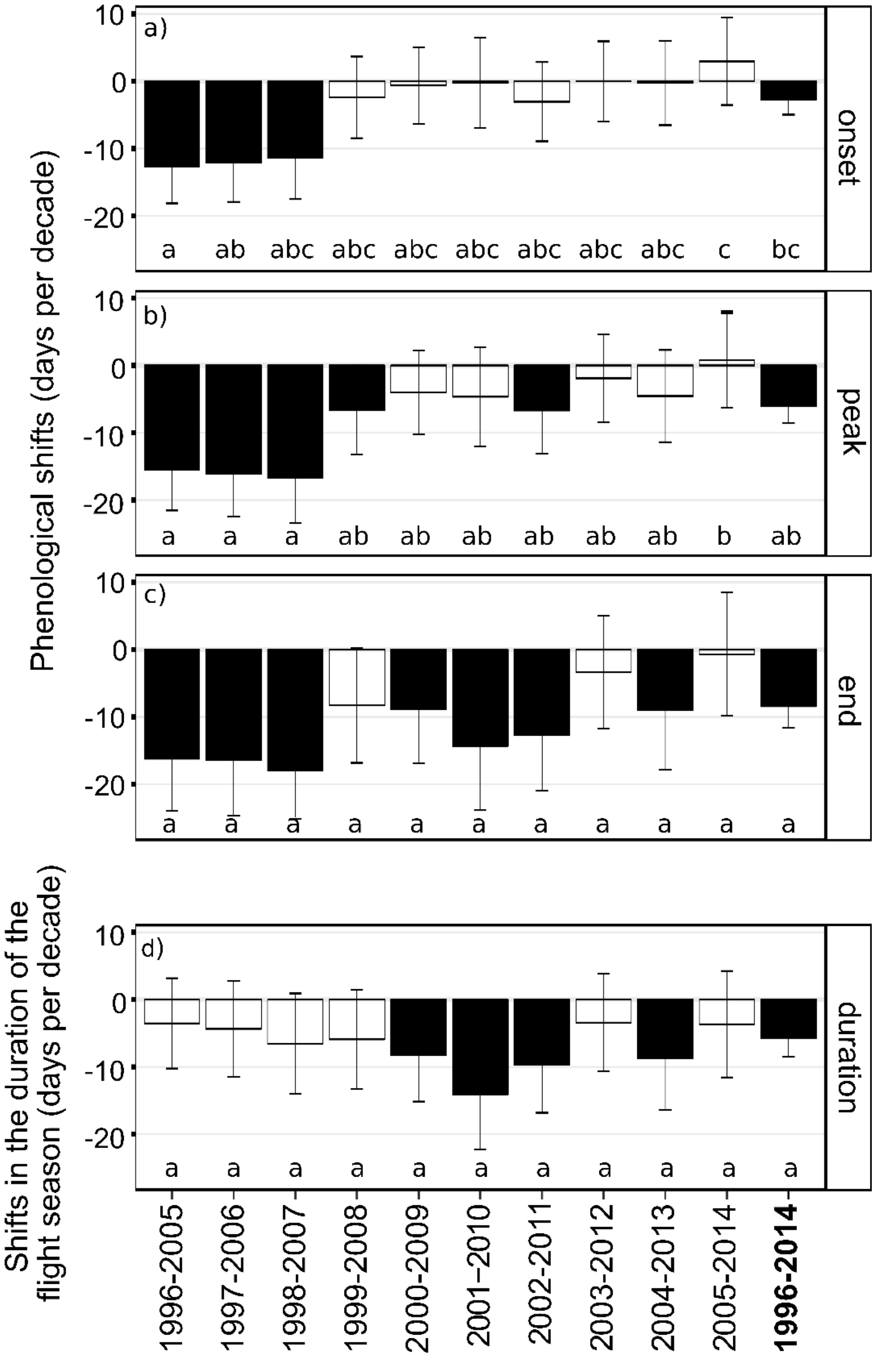

Figure 3-3 Phenological shifts in the onset (a), peak (b), and end of flight season (c), and the resulting shifts in the duration of the flight season (d) for 12 muscid fly species at Zackenberg for the 10 different decadal time windows between 1996 and 2014, compared to phenological shifts calculated over the study period (last column in bold). Bars represent mean, and error bars represent SEs. For each phenological event, bars with different letters indicate significantly

different values of shifts between window periods (*p*<0.05 as determined by Tukey-Kramer for pairwise comparisons). Black bars represent significant shifts over time (*p*<0.05).

3.4.2 Species' traits and species phenology

Body size significantly influenced phenological shifts of muscid flies (Table 3-1). Small species have advanced the timing of their activity and reduced the duration of their flight period to a greater extent than large species over the study period (Figure 3-4). Phenological niche, rates of flower visitation, and temperature sensitivity were not significantly associated with the timing or the duration of the flight season of muscid species (Table 3-1). Interaction terms were not significant and were removed from the final model.

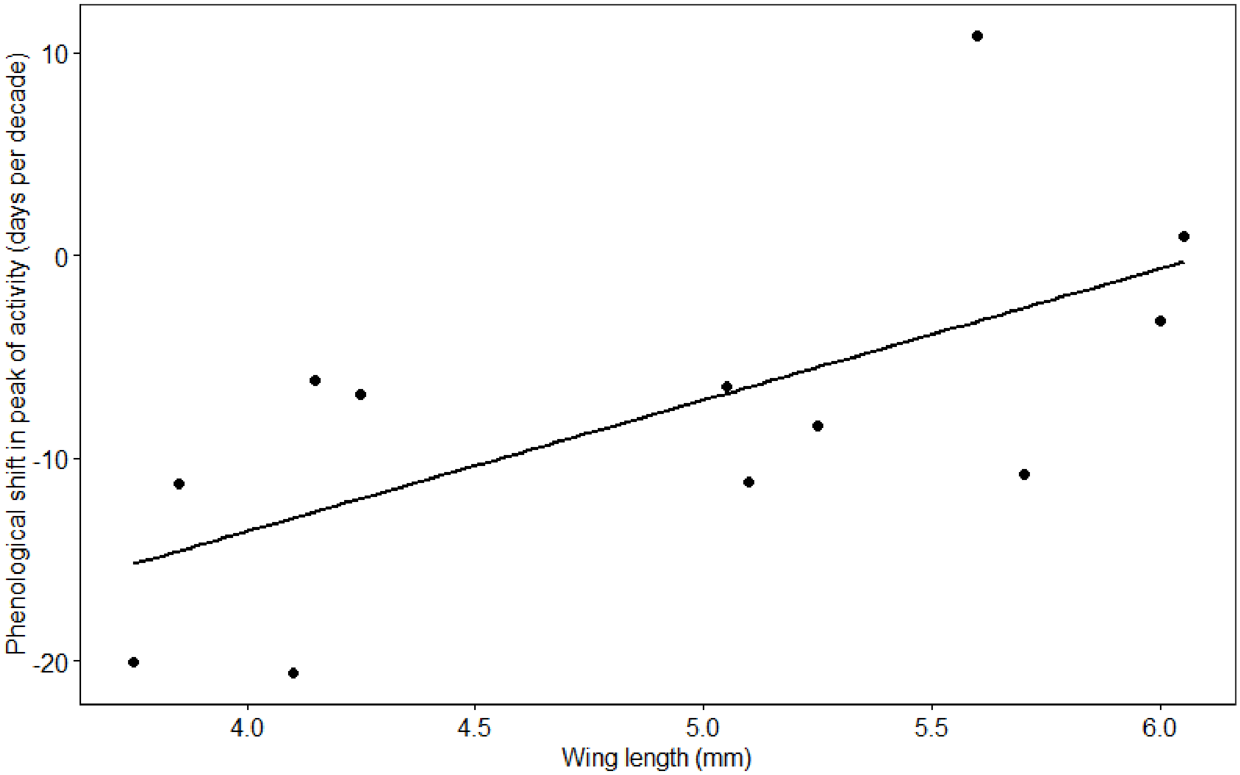

Figure 3-4 Temporal shifts of the peak of activity of muscid flies at Zackenberg between 1996 and 2014 as a function of wing length, a proxy of species body size ($F_{1,145}$= 1.34, p<0.001).

Table 3-1 Results of the linear mixed model testing the effects of body size, phenological niche, anthophilous habits, and temperature sensitivity on phenological shifts of muscid fly species at Zackenberg. These results were obtained with estimates of phenological shifts of the peak of the flight period as dependent variable. Similar results were obtained with temporal shifts in the duration of the flight period and are not presented.

Effect	Estimate ± s.e.	df	t-value	*p*
Body size	0.17 ± 0.07	145	2.65	**<0.001**
Phenological niche	2.19e-04 ± 8.55e-03	154	0.03	0.98
Anthophilous habits	0.04 ± 0.13	144	0.32	0.75
Temp. sensitivity	0.04 ± 0.03	91	1.43	0.16

3.4.3 Species phenology and abundance

No relationship was found between phenology and species abundance of the same year or two summers later (Table 3-2). However, the abundance of muscid flies was significantly lower one year after an early onset of emergence in the spring or an earlier end of active season (Table 3-2).

Table 2-2 Summary results of linear mixed models of muscid phenology (peak or duration) against muscid fly abundance. Abundancet: relationship between abundance and phenology of the same year, Abundancet-1: relationship between abundance and phenology of the previous year and, Abundance$_{t-2}$: relationship between abundance and phenology two years earlier.

Phenological event	Annual abundance	Sum Sq	df	F-value	*p*
Onset	Abundance$_t$	5.39	95	0.10	0.75
	Abundance $_{t-1}$	**236.42**	**68**	**5.20**	**0.03**
	Abundance $_{t-2}$	30.28	68	0.60	0.44
Peak	Abundance$_t$	107.15	96	1.44	0.23
	Abundance $_{t-1}$	225.19	69	3.77	0.06
	Abundance $_{t-2}$	8.63	69	0.14	0.71
End	Abundance$_t$	0.68	96	0.01	0.93
	Abundance $_{t-1}$	**477.59**	**69**	**6.32**	**0.01**
	Abundance $_{t-2}$	0.51	69	0.01	0.94
Duration	Abundance$_t$	42.78	96	0.52	0.47

	Abundance $_{t-1}$	43.01	69	0.99	0.32
	Abundance $_{t-2}$	35.17	69	0.90	0.35

However, at the species level, the signal of a relationship between phenology and abundance was absent for most species, probably because of a low statistical power (Table 3-3).

Table 3-3 Summary results of multivariate generalized linear mixed models of species timing of emergence phenology activity (onset, peak and end) against abundance. Abundance$_{t}$: relationship between abundance and phenology of the same year, Abundance$_{t-1}$: relationship between abundance and phenology of the previous year and, Abundance$_{t-2}$: relationship between abundance and phenology two years earlier.

Species	Phenological event	Annual abundance	Sum Sq	df	F-value	*p*
Drymeia segnis	Onset	Abundance$_{t}$	11.06	16	0.17	0.68
		Abundance $_{t-1}$	14.03	13	0.25	0.63
		Abundance $_{t-2}$	25.55	13	0.46	0.51
	Peak	Abundance$_{t}$	6.34	16	0.06	0.82
		Abundance $_{t-1}$	2.71	13	0.03	0.86
		Abundance $_{t-2}$	30.93	13	0.48	0.50
	End	Abundance$_{t}$	1.64	16	0.02	0.89
		Abundance $_{t-1}$	19.38	13	0.28	0.61
		Abundance $_{t-2}$	15.05	13	0.21	0.66
	Duration	Abundance$_{t}$	0.10	16	0.00	0.99
		Abundance $_{t-1}$	0.43	13	0.01	0.92
		Abundance $_{t-2}$	1.38	13	0.03	0.87
Spilogona almqvistii	Onset	Abundance$_{t}$	1.70	10	0.02	0.88
		Abundance $_{t-1}$	1.45	8	0.02	0.90
		Abundance $_{t-2}$	2.15	9	0.03	0.87
	Peak	Abundance$_{t}$	2.54	11	0.05	0.83
		Abundance $_{t-1}$	0.15	9	0.00	0.96
		Abundance $_{t-2}$	90.01	10	1.84	0.21
	End	Abundance$_{t}$	0.70	11	0.01	0.93
		Abundance $_{t-1}$	61.27	9	0.73	0.42
		Abundance $_{t-2}$	190.22	10	2.97	0.12
	Duration	Abundance$_{t}$	16.50	11	0.16	0.70
		Abundance $_{t-1}$	28.68	9	0.27	0.61
		Abundance $_{t-2}$	61.82	10	0.66	0.44

Spilogona dorsata	Onset	Abundance$_{t}$	3.69	14	0.07	0.79
		Abundance $_{t-1}$	117.70	13	3.12	0.10
		Abundance $_{t-2}$	28.63	13	0.56	0.47
	Peak	Abundance$_{t}$	100.14	14	1.36	0.26
		Abundance $_{t-1}$	83.98	13	1.18	0.30
		Abundance $_{t-2}$	122.38	13	1.58	0.23
	End	Abundance$_{t}$	31.46	14	0.26	0.62
		Abundance $_{t-1}$	315.64	13	3.21	0.10
		Abundance $_{t-2}$	148.01	13	1.28	0.28
	Duration	Abundance$_{t}$	13.61	14	0.25	0.62
		Abundance $_{t-1}$	49.11	13	0.89	0.36
		Abundance $_{t-2}$	49.23	13	1.36	0.26
Spilogona sanctipauli	Onset	Abundance$_{t}$	43.65	16	0.80	0.38
		Abundance $_{t-1}$	2.74	16	0.05	0.83
		Abundance $_{t-2}$	33.10	16	0.60	0.45
	Peak	Abundance$_{t}$	**472.50**	**16**	**5.46**	**0.03**
		Abundance $_{t-1}$	130.08	16	1.20	0.29
		Abundance $_{t-2}$	2.44	16	0.02	0.89
	End	Abundance$_{t}$	0.81	16	0.00	0.95
		Abundance $_{t-1}$	111.06	16	0.63	0.44
		Abundance $_{t-2}$	0.33	16	0.00	0.97
	Duration	Abundance$_{t}$	56.38	16	0.64	0.43
		Abundance $_{t-1}$	78.92	16	0.92	0.35
		Abundance $_{t-2}$	40.03	16	0.45	0.51
Spilogona tundrae	Onset	Abundance$_{t}$	126.98	13	4.73	0.05
		Abundance $_{t-1}$	97.72	12	3.45	0.09
		Abundance $_{t-2}$	15.47	12	0.41	0.53
	Peak	Abundance$_{t}$	**182.82**	**13**	**5.55**	**0.03**
		Abundance $_{t-1}$	**213.74**	**12**	**7.10**	**0.02**
		Abundance $_{t-2}$	0.71	12	0.01	0.90
	End	Abundance$_{t}$	**190.18**	**13**	**5.69**	**0.03**
		Abundance $_{t-1}$	98.26	12	2.48	0.14
		Abundance $_{t-2}$	0.08	12	0.00	0.96
	Duration	Abundance$_{t}$	4.70	13	0.22	0.65
		Abundance $_{t-1}$	0.03	12	0.00	0.97
		Abundance $_{t-2}$	21.32	12	0.98	0.34
Spilogona zaitzevi	Onset	Abundance$_{t}$	57.01	16	1.00	0.33

	Abundance $_{t-1}$	67.23	14	1.23	0.29
	Abundance $_{t-2}$	0.33	13	0.01	0.95
Peak	Abundance$_t$	99.77	16	1.50	0.24
	Abundance $_{t-1}$	56.43	14	0.78	0.39
	Abundance $_{t-2}$	22.07	13	0.28	0.61
End	Abundance$_t$	159.43	16	1.51	0.24
	Abundance $_{t-1}$	179.70	14	1.68	0.22
	Abundance $_{t-2}$	66.95	13	0.57	0.46
Duration	Abundance$_t$	25.77	16	1.12	0.30
	Abundance $_{t-1}$	27.10	14	1.18	0.30
	Abundance $_{t-2}$	57.84	13	3.27	0.10

3.5 Discussion

The objective of this research was to document temporal trends in phenology for 12 species of Arctic muscid flies and to test for a potential relationship between population declines and phenological shifts. Our results show that in Greenland, Zackenberg, muscid flies were active earlier in the spring and for a shorter period of time between 1996 and 2014. The loss of almost one-fifth of the total active season for these flies is remarkable, especially considering that northern species already have a short activity period at these latitudes.

