CHIRONOMIDS THEN AND NOW: CLIMATE CHANGE EFFECTS ON A TUNDRA FOOD WEB IN THE ALASKAN ARCTIC

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

Although climate change is a global phenomenon, the Arctic is warming faster than any other region on earth. These climatic changes have driven rapid regional changes over the past half-century in both the physical landscape and the ecosystems therein. One such ecological interaction is between migratory shorebird survival and local insect emergence. Annually, tens of millions of migratory shorebirds travel to the Arctic to rear their young in the relative absence of predators, but in a relative abundance of food (insects). Over evolutionary time, these trophic levels have coupled: shorebird chicks tend to hatch during the period of highest terrestrial insect availability. However, climate change is currently uncoupling this food-web synchrony, creating potential for *trophic mismatch*. In the High Arctic near Utqiaġvik (formerly Barrow), Alaska, trophic mismatch between nesting shorebirds and their insect food base is already detectable. In this ecosystem, flies in the Family Chironomidae (non-biting midges) dominate the prey trophic level in the avian food web. We have found that the pre-emergence development of one particular midge, *Trichotanypus alaskensis*, defies conventional wisdom of the Family, as this species molts to an additional fifth larval instar prior to pupation and emergence (all other chironomids are known to have four larval instars). We discovered an Utqiaġvik midge that reproduces asexually, a species that was not documented in the 1970s. Utilizing controlled temperature rearings of Utqiaġvik midge larvae, we discovered that as temperatures rise, emerging chironomid adults are generally smaller in size. We have found that chironomid pre-emergence developmental rates follow a positive exponential relationship as temperatures increase, can vary by taxon, yet are consistent across field and lab settings for a given taxon. At Utqiaġvik in the 2010s, chironomid emergence occurs 8-12 days earlier than it did in the 1970s. These findings shape our understanding of trophic mismatch in this arctic food web.

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DEDICATION

This work is dedicated to Ewelina.

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LIST OF ABBREVIATIONS

ACP.....Arctic Coastal Plain

HC.....Head Capsule

HCW.....Head Capsule Width

HCL.....Head Capsule Length

PE.....Pupal Exuviae

LE.....Larval Exuviae

CI.....Confidence Interval

DH.....Degree Hours

TSR.....Temperature-Size Rule

GLM.....General Linear Model

GLIM.....Generalized Linear Model

BEO.....Barrow Environmental Observatory

IBP.....International Biological Program

AICc.....Akaike's Information Criterion

BIC.....Bayesian Information Criterion

1. INTRODUCTION

1.1. Ecological Background

1.1.1. Field Site

The field work for this dissertation was conducted in the High Arctic near Utqiaġvik (formerly Barrow), Alaska (71°17'27.5"N 156°47'18.5"W). This village of Utqiaġvik is a part of Alaska's Arctic Coastal Plain (ACP), a low-relief, ecosystem north of the Brooks Range known for its vast wetland ecosystem comprised of tundra pond mosaics, thermokarst basins, inundated vegetation, ice wedge polygons, and semiterrestrial tundra that rests on continuous permafrost (Muster *et al*. 2013; Arp *et al*. 2015; Liljedahl *et al*. 2016; McEwen and Butler 2018).

1.1.2. Climate Change

Climate change over the past several decades has resulted in global ecological changes (Walther *et al*. 2002; Visser and Both 2005; Chen *et al*. 2011; Bowden *et al*. 2015), with many species predicted to go extinct because of insufficient adaptation rates (Davis and Shaw 2001). In the Arctic, longer growing seasons, higher average temperatures, and increased variation in temperatures have been documented (IPCC 2014; Osborne *et al*. 2018). In fact, the Arctic is the fastest warming region on earth in the current climate change era, and in 2018 this region was consistently > 4ºC warmer than the 1981-2010 baseline (Osborne *et al*. 2018). Annual mean temperature anomalies in the Arctic are more than two times greater than the global average, and these arctic values for the past five consecutive years (2014-2018) have all been greater than any other year since records began in 1900 (Osborne *et al*. 2018). This record-setting rate of warming is largely due to the anthropogenic release of greenhouse gases from burning fossil fuels (Baes *et al*. 1977; Gardner *et al*. 2011; Sheridan and Bickford 2011; IPCC 2014; Notz and Stroeve 2016).

Indeed, polar surfaces in general are warming at rates two to three times faster than the global average, a phenomenon known as polar warming amplification (Taylor *et al*. 2013). This is partially because in the late 20th Century, the Arctic switched from being a carbon dioxide sink (as it was over recent geological time), to a carbon dioxide source that releases substantial amounts of previously-stored greenhouse gases as it melts (Oechel *et al*. 1993). In addition, the surface albedo in polar regions is decreasing significantly; as ice and snow melt over sea and land, darker surfaces like liquid water and rock are exposed, absorbing heat, instead of reflecting it, thus creating a positive feedback loop for accelerated warming (Taylor *et al*. 2013). This process releases more moisture into the atmosphere causing more precipitation, greater cloud cover, and an even stronger greenhouse effect (Osborne *et al*. 2018). These are net cloud feedback loops, and they have increased during the anthropogenic climate change era. Now the poles act as a sink for highly energetic storm systems, a phenomenon known as 'forcing', where these systems tend to dump their energy at the poles (Taylor *et al*. 2013). Moreover, arctic sea ice is younger on average, resulting in thinner and darker ice packs that absorb more heat. In the mid-1980s multiyear ice made up ~70% of the winter sea ice extent, while in 2012 only 20% of the winter pack was older, more reflective ice (Tschudi *et al*. 2016). This additional energy increases the rate at which the poles' atmospheric temperatures increase. In sum, global warming is happening extremely rapidly in the Arctic, and this localized, amplified warming has cascading effects on the rest of the world (Osborne *et al*. 2018).

At the Barrow Peninsula, mean air temperatures have increased at a rate of 0.7ºC decade^{-1} since the 1960s (Hobbie *et al*. 1999). Temperature changes have been documented in the tundra pond habitat. In a well-studied low-centered polygon pond, mean water temperature during the growing season warmed at a rate of 0.5ºC decade^{-1} from 1971 to 2012 (McEwen and Butler

2018). Although thaw date did not change significantly, the heat sum during the first month of the growing season did increase significantly. The length of the growing season increased by ~2 weeks as the date of pond freeze averaged later. Other low-centered polygon ponds on the Utqiaġvik Peninsula exhibited similar thermal patterns based on contemporary temperature measurements alone, suggesting similar climatic changes have taken place in tundra pond ecosystems across Alaska's Arctic Coastal Plain.

These climatic changes not only affect heat budgets and freeze-thaw cycles in the Arctic, but also have changed the physical landscape. Such landscape evolution is increasingly apparent and has been documented since 1948. Andresen and Lougheed (2015), documented a net decrease of about 30% in the visible surface area of water on the Utqiaġvik Peninsula from 1948 to 2013. This decrease in open water is largely due to increased macrophytic vegetation (*i.e.* standing water grasses and sedges) that has encroached along the periphery of ponds. In addition to these vegetation changes, current climatological trends have caused upper layers of permafrost to melt. On the ACP, much of the underlying permafrost is riddled with ice wedge polygons that are now degrading as the climate warms (Liljedahl 2012; Liljedahl *et al*. 2016). This degradation ultimately leads to a decrease in inundated tundra and low-centered polygon ponds, but an increase in high-centered polygon trough ponds, hydroconnectivity, and total vegetation - in short, an overall draining of the tundra landscape (Gangodagamage 2014; Liljedahl *et al*. 2016). These changes to the landscape could have significant effects on the endemic fauna. For example, as the ratio of vegetation to open water increases (Andresen and Lougheed 2015; Lijedahl *et al*. 2016), there may be more 'keystone' habitats for invertebrates (Tews *et al*. 2004). On the other hand, many freshwater habitats are eroding away. Coastal erosion at Utqiaġvik has accelerated since the 1970s by about 50% on average, with shoreline recession ranging 0.86-2.75

m year^{-1} (Brown *et al*. 2003). These landscape-level changes may add another dimension of unpredictability in our attempt to understand how this arctic ecosystem will be altered by further climate change.