Changes in the duration of activity period and life span of insects depend on species voltinism (Forrest, 2016; Gallinat, Primack, & Wagner, 2015; Hassall, Thompson, French, & Harvey, 2007). Multivoltine species from temperate and tropical regions may extend their period of activity by adding generations. In contrast, univoltine species, such as most northern species (Zeuss, Brunzel, & Brandl, 2017), are predicted to have a shorter period of activity and accelerated development under rapid warming as temperature increases (Forrest, 2016). Muscid species in the genera *Spilogona* and *Drymeia* are most likely univoltine in the Arctic (Skidmore, 1985), so the reduction of their flight period is likely a signal of an accelerated development under rapid warming. An accelerated development in ectotherms result in smaller individuals (Buckley, Arakaki, Cannistra, Kharouba, & Kingsolver, 2017; Bowden et al., 2015; Kingsolver et al., 2011) and lower individual fitness (Bennett, Severns, Parmesan, & Singer, 2015). If this is the case at Zackenberg, this mechanism could explain the correlation we found between phenology in a given year and low abundance in the subsequent years for early active species such as *S. tundrae, S.dorsata* and *S. zaitzevi*, but not for *S. almqvistii* for which the opposite correlation was found. However, phenological shifts can also affect species abundance indirectly by changing inter- and intra-specific interactions. For example, the declines in abundance of some muscid flies might result from an increased intraspecific density induced by the contraction of the flight period (Nowicki, Bonelli, Barbero, & Balletto, 2009). Disentangling the direct and indirect effects of multiple drivers, abiotic and biotic, of population decline is complex and will require a deeper knowledge of species' life cycle and ecology. Even if our study is limited taxonomically and geographically, our results suggest that further studies on insect abundance changes over time take into account phenology to potentially provide new insights on the nature and extent of the relationship between phenology, voltinism and demography.

The mean advancement of 6.3 days per decade reported here for the peak of activity of muscid flies at Zackenberg is less pronounced than the average of 14.5 days per decade previously reported for multiple arthropod, bird and plant taxa at the same site (Høye, Post, Meltofte, Schmidt, & Forchhammer, 2007). However, this contrast is likely attributable to differences in the study period as changes in muscid phenology were generally more pronounced between 1996 and 2005 than in the following decade. The observed shifts in Arctic muscid phenology reported here between 1996 and 2014 are higher than the global average shifts (2.88 days per decade) documented across taxa since 1950 (Cohen et al., 2018). They are also greater than those observed for other pollinators at lower latitudes such as bees in North America, with 0.8 day per decade (Bartomeus et al., 2011), or syrphid flies in Colorado Mountains with 2.3 days per decade (Iler, Inouye, et al., 2013). Our results support the general observation that phenological shifts are greater at high latitudes where climate change is more pronounced (Buckley, Arakaki, Cannistra, Kharouba, & Kingsolver, 2017; Parmesan, 2007; Valtonen et al., 2014).

Pollinator species in our muscid assemblage showed similar phenological shifts than irregular flower visitor species. Considering that plant species have advanced their flowering by 9.1 days per decade on average at Zackenberg since 1996 (Iler, Høye, et al., 2013), we predict a reduction in synchrony between flowering and the activity of flower-visiting muscids in a warmer future. Prior studies have noted the potential impact of a reduced temporal overlap between flowering time and pollinator activities on Arctic pollinator abundance through food limitation (Høye, Schmidt, Trøjelsgaard, & Forchhammer, 2013; Schmidt, Mosbacher, et al., 2016). We can also presume that increasing asynchrony between flowering and pollinators can have an impact on plants, especially at Zackenberg, where the plant flowering season lasts only one month (Høye et al., 2015). The significant reduction in the active period of *S. sanctipauli* is of particular concern because the reproductive success of *Dryas* sp., a common and abundant perennial plant in the Arctic, is primarily influenced by the presence of this species (Tiusanen, Hebert, Schmidt, & Roslin, 2016). Pollination might also be reduced as a result of lower pollinator abundance (Loboda et al., 2018), and the potential body-size shrinkage of pollinators induced by warmer temperature (Bowden et al., 2015) might affect the amount of pollen transferred and the dispersal abilities of pollinators in the future (Oliveira, Freitas, Scheper, & Kleijn, 2016). Given the cumulative influence of these effects on Arctic pollinators in a warming climate, it is crucial to monitor not just abundance, diversity and phenology of pollinator species in the Arctic, but also their morphology over time, and to investigate how these ecological responses could influence the fitness of pollinators and the reproductive success of plants.

In the Arctic, the growth and survival of migratory bird offspring depend the food availability such as the peak of arthropod abundance (McKinnon, Picotin, Bolduc, Juillet & Bêty, 2012; Senner, Stager, & Sandercock, 2017). A potential trophic mismatch between the timing of reproduction of migratory birds and the peak of abundance in arthropods has been hypothesized as an important effect of climate change in the Arctic (Tulp & Schekkerman, 2008). However, relatively few evidence support this hypothesis (Smith et al., 2020). Reneerkens et al. (2016) have found that the growth of sanderling chicks at Zackenberg was higher when the peak in arthropod abundance was broad, even in presence of temporal mismatch. If the reduced duration of the active period is occurring in multiple arthropod taxa such as documented here for muscid flies, the impact of these phenological changes on higher trophic levels could be more important than a potential temporal mismatch.

Temporal changes in the timing of muscid activity reported here varied greatly by species but were not correlated with their phenological niche, contrary to previous studies on Arctic species or insect species (Iler et al., 2013; Maurer et al., 2018); early-active species did not show higher values of phenological shifts than late-active species. In fact, body size better explains the differences in direction and magnitude of species phenological shifts and smaller species advanced the timing of their activity more than larger ones. This result is similar to findings from a global meta-analysis on phenological shifts in animals, which has found that small species have greater advancements over time in response to temperature increase (Cohen et al., 2018).

However, body size is a complex life history trait associated with many ecological aspects of a species, such as life cycle and dispersal. As such, a relationship between phenology and body size might arise through the direct effect of thermoregulation (Cohen et al., 2018) or indirectly through dispersal capacity (Perry, Low, Ellis, & Reynolds, 2005).

Overall, we found evidence for an earlier and shorter period of activity for muscid flies in the High-Arctic as a consequence of rapid warming in the last two decades. Our study is the first to demonstrate that phenological shifts induced by warming could be associated with insect decline. Whether the abundance decline in insects observed at Zackenberg is due to the direct effect of temperature on developmental rate or indirect effect through changes in interactions due to phenological shifts is a promising avenue for future research. This study highlights the high value of long-term monitoring in detecting species responses to rapid and recent climate change.

3.6 Acknowledgements

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Linking statement

In Chapter 3, I examined how species phenology has changed over two decades of rapid warming and how these changes could affect species abundance over time. I investigated how phenological shifts induced by rapid warming varied by species and whether this variation could be explained by species traits (body size, anthophilous habits and phenological niche). Despite an extended growing season at Zackenberg, the flight periods of muscid flies have advanced and shortened between 1996 and 2014, particularly for small species. The contraction of the flight period has been associated with abundance changes over time for some species, suggesting that time for reproduction might be limited. Thus, in Chapter 4, I investigated sex-specific changes in phenology and abundance and the relationship between these responses and the decline in species abundance.

Chapter 4 Declining abundance of High-Arctic muscid flies (Diptera: Muscidae) associated with a reduction of the reproductive period over two decades of rapid warming

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4.1 Abstract

Climate change affects insect population abundance and phenology, especially in the Arctic where warming is double the rate of the global average. However, little is known about how Arctic species' sex-specific phenology and abundance respond to temperature increases. We used 18 years of data from six species of muscid flies (Diptera: Muscidae) sampled at Zackenberg, High-Arctic Greenland, between 1996 and 2014 to explore temporal trends in timing and duration of the flight period and abundance of males and females, and the influence of sex-specific temperature sensitivities on these trends. We also investigated temporal changes in the length of the reproductive period, determined as the temporal overlap between the male and the female flight periods and tested if these changes could be correlated with species abundance changes over the study period. The sex ratio changed in three species over the study period despite the fact that males and females had similar sensitivities to temperature and comparable rates of decline over time. Warmer temperatures were associated with earlier and shorter periods of activity for both males and females. Similar rates of phenological change over the study period were found for both sexes, so no evidence of temporal mismatch between males and females were observed. However, the length of the reproductive period was significantly reduced by 5.67 days per decade on average and this reduction in a given year was associated with lower

population abundance the following years, suggesting a reduced fitness due to a shorter life span or an increasing rate of mate-finding failure through density-dependent processes. These findings raise intriguing questions regarding the mechanism behind the association of insect population size and variations in reproductive phenology induced by rapid warming.

4.2 Introduction

Climate change has severe impacts on insects by modifying diversity (Fernandez-Triana et al., 2011; Maxwell, Fuller, Brooks, & Watson, 2016), species distribution ranges (Brown et al., 2016; Perry, Low, Ellis, & Reynolds, 2005), timing and duration of activity period (Cohen, Lajeunesse, & Rohr, 2018; Thackeray et al., 2016; and see Chapter 3), voltinism (Altermatt, 2009; Forrest, 2016) and body size (Gardner, Peters, Kearney, Joseph, & Heinsohn, 2011; Sheridan & Bickford, 2011). Considerable decline in global terrestrial insect abundance and biomass has been reported since 1950 (van Klink et al., 2020), including estimates of up to 75% loss of biomass in Europe (Hallmann et al., 2017; Martay et al., 2017; Shortall et al., 2009), even in the Arctic (Gillespie et al., 2020; Høye et al., 2020; Loboda, Savage, Buddle, Schmidt, & Høye, 2018). This is of significant concern because of the ecological and economic roles of insects in ecosystems (Cardoso et al., 2020; Nebel, Mills, McCracken, & Taylor, 2010; Noriega et al., 2018). Multiple drivers likely underpin these declines, especially habitat loss, fragmentation, and pesticides, but in pristine areas, climate change is the most likely threat (Cardoso et al., 2020; Hodkinson et al., 2013; Maxwell et al., 2016). The Arctic is arguably one of the last pristine ecosystems, and it represents an ideal region to study the role of climate change on insect declines. Direct human pressures are less common at higher latitudes (CAFF, 2013), and the rate of climate warming is twice as fast as in the rest of the world (IPCC, 2019).

Thermal acclimation in ectotherm organisms such as insects, depends greatly on body size (Rohr et al., 2020). Since insects with large body sizes are more resistant to thermal stress than small-bodied insects (Bishop & Armbruster, 1999; Rohr et al., 2020), this inter-specific difference in thermal resistance can also lead to biased intra-specific mortality between males and females where sexual dimorphism is present (females are usually larger than males). This mechanism is thought to be at the origin of biased sex ratio towards females in beetle populations for example (Bonal, Hernández, Espelta, Muñoz, & Aparicio, 2015). The effects of climate change on sex-specific sensitivity and sex ratio has been widely studied in reptiles for which sex determination is temperature-dependent (Hays, Mazaris, & Schofield, 2014). Nevertheless, rapid changes in temperature or precipitation can also modify sex ratio in other taxa such as mammals (Mysterud, Yoccoz, Stenseth, & Langvatn, 2000; Peeters et al., 2017; Post, Forchhammer, Stenseth, & Langvatn, 1999), plants (Petry et al., 2016) and insects (Bonal et al., 2015). Effects of climate change on sex-specific mortality, sensitivity and sex-ratio in insect species could help better understand how climate change could alter species demography.

Sex-specific sensitivity to climate can also lead to differences in phenological shifts between males and females. Temporal mismatches between interacting species in the context of climate change can affect ecosystems' functions and species demography (Bartomeus et al., 2011; Kharouba et al., 2018; Plard et al., 2014; Ren et al., 2020; Renner & Zohner, 2018). Temporal mismatches between males and females of the same species induced by rapid environmental change have been observed in some species of migratory birds for which males have shifted their timing of arrival more than females under rapid warming (Gordo, Tryjanowski, Kosicki, & Fulín, 2013; Møller, 2004). Protandry, i.e., the earlier arrival or emergence of males, is frequently observed in many species of insects and birds (Møller, 2004; Teder, 2014). However, in some natural populations of protandrous insects, a significant portion of females can die unmated as a result of reproductive asynchrony (Calabrese et al., 2008; Larsen, Calabrese, Rhainds, & Fagan, 2013), especially when the degree of protandry is too high (Larsen et al., 2013). Under rapid climate change, the reproductive asynchrony could be enhanced, with the potential to affect mate availability and population fitness (CaraDonna et al., 2018; Larsen et al., 2013; Miller-Rushing, Høye, Inouye, & Post, 2010).

True flies (Diptera) are the most abundant and diverse group of insects at northern latitudes, contributing to several ecological functions such as pollination and decomposition. In the Arctic, the larvae of flies in the family Muscidae (e.g., house flies and their relatives) are mostly predators of other fly larvae or saprophagous in tundra soils while the adults are predaceous (*Spilogona* spp.) and/or visit flowers to feed on nectar and occasionally pollen (Michelsen, 2015). Muscid flies are important pollinators in the Arctic, where syrphid flies and bumblebees are less dominant than in southern ecosystems (Elberling & Olesen, 1999; Tiusanen, Hebert, Schmidt, & Roslin, 2016). In Greenland, muscid flies have shown significant population abundance declines over the last two decades (Loboda et al., 2018) as well as shorter and earlier periods of activity in the spring as a consequence of rapid warming (see Chapter 3). The reduction of the temporal overlap of these pollinators with their flower resources has been suggested to be a contributor to these large population declines (Høye, Schmidt, Trøjelsgaard, & Forchhammer, 2013; Schmidt, Mosbacher, et al., 2016).

The objective of this study is to investigate the potential influence of sex-specific sensitivity, abundance changes and phenological shifts on temporal variations in muscid abundance. First, we tested whether the rates of abundance change over time varied for males and females of six species of muscid flies collected at Zackenberg, Greenland, between 1996 and 2014 and we also calculated how the sex ratio of each species changed over the study period. Second, we quantified whether differences in the magnitude of phenological shifts over the study period between males and females could have resulted in a change in the reproductive period. We then tested whether differences in the temporal trends in abundance and phenology result from a difference in temperature sensitivity between males and females. Finally, we analyzed the potential relationship between species abundance and change in reproductive phenology with a time-lagged approach to account for the length of the life cycle.

4.3 Material and methods

4.3.1 Study site and data collection

In the Zackenberg Valley, in North-East Greenland, insects are monitored each summer, from snowmelt (in May-June) to snowfall (in September) since 1996 as part of the Zackenberg Ecological Research Operation (Schmidt, Hansen, Hansen, Berg, & Meltofte, 2016). Zackenberg has experienced a significant increase in summer and spring temperatures between 1996 and 2014 (Loboda et al., 2018). At Zackenberg, muscid flies were captured in three habitats (a wet fen, a mesic heath, and an arid heath) with two yellow pitfall traps per habitat. Yellow pitfall traps measured 10 cm in diameter and 8 cm deep (Schmidt, Hansen, et al., 2016). In each habitat, each pitfall trap was randomly placed within a plot of 5 x 5 meters, so the average distance between the two traps was 5 meters. Traps were emptied every week during the active season (see details in Schmidt, Hansen, et al., 2016). A total of 18,385 adult muscid flies was sampled and identifies to species between 1996 and 2014. No specimens were available for examination in

2010, as samples from that year were lost in transit. All individuals were identified to species using morphology, sexed, and species identities were confirmed with DNA barcoding (Collin, 1930; Huckett, 1965; Loboda et al., 2018; Michelsen, 2006, 2015).

4.3.2 Statistical analyses

4.3.2.1 Temporal trend in sex-specific abundance and trends in sex ratio

We focused the analyses on species for which total annual abundance was minimum 10 for at least 13 years to maximize the statistical power to detect a non-random trends (see Didham et al., 2020). Based on this criteria, we thus analyzed trends in sex-specific abundance and phenology for the six most common species of muscids in the samples from Zackenberg: *Drymeia segnis* (Holmgren), *Spilogona sanctipauli* (Malloch), *Spilogona zaitzevi* (Schnabl), *Spilogona almqvistii* (Holmgren), *Spilogona dorsata* (Zetterstedt) and *Spilogona tundrae* (Schnabl) (Loboda et al., 2018). Total number of active trapping days per summer differed between years so we first standardized abundance from each trap and each sampling period (usually one week) as a number of individuals per trap day. Temporal trends in standardized abundance of females and males of each muscid fly species were assessed using Mann-Kendall trend tests corrected for temporal autocorrelation. We then evaluated if the rate of temporal change in abundance was different between males and females of each species by modeling abundance (log-transformed for *D. segnis*, *S. sanctipauli* and *S. zaitzevi* and Tukey transformed for *S. almqvistii*, *S. dorsata* and *S. tundrae* with the function *transformTukey* of the rcompanion package (Venables & Ripley, 2002). We then used the Mann-Kendall trend test corrected for temporal autocorrelation when detected by the Ljung-Box test to calculate temporal trends in sex ratio over the study period for each species, calculated as the proportion of males (annual abundance of males/annual abundance of all individuals of the species).

To evaluate whether temporal trends in abundance differ between males and females across species, we used one linear mixed-effect model with time (Year), sex as independent factors and standardized abundance (Tukey-transformed) as the dependent variable, the interaction between these two factors (Year*Sex), and species as a random effect to control for non-independency of data within species. A generalized mixed-effect model with binomial distribution was also used to model the sex ratio as a function of time (Year) with species as a random effect to determine the general temporal trend in sex ratio.

4.3.2.2 Temporal trends in sex-specific phenology and trends in the reproductive period

We measured the timing of the yearly flight period for males and females of each species as the date of onset, peak, end, and duration of the active period calculated as the number of days between the onset and the end date of the flight period. In order to remove the effects of variation in trapping effort within and between seasons, weekly abundance values were standardized as a number of individuals per day per trap. We modeled seasonal variation in the standardized abundance of males and females using the smoothing method with generalized additive models (GAMs) with the quasi-Poisson distribution, between early June (Day of year 152) and early September (Day of year 245). Annual abundance for each sex was modeled with GAM only if 1) at least 20 individuals were collected in total between 1996 and 2014; 2) if individuals were captured at least three times within the season (minimum of 3 individuals within each season); and 3) if the peak of the flight period could be modeled completely (for example, when sampling started too late or stopped too early in a season, we did not have enough information at the beginning of the season for early-active species or the end of the season for late-active species, and we were not able to model the seasonal abundance properly). Each GAM was inspected visually to see if it fits the data well, normality of model residuals was assessed with QQ plots, and the k-index was used to evaluate how adequate the basis dimension was for each GAM (*gam.check* function from the mgcv package (Wood, 2011)). We then determined the dates at which 10%, 50%, and 90% of the area under the curve obtained with the GAMs were reached, representing the annual timing of the onset, peak, and end of the flight period, respectively. Temporal trends in phenology (onset, peak, end, and duration of the flight period) of females and males were analyzed by species with Mann-Kendall trend test corrected for temporal autocorrelation as assumptions for parametric tests were not met (Patle, Libang, & Ahuja, 2016). Finally, for each species, we modeled phenology (onset, peak, end, and duration of the flight period) as a function of time (Year), sex, and the interaction between these two factors (Year*Sex). A significant interaction term in the linear model would indicate that males and females have different temporal trends in phenology. The length of the reproductive period was measured annually as the number of days when males and females were both active during the season. We evaluated temporal changes in the length of the reproductive period (number of days) with linear regression since all assumptions were satisfactory (tested with the *gvlma* function (Peña & Slate, 2006)).

We examined whether temporal trends in phenology differ between sexes across species with mixed-effect models (LMMs) using the time (Year) and Sex as fixed-effects and Species as a random effect to account for non-independence of data within species. We also included the interaction between Year and Sex to test for differences between males and females in temporal trends (Year*Sex in Tables). We fitted generalized linear mixed-effect models (gamma distribution with link log) for phenology (onset, peak, end or duration of the flight period) and then one generalized linear mixed-effect models using penalized quasi-likelihood (*glmmPQL* function in the MASS package, quasi-Poisson family (Venables & Ripley, 2002)) for the length of the reproductive period.

4.3.2.3 Sex-specific sensitivity of phenology and abundance to temperature

We used a sliding window approach to determine which climate window best relates to muscid fly phenology for each species (Bailey & Van De Pol, 2016; van de Pol et al., 2016). The mean temperature during the period that was best correlated with the peak of the flight period of each species is referred to as the ‘climate window’ of a species. To run the sliding window analyses, we used the average date of peak activity of a species across the years as the date of reference (calculated in Chapter 3) and restricted the possible starting date of the window to vary from 0 to 300 days before this reference. AICc values were calculated for each model and compared to find the best climate window. Because multiple testing can inflate the risk of type I errors (false positive; see Bailey & Van De Pol (2016)), we apply the same analysis to a number of randomizations of the phenological data and used the P_c probability statistic developed by Van de Pol et al. (2016) to identify models with high likelihood that the relationship between temperature and phenology was not due to chance.

Once we found the best temperature window for each species, we calculated the annual mean temperature within that time window. We then extracted residuals of the simple linear regression of peak of emergence, abundance, and temperature against time. This technique allowed us to obtain ‘detrended’ variables and avoid spurious relationships between time series (Iler, Inouye, Schmidt, & Høye, 2017). We used two linear models per species first to test the relationship between the timing of peak emergence and temperature (both detrended) with sex as a fixed factor for each species, and then, the relationship between abundance and temperature in the same way. A significant interaction between sex and temperature would indicate significant differences in temperature sensitivity between males and females. To determine general sensitivity, we finally modeled two mixed-effect models, one with detrended phenology as the dependent variable, the other with detrended abundance, as a function of detrended temperature, sex, and the interaction between the two independent variables, with species as a random factor.

4.3.2.4 Relationship between reproductive phenology and population abundance

The effect of the length of the reproductive period on species abundance was examined using linear distributed lag functions for lags of one, two, three, and four years. For each species, we built four linear regression models of abundance in function of the length of the reproductive period with the distributed lag matrices created with different lags (t-1, t-2, t-3, and t-4). We did not include *S. almqvistii* in this analysis because the data was not sufficient to calculate lagged effects. We then selected the lagged predictor variable based on the highest R^2_{adj} of the model and the *p*-value associated with the lagged variable (Results of each model can be found in Supplementary Table 4-1). Once we found the best-lagged predictor variables for each species, we built a linear model using generalized least squares with abundance as the dependent variable and the lagged length of the reproductive period as the predictor, and time (Year) to account for the temporal trend. We also specified the correlation structure (temporal autocorrelation within time series) with the correlation object ‘corARMA.’ Finally, we pooled data to build a linear mixed-effect model of abundance (log-transformed) in year t as a function of lagged length of the reproductive period (t-1 and t-2), with species as a random effect and the ‘corARMA’ structure for temporal autocorrelation structure of data.

All statistical analyses were performed in the R 3.6.1 platform (R Core Team, 2019) using the fume package for the corrected Mann-Kendall trend test (Santander Meteorology Group, 2012), the lme4 and nlme packages to fit mixed-effect models and linear models using generalized least squares (Bates, Maechler, Bolker, & Walker, 2015; Pinheiro, Bates, Debroy, Sarkar, & Team, 2019), the climwin package for the sliding window analyses (Bailey & Van De Pol, 2016), the dlnm package for linear distributed lag matrices (Gasparrini, 2011) and the ggplot2 package to create figures (Wickham, 2016).

4.4 Results

4.4.1 Temporal trend in sex-specific abundance and sex ratio

With the exception of *D. segnis* males, the abundance of males and females of all species showed a significant decline over the study period (detailed temporal trends of each sex per species are presented in Supplementary Table 4-2). We found no significant differences in the rates of abundance change over the study period between sexes for each species individually (Figure 4-1 left column, see details in Supplementary Table 4-3,) or across species with the mixed-effect model ($F_{1,183}$=3.88, *p*=0.0506).

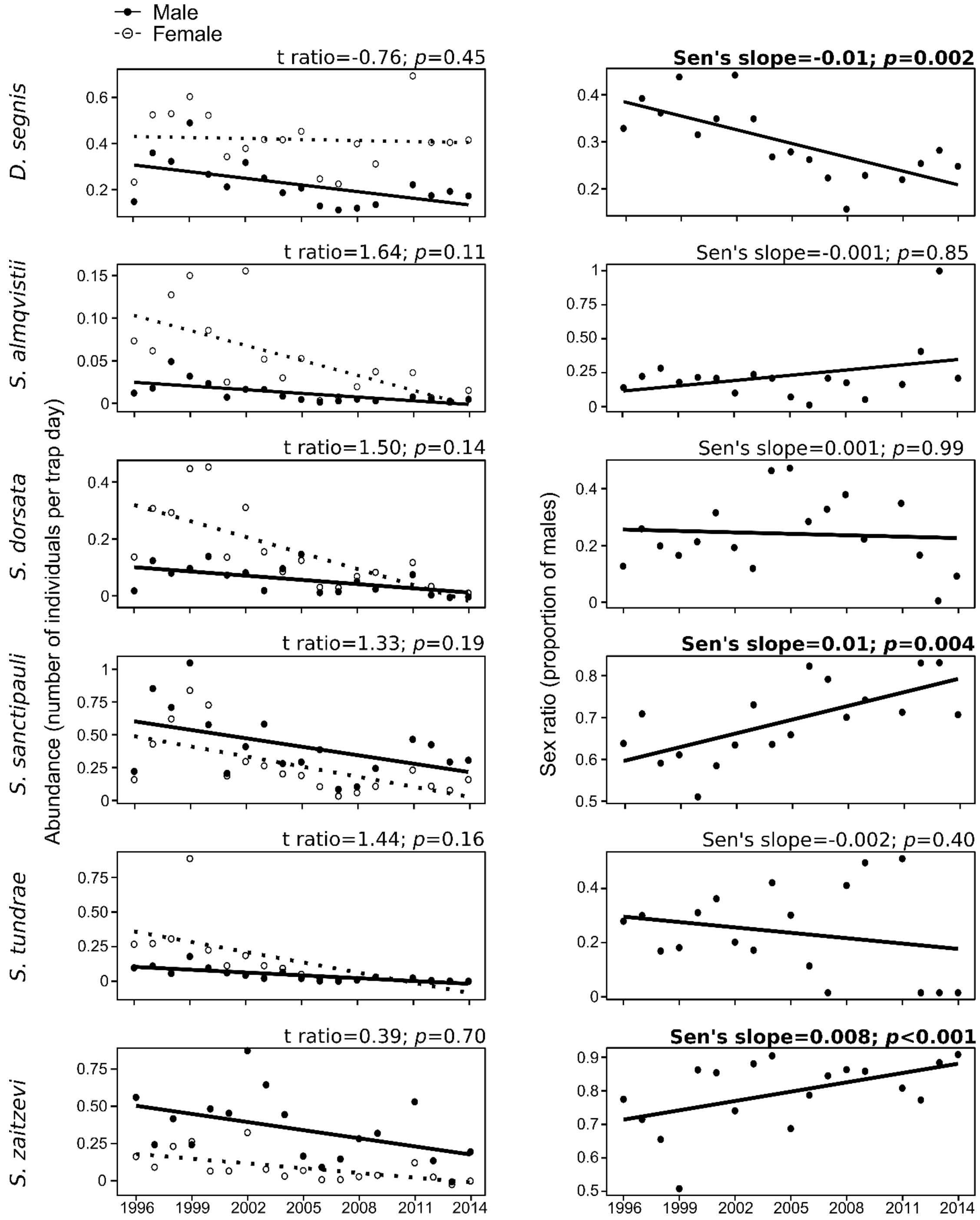

Figure 4-1 Temporal trends in sex-specific abundance of six muscid fly species between 1996 and 2014 (left column), and the associated temporal trend in sex ratio (right column). The effect of sex on temporal trends in abundance is indicated above the figure in the left column (Details on the linear regression of abundance against time and sex are presented in Supplementary Table 4-3). Temporal trends in sex ratio are indicated above the figure in the right column (calculated with the Mann Kendall trend test corrected for temporal autocorrelation). Values of the temporal trends in sex-specific abundance are presented in the Supplementary Table 4-2.

Across species, sex ratio did not change significantly over time ($F_{1,108}$=1.80, *p*=0.33). However, this result masked considerable variation among species. *Spilogona zaitzevi* (tau=0.36, Sen's slope=0.008, *p*<0.001) and *S. sanctipauli* (tau=0.50, Sen's slope=0.01, *p*=0.004) were the two species for which the proportion of males increased significantly over time (Figure 4.1). The proportion of males of *D. segnis* decreased significantly during the same period (tau=-0.54, Sen's slope=-0.01, *p*=0.002; Figure 4-1).

4.4.2 Temporal trends in sex-specific phenology and the reproductive period

Slopes of temporal trends of muscid phenology of males and females at the species level were all negative, indicating that timing of the flight period advanced over time for both segments of the population (Figure 4-2 left column, see details Supplementary Table 4-4). When testing with mixed-effect models, significant advancements in the peak and the end of the flight period were found, with a significant reduction of the length of the flight period (Table 4-1). Sex had a significant effect on phenology because males were active before females for most species (Figure 4-2, left column), and males had in general a shorter flight period than females (See 'Sex' in Table 4-1, the average duration of the flight period across years and species was 23.3 days for males and 31.3 days for females). However, no differences in temporal trends in phenology between males and females were observed at the species level (Figure 4-2, left column for the peak of activity) or across species (see interaction term Year*Sex in Table 4-1).

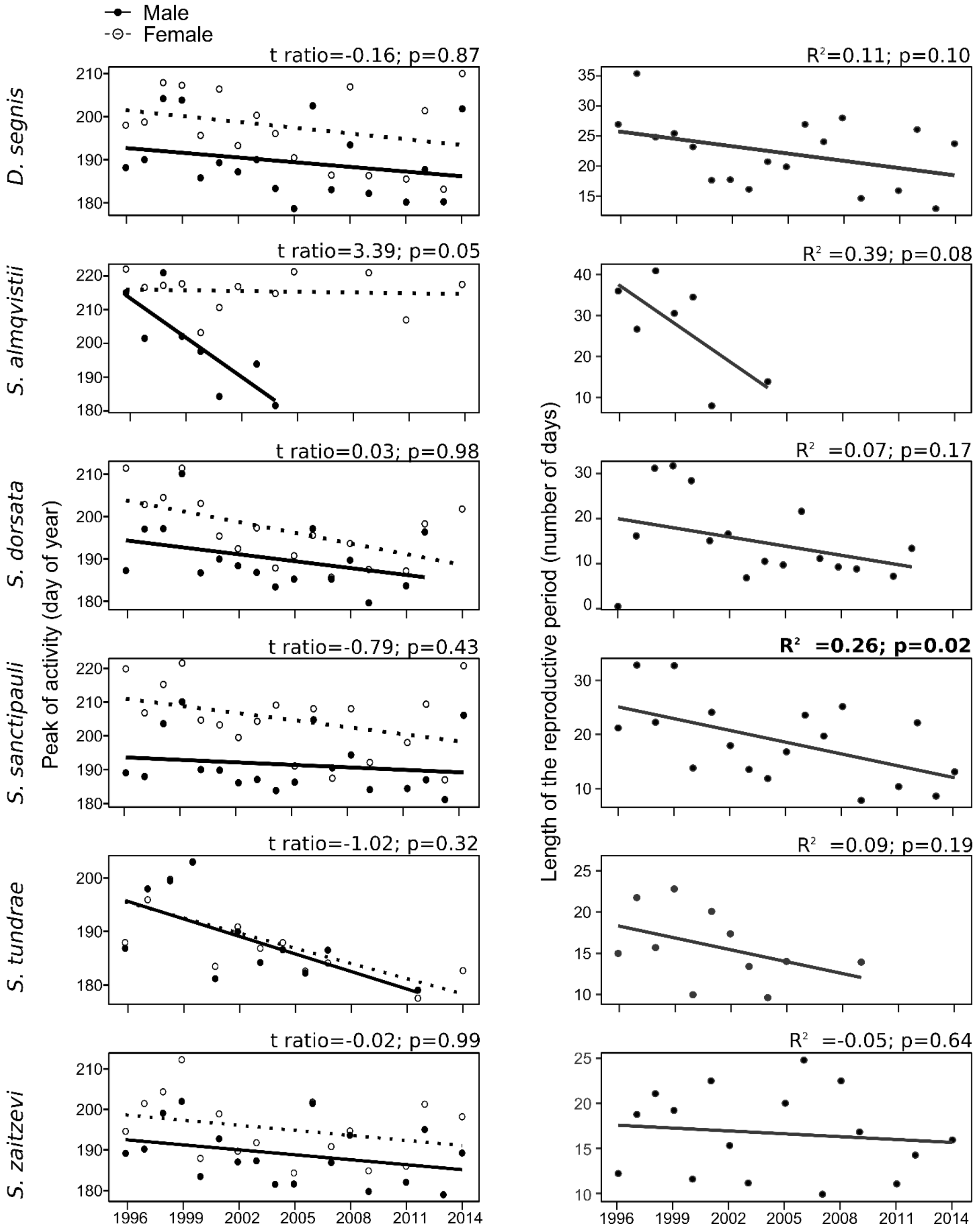

Figure 4-2 Temporal trends in the peak of activity for males and females (left column), and in the length of the reproductive period (right column) of the six muscid fly species between 1996 and 2014. Values of the temporal trends of sex-specific phenology (onset, peak, end, and duration of the flight period) are presented in the Supplementary Table 4-4 Results from the comparison of sex-specific temporal trends in the peak of activity performed with linear regression (interaction term Year*Sex) are presented above each panel on the left column. Comparisons of temporal trends in sex-specific phenology for the onset, end, and duration of the flight period are presented in Supplementary Table 4-5. Results of the linear regression of the length of the reproductive period over time are presented above each panel of the right column. Detailed results of the linear

regression of the length of the reproductive period over time per species are presented in Supplementary Table 4-6.

Table 4-1 Results of the generalized mixed-effect models of phenology (onset, peak, end, and duration of the flight period) as a function of time (Year) and sex of muscid fly species caught at Zackenberg, Greenland, between 1996 and 2014. Negative estimate values for 'Sex' indicates that males are active before females. Detailed values of temporal trends in onset, peak, end, and duration of the flight period of males and females of each muscid species are presented in Supplementary Table 4-4.