1.1.3. Predators and Prey

The Arctic Coastal Plain is well known as a hotspot of avian abundance, seasonally hosting tens of millions of migratory shorebirds alone (Andres *et al*. 2012; Bart *et al*. 2012a) during the spring and summer (Custer and Pitelka 1978; Tulp and Schekkerman 2008; Lanctot *et al*. 2015; Weiser *et al*. 2018). The avian community is comprised of at least 185 species in Alaska's North Slope alone, and 151 species in the Utqiaġvik region (Pitelka 1974). Numerous waterbird species have evolved to rear their chicks in this remote environment because of the relative lack of predators, parasites, and pathogens, coupled with an ephemeral overabundance of prey that enhances survival and growth rates of their offspring (Carey 1986; Helmers and Gratto-Trevor 1996; Lepage *et al*. 1998; Andreev 1999; Schekkerman *et al*. 2003; Tulp and Schekkerman 2008). Approximately 95% of this avifauna is migratory (Johnson and Herter 1990), including at least 33 species of shorebirds (Charadriiformes) (Pitelka 1974). In fact, shorebirds are the dominant avifauna in the Arctic, with ~50 % of North American's breeding shorebird diversity found in Northern Alaska (Boyd and Madsen 1997; Johnson and Herter 1989; Morrison et al. 2006; Andres *et al*. 2012; Andres, Smith *et al*. 2012). These species migrate for thousands, even tens of thousands, of kilometers (Conklin *et al*. 2010) to rear their young in this region.

The prey for these birds is almost entirely insects (Holmes and Pitelka 1968; Pitelka 1974; Høye and Forchhammer 2008). Although the abundance of insect biomass at Utqiaġvik is astounding, the diversity is not as impressive as that found intemperate zone habitats (Holmes

and Pitelka 1968; Ferrington 2007). Besides a few insect species in the Orders Ephemeroptera, Phthiraptera (Gressitt and Yoshimoto 1974), Neuroptera (Gressitt and Yoshimoto 1974), Lepidoptera (Dyar 1897; Gressitt and Yoshimoto 1974), Plecoptera, Hemiptera, Coleoptera, Trichoptera, and Hymenoptera, most insects (~95% of the total abundance) this far north belong to the Diptera (Holmes and Pitelka 1968; MacLean and Pitelka 1971; Pitelka 1974). In this order two families, the Tipulidae (3 species) and Chironomidae (40-50 species), make up 80% of the food consumed by shorebirds (Holmes 1966; Holmes and Pitelka 1968).

Among these dipterans, the Family Chironomidae prevails in aquatic habitats, in both total abundance and species diversity (Butler 1982b; Lougheed *et al*. 2011; Cranston *et al*. 2012). Indeed, on a global scale, chironomids are often the most abundant and speciose macroinvertebrates in freshwater (Armitage *et al.* 1995). Within tundra ponds at Utqiaġvik there are at least 31 chironomid species, many of which lack formal species names (Lougheed *et al*. 2011). Nonetheless, about 12 of these chironomid taxa are estimated to comprise 90% of the biomass emerging from ponds on an annual basis (Lougheed *et al*. 2011; Butler *et al*. in prep). The dominance of terrestrial insect biomass by Chironomidae at Utqiaġvik is also impressive (Holmes and Pitelka 1968; MacLean and Pitelka 1971; Saalfeld *et al*. 2019), given theaquatic habitat of most species as larvae (Armitage *et al*. 1995). Even biomass of the much larger-bodied crane fly (Tipulidae) species at Utqiaġvik rarely equals that of Chironomidae in terrestrial arthropod samples of the tundra (Holmes 1966; MacLean and Pitelka 1971). Overall, chironomids are crucial components of the Utqiaġvik tundra ecosystem, both aquatically and terrestrially.

Chironomids at Utqiaġvik have highly synchronized emergence during the already brief (~3 month) growing season (Butler 1980a; Butler 1982a; Braegelman 2016). Most chironomids

spend almost their entire existence as aquatic larvae, but then complete their life cycle by metamorphosing and emerging as adults that take on a brief terrestrial existence during which reproduction takes place. At Utqiaġvik, chironomid life cycles range from 1-7 years depending on the species (Butler 1982a; Lackmann and Butler 2018; Butler and Braegelman 2018). Taken together, the pupal and adult stage of their development only lasts about a week and always occurs in their final year of life. As adults chironomids tend to live just 2-3 days (Armitage *et al*. 1995; Lackmann *et al*. in prep).

At this and other arctic sites, it appears selection has acted strongly to maintain population synchrony of this reproductive stage (Danks and Oliver 1972; Butler 1982a; Hodkinson *et al*. 1996; Høye and Forchhammer 2008). With sustained low temperatures, consistent winds, and a relatively shelterless landscape (*e.g.* there are no trees; even no shrubs over 0.3 m tall), Utqiaġvik is a very inhospitable environment for an insect, especially for those that reproduce sexually (require mate discovery) and use flight for dispersal. Thus, having synchronized emergence increases the probability that successful recruitment takes place. Indeed, most emergence of any one species occurs within a week, and the entire chironomid phenology is largely completed in 3-4 weeks in any given year (Butler 1980a; Braegelman 2016).

Chironomid emergence at Utqiaġvik is largely protandrous (males emerge slightly earlier than females) (Butler 1980a), another known adaptation that increases the likelihood of successful reproduction (Fagerström and Wiklund 1982). In protandrous systems, females arrive to the scene after environmental stochasticity has already run its course on males, and thus 'better' males tend to be left and are ready to mate when females emerge (Morbey and Ydenberg 2001; Petit *et al*. 2001). This minimizes a female's time being unmated, and thus increases the

likelihood of successful mating (Fagerström and Wiklund 1982). Furthermore, males that have made it through this 'waiting period' tend to have more mating opportunities, and individuals are less likely to inbreed: males have had time to disperse prior to the emergence of females and thus populations are more likely to intermix (Morbey and Ydenberg 2001). The effects of rising temperatures on protandry are not well known (Møller 2004), but if different sex-specific developmental rate responses exist within a species, they could theoretically disrupt these chironomid mating systems.

For migratory shorebirds, this highly synchronous, terrestrial pulse of insect biomass is crucial to the success of their reproductive effort (Holmes 1966; Holmes and Pitelka 1968; MacLean and Pitelka 1971). Shorebird young are precocial *i.e.* they are immediately capable of mobility and foraging post-hatch. Shorebird chicks in the High Arctic predominantly feed on chironomid adults they find on tundra vegetation as they rarely probe substrate because their beaks are immature in size and not hardened (Holmes 1966). Once chicks reach the fledgling stage they transition to feed on aquatic and semiterrestrial larvae (Chironomidae and Tipulidae) (Holmes 1966; Holmes and Pitelka 1968; MacLean and Pitelka 1971). It is thus very important that chironomid adults are available during the hatch-to-fledge interval, a 2-3 week period depending on the species (Holmes 1966). As the time between shorebird arrival at the breeding site and egg hatch is about 1 month (Holmes 1966), the phenological linkage of these two trophic levels depends on consistency in both migration timing and climate at the tundra breeding site.

1.2. Ecological Theory

1.2.1. Trophic Mismatch

It is likely that climate change is disrupting tundra pond food webs in the Alaskan Arctic by shifting phenologies of local prey away from the seasonal timing of migratory predators.