Phenological event	Variable	Estimate	Std. Error	t value	*p*
Onset	Year	-0.01	0.00	-1.36	0.17
	Sex	**-0.03**	**0.01**	**-4.29**	**<0.01**
	Year*Sex	0.00	0.01	-0.47	0.64
Peak	**Year**	**-0.02**	**0.00**	**-3.47**	**<0.01**
	Sex	**-0.05**	**0.01**	**-6.90**	**<0.01**
	Year*Sex	0.00	0.01	-0.04	0.97
End	**Year**	**-0.02**	**0.00**	**-3.08**	**<0.01**
	Sex	**-0.06**	**0.01**	**-8.75**	**<0.01**
	Year*Sex	-0.01	0.01	-1.56	0.12
Duration	**Year**	**-0.08**	**0.03**	**-2.82**	**<0.01**
	Sex	**-0.30**	**0.04**	**-7.46**	**<0.01**
	Year*Sex	-0.08	0.04	-1.89	0.06

Changes in the length of the reproductive period over the study period was only significant for *S. sanctipauli* (Figure 4-2, right column). However, results from mixed-effect models showed a significant reduction in the length of the reproductive period by 5.67 ± 1.58 days per decade (X_{86}=13.31, p<0.001) across species, a consequence of a reduced activity period for both, males and females (the average length of the reproductive period across species and years was 19.47 days).

4.4.3 Sex-specific temperature sensitivities

As the temperature increased, the activity of muscid flies was earlier ($F_{1,173}$=211.09; *p*<0.001), and abundance decreased ($F_{1,199}$=7.83, *p*=0.006). However, we did not find differences in temperature sensitivities of phenology for males and females at the species level (Figure 4-3, left column) or across species with the mixed-effect models ($F_{1, 173}$=0.35; *p*=0.56). Furthermore, no difference in temperature sensitivity of abundance for males and females was found across species neither ($F_{1,199}$=2.97; *p*=0.09). Only individuals of *S. tundrae* showed sex-specific temperature sensitivities of abundance (Figure 4-3, right column). The abundance of females *S. tundrae* declined faster than the abundance of males as the temperature increased.

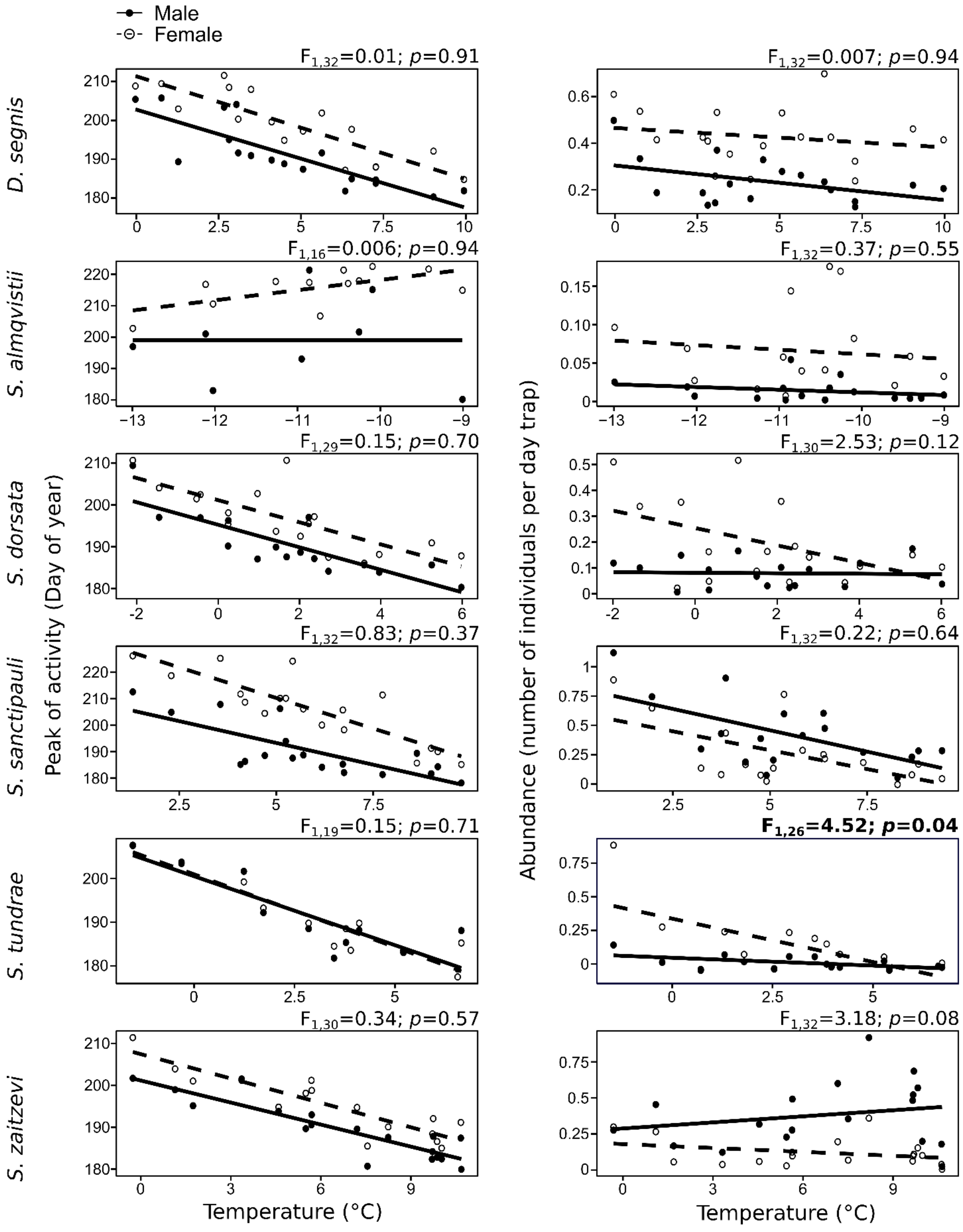

Figure 4-3 Relationships between the timing of the peak of activity (left column) and the abundance (right column) with temperature for males and females of six muscid fly species sampled at Zackenberg, Greenland, between 1996 and 2014. Significant effect of sex on temperature sensitivity is indicated in bold.

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4.4.4 Relationship between the reproductive phenology and species abundance over time

Significant associations between the length of the reproductive period and the annual abundance the following years were found only for *D. segnis* and *S. sanctipauli* (Table 4-2). After pooling data for all species, the linear mixed-effect model identified a positive and significant relationship between abundance in a given year and the length of the reproductive period in the two previous years (t-1:t value= 2.36; *p*=0.02; t-2: t value=2.71, *p*=0.01; Figure 4-4 for t-1).

Table 4-2 Results of the linear models of population abundance as a function of time and length of the reproductive period using generalized least squares for five species of muscid flies from Zackenberg. Temporal autocorrelation was accounted for in the models. Detailed results of cross-correlation tests for the relationship between abundance and length of the reproductive period are presented in Supplementary Figure 4-1. Results of regressions of species abundance against the lagged length of the reproductive period used to identify the best-lagged effect for the final model are presented in Supplementary Table 4-1.

Species	Variable	Value	Std. error	t-value	*p*	Chi
D. segnis	**Length$_{t-2}$**	**0.03**	**0.01**	**2.42**	**0.04**	**5.84**
S. dorsata	Length$_{t-1}$	-0.01	0.01	-0.67	0.52	0.45
S. sanctipauli	**Length$_{t-1}$**	**0.04**	**0.01**	**3.31**	**0.01**	**10.93**
S. tundrae	Length	0.01	0.03	0.27	0.80	0.07
S. zaitzevi	Length$_{t-2}$	-0.03	0.02	-1.62	0.20	2.61

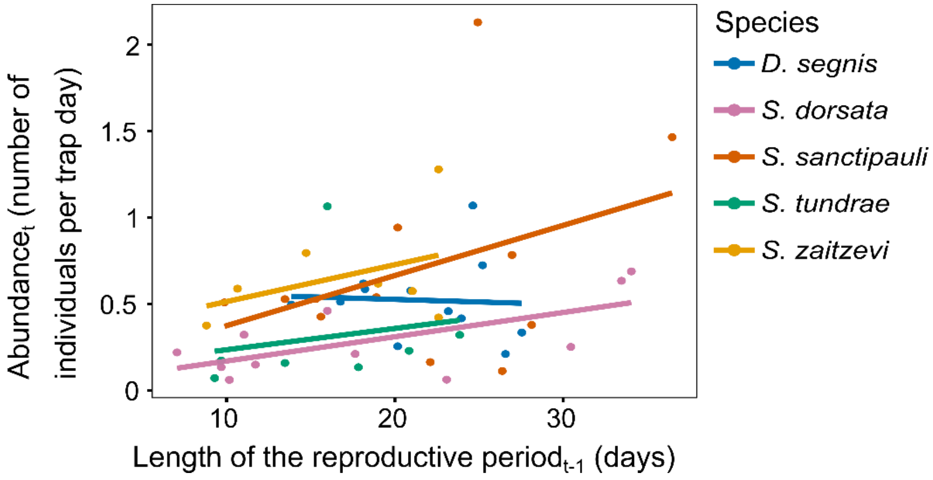

Figure 4-4 Relationships between abundance and the length of the reproductive period for five species of muscid flies from Zackenberg, Greenland, between 1996 and 2014.

4.5 Discussion

The objective of this study was to document sex-specific abundance changes and phenological shifts of six species of Arctic muscid flies between 1996 and 2014 at Zackenberg. Male and female abundance of muscid flies decreased significantly over the study period, and at a similar rate for both sexes. However, significant changes in the sex ratio for three species indicated that some differences in male vs. female responses exist, and we were unable to reject the null hypothesis that changes in abundance were similar for both sexes with the data used. The flight period of males and females have advanced and shortened significantly between 1996 and 2014. The shrinkage of the flight period for both sexes has resulted in shorter reproductive period of muscid flies over time, and this reduction was associated with a subsequent decrease in species abundance over time for some species.

We investigated whether sex differences in the magnitude of phenological shifts over the study period could have resulted in a change in the reproductive period. We found that males and females muscid flies advanced their period of activity at a similar rate over time, resulting in no temporal mismatch between males and females. This finding is contrary to some previous studies on migratory birds and insects suggesting that in protandrous species, males could advance their phenology more than females on warmer years (Gordo et al., 2013; Larsen et al., 2013; Møller, 2004). A possible explanation for this might be that male and female muscids have similar temperature sensitivities. However, the reproductive period of these flies has been reduced by 5.67 days per decade, not because of a change in the degree of protandry over time, but because of a contraction in the duration of the flight periods of both sexes. This shrinkage was associated with species abundance decline the following year and the one after that across species. There are several likely mechanisms behind this association. First, this correlation could result from a reduction in the flies' longevity, which limits the number of eggs produced by the female and, thus, the overall fitness of a population (Moon, 2019). Second, under warmer temperatures, insects tend to accelerate their development, thereby sacrificing a portion of the body size they reach as adults (Bowden, Eskilden, Hansen, Olsen, Kurle, & Høye, 2015; Daly, 2018; Gardner et al., 2011; Sheridan & Bickford, 2011). This fast development could result in a shorter life span and reduced fecundity because of the smaller body size (Berger, Walters, & Gotthard, 2008). Thus, in this case, the abundance of a population would depend more on the duration of the active period of females than on the reproductive period per se. Third, the significant relationship between abundance and length of the reproductive period could result from a density-dependent competition increase. A more condensed period of activity can increase intra- and inter-specific competition for limited resources, which could in turn influence population size over time. Muscid flies in the *Spilogona* genus are predators of soft-bodied insects like simuliids and chironomids (Michelsen, 2015), two families of flies in decline at Zackenberg between 1996 and 2009 at Zackenberg (Høye et al., 2013). The drastic drop in prey abundance, and the potential increase in competition offer a possible explanation for the decline in *Spilogona* spp compared to relatively stable abundance over time in *Drymeia* spp at Zackenberg (Gillespie et al., 2020; Loboda et al., 2018).

Fourth, and finally, population sizes and densities could be small enough to increase the chances that a mate won't be found, a mechanism that is part of the phenomenon called the Allee effect (Allee & Bowen, 1932). Failure to find a mate could also result from a biased sex ratio. In muscid flies, the sex ratio of adults emerging from the pupae is expected to be 1:1, regardless of larval conditions (Moon, 2019). For the two species with more males than females in the samples (*S. sanctipauli* and *S. zaitzevi*), we observed a significant increase in the male-biased sex ratio and a significant reduction in the reproductive period indicating that mating could indeed be limited by unsuccessful mating. Unlike *S. sanctipauli* males who use flowers as lookouts to detect females in flight (Michelsen, 2006), males *D. segnis* are known to make swarms during the breeding season to intercept females for mating (Michelsen, 2015). *Drymeia segnis* is the only species with no significant temporal decline in abundance over the study period at Zackenberg. The reproductive behavior of this species might spare them the impact of temporal change in sex ratio on mating.

The current study found that male and female abundance of muscid flies declined over time at a similar rate. A note of caution is due here since this finding is somewhat limited by the length of the time series and the missing data (see Didham et al., 2020). White (2019) showed that temporal trends of abundance at a single site should be measured with at least 15 data points to have enough statistical power to tease apart a trend from natural variations in population sizes.

Even if we did not measure sex-specific abundance trends with less than 15 data points, adding data points in the future might modify some of the current conclusions. The decline in muscid abundance at the family level found in the arid heath between 1996 and 2014 (Loboda et al., 2018) was no longer significant when investigated on a longer study period (1996-2018, Høye et al., 2020). It seems that the combination of warmer temperatures in the fall and higher summer precipitation since 2014 at Zackenberg led to high abundance in many Diptera families in that dry habitat between 2014 and 2018 (Høye et al., 2020). This result illustrates how the length of time series can influence conclusions but also how continued monitoring is crucial for investigating the decline and recovery of insect populations.

Significant changes in sex ratio were found for three species even though the rate of male abundance change over time was not different from the trend in female abundance at Zackenberg, potentially because of limited data (marginally not significant with a *p*-value of 0.0506 see section 4.4.1). For two species of *Spilogona*, we found significant increase in sex-ratio between 1996 and 2014, while for *D. segnis*, we found a significant reduction in the proportion of males over the study period. Different energetic needs and behavior between males and females could lead to differences in mortality. Male-biased mortality could happen because of lower thermoregulatory capacities implied by smaller body size (Bishop & Armbruster, 1999; Rohr et al., 2020) as Arctic muscid species are sexually dimorphic (Michelsen, 2015). If it is the case, male-biased mortality could be amplified in a warmer future since body size is shrinking with temperature increase (Gardner et al., 2011; Sheridan et al., 2011), and more so for males than females in one Arctic spider species (Høye, Hammel, Fuchs, & Toft, 2009). At Zackenberg, female-biased mortality of adult muscid flies could result from the presence of pathogenic fungi. During morphological identifications in the laboratory, we noticed many *Spilogona* and *Drymeia* females with one or two circular holes in the abdomen, a phenomenon induced by *Strongwellsea* sp., pathogenic fungi known from Zackenberg since the beginning of the sampling in 1996 (Meltofte & Thing 1997). Lamb and Foster (1986) also noticed that *Strongwellsea castrans* infected more females than males *Delia* (Anthomyiidae). *Strongwellsea spp*. cause abdominal holes in the host while still alive, from which spores are disseminated (Goettel, Eilenberg, & Glare, 2010), it is therefore plausible that we had a high percentage of infected individuals in our samples without noticing the presence of the fungi. Further work is required to establish how the rise in temperature in the Arctic, parasitism, or changes in body size affect sex-specific abundance of insect species.

In this study, we have found that rapid warming has reduced the length of the reproductive period of some insect species, which could, in turn, indirectly affect population abundance over time. The present results raise the possibility of sex-biased mortality over time for some species of muscid flies, accelerated development of Arctic insects, and the associated reduction in body size. Indirect and complex effects of climate change on population dynamics will surface as the temperature will continue to rise in the future, and these effects will be more apparent in the Arctic region (CAFF, 2013; IPCC, 2019). Developing models of insect population dynamics for some Arctic insect species which consider experiments and field observations to forecast and predict population size changes over time could be of valuable help when estimating the role of climate change on insect decline in temperate and tropical regions.

Our results illustrate how sex-specific responses to climate change could contribute to the great variation in species responses observed within a community, including the worrying decline in insects. Understanding the contribution of sex-specific responses to temporal patterns at higher biological scales in the context of climate change is a promising and challenging avenue of research that will require the combination of experiments and long-term monitoring data. Experiments on the survival of different life stages of males and females, and the different mechanisms of mate limitation through phenological shifts and sex ratio changes should be prioritized. Our study, one of the first to investigate sex-specific responses of multiple Arctic insect species to rapid environmental changes, highlights the need to account for intra-specific responses to better understand the effects of climate change on Arctic biodiversity.

4.6 Acknowledgments

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Chapter 5 General discussion

5.1 Effects of climate change on insect abundance

Insect populations are in decline in many habitats and regions of the world because of intensive agriculture and habitat fragmentation, but climate change is also a significant factor. The Arctic region provides a unique opportunity to examine the effects of climate change on insect demographics in a simplified system far from direct anthropogenic stressors. Using data from Zackenberg, Greenland, I find that the abundance of muscid flies from three different habitats were in decline between 1996 and 2014 (Chapter 2), and that this decline is associated with summer temperature increase over the study period (Table 2-1). Population decline was more pronounced in the wet fen than in the arid and mesic heath suggesting that the direct effect of temperature on insects is modulated by factors at a small spatial scale (Bowden, Hansen, Olsen, & Høye, 2015). However, my study is restricted geographically and taxonomically. The lack of replication (only one site) makes generalizations to other regions, or other parts of the Arctic region, or other taxa difficult. As it is now, the paucity of monitoring programs in the Arctic following insects and other invertebrates properly prevents comparisons of species-level temporal patterns with other Arctic sites. However, the standardization of Arctic monitoring programs proposed by the Conservation of the Arctic Flora and Fauna (CAFF) working groups is slowly being set up across the Arctic region and will provide valuable comparative data across sites and habitats for future research (Gillespie, Alfredsson, Barrio, Bowden, Convey, Coulson, et al., 2020; Gillespie, Alfredsson, Barrio, Bowden, Convey, Culler, et al., 2020; Petersen, Zockler, & Gunnarsdottir, 2004). Moreover, temporal patterns of muscid fly species abundance observed at Zackenberg would have been different in southern regions where multiple direct and indirect stressors can have additive and interactive (synergistic and antagonistic) effects on biodiversity.

Comparisons of temporal patterns of biodiversity change in the Arctic with those in temperate or tropical regions might help elucidate the relative role of climate change on insect decline.

Temperature increase can affect population size indirectly by altering the development of individuals, particularly ectotherms such as insects. The flight period of Arctic muscid flies at Zackenberg has both advanced and shortened between 1996 and 2014 (Chapter 3) and these phenological shifts were associated to subsequent declines in abundance. These results suggest that insect development has accelerated under warmer temperatures, especially for small insects with lower thermoregulation capacities. Even if the two other traits tested (i.e. phenological niche and anthophilous habits) are not significantly related to phenology, trait-based investigations of population changes over time are promising, providing prior warnings of population collapse (Clements & Ozgul, 2016); however, these investigations are only as good as their information on traits. In the Arctic, as in most part of world, ecological information on insect species of no economic or medical relevance is very limited and many traits that could be of interest (i.e. hibernation strategy, length of life cycle) could not be tested. On the other hand, the reduced number of species in the Arctic region provides a favorable context to document behavior, survival, life cycle, reproduction, and fitness of multiple insect species with minimal observations and experiments (Gillespie, Alfredsson, Barrio, Bowden, Convey, Coulson, et al., 2020; Gillespie, Alfredsson, Barrio, Bowden, Convey, Culler, et al., 2020).

Temperature increase can also influence insect population size by reshaping population structure. I found that the sex ratio in three species of muscid flies at Zackenberg has changed significantly between 1996 and 2014 (Chapter 4). How the sex ratio of a population could be affected by warmer temperatures has been rarely investigated except for species with temperature-dependent sex-determination such as tortoises. This result suggests that males and females are affected by different climate variables and/or have different sensitivities to the same climate variable. Drivers behind the reported patterns could not be identified because my research was mainly descriptive. By investigating changes in abundance and phenology in muscid flies and exploring relationships between patterns and temperature changes, I provided valuable relationships between elements of the ecosystems. However, correlations do not necessarily indicate causal relationships (Berteaux et al., 2006; Krebs & Berteaux, 2006) and these relationships need to be tested to find the underlying mechanism in order to forecast and make projections of insect abundance and biomass changes over time.

5.2 Recommendations for future research

Research projects often lead to more questions than they answer, and this is the case with my work. Future research could evaluate species-level temporal patterns of abundance and phenology of other taxonomic groups from the Zackenberg collection. Taking advantage of this unique collection of arthropods, species-level patterns of abundance could be investigated in multiple taxa with different feeding habits, body sizes, and life cycle, providing helpful information regarding the predictability of traits and the contribution of interspecific interactions in the observed patterns.

It would be interesting to obtain more information on how the life cycle and voltinism of Arctic insects are affected by climate change, and if there is a risk of a developmental trap in this region that could drive populations to local extinction. My results imply that warmer temperatures could induce faster development of flies, resulting in smaller body sizes of individuals and lower fecundity. This hypothesis could be partially tested by measuring individuals present in the Zackenberg collection (this was done for butterflies (Bowden, Eskilden, et al., 2015)), and measuring and counting eggs. While examining muscid samples I often found intact gravid females with eggs in the abdomen. Since the Zackenberg insect collection is kept in ethanol (and not dried like it is often the case for Diptera), counting eggs is feasible and these measures could provide valuable data on the fecundity of Arctic fly species over time. An experiment on how sex ratio influences fecundity could also help to elucidate the role of sex ratio changes on population abundance changes over time.

Holometabolous insects have a complex life cycle, and different stages can inhabit different habitats, experiencing different environmental conditions at each stage (Kingsolver et al., 2011). The results in Chapter 2 indicated a potential low survival of early stages (eggs, larvae, and pupae) of species with aquatic and semi-aquatic preferences in the tundra. It would be interesting to determine the vulnerability of the different life stages to different climate factors, including temperature, humidity, and snow depth.

Recent publications on trophic and community changes in the Arctic region based on barcoding and meta-barcoding have promoted the use of genetic tools to document biodiversity modifications over time with high taxonomic resolution and low costs (Pentinsaari et al., 2020; Wirta et al., 2016; Wirta et al., 2015). My work, however, shows that morphological identification produces high-resolution datasets allowing the documentation of sex-specific changes over time, an aspect of biodiversity change that cannot be investigated using metabarcoding, calling attention to the complementarity and the necessity of both methods to document insect responses to climate change in the projected warmer future.

Van Klink et al., (2020) have documented a decline of 11% in terrestrial insect abundance globally since 1950, but they also found a 9% increase in aquatic insects during the same period, a sign that strategies to improve water quality in the last decades were adequate. Therefore, to improve strategies to mitigate changes in terrestrial insect assemblages, future research could develop into comprehensive population dynamic models that include multiple factors influencing births, deaths, immigration, and emigration of individuals to unwind the "baseline" from the "unusual" variability during the Anthropocene.

Chapter 6 Summary and conclusions

The presence of insects is necessary for the planet, but also for human well-being directly, through their contributions to ecosystem services such as pollination and food production (Noriega et al., 2018). However, the abundance decline of these tiny creatures, rarely considered charismatic, has largely been ignored. There has been recent interest in this topic, and the results are alarming: van Klink et al (2020) showed that insect abundance and biomass are dropping by 9% per decade in terrestrial ecosystems worldwide since 1950. This is a manifestation of the new geological epoch, the Anthropocene, marked by the sixth mass extinction (Dirzo et al., 2014; McGill, Dornelas, Gotelli, & Magurran, 2015). Understanding the relative roles of different stressors and disturbances (direct or indirect) on insect abundance decline is a pivotal step to develop strategies to mitigate the impacts on ecosystems.

My objective was to study the effects of climate change on insect assemblages and populations in the Arctic region, where the common direct human disturbances are less prevalent, but climate change is acute (Arctic Monitoring and Assessment Programme, 2019). True flies (Diptera) dominate Arctic terrestrial insect communities by their diversity, abundance, and biomass, making them arguably ideal targets to study the change in abundance over time at high latitudes (Hodkinson et al., 2013). Muscidae, one family of flies particularly diverse in cold regions, has been shown to decline in abundance since 1996 at Zackenberg, North-Eastearn Greenland, where the most extensive ecosystem monitoring program in the Arctic region is established since 1995 (Høye, Schmidt, Trøjelsgaard, & Forchhammer, 2013).

Taking advantage of this unique dataset, the first objective of this thesis was to investigate the decline in muscid flies at Zackenberg between 1996 and 2014 at the species level and its impacts on assemblage structure (Chapter 2). I identified more than 18, 000 muscid specimens to species, collected annually in three habitats of the Zackenberg Valley and found 16 species. The summer temperature increase was associated with an 80% drop in muscid fly abundance over the study period. The relative abundance of most species has significantly changed between 1996 and 2014, modifying the diversity and the composition of muscid fly assemblages at Zackenberg. Abundance decline and compositional change were more pronounced in the wet habitat, possibly because the top of the soil in the Arctic region got drier over the study period. Composition in each habitat changed over the study period, but no biotic homogenization was observed, suggesting that assemblages in each habitat are still unique despite significant changes in species composition in the wet fen.

Focusing on the 12 most abundant species of muscid flies in the dataset, I explored species-specific changes in phenology and abundance over time and the potential relationship between the two types of responses (Chapter 3). The flight period of muscids has advanced and shortened over the study period as a consequence of temperature increase. These phenological shifts were associated with a decline in species abundance and were higher for small species indicating that bigger ectotherms might be more resilient to temperature changes.

Finally, I explored how sex-specific phenological shifts and abundance changes over time could be related to change in species abundance in the six most common species of muscid flies collected at Zackenberg (Chapter 4). The reproductive period of muscid species, measured as the time window where males and females are active simultaneously, has shortened over the study period as a result of the reduction in the activity period of both sexes, and not because of temporal sexual mismatch. Males and females had similar temperature sensitivities, but we found some indications that female survival could be altered more than male survival under warmer temperatures. The reduction of the reproductive period was associated with the abundance decline over time for some muscid species at Zackenberg.

In summary, temperature increase at Zackenberg could be related to insect demography through possible impacts on the survival of individuals with semi-aquatic preferences, but also through phenological shifts of the flight and the reproductive period.

"We love what amazed us, and we protect what we love"
Jacques Yves Cousteau

Insect decline is an important issue that has been finally acknowledged and talked about in the public media. Building on this momentum, I believe it is now time to consider these little creatures that run the word when developing conservation strategies. The research presented in this thesis should raise alarm bells and signal significant concerns to a range of stakeholders, from entomologists to conservation biologists, policy-makers, politicians, and for the peoples living in Polar Regions. I hope this work also provides inspiration for ongoing research on Arctic insects and their vulnerable ecosystems, and stimulates curiosity for Nature, and insects in particular. As Terry Wheeler liked to say "Go have a look. Explore it. Poke Nature."

Chapter 7 Supplementary material

7.1 Supplementary material for Chapter 2

Supplementary Table 2-1 Number of individual muscid flies identified per species, collected at Zackenberg, NE Greenland, in three habitats between 1996 and 2014 with 4 yellow pitfall traps per habitat in 1996 and 2 yellow pitfall traps in each habitat, each year between 1997 and 2014.

genus	species	Arid heath	Mesic heath	Wet fen
Drymeia	*groenlandica* (Lundbeck, 1901)	53	17	2
	segnis (Holmgren, 1883)	1534	2246	588
Limnophora	*groenlandica* (Malloch, 1920)	5	3	20
Phaonia	*bidentata* (Ringdahl, 1933)	82	2	8
Spilogona	*almqvistii* (Holmgren, 1880)	101	167	214
	deflorata (Holmgren, 1872)	0	0	60
	dorsata (Zetterstedt, 1845)	123	168	1333
	malaisei (Ringdahl, 1920)	26	5	130
	megastoma (Boheman, 1866)	25	79	2
	melanosoma (Huckett, 1932)	0	0	15
	micans (Ringdahl, 1918)	3	4	6
	novaesibiriae (Frey, 1915)	0	0	372
	sanctipauli (Malloch, 1921)	2856	2752	212
	tornensis (Ringdahl, 1926)	2	1	0
	tundrae (Schnabl, 1915)	1	3	1259
	zaitzevi (Schnabl, 1915)	6	38	3862
		4817	**5485**	**8083**

Supplementary Table 2-2 Statistics (estimates, standard error and p-values) of simple linear regression of species-specific abundance in each assemblage over time at Zackenberg. The * indicates that regression values have been corrected for a first-order temporal autocorrelation using the cochrann-orcutt procedure. Bold values indicates significant trend over time (*p* <0.05).

Assemblage	Species	Estimate	Std-error	*p*-value
Across habitats	*Spilogona zaitzevi*	-10.00	4.53	**0.04**
	Spilogona sanctipauli	-11.74	7.61	0.14
	Drymeia segnis	0.63	3.73	0.87
	other species *	-24.19	5.83	**<0.001**
Arid heath	*Drymeia segnis*	-0.66	2.53	0.80
	Spilogona sanctipauli	-6.01	4.15	0.17
	other species	-1.40	0.96	0.16
Mesic heath	*Drymeia segnis*	-0.33	2.17	0.88
	Spilogona sanctipauli	-5.15	4.82	0.30
	other species	-3.40	1.54	**0.04**
Wet fen	*Spilogona zaitzevi*	-9.68	4.54	**0.05**
	Spilogona dorsata *	-7.30	1.35	**<0.001**
	Spilogona tundrae *	-8.14	2.19	**<0.001**
	other species	-3.61	1.60	**0.04**

Supplementary Table 2-3 Statistics (estimates, standard error, and *p*-values) of simple linear regression of diversity measures (Hill numbers 0D,1D, and 2D) in each muscid assemblage studied (across habitats, arid heath, mesic heath, and wet fen) over time at Zackenberg. The * indicates that regression values have been corrected for a first-order temporal autocorrelation using the Cochrane-Orcutt procedure. Bold values indicate significant trends over time ($p<0.05$).

Assemblage	Diversity measure	Estimate	Std-error	*p*-value
Across habitats	Hill ^{0}D	-0.21	0.10	0.06
	Hill ^{1}D	-0.15	0.03	**<0.001**
	Hill ^{2}D	-0.11	0.03	**<0.001**
Arid heath	Hill ^{0}D	-0.22	0.09	**0.04**
	Hill ^{1}D	-0.02	0.02	0.31
	Hill ^{2}D	0.00	0.02	0.99
Mesic heath	Hill ^{0}D	-0.29	0.09	**0.01**
	Hill ^{1}D	-0.06	0.02	**0.01**
	Hill ^{2}D	-0.02	0.01	0.06
Wet fen	Hill ^{0}D*	-0.29	0.13	**0.04**
	Hill ^{1}D*	-0.13	0.03	**<0.001**
	Hill ^{2}D*	-0.12	0.03	**<0.001**

7.2 Supplementary material for Chapter 3

Supporting Table 3-1 Detailed information on the phenological niche, body size, anthophilous habit, and mean timing of activity of the 12 species of muscid flies from Zackenberg. Nb of years: number of years for which phenological dates were calculated.

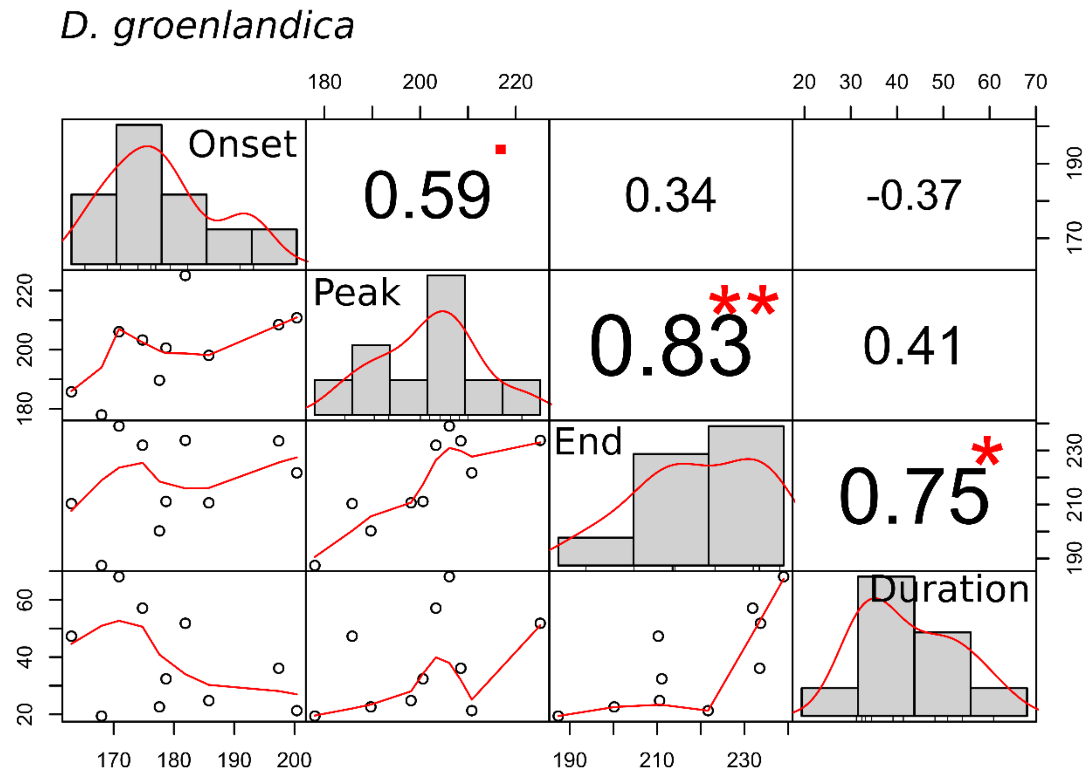
Species	Onset (DOY)	Peak (DOY)	End (DOY)	Wing length (mm)	Anthophilous habits	Total number of ind. collected	Nb of years
Drymeia groenlandica	179.85	200.60	217.94	5.70	irregular	72	10
Drymeia segnis	181.95	198.36	216.03	6.00	frequent	4368	18
Phaonia bidentata	180.30	193.21	206.11	5.60	irregular	92	9
Spilogona almqvistii	192.17	216.7	232.75	6.05	frequent	482	13
Spilogona deflorata	187.19	196.29	210.19	3.75	irregular	60	7
Spilogona dorsata	186.04	198.92	214.53	5.10	frequent	1624	17
Spilogona malaisei	186.66	201.03	218.92	3.85	irregular	161	14
Spilogona megastoma	188.96	201.93	214.84	4.10	frequent	106	7
Spilogona novaesibiriae	178.18	188.50	199.64	4.15	NA	372	11
Spilogona sanctipauli	181.96	197.62	218.75	4.25	frequent	5820	18
Spilogona tundrae	183.22	191.64	199.57	5.25	irregular	1263	15
Spilogona zaitzevi	178.57	188.42	200.19	5.05	irregular	3906	18

Supporting Table 3.2 Results of Durbin-Watson tests to examine the temporal autocorrelation of species' peak of activity and duration of the active period for six species of muscid flies. A p-value >0.05 indicates that the variable is not autocorrelated at a certain lag.