Along with reduced body size and range shifts to higher latitudes and altitudes, phenological shifts are one of the three universal responses of biota to climate change (Daufresne *et al*. 2009). The idea that trophic levels could decouple due to shifting phenologies (*i.e.* a trophic mismatch) began before the global consciousness of climate change was established. Hjort first described potential match/mismatch dynamics in spawning fish phenologies (1914), and Cushing (1969) later expounded on this idea. Since anthropogenic climate change became well documented, there has been an intellectual surge in trying to understand the nature of the resulting trophic mismatches (Durant *et al*. 2007).

Visser *et al*. (1998) discovered that despite climate-driven advances in vegetation growth and caterpillar peak densities in the Netherlands, lay dates of great tits had not changed (. They hypothesized that a selection differential now exists on great tits to lay earlier with advancing springs, and that the selective forces are just beginning to act on the population. In another study, Strode (2003) found that for 13 of 16 species of wood warblers, migratory patterns did not change in response to earlier pulses of caterpillars. In another study, Dunn *et al*. (2011) discovered that although tree swallows tend to lay earlier across several decades, it was not because of shifts in the seasonal pulse of their prey. Instead, lay date of these insectivorous swallows was strongly correlated with the appearance of flying insect biomass, despite there being increasingly more food as the season progressed. These authors suggest that the trophic mismatch hypothesis may not apply to systems where food supply is relatively constant across the growing season. In a study on low arctic shorebirds and passerines, Leung *et al*. (2018) detected no evidence of trophic mismatch. As intuitive as this phenomenon may seem, phenological shifts may not always lead to temporary mismatches between trophic levels, even when one trophic level is migratory.

McKinnon *et al*. (2012) documented declining nesting success for High Arctic shorebird species where the hatch-to-fledge period was later than the pulse of arthopodan biomass. At Utqiaġvik the phenological pulse of Chironomidae has shifted earlier by about one week on average, from the mid-1970s to the early 2010s (Braegelman 2016). Avian ecologists working at Utqiaġvik have found that several species of migratory shorebirds showed evidence of decreased chick growth rates when minimal arthropodan biomass was available (Saalfeld *et al*. 2019). Taken together, the potential for trophic mismatch among migratory shorebirds and their prey, especially in the High Arctic, warrants further study.

1.2.2. Growth and Development of Insects

Despite the dominant role both climate change and chironomids play in the tundra pond ecosystem at Utqiaġvik (Hobbie et al. 1999; Lougheed *et al*. 2011; McEwen and Butler 2018; Holmes 1966; Butler 1980a; Saalfeld *et al*. 2019), no study has experimentally tested the impacts of increased temperature on both growth and development of these taxa. One species (*Trichotanypus alaskensis*) has been investigated by Braegelman (2016), who assessed developmental rates across temperature treatments. This species makes up ~2% of the total emerging biomass in the chironomid phenology (Lougheed et al. 2011). The paucity of information on this matter calls for further study as there are at least 31 species in the tundra pond ecosystem (Lougheed *et al*. 2011), and estimates of up to 50 species for the entire Utqiaġvik region (Holmes 1966).

Temperature plays a major role in the growth and development of insects, but the effects are usually not linear, nor are these two processes affected the same way by temperature (Sharpe and DeMichele 1977; Frouz *et al*. 2002; Baek *et al*. 2012). Nonetheless, at least three common themes exist: insects are smaller, they develop faster, and they are less fecund at warmer

temperatures (Kingsolver and Huey 2008). At reduced size, insects (including chironomids) have lower fitness (Butler and Walker 1992; Armitage *et al*. 1995; Kingsolver and Huey 2008), although there is a selective advantage of being smaller in a warming climate (Daufresne *et al*. 2009). If development is slowed down at lower temperatures more than is growth rate, then a given insect will emerge at a larger adult body size. Perhaps this theoretical explanation is the basis for why insects are getting smaller, even though the precise mechanisms are not yet known (Angilletta and Dunham 2003).

1.3. Life Cycles and Histories

The ability to make ecological inferences is limited by our understanding of the individual components of which the ecosystem is comprised (Marcus 1998). In this era of the internet, citizen science, ecosystem modeling, and big data, basic knowledge of an organism is often assumed to be known. Biotic uncertainty is easily misrepresented, in part because ecological models based on large datasets tend to add noise and obscure the importance of actual life history diversity (Marcus 1998; La Sorte *et al*. 2018). The wealth of digital misinformation that is easily accessible online can obscure the truth in all aspects of human society, including the sciences (Metzger 2007; Del Vicario *et al*. 2016; Vosoughi *et al*. 2018). It is as important as ever that we attempt to understand biological mysteries that remain at the life history level.

Life history diversity is crucial in understanding the way populations replace themselves (Marcus 1998), and the importance of recognizing such diversity is well illustrated by our study system in northern Alaska. Prior work on chironomids at Utqiaġvik produced unexpected findings, and this community likely harbors additional surprises. Butler (1982a) discovered and then described (1982c) two congeneric *Chironomus* species that have 7-year life cycles, the longest for any chironomid known (Armitage *et al*. 1995). In addition, Butler (2000) described a

pair of *Tanytarsus* species, both exhibiting 2-year life cycles but emerging at different times in the chironomid phenology. Butler's life history analyses were based in part on morphological analyses of developing structures in fourth instar larvae (see Wülker and Götz 1968; Ineichen *et al*. 1983), which indicate these congeneric species overwinter at different developmental stages in their final year. More recently, Butler and Braegelman (2018) documented that one of the earliest-emerging chironomids at Utqiaġvik is not an 'absolute spring species', contradicting that hypothesis put forth by Danks and Oliver (1972) for high arctic midges.

These life history discoveries are important in framing the context for this analysis of chironomids as a "prey trophic level" in a system currently experiencing climate change. For example, life cycle durations for Utqiaġvik chironomids may be shortening, perhaps altering population synchrony and phenological order in ways that would be otherwise unpredictable without knowledge of multiyear life cycles or overwintering states. If pre-emergence growth and development of Utqiaġvik chironomids do not conform to the absolute spring species hypothesis of Danks and Oliver (1972), emergence synchrony and seasonal timing may not be as predetermined as previously thought for high arctic midges. Since only a small percentage of the chironomid species at Utqiaġvik have been investigated closely, many other life history discoveries are likely to be made.

1.4. References

Andreev, A. V. (1999). Energetics and survival of birds in extreme environments. *Ostrich*, 70(1), 13-22.

Andres, B. A., Johnson, J. A., Brown, S. C., & Lanctot, R. B. (2012). Shorebirds breed in unusually high densities in the Teshekpuk Lake Special Area, Alaska. *Arctic*, 411-420.

Andres, B. A., Smith, P. A., Morrison, R. G., Gratto-Trevor, C. L., Brown, S. C., & Friis, C. A. (2012). Population estimates of North American shorebirds. *Wader Study Group Bull*, 119(3), 178-194.

Andresen, C. G., & Lougheed, V. L. (2015). Disappearing Arctic tundra ponds: Fine-scale analysis of surface hydrology in drained thaw lake basins over a 65 year period (1948–2013). *Journal of Geophysical Research: Biogeosciences*, 120(3), 466-479.

Angilletta, Jr, M. J., & Dunham, A. E. (2003). The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. *The American Naturalist*, *162*(3), 332-342.

Armitage, P., Cranston, P. S., & Pinder, L. C. V. (1995). The Chironomidae: The biology and ecology of non-biting midges. 1. Chapman and Hall, London: 572 pp.

Arp, C. D., Jones, B. M., Liljedahl, A. K., Hinkel, K. M., & Welker, J. A. (2015). Depth, ice thickness, and ice-out timing cause divergent hydrologic responses among Arctic lakes. *Water Resources Research*, 51(12), 9379-9401.

Baek, M. J., Yoon, T. J., & Bae, Y. J. (2012). Development of Glyptotendipes tokunagai (Diptera: Chironomidae) under different temperature conditions. *Environmental entomology*, *41*(4), 950-958.

Baes, C. F., Goeller, H. E., Olson, J. S., & Rotty, R. M. (1977). Carbon Dioxide and Climate: The Uncontrolled Experiment: Possibly severe consequences of growing CO 2 release from fossil fuels require a much better understanding of the carbon cycle, climate change, and the resulting impacts on the atmosphere. *American Scientist*, 65(3), 310-320.

Bart, J., Brown, S., Andres, B.A., Platte, R., and Manning, A. (2012a). North Slope of Alaska. Chapter 4. In: Bart, J.R., and Johnston, V., eds. Arctic shorebirds in North America: A

decade of monitoring. Studies in Avian Biology 44. Berkeley: Cooper Ornithological Society and University of California Press. 37– 96.

Bowden, J. J., Eskildsen, A., Hansen, R. R., Olsen, K., Kurle, C. M., & Høye, T. T. (2015). High-Arctic butterflies become smaller with rising temperatures. *Biology Letters*, *11*(10), 20150574.

Boyd, H., & Madsen, J. (1997). Impacts of global change on Arctic-breeding bird populations and migration. "Global change and Arctic terrestrial ecosystems" (pp. 201-217). Springer, New York, NY.

Brown, J., Jorgenson, M. T., Smith, O. P., & Lee, W. (2003). Long-term rates of coastal erosion and carbon input, Elson Lagoon, Utqiaġvik, Alaska. In *Eighth International Conference on Permafrost* (Vol. 21, p. 25).

Butler, M. G. (1980a). Emergence phenologies of some arctic Alaskan Chironomidae. In *Chironomidae* (pp. 307-314).

Butler, M. G. (1980b). The population ecology of some arctic Alaskan Chironomidae (Diptera).

Butler, M. G. (1982a). A 7-year life cycle for two Chironomus species in arctic Alaskan tundra ponds (Diptera: Chironomidae). *Canadian Journal of Zoology*, *60*(1), 58-70.

Butler, M. G. (1982b). Production dynamics of some arctic Chironomus larvae. *Limnology and Oceanography*, 27(4), 728-736.

Butler, M. G. (1982c). Morphological and phenological delimitation of Chironomus prior sp. n. and C. tardus sp. n. (Diptera: Chironomidae), sibling species from arctic Alaska. *Aquatic Insects*, 4(4), 219-235.

Butler, M. G., & Walker, L. (1992). Fecundity, reproductive effort, and pupal size in the profundal midge Chironomus cuccini (Diptera: Chironomidae). *Netherland Journal of Aquatic Ecology*, 26(2-4), 263-267.

Butler, M. G. (2000). Tanytarsus aquavolans, spec. nov. and Tanytarsus nearcticus, spec. nov., two surface-swarming midges from arctic tundra ponds. *Spixiana*. 23: 211–218.

Butler, M. G., & Braegelman, S. D. (2018). Pre-emergence growth and development in the arctic midge Trichotanypus alaskensis Brundin. *Journal of Limnology*.

Braegelman, S. D. (2016). Seasonality of some arctic Alaskan chironomids (Order No. 10147072). Available from Dissertations & Theses @ North Dakota State University; ProQuest Dissertations & Theses Global. (1830464132).

Carey, C. (1986). Avian reproduction in cold climates. In *Proceedings of the International Ornithological Congress* (Vol. 19, pp. 2708-2715).

Chen, I. C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, *333*(6045), 1024-1026.

Conklin, J. R., Battley, P. F., Potter, M. A., & Fox, J. W. (2010). Breeding latitude drives individual schedules in a trans-hemispheric migrant bird. *Nature Communications*, 1, 67.

Cranston, P. S., Hardy, N. B., & Morse, G. E. (2012). A dated molecular phylogeny for the Chironomidae (Diptera). *Systematic Entomology*, *37*(1), 172-188.

Cushing, D. H. (1969). The regularity of the spawning season of some fishes. ICES *Journal of Marine Science*, 33(1), 81-92.

Custer, T. W., & Pitelka, F. A. (1978). Seasonal trends in summer diet of the Lapland Longspur near Utqiaġvik, Alaska. *The Condor*, 80(3), 295-301.

Danks, H. V., & Oliver, D. R. (1972). Seasonal emergence of some high arctic Chironomidae (Diptera). *The Canadian Entomologist*, 104(5), 661-686.

Daufresne, M., Lengfellner, K., & Sommer, U. (2009). Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences*, *106*(31), 12788-12793.

Davis, M. B., & Shaw, R. G. (2001). Range shifts and adaptive responses to Quaternary climate change. *Science*, *292*(5517), 673-679.

Del Vicario, M., Bessi, A., Zollo, F., Petroni, F., Scala, A., Caldarelli, G., & Quattrociocchi, W. (2016). The spreading of misinformation online. *Proceedings of the National Academy of Sciences*, 113(3), 554-559.

Dunn, P. O., Winkler, D. W., Whittingham, L. A., Hannon, S. J., & Robertson, R. J. (2011). A test of the mismatch hypothesis: How is timing of reproduction related to food abundance in an aerial insectivore?. *Ecology*, 92(2), 450-461.

Durant, J. M., Hjermann, D. Ø., Ottersen, G., & Stenseth, N. C. (2007). Climate and the match or mismatch between predator requirements and resource availability. *Climate research*, *33*(3), 271-283.

Dyar, H. G. (1897). Note on Larvae of Gynaephora Groenlandica and S. Rossii. Psyche: A Journal of Entomology, 8(260), 153-153.

Fagerström, T., & Wiklund, C. (1982). Why do males emerge before females? Protandry as a mating strategy in male and female butterflies. *Oecologia*, 52(2), 164-166.

Ferrington, L. C. (2007). Global diversity of non-biting midges (Chironomidae; Insecta-Diptera) in freshwater. In Freshwater Animal Diversity Assessment (pp. 447-455). Springer, Dordrecht.

Frouz, J., Ali, A., & Lobinske, R. J. (2002). Influence of temperature on developmental rate, wing length, and larval head capsule size of pestiferous midge Chironomus crassicaudatus (Diptera: Chironomidae). *Journal of Economic Entomology*, 95(4), 699-705.

Gardner, J. L., Peters, A., Kearney, M. R., Joseph, L., & Heinsohn, R. (2011). Declining body size: a third universal response to warming?. *Trends in ecology & evolution*, *26*(6), 285-291.

Gangodagamage, C., Rowland, J. C., Hubbard, S. S., Brumby, S. P., Liljedahl, A. K., Wainwright, H., Wilson, C. J., Altmann, G. L., Dafflon, B., Peterson, J., Ulrich, C., Tweedie, C. E., and Wullschleger, S. D. (2014). Extrapolating active layer thickness measurements across Arctic polygonal terrain using LiDAR and NDVI data sets. *Water Resources Research*, 50(8), 6339-6357.

Gressitt, J. L., & Yoshimoto, C. M. (1974). Insect dispersal studies in northern Alaska. *Pacific Insects*, 16(1), 11-30.

Helmers, D. L., & Gratto-Trevor, C. L. (1996). Effects of predation on migratory shorebird recruitment. In *Transactions of the North American Wildlife and Natural Resources Conference* (Vol. 61, pp. 50-61). Wildlife Management Institute.

Hjort, J. (1914). Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. *ICES*.

Hobbie, J. E., Peterson, B. J., Bettez, N., Deegan, L., O'Brien, W. J., Kling, G. W., ... & Hershey, A. E. (1999). Impact of global change on the biogeochemistry and ecology of an Arctic freshwater system. *Polar Research*, 18(2), 207-214.

Hodkinson, I. D., Coulson, S. J., Webb, N. R., Block, W., Strathdee, A. T., Bale, J. S., & Worland, M. R. (1996). Temperature and the biomass of flying midges (Diptera: Chironomidae) in the high Arctic. *Oikos*, 241-248.

Holmes, R. T. (1966). Feeding ecology of the red-backed sandpiper (Calidris alpina) in Arctic Alaska. *Ecology*, 47(1), 32-45.