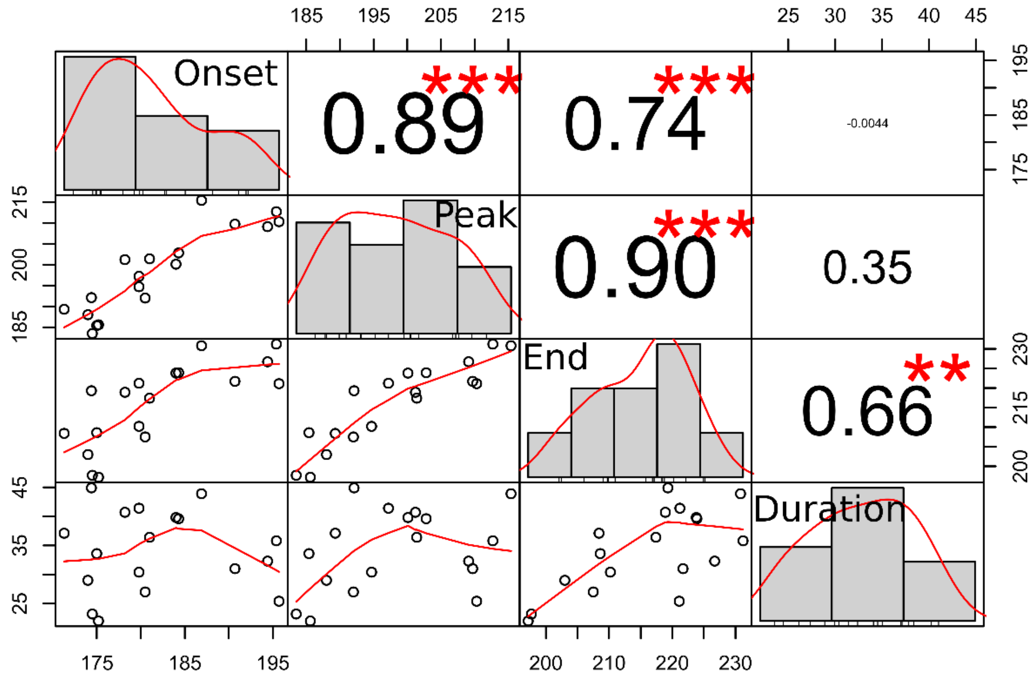
Species	Variable	Lag	Autocorrelation	D-W Statistic	*p*
D. segnis	Peak	1	-0.12	1.86	0.86
		2	-0.11	1.83	0.86
		3	-0.05	1.48	0.75
		4	-0.13	1.52	0.83
		5	0.10	0.95	0.23
	Duration	1	-0.12	1.86	0.94
		2	-0.11	1.84	0.89
		3	-0.05	1.48	0.64
		4	-0.12	1.51	0.75
		5	0.10	0.94	0.23
S. almqvistii	Peak	1	-0.59	3.01	0.09
		2	-0.04	1.80	0.90
		3	0.30	0.83	0.22
		4	-0.21	0.98	0.90
		5	0.09	0.10	0.21
	Duration	1	-0.27	2.50	0.46
		2	-0.03	1.92	0.71
		3	-0.22	2.21	0.13
		4	0.02	0.20	0.21
		5	-0.01	0.14	0.38
S. dorsata	Peak	1	-0.39	2.54	0.34
		2	0.05	1.65	0.77
		3	0.29	1.07	0.28
		4	-0.49	2.62	0.03
		5	0.29	0.64	0.09
	Duration	1	-0.36	2.49	0.31
		2	0.18	1.40	0.35
		3	0.11	1.47	0.55
		4	-0.42	2.55	0.04
		5	0.30	0.69	0.12
S. sanctipauli	Peak	1	-0.15	2.02	0.81
		2	-0.14	2.00	0.89
		3	0.03	1.50	0.71
		4	-0.22	1.87	0.64
		5	0.04	1.07	0.45
	Duration	1	-0.05	1.77	0.77

		2	-0.13	1.91	0.96
		3	-0.10	1.73	0.86
		4	-0.11	1.59	0.99
		5	-0.02	1.17	0.55
S. tundrae	Peak	1	-0.40	2.75	0.12
		2	-0.27	2.45	0.23
		3	0.24	0.86	0.14
		4	-0.17	1.51	0.97
		5	0.23	0.58	0.09
	Duration	1	-0.40	2.80	0.08
		2	-0.11	2.21	0.33
		3	0.04	1.43	0.79
		4	-0.14	1.34	0.74
		5	0.20	0.61	0.08
S. zaitzevi	Peak	1	-0.19	2.23	0.53
		2	-0.23	2.16	0.63
		3	-0.18	1.95	0.59
		4	0.18	1.22	0.35
		5	-0.27	2.09	0.21
	Duration	1	-0.15	2.14	0.67
		2	-0.34	2.36	0.35
		3	-0.09	1.75	0.95
		4	0.14	1.29	0.44
		5	-0.18	1.89	0.38

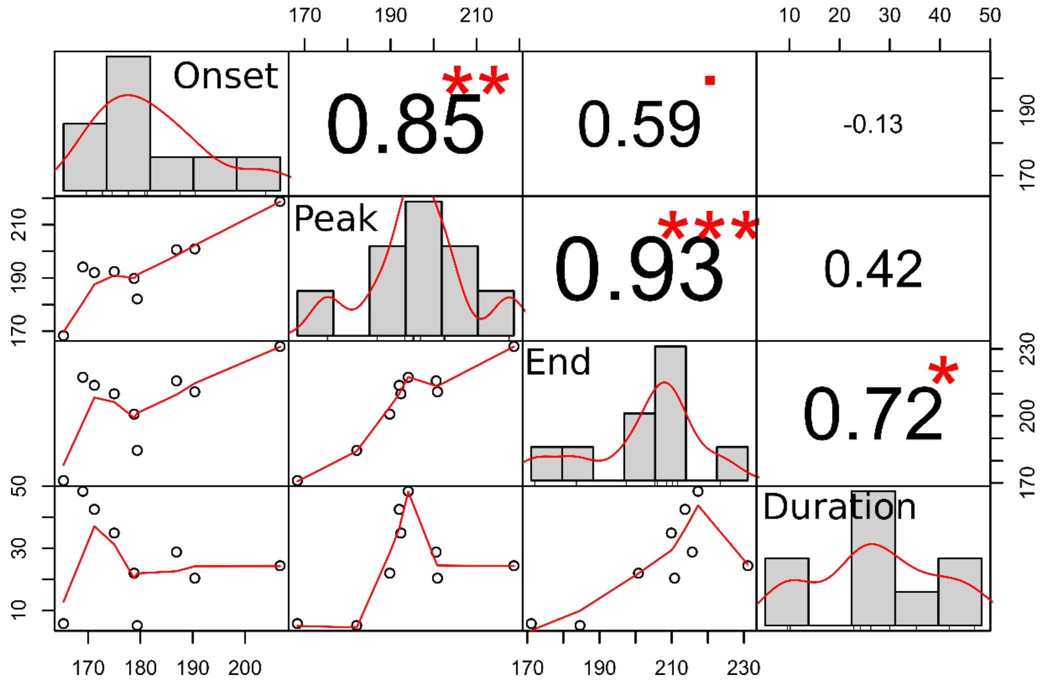
Supporting Figure 3-1 Correlation matrix of the annual onset, peak, end of the activity and, duration of the flight period for each of the 12 species of muscid fly caught at Zackenberg between 1996 and 2014. Numbers are the correlation coefficient from the Pearson's correlation test. Significant correlations are indicated by asterisks (* when *p*<0.05,** when *p*<0.01, *** when *p*<0.001)