Holmes, R. T., & Pitelka, F. A. (1968). Food overlap among coexisting sandpipers on northern Alaskan tundra. *Systematic Zoology*, 17(3), 305-318.

Høye, T. T., & Forchhammer, M. C. (2008). Phenology of high-arctic arthropods: effects of climate on spatial, seasonal, and inter-annual variation. *Advances in Ecological Research*, 40, 299-324.

Ineichen, H., Meyer, B., Lezzi, M., (1983). Determination of the developmental stage of living fourth instar larvae of Chironomus tentans. *Developmental Biology* 98: 278-286.

Intergovernmental Panel on Climate Change (IPCC). Fifth Assessment Report (2014).

Johnson, S. R., & Herter, D. R. (1989). The birds of the Beaufort Sea. Anchorage: British Petroleum Exploration (Alaska).

Johnson, S. R., & Herter, D. R. (1990). Bird migration in the Arctic: a review. In *Bird Migration* (pp. 22-43). Springer, Berlin, Heidelberg.

Kingsolver, J. G., & Huey, R. B. (2008). Size, temperature, and fitness: three rules. *Evolutionary Ecology Research*, *10*(2), 251-268.

La Sorte, F. A., Lepczyk, C. A., Burnett, J. L., Hurlbert, A. H., Tingley, M. W., & Zuckerberg, B. (2018). Opportunities and challenges for big data ornithology. *The Condor*, 120(2), 414-426.

Lackmann, A. R., & Butler, M. G. (2018). Breaking the rule: Five larval instars in the podonomine midge Trichotanypus alaskensis Brundin from Utqiaġvik, Alaska. *Journal of Limnology*.

Lackmann, A. R., McEwen D. C., & Butler, M. G. (in prep). Evidence of Parthenogenetic Paratanytarsus laccophilus (Diptera: Chironomidae) populations from the Alaskan Arctic

Lanctot, R. B., Weiser, E. L., Sandercock, B. K., & Brown, S. C. (2015). 2010–2014 Final report: Using a Network of Sites to Evaluate How Climate-mediated Changes in the Arctic Ecosystem are Affecting Shorebird Distribution, *Ecology and Demography*.

Lepage, D., Gauthier, G., & Desrochers, A. (1998). Larger clutch size increases fledging success and offspring quality in a precocial species. *Journal of Animal Ecology*, 67(2), 210-216.

Leung, M. C. Y., Bolduc, E., Doyle, F. I., Reid, D. G., Gilbert, B. S., Kenney, A. J., Charles, J. K., & Bêty, J. (2018). Phenology of hatching and food in low Arctic passerines and shorebirds: is there a mismatch?. *Arctic Science*, (0), 1-19.

Liljedahl, A. K., Hinzman, L. D., & Schulla, J. (2012). Ice-wedge polygon type controls low-gradient watershed-scale hydrology. In *Proceedings of the Tenth International Conference on Permafrost* (Vol. 1, pp. 231-236). The Northern Publisher: Salekhard, Russia.

Liljedahl, A. K., et al. (2016). Pan-Arctic ice-wedge degradation in warming permafrost and its influence on tundra hydrology. *Nature Geoscience*, 9(4), 312-318.

Lougheed, V. L., Butler, M. G., McEwen, D. C., & Hobbie, J. E. (2011). Changes in tundra pond limnology: Re-sampling Alaskan ponds after 40 years. *Ambio*, 40(6), 589-599.

MacLean Jr, S. F., & Pitelka, F. A. (1971). Seasonal patterns of abundance of tundra arthropods near Utqiaġvik. *Arctic*, 19-40.

Marcus, N. H. (1998). Minireview: The importance of benthic-pelagic coupling and the forgotten role of life cycles in coastal aquatic systems. *Limnology and Oceanography*, 43(5), 763-768.

McEwen, D. C., & Butler, M. G. (2018). Growing-Season Temperature Change across Four Decades in an Arctic Tundra Pond. *Arctic*, 71(3), 281-291.

McKinnon, L., Picotin, M., Bolduc, E., Juillet, C., & Bêty, J. (2012). Timing of breeding, peak food availability, and effects of mismatch on chick growth in birds nesting in the High Arctic. *Canadian Journal of Zoology*, 90(8), 961-971.

Metzger, M. J. (2007). Making sense of credibility on the Web: Models for evaluating online information and recommendations for future research. *Journal of the American Society for Information Science and Technology*, 58(13), 2078-2091.

Møller, A. P. (2004). Protandry, sexual selection and climate change. *Global Change Biology*, 10(12), 2028-2035.

Morbey, Y. E., & Ydenberg, R. C. (2001). Protandrous arrival timing to breeding areas: a review. *Ecology letters*, 4(6), 663-673.

Morrison, R. G., McCaffery, B. J., Gill, R. E., Skagen, S. K., Jones, S. L., Page, G. W., ... & Andres, B. A. (2006). Population estimates of North American shorebirds. Bulletin-Wader Study Group, 111, 67.

Muster, S., Heim, B., Abnizova, A., & Boike, J. (2013). Water body distributions across scales: a remote sensing based comparison of three arctic tundra wetlands. *Remote Sensing*, 5(4), 1498-1523.

Notz, D., & Stroeve, J. (2016). Observed Arctic sea-ice loss directly follows anthropogenic CO2 emission. *Science*, 354(6313), 747-750.

Oechel, W. C., Hastings, S. J., Vourlrtis, G., Jenkins, M., Riechers, G., & Grulke, N. (1993). Recent change of Arctic tundra ecosystems from a net carbon dioxide sink to a source. *Nature*, 361(6412), 520.

Osborne, E., Richter-Menge J., Jeffries, M. O., (2018). Arctic report card 2018.

Petit, S., Moilanen, A., Hanski, I., & Baguette, M. (2001). Metapopulation dynamics of the bog fritillary butterfly: movements between habitat patches. *Oikos*, 92(3), 491-500.

Pitelka, F. A. (1974). An avifaunal review for the Utqiaġvik region and North Slope of Arctic Alaska. *Arctic and Alpine Research*, 6(2), 161-184.

Saalfeld, S. T., McEwen, D. C., Kesler, D. C., et al. (2019). Phenological mismatch in Arctic-breeding shorebirds: Impact of snowmelt and unpredictable weather conditions on food availability and chick growth. *Ecology and Evolution*, 9, 6693–6707.

Schekkerman, H., Tulp, I., Piersma, T., & Visser, G. H. (2003). Mechanisms promoting higher growth rate in arctic than in temperate shorebirds. *Oecologia*, 134(3), 332-342.

Sharpe, P. J., & DeMichele, D. W. (1977). Reaction kinetics of poikilotherm development. *Journal of Theoretical Biology*, 64(4), 649-670.

Sheridan, J. A., & Bickford, D. (2011). Shrinking body size as an ecological response to climate change. *Nature climate change*, *1*(8), 401.

Strode, P. K. (2003). Implications of climate change for North American wood warblers (Parulidae). *Global Change Biology*, 9(8), 1137-1144.

Taylor, P. C., Cai, M., Hu, A., Meehl, J., Washington, W., & Zhang, G. J. (2013). A decomposition of feedback contributions to polar warming amplification. *Journal of Climate*, 26(18), 7023-7043.

Tews, J., Brose, U., Grimm, V., Tielbörger, K., Wichmann, M. C., Schwager, M., & Jeltsch, F. (2004). Animal species diversity driven by habitat heterogeneity/diversity: the importance of keystone structures. *Journal of biogeography*, 31(1), 79-92.

Tschudi, M. A., Stroeve, J. C., & Stewart, J. S. (2016). Relating the age of Arctic sea ice to its thickness, as measured during NASA's ICESat and IceBridge campaigns. *Remote Sensing*, 8(6), 457.

Tulp, I., & Schekkerman, H. (2008). Has prey availability for arctic birds advanced with climate change? Hindcasting the abundance of tundra arthropods using weather and seasonal variation. *Arctic*, 48-60.

Visser, M. E., Van Noordwijk, A. J., Tinbergen, J. M., & Lessells, C. M. (1998). Warmer springs lead to mistimed reproduction in great tits (Parus major). *Proceedings of the Royal Society B: Biological Sciences*, *265*(1408), 1867-1870.

Visser, M. E., & Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society of London B: Biological Sciences*, *272*(1581), 2561-2569.

Vosoughi, S., Roy, D., & Aral, S. (2018). The spread of true and false news online. *Science*, 359(6380), 1146-1151.

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J., Fromentin, J.M., Hoegh-Guldberg, O. and Bairlein, F., (2002). Ecological responses to recent climate change. *Nature*, *416*(6879), p.389.

Weiser, E. L., Brown, S. C., Lanctot, R. B., Gates, H. R., Abraham, K. F., Bentzen, R. L., ... & English, W. B. (2018). Effects of environmental conditions on reproductive effort and nest success of Arctic-breeding shorebirds. *Ibis*, 160(3), 608-623.

Wülker, W., Götz, P. (1968). Die Verwendung der Imaginalscheiben zur Bestimmung des Entwicklungszustandes von Chironomus-Larven (Dipt.). *Zoomorphology*. 62(4): 363-388.

2. BREAKING THE RULE: FIVE LARVAL INSTARS IN THE PODONOMINE MIDGE *TRICHOTANYPUS ALASKENSIS* BRUNDIN FROM BARROW, ALASKA[1]

2.1. Abstract

Chironomid larvae are generally reported to pass through four larval instars between egg and pupal stages. We have observed a fifth larval instar to be a standard life-cycle feature of the podonomine *Trichotanypus alaskensis* Brundin 1966 in tundra ponds on the Arctic Coastal Plain near Utqiaġvik, Alaska. *T. alaskensis* has a one-year life cycle in these arctic ponds. Adults emerge in June ~2-3 weeks after pond thaw, then mate and oviposit; most newly-hatched larvae reach instar IV by October when pond sediments freeze. Overwintering larvae complete instar IV within a few days of thaw, then molt again to a fifth larval instar. Imaginal discs, normally seen only during instar IV in Chironomidae, develop across both instars IV & V prior to pupation and adult emergence. While monitoring larval development post-thaw in 2014, we noticed freshly-molted *T. alaskensis* larval exuviae a week or more prior to any pupation by that species. In 2015-16 we reared overwintering instar IV larvae from single pond sources, individually with daily monitoring, through molts to instar V, pupa, and adult. Some overwintering instar II and III larvae were reared as well, but were few in number. During 2016 we also reared *T. alaskensis* progeny (from eggs) through instar II, thus documenting head capsule size ranges for all five instars in a single pond's population. Without individual rearings, the fifth larval instar was not

[1] The material in this chapter was co-authored by Alec Lackmann and Malcolm Butler, and published in the *Journal of Limnology* (see references). Alec Lackmann had primary responsibility for data collection and the conclusions developed here. Alec Lackmann also drafted and revised all versions of this chapter. Malcolm Butler served as proofreader, contributed to data collection, and assisted development of conclusions.

readily apparent for two reasons: 1) The molt itself occurs immediately after thaw and is so synchronous it is difficult to discern in daily field samples. 2) The head capsule size increment between instars IV-V is much lower than the ratio predicted by the Brooks-Dyar Rule. Up through instar IV, the Brooks-Dyar ratio for *T. alaskensis* ranged 1.30-1.61, but during the IV-V molt head capsule dimensions increased by a ratio of 1.09 – comparable to the magnitude of sexual dimorphism in head capsule size. Individual rearings coupled with 2014-2016 field surveys in nine other ponds suggest that five larval instars is an obligatory trait of this species at this location. As this is the first known case of five larval instars in a chironomid, the phylogenetic uniqueness of this trait needs further investigation.

2.2. Introduction

Chironomids are often the most abundant macroinvertebrates in freshwater, and this ubiquity has strongly encouraged their global study over the past century. The chironomid life-cycle consists of four different stages: the egg, the larval instars, the pupa, and the imago (Armitage *et al.*, 1995), and for decades it is has been a general rule that chironomids pass through four larval instars prior to pupation (Thienemann, 1954; Ford, 1959; Oliver, 1971) based on evidence from the subfamilies Chironominae, Orthocladiinae, Tanypodinae and Diamesinae (McCauley, 1974).

Nevertheless, there have been unconfirmed reports of more than four larval instars in the Orthocladiinae (Kettisch, 1937; Styczynski and Rakusa-Suszczwski, 1963). Thienemann (1954) discounted Kettisch's (1937) report of seven larval instars in an orthoclad because the result was not based on individual larval rearings. The report of five larval instars in a tanypod (Sæther, 1968; Oliver, 1971; Armitage *et al.*, 1995) appears to be misconstrued. The actual source (Styczynski and Rakusa-Suszczwski, 1963) does not mention any tanypodine, but instead

documents two orthoclad species with a fifth larval instar group. Their method of determining larval instar groups is poorly described, and is likely inaccurate because they did not individually rear larvae, nor did they account for multiple habitat sources (Usher and Edwards, 1984) or sexual dimorphism (Ford, 1959). Similarly, it was once claimed that the Antarctic midge (Orthocladiinae: *Belgica antarctica* Jacobs) had six larval instars after larvae were collected from different sources and sorted into instar groups based on head capsule size (Peckham, 1971). The author was aware that habitat-specific size differences could possibly confound the results, but still concluded that the species had six larval instars. *B. antarctica* was later documented to have four larval instars (Sugg *et al*., 1983; Usher and Edwards, 1984). Thus, there has not been a confirmed report of a chironomid passing through anything other than four larval instars. Here we present evidence of five larval instars in the podonomine midge *Trichotanypus alaskensis* Brundin based on collections and rearings from a single pond on Alaska's Arctic Coastal Plain (ACP).

2.3. Methods

The main study site is located near Utqiaġvik (Barrow), Alaska (71°17'27.5"N 156°47'18.5"W) at Pond OH (71°16'35.4"N 156°38'28.0"W) approximately 5.5 km ESE from the village. Pond OH is a roughly circular, low-centered polygon pond (~15 m in diameter) with a maximum depth of about 35 cm. The emergent sedge *Carex aquatilis* Wahlenberg surrounds the pond's edge, and in the open water lies flocculent peat-rich sediment with an active layer reaching ~15 cm maximum, beneath which is permafrost. Like most tundra ponds on the ACP, Pond OH freezes completely during the winter. In 2015 we reared *T. alaskensis* larvae from OH, while in 2016 we used larvae collected from both Pond OH and Bear Pond (71°16'37.1"N 156°38'23.0"W, 50 m from OH).

In addition to Ponds OH and Bear, we sampled eight other ponds across years 2014-2016 (Icy and Humpback, 130 m from OH, and Ponds A, C, E, J, G, and Kaleak within 3.1 km of OH). We sampled all ten ponds for *T. alaskensis* larvae, pupae, and emerging adults (by presence of pupal exuviae [PE]) on an every-other-day basis, from pond thaw until emergence. We made collections by sweeping through aquatic vegetation along the edge of ponds with a hand-held dip net, immediately fixing collected specimens in Kahle's fluid (59% water, 28% EtOH, 11% formalin, and 2% glacial acetic acid) (Winterbourn *et al*., 1989). This fixative highlights pupal/adult primordia (imaginal discs) (e.g. developing legs and wings in the thoracic segments), facilitating accurate analysis of developmental phases (*sensu* Wülker and Götz, 1968).