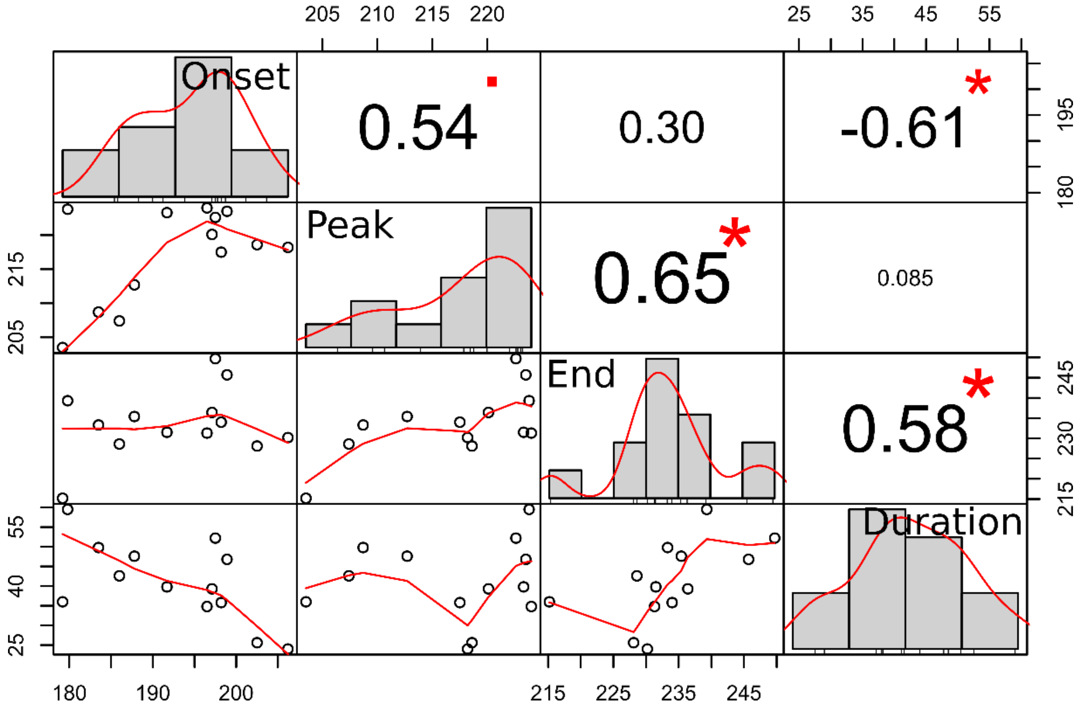
D. segnis



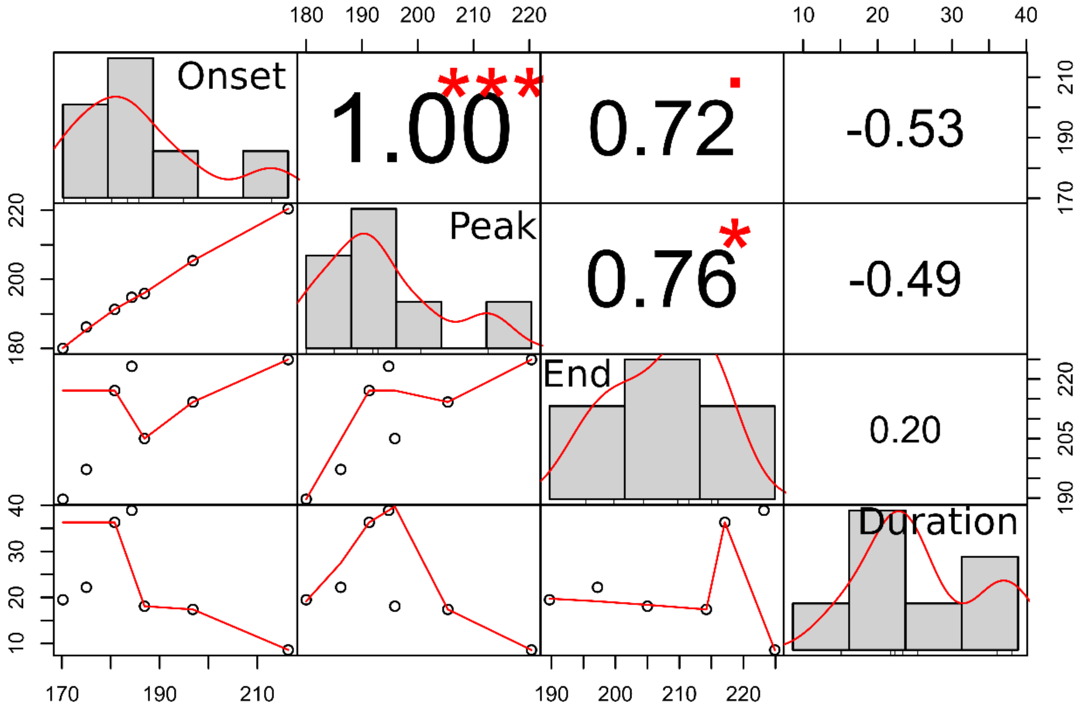
P. bidentata

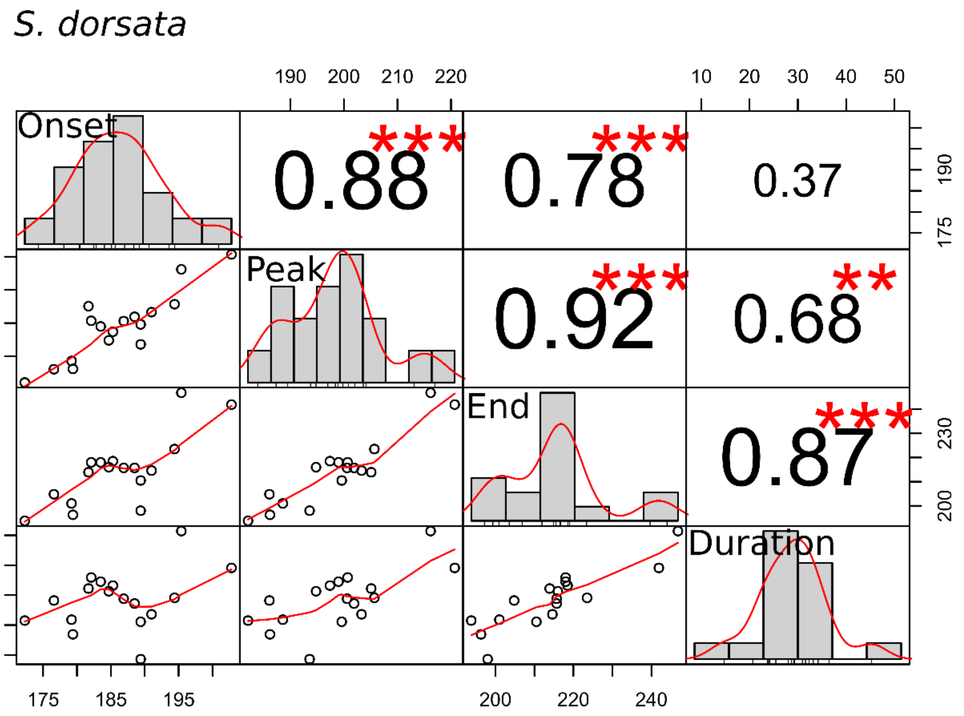


S. almqvistii

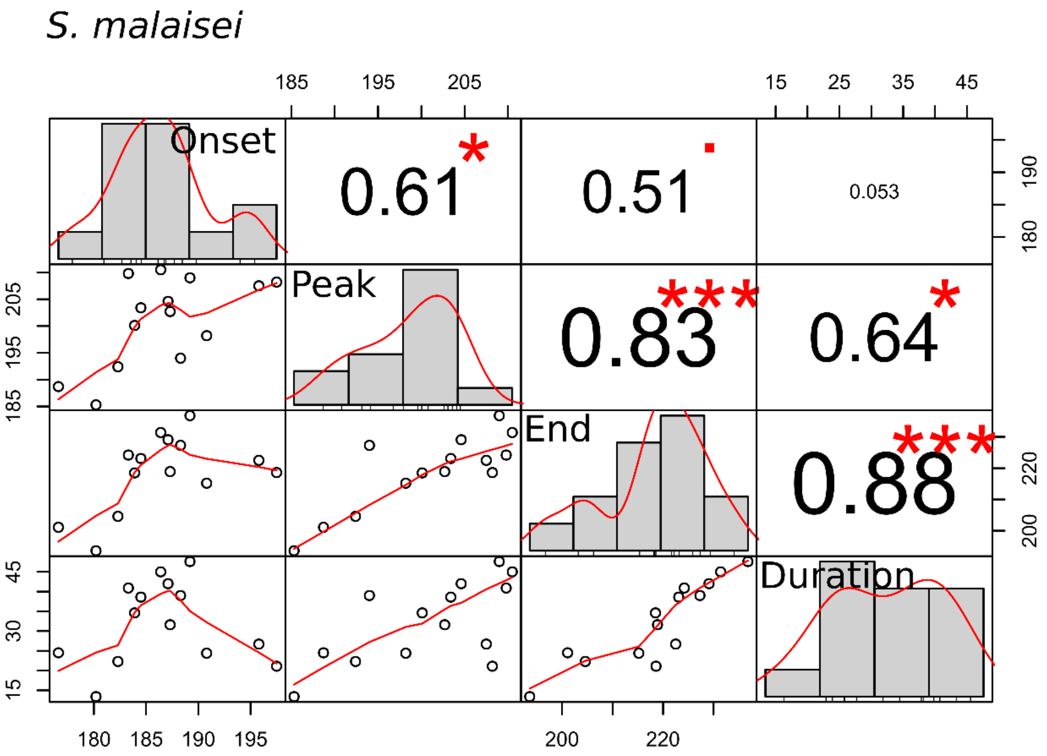


S. deflorata



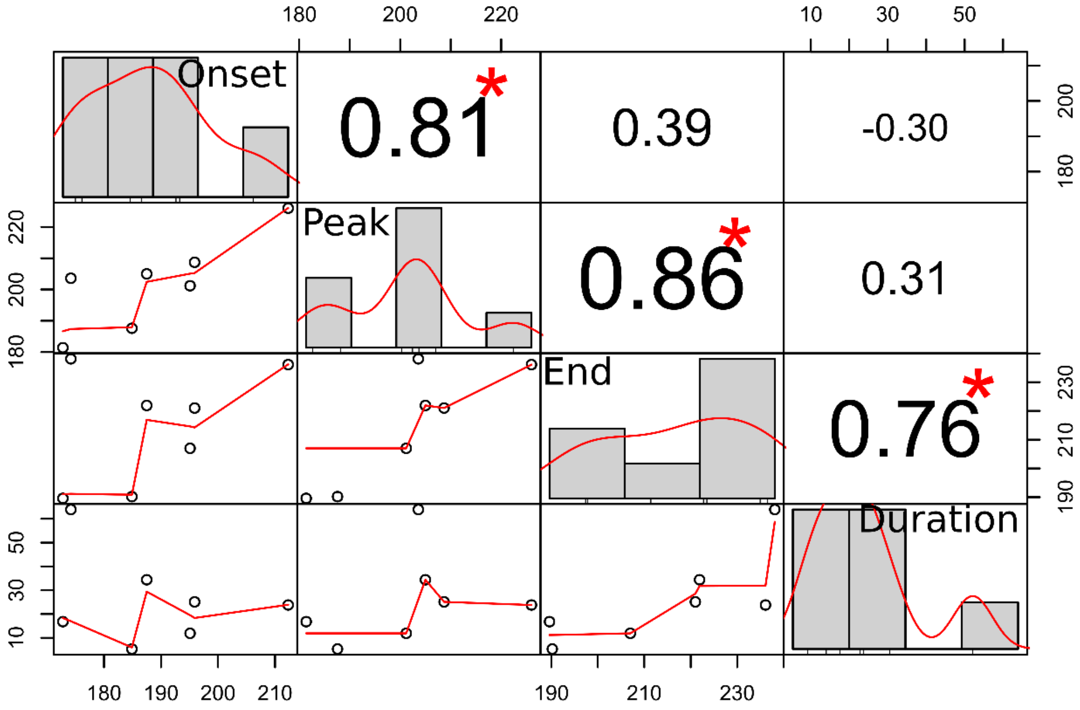


S. dorsata

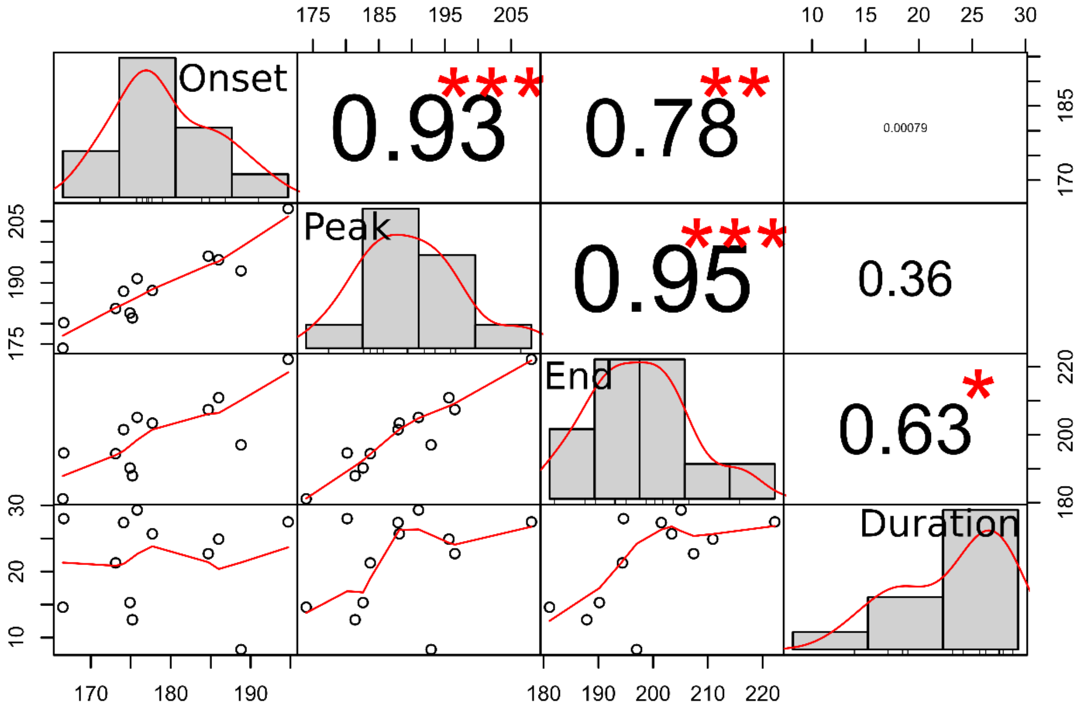


S. malaisei

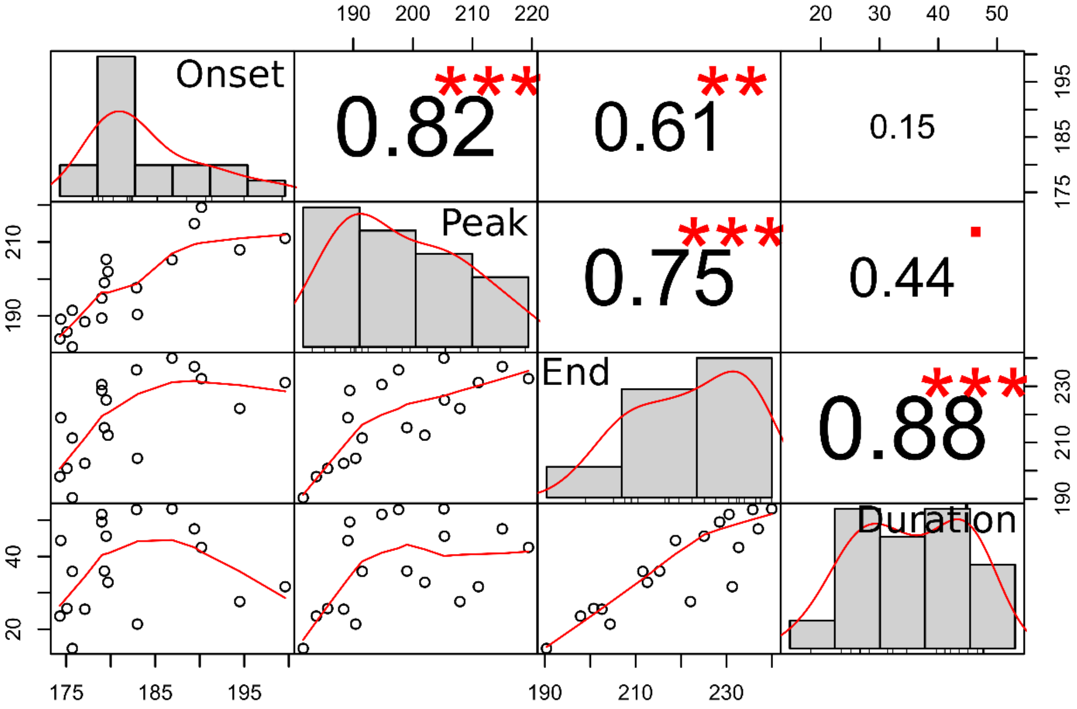
S. megastoma



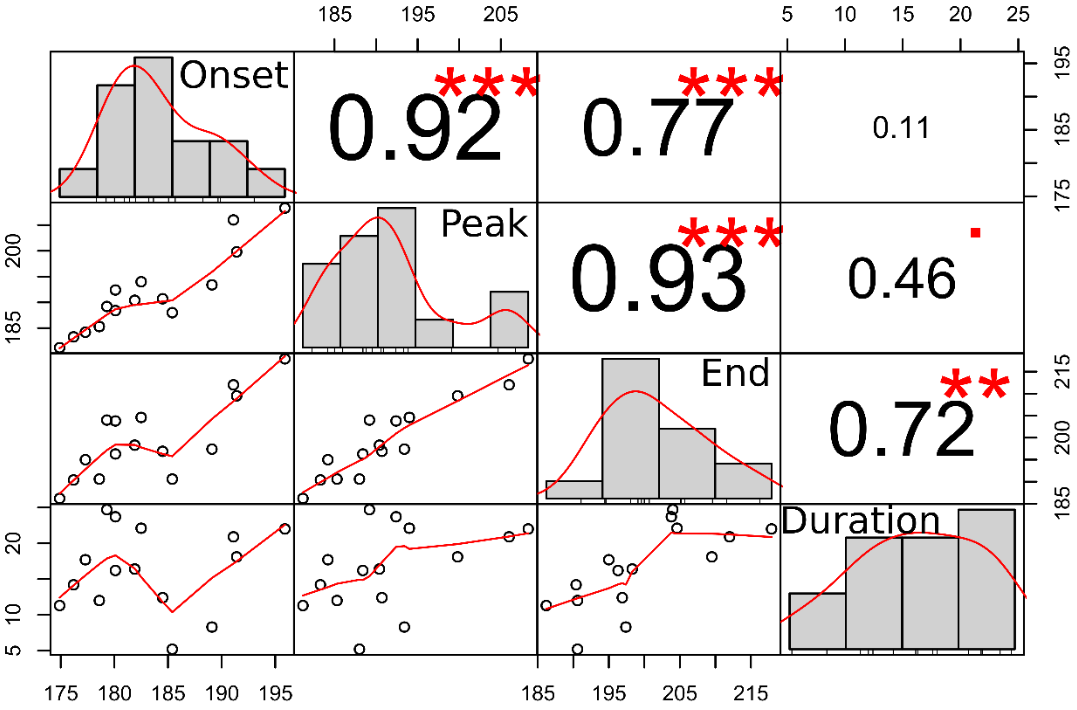
S. novaesibiriae



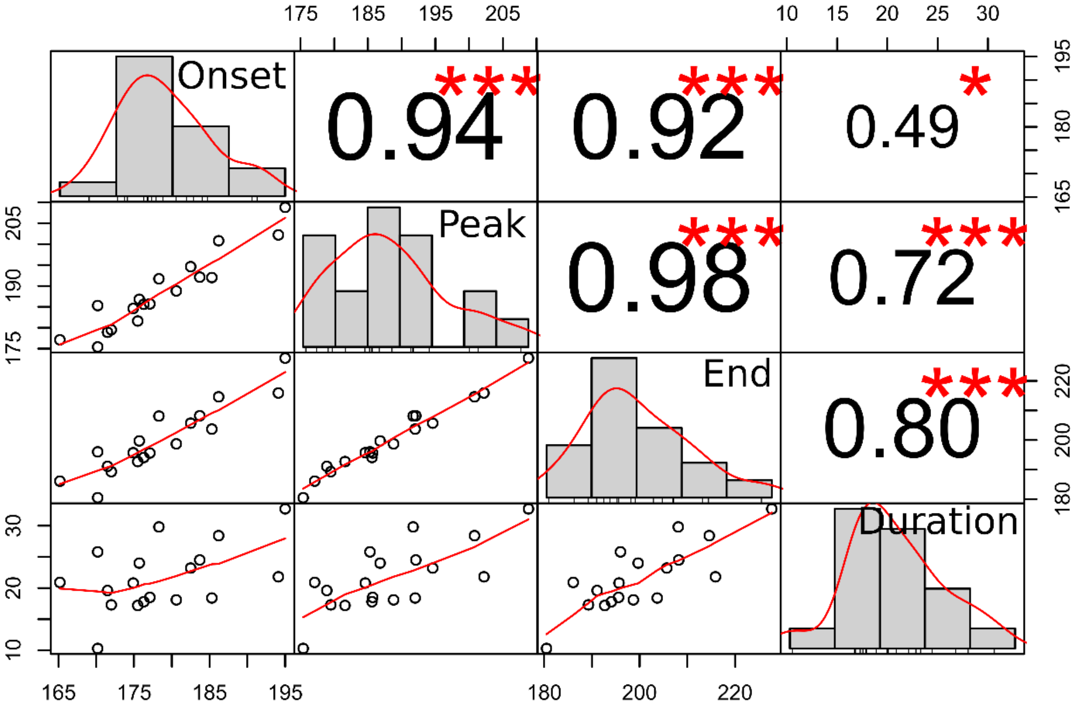
S. sanctipauli



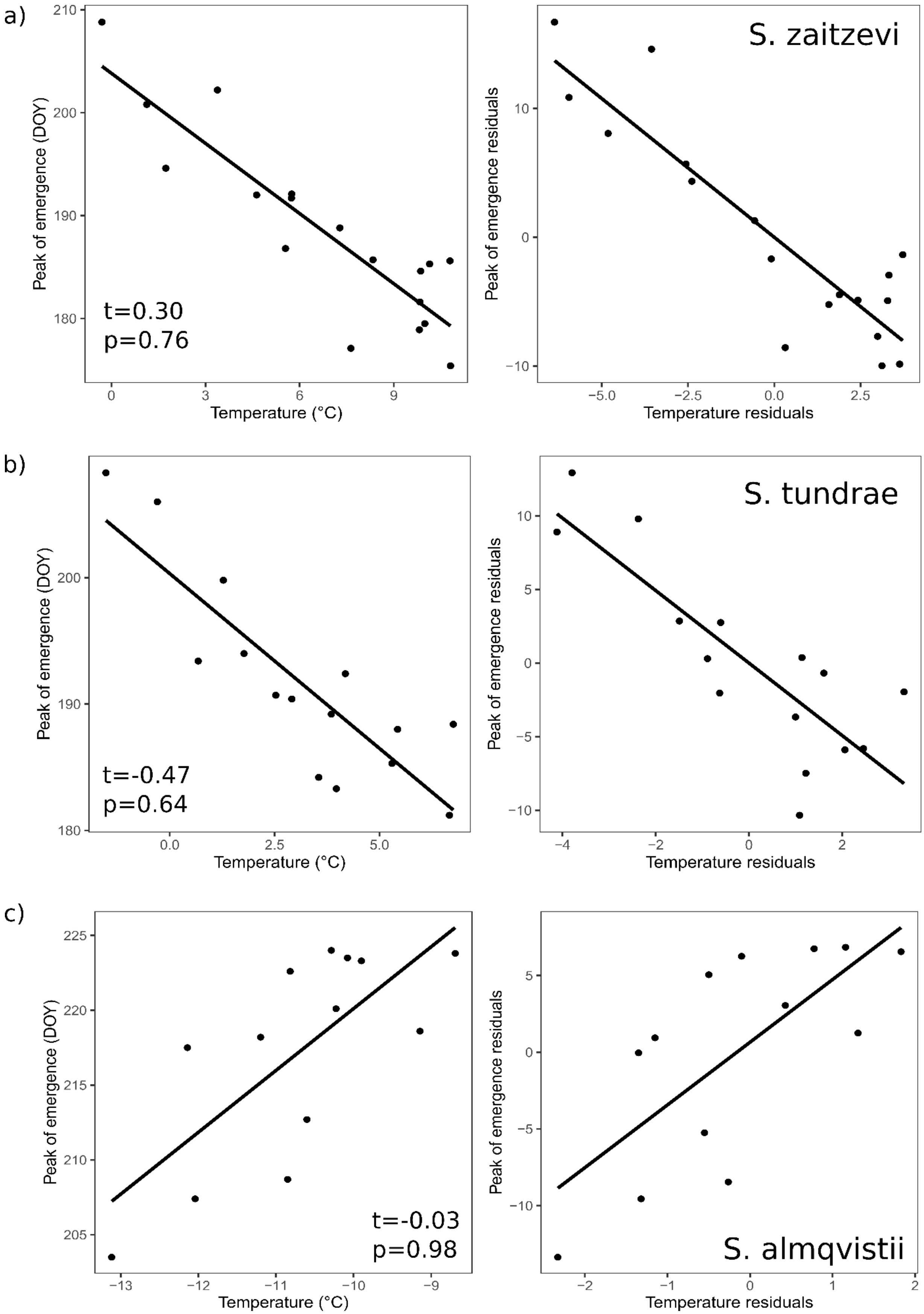
S. tundrae

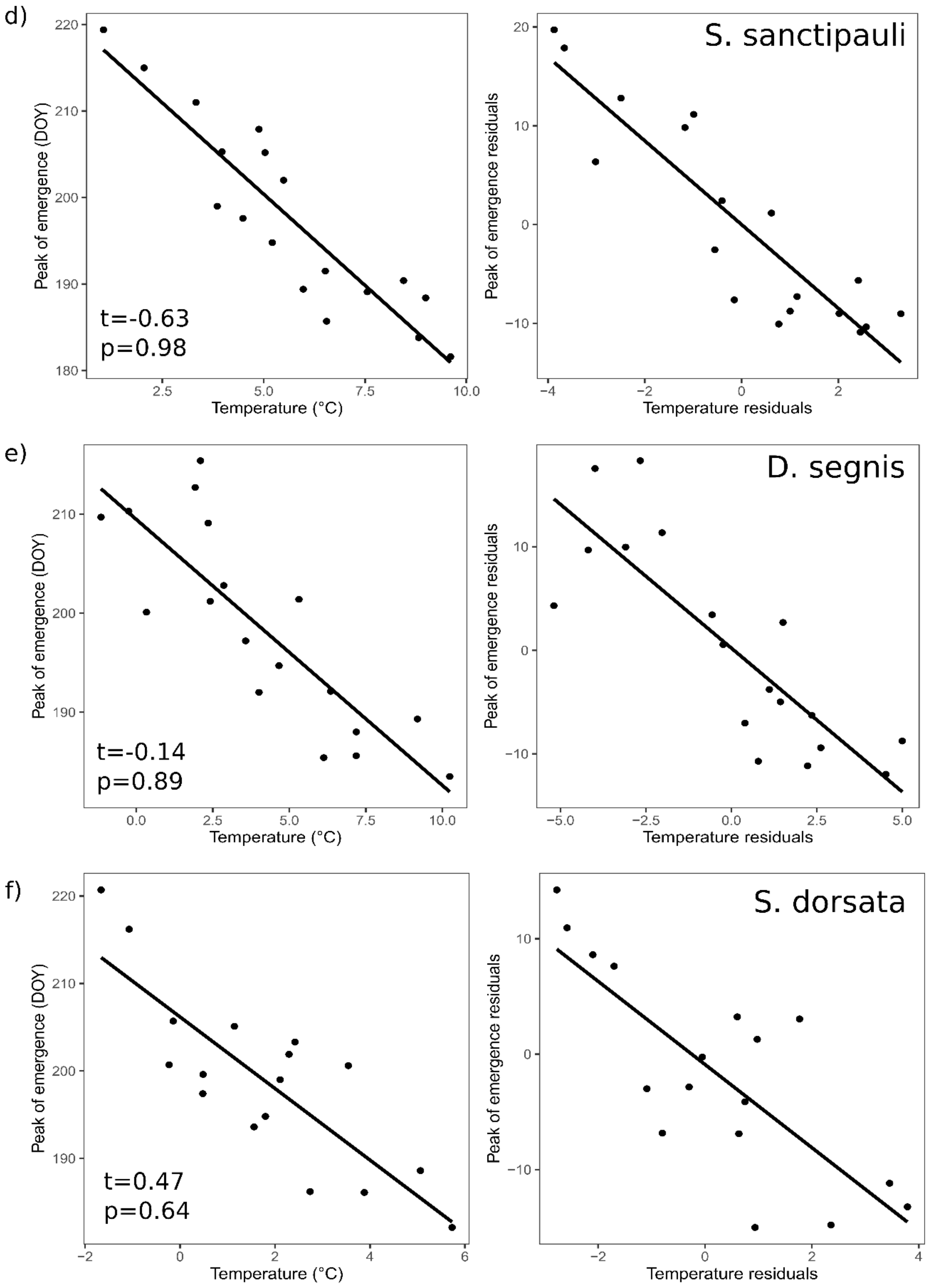


S. zaitzevi


Onset
Peak
End
Duration
0.94 ***
0.92 ***
0.49 *
0.98 ***
0.72 ***
0.80 ***
175
185
195
205
10
15
20
25
30
195
180
165
205
190
175
220
200
180
30
20
10
165
175
185
195
180
200
220

Supporting Figure 3-2 Linear regressions of phenology (peak of activity) with the average temperature within the time window identified with the sliding window analysis, with original variables in the left column and detrended variables in the right column for species of muscid flies caught at Zackenberg between 1996 and 2014. a) *Spilogona zaitzevi*, b) *Spilogona tundrae*, c) *Spilogona almqvistii*, d) *Spilogona sanctipauli*, e) *Drymeia segnis*, and f) *Spilogona dorsata*. Comparisons of slopes of regression with original data and detrended data were performed with two-tailed t-tests, t values and *P* values are presented for each species in the left figure. Slopes of regression were used as a measure of species temperature sensitivity.


a)
S. zaitzevi
t=0.30
p=0.76
Peak of emergence (DOY)
210
200
190
180
0
3
6
9
Temperature (°C)
Peak of emergence residuals
10
0
−10
−5.0
−2.5
0.0
2.5
Temperature residuals
b)
S. tundrae
t=-0.47
p=0.64
Peak of emergence (DOY)
200
190
180
0.0
2.5
5.0
Temperature (°C)
Peak of emergence residuals
10
5
0
−5
−10
−4
−2
0
2
Temperature residuals
c)
S. almqvistii
t=-0.03
p=0.98
Peak of emergence (DOY)
225
220
215
210
205
−13
−12
−11
−10
−9
Temperature (°C)
Peak of emergence residuals
5
0
−5
−10
−2
−1
0
1
2
Temperature residuals


d)
S. sanctipauli
t=-0.63
p=0.98
Peak of emergence (DOY)
Temperature (°C)
Peak of emergence residuals
Temperature residuals
220
210
200
190
180
2.5
5.0
7.5
10.0
20
10
0
−10
−4
−2
0
2
e)
D. segnis
t=-0.14
p=0.89
Peak of emergence (DOY)
Temperature (°C)
Peak of emergence residuals
Temperature residuals
210
200
190
0.0
2.5
5.0
7.5
10.0
10
0
−10
−5.0
−2.5
0.0
2.5
5.0
f)
S. dorsata
t=0.47
p=0.64
Peak of emergence (DOY)
Temperature (°C)
Peak of emergence residuals
Temperature residuals
220
210
200
190
−2
0
2
4
6
10
0
−10
−2
0
2
4

7.3 Supplementary material for Chapter 4

Supplementary Table 4-1 Results of linear models of abundance against distributed lagged matrices of length of the reproductive period with different lag values (t-1, t-2 and t-3) for five species of muscid flies caught at Zackenberg between 1996 and 2014.

Species	Lag	Sum Sq	Mean Sq	F value	*p*	R^2_{adj}
D. segnis	t-1	0.01	0.00	0.07	0.93	0.27
	t-2	0.20	0.10	3.14	0.09	0.36
	t-3	0.17	0.08	2.05	0.19	0.23
S. dorsata	t-1	0.02	0.01	0.42	0.68	0.45
	t-2	0.03	0.02	1.35	0.34	0.28
	t-3	0.07	0.04	3.36	0.14	0.42
S. sanctipauli	t-1	0.19	0.09	0.52	0.61	0.43
	t-2	1.87	0.93	4.80	0.03	0.41
	t-3	1.49	0.75	3.21	0.09	0.30
S. tundrae	t-1	0.09	0.04	0.58	0.60	0.14
	t-2	0.40	0.20	2.67	0.18	0.25
S. zaitzevi	t-1	0.07	0.04	0.41	0.68	0.05
	t-2	0.22	0.11	1.70	0.27	0.29
	t-3	0.19	0.09	0.82	0.52	-0.25

Supplementary Table 4-2 Results of temporal trends in male and female abundance of six species of muscid fly species caught at Zackenberg, Greenland, between 1996 and 2014. Trends were calculated with the Mann-Kendall trend test corrected for temporal autocorrelation when detected with the Ljung-Box test.

Species	Sex	z value	tau	Sen's slope	*p*
D. segnis	**Female**	**-2.58**	**-0.45**	**-0.46**	**0.01**
	Male	-0.48	-0.08	-0.16	0.63
S. almqvistii	**Female**	**-3.15**	**-0.55**	**-0.06**	**<0.001**
	Male	**-3.26**	**-0.57**	**-0.25**	**<0.001**
S. dorsata	**Female**	**-1.85**	**-0.34**	**-0.21**	**0.06**
	Male	**-2.92**	**-0.53**	**-0.79**	**<0.001**
S. sanctipauli	**Female**	**-2.05**	**-0.36**	**-1.01**	**0.04**
	Male	**-2.80**	**-0.49**	**-0.91**	**0.01**
S. tundrae	**Female**	**-3.72**	**-0.67**	**-0.24**	**<0.001**
	Male	**-4.49**	**-0.81**	**-0.76**	**<0.001**
S. zaitzevi	**Female**	**-3.05**	**-0.33**	**-0.57**	**<0.001**
	Male	**-3.18**	**-0.56**	**-0.29**	**<0.001**

Supplementary Table 4-3 Results of linear regression of abundance of six muscid fly species caught at Zackenberg, Greenland, between 1996 and 2014, as a function of time and sex. Results are from linear models of the log-transformed standardized abundance for *D. segnis*, *S. sanctipauli* and *S. zaitzevi*, and a Tukey transformation of abundance for *S. almqvistii*, *S. dorsata* and *S. tundrae*. Linear models were corrected for temporal autocorrelation with the Cochrane-orcutt procedure when detected with the Ljung-Box test.