Our 2014 observations on *T. alaskensis* larvae collected in the week following ice-out prompted us to conduct controlled rearings in 2015-2016. Among the 4,171 Kahle's-fixed *T. alaskensis* larvae we collected early in June of 2014, we found numerous specimens preserved in mid-molt, but showing an advanced state of thoracic primordial development. We also found hundreds of fresh larval exuviae (LEs) in these samples, with no pupae present and seemingly no increase in mean larval size relative to earlier samples. We hypothesized that if we were to rear larvae individually immediately after thaw, penultimate and ultimate instar LEs could be collected from each larva.

In both 2015 and 2016 we collected *T. alaskensis* larvae shortly after pond thaw to rear under semi-artificial conditions (Fig. 2.1) where we could monitor larval and pupal molting. Upon our arrival at the field site on 2 June 2015, much of Pond OH had already thawed over the prior 1-2 days. We collected *T. alaskensis* larvae from *Carex* in a part of the pond where much bedfast ice remained. On 26 May 2016, we again collected larvae from the same part of Pond OH, but that year only the pond's periphery had thawed, forming a moat that surrounded solid

ice remaining in the pond's center. With most of Bear Pond still frozen on 28 May 2016, we collected *T. alaskensis* larvae from a newly thawed area of emergent *Carex* habitat. We suspect that these larvae collected early post-thaw were indicative of the developmental stage at which they overwintered, as Butler and Braegelman (2018) show insignificant growth or development by this species in the first days following thaw.

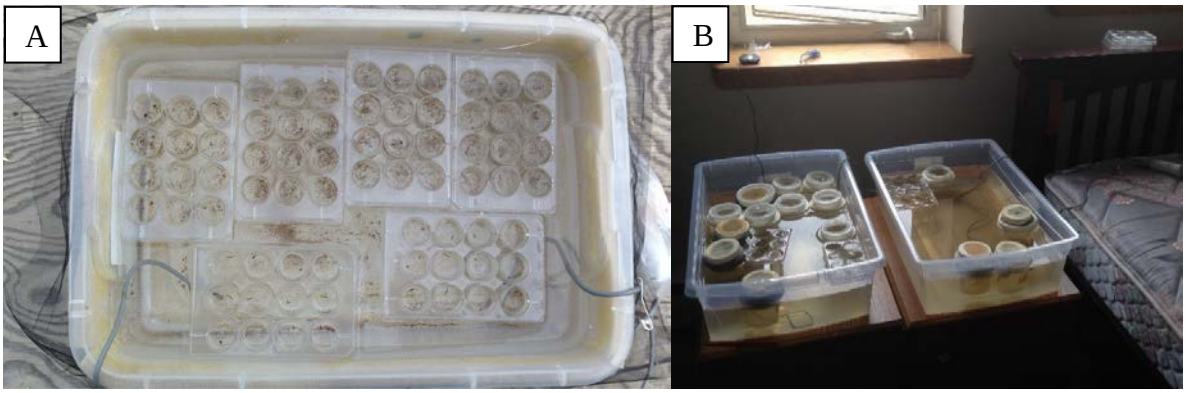

Figure 2.1. Rearing setups used in 2015 (A) and 2016 (B).
See Methods for details.

In 2015, each of 184 *T. alaskensis* larvae, freshly collected from Pond OH, was placed in its own well within a 12-well culture plate; these plates were placed in one of two tubs filled with pond water (Fig. 2.1A), each well receiving about 0.25 ml of detritus slurry via pipette. We placed tubs outdoors near our residence and monitored water temperature using ONSET® HOBOware data loggers, checking rearings at least daily for molting (as indicated by shed LEs). All LEs recovered were preserved individually in vials of 70% ethanol. We often found a freshly-shed LE along with a teneral larva whose head capsule was still white/translucent, indicating a recent molt. We continued to track larvae for further ecdysis to pupal and adult stages, in which case we collected another LE or PE from that individual.

In 2016, recently-thawed *T. alaskensis* larvae collected from Pond OH and Bear Pond were group-reared in buoyant, meshed-sided cups maintained in separate pond-specific tubs,

each containing a water-temperature logger (Fig. 2.1B). We placed approximately 20 larvae, along with detritus as food, into each rearing cup, and maintained the tubs indoors in an unheated room with open windows (Fig. 2.1B) so the insects experienced near-natural temperature and light conditions. This rearing setup improved on our 2015 method in that it buffered the insects from extreme weather events. We checked all rearing cups two or more times daily for insects undergoing a life-stage change, removing and preserving LEs as they were discovered. We transferred all teneral, white-headed larvae to individual rearing cells within floating six-well plates (Fig. 2.1B). We tracked subsequent development during the fifth larval instar under two treatments: one with, and one without food. To the "with food" wells we added detritus, adding only settled pond water (pipetted from the clear surface water of the rearing tub) to the "no food" wells. We subsequently tracked these instar V larvae to pupation (collecting their LEs), and then to adult eclosion (collecting PEs). In 2016, we left nine of the initially-collected instar IV larvae in rearing cups to encourage reproduction by emerging adults within a contained area. Emerging adults did produce viable egg masses that we reared individually, and monitored for development several times daily. We preserved (in 70% ethanol) a subset of the larvulae soon after hatching. Once we observed the first larval molt, we also preserved a subset of instar II larvae, along with their instar I LEs. We preserved surviving larvae, all still in instar II, on 31 July 2016 when we left Utqiaġvik.

To discriminate larval instars quantitatively, we took photographs of the shed LEs under an Olympus SZH10 microscope at 70X magnification using SPOT microscopy imaging software. LEs were placed in 70% EtOH in a glass petri dish with a tiny amount of petroleum jelly on the bottom. The jelly works as an adhesive to hold the head capsules in proper orientation (ventral side up) for consistent length and width measurements. This method avoids

the compressional forces of mounting head capsules on slides, maintaining the head capsule's three-dimensionality. Head capsule length (HCL) and head capsule width (HCW) were measured on LEs that were intact (Fig. 2.2A-B). Furthermore, previously preserved larvae from 2014 field samples in Ponds OH, Icy and Humpback were photographed for thoracic imaginal disk primordia (Fig. 2.2C-E) to document their developmental stage at the time of molting to the final larval instar.