Species	Variable	Estimate	Std. Error	t value	*p*
D. segnis	Intercept	9.66	12.19	0.79	0.43
	Year	0.00	0.01	-0.74	0.46
	Sex	12.37	16.64	0.74	0.46
	Year*Sex	-0.01	0.01	-0.76	0.45
S. almqvistii	**Intercept**	**42.66**	**9.29**	**4.59**	**<0.001**
	Year	**-0.02**	**0.00**	**-4.55**	**<0.001**
	Sex	-20.85	12.65	-1.65	0.11
	Year*Sex	0.01	0.01	1.64	0.11
S. dorsata	**Intercept**	**46.91**	**9.75**	**4.81**	**<0.001**
	Year	**-0.02**	**0.00**	**-4.74**	**<0.001**
	Sex	-20.02	13.27	-1.51	0.14
	Year*Sex	0.01	0.01	1.50	0.14
S. sanctipauli	**Intercept**	**38.01**	**14.56**	**2.61**	**0.01**
	Year	**-0.02**	**0.01**	**-2.56**	**0.02**
	Sex	-26.63	20.04	-1.33	0.19
	Year*Sex	0.01	0.01	1.33	0.19
S. tundrae	**Intercept**	**83.70**	**12.40**	**6.75**	**<0.001**
	Year	**-0.04**	**0.01**	**-6.71**	**<0.001**
	Sex	-24.55	16.97	-1.45	0.16
	Year*Sex	0.01	0.01	1.44	0.16
S. zaitzevi	**Intercept**	**35.86**	**13.38**	**2.68**	**0.01**
	Year	**-0.02**	**0.01**	**-2.65**	**0.01**
	Sex	-6.95	18.26	-0.38	0.71
	Year*Sex	0.00	0.01	0.39	0.70

Supplementary Table 4-4 Results of temporal trend of phenology (onset, peak, end and duration of activity) of males and females of six muscid fly species caught at Zackenberg, Greenland, between 1996 and 2014. Results are from Mann-Kendall trend tests corrected for temporal autocorrelation when necessary.

Species	Sex	Phenological event	Sen's slope	tau	*p*
D. segnis	Male	Onset	-0.45	0.01	1.00
		Peak	-0.68	-0.27	0.12
		End	-1.19	-0.27	0.13
		Duration	-0.50	-0.27	0.13
	Female	Onset	-0.30	-0.15	0.40
		Peak	-1.12	-0.24	0.06
		End	-0.56	-0.06	0.76
		Duration	-0.33	-0.12	0.50
S. almqvistii	Male	Onset	-2.70	-0.36	0.27
		Peak	**-4.38**	**-0.71**	**0.02**
		End	**-4.30**	**-0.64**	**0.04**
		Duration	-0.92	-0.36	0.11
	Female	Onset	0.25	0.12	0.63
		Peak	-0.03	-0.06	0.84
		End	-0.34	-0.20	0.41
		Duration	-0.23	-0.12	0.63
S. dorsata	Male	Onset	-0.80	-0.25	0.19
		Peak	-0.86	-0.30	0.11
		End	**-1.93**	**-0.42**	**0.03**
		Duration	-0.70	-0.27	0.16
	Female	Onset	-0.65	-0.24	0.20
		Peak	**-1.28**	**-0.43**	**0.02**
		End	**-1.62**	**-0.64**	**0.00**
		Duration	**-0.96**	**-0.43**	**0.02**
S. tundrae	Male	Onset	-1.65	-0.33	0.18
		Peak	-2.09	-0.45	0.06
		End	**-2.60**	**-0.53**	**0.03**
		Duration	**-1.20**	**-0.49**	**0.04**
	Female	**Onset**	**-1.49**	**-0.53**	**0.02**
		Peak	**-2.13**	**-0.53**	**0.02**
		End	**-2.65**	**-0.55**	**0.02**
		Duration	-0.74	-0.42	0.06
S. sanctipauli	Male	Onset	-0.14	-0.02	0.94
		Peak	-0.43	-0.19	0.15

		End	-1.38	-0.28	0.11
		Duration	**-1.14**	**-0.56**	**<0.001**
	Female	Onset	-0.30	-0.05	0.82
		Peak	-1.15	-0.25	0.16
		End	-0.52	-0.15	0.40
		Duration	-0.40	-0.21	0.24
S. zaitzevi	Male	Onset	-0.60	-0.32	0.07
		Peak	-0.55	-0.23	0.20
		End	-0.84	-0.28	0.11
		Duration	-0.33	-0.12	0.52
	Female	Onset	-0.30	-0.17	0.39
		Peak	-0.73	-0.18	0.34
		End	-1.07	-0.23	0.22
		Duration	-0.49	-0.16	0.42

Supplementary Table 4-5 Results of the interaction between time (year) and sex from the linear regressions of phenology (onset, peak, end, or duration of the activity period) as a function of time (year) and sex for each species of muscid fly caught at Zackenberg, Greenland, between 1996 and 2014. A significant interaction term indicates that the rate of temporal change in phenology is different between males and females.

Species	Phenological event	Estimate	Std. Error	df	t ratio	*p*
D. segnis	Onset	-0.13	0.48	32.00	-0.28	0.78
	Peak	-0.10	0.58	32.00	-0.16	0.87
	End	-0.28	0.34	32.00	-0.82	0.42
	Duration	0.26	0.42	32.00	0.61	0.55
S. almqvistii	Onset	2.52	1.47	16.00	1.72	0.11
	Peak	**3.89**	**1.15**	**12.20**	**3.39**	**0.01**
	End	**3.55**	**1.07**	**16.00**	**3.32**	**<0.001**
	Duration	1.03	1.33	16.00	0.78	0.45
S. dorsata	Onset	0.13	0.67	29.00	0.19	0.85
	Peak	0.02	0.72	29.00	0.03	0.98
	End	-0.83	0.92	29.00	-0.90	0.38
	Duration	-0.29	0.52	29.00	-0.56	0.58
S. sanctipauli	Onset	-0.28	0.44	32.00	-0.62	0.54
	Peak	-0.52	0.66	32.00	-0.79	0.43
	End	-0.48	0.42	32.00	-1.14	0.27
	Duration	0.74	0.43	32.00	1.72	0.10
S. tundrae	Onset	-0.55	0.71	19.00	-0.78	0.44
	Peak	-0.63	0.61	19.00	-1.02	0.32
	End	-0.72	0.68	19.00	-1.07	0.30
	Duration	-0.26	0.57		-0.46	0.65
S. zaitzevi	Onset	0.17	0.43	30.00	0.39	0.70
	Peak	-0.01	0.53	30.00	-0.02	0.99
	End	0.17	0.63	30.00	0.28	0.78
	Duration	0.01	0.33	30.00	0.02	0.99

Supplementary Table 4-6 Results of the linear regressions of the temporal changes in the length of the reproductive period against time for six species of muscid fly caught at Zackenberg, Greenland, between 1996 and 2014.

Species	Estimate	Std. Error	t value	*p*
D. segnis	-0.38	0.22	-1.75	0.10
S. almqvistii	-3.59	1.63	-2.20	0.08
S. dorsata	-0.75	0.51	-1.47	0.17
S. sanctipauli	**-0.83**	**0.31**	**-2.65**	**0.02**
S. tundrae	-0.55	0.39	-1.41	0.19
S. zaitzevi	-0.12	0.25	-0.47	0.64

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