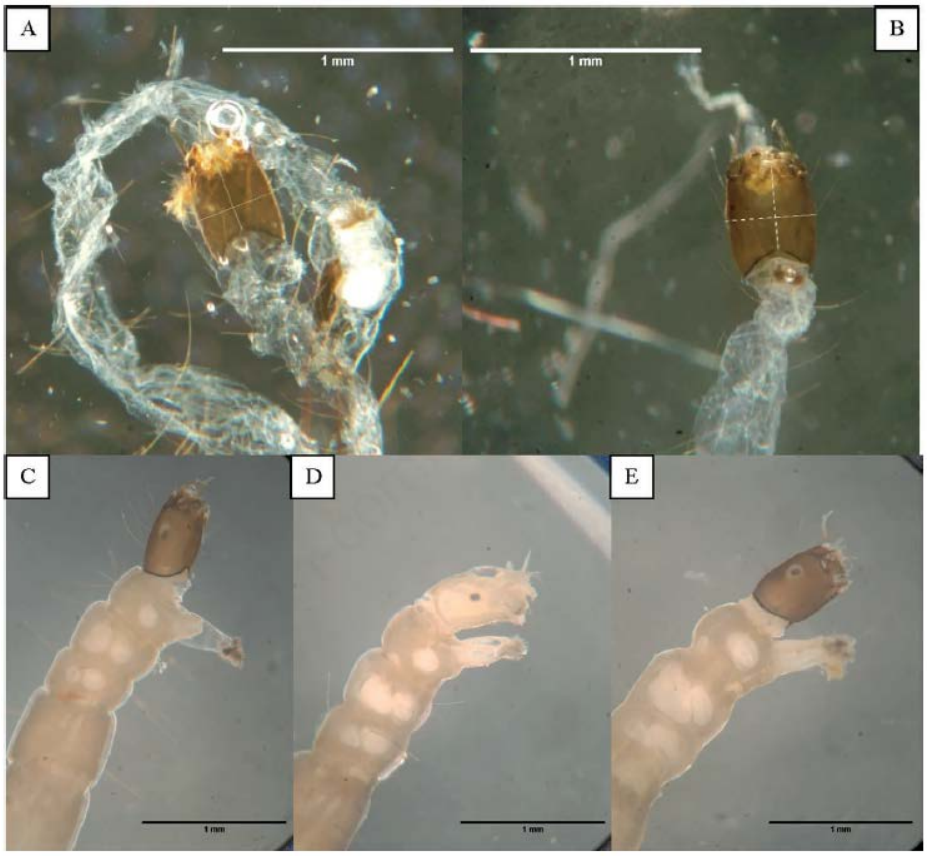

Figure 2.2. *Trichotanypus alaskensis* larvae.
Fourth (A) and fifth (B) instar larval exuviae from a single *T. alaskensis* individual. Fine white lines show measurement of head capsule length (HCL) and width (HCW). Here HCL increased by 7.2 and HCW by 9.3 % from larval instar IV to V, more than 20 % less than predicted by the Brook's-Dyar rule, and nearly identical to the magnitude of sexual dimorphism in head capsule size within instars IV & V. (C-E): Three female *T. alaskensis* larvae collected from HB pond on 20 June 2014, and immediately fixed in Kahle's fluid in the field. Relative developmental phase increases from left to right. (C) Instar IV nearing ecdysis to larval instar V (note retracting eyespot). (D) Teneral instar V larva. (E) Instar V larva at a yet later stage of development as evidenced by enlarged leg, wing, haltere, and respiratory organ primordia.

We analyzed head capsule images with ImageJ software, making linear measurements to the nearest micrometer. We defined HCL as the medial distance from the posterior margin of the gula to the anterior-most point of the mentum, and HCW as the maximum width of the HC orthogonal to its longitudinal axis (Fig. 2.2A-B). We used JMP 13 Statistical Discovery™ for statistical analysis and graphical output.

2.4. Results

Our 2015 rearings began with 184 *T. alaskensis* larvae from Pond OH, 129 of which had large head capsules (presumed instar IV) and 55 were earlier instars (with smaller HCs and without thoracic primordia). An enormous molting event occurred on 7 June 2015, when we collected all but two of the 116 penultimate (instar IV) LEs from the 129 larger HC larvae (Fig. 2.3). The other 13 larger larvae either failed in mid-molt to the fifth larval instar (10), escaped (2), or died before molting (1). This evidence strongly suggests the fifth larval instar is obligatory for this species. For those larvae that successfully reached the fifth instar, 49 (42%) pupated, and the other 58% failed in their molt to the pupal stadium. Only three pupae eclosed to the adult stage, the rest perished as pupae (Fig. 2.3A). This low molting success late in the life cycle in 2015 may have resulted from high water temperatures (some over 20ºC) and the small rearing wells used that year, relative to our rearings in 2016 (Figs. 2.1 and 2.3). Overall, in 2015 we tracked individual growth in HCL for 27 larvae, from the penultimate through the ultimate larval instar (27 of the 105 instar IV data & all instar V data in Fig. 2.4), excluding LEs with damaged head capsules.

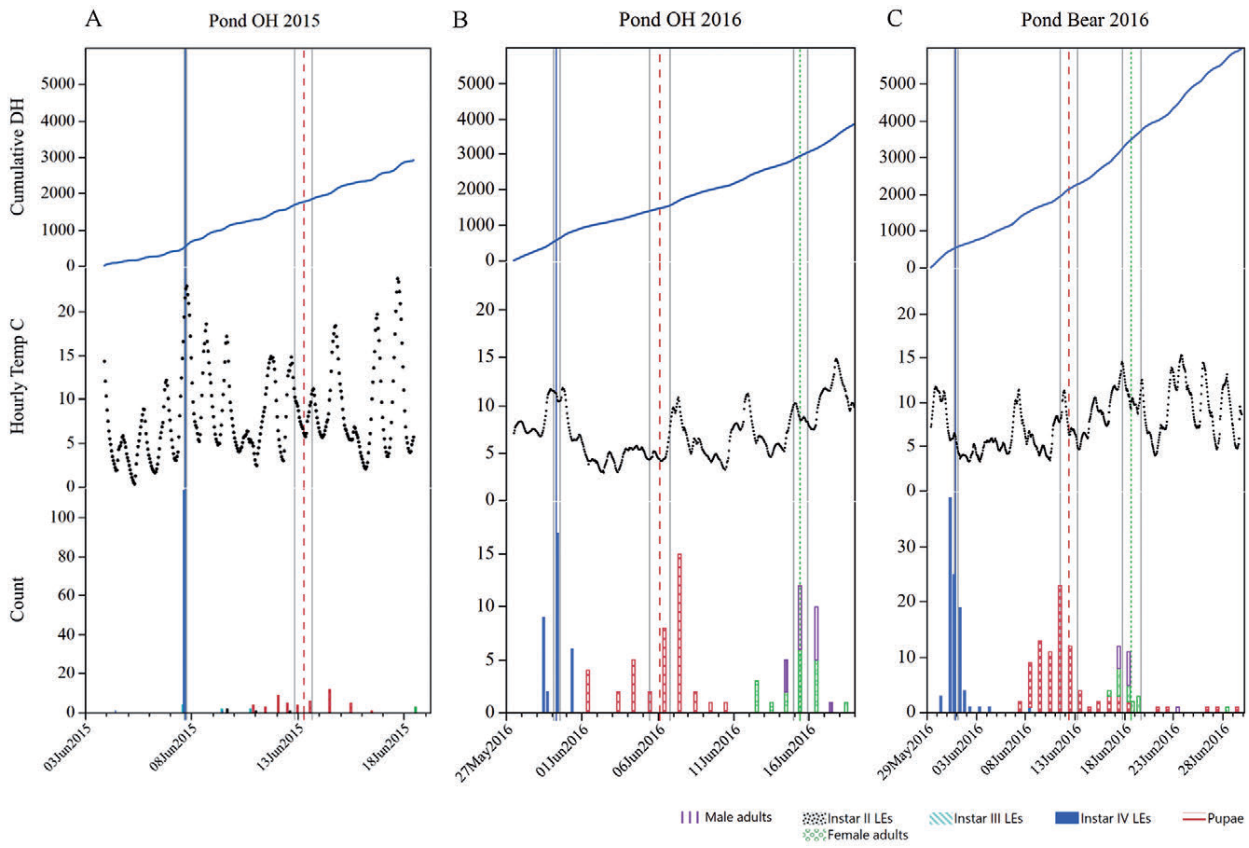

Figure 2.3. Summary of *T. alaskensis* larvae reared in 2015 (A) and 2016 (B-C). Counts are number of individuals molting to instar III (striated black bars), instar IV (teal diagonally-hatched bars), instar V (solid blue bars), pupa (horizontally-hatched bars), and adult (♀ green circled bars, ♂ purple vertically-hatched bars). Vertical lines show the mean date of ecdysis for the molt to the final larval instar, the pupa, and the imago – with grey lines indicating 95% CI (adult sexes combined). Hourly temperatures (middle panel) and cumulative degree hours (DH) (upper panel) are plotted.

Because we reared individual larvae to pupation or eclosion, we know that the LE measurements shown as instar IV and instar V in Fig. 2.4 represent penultimate and ultimate instars. Most (78%) of the 55 larvae in the "small HC group" perished before any molt was observed, but we collected 12 additional LEs from larvae that did molt successfully. From HCL measurements, we proposed that four of these larvae had overwintered as instar II, and eight as instar III (shown as triangles for instar II data points, plus all instar III data points in Fig. 2.4). We later confirmed these instar II and III determinations by rearing F1 progeny of emerging

adults in 2016 (see below). None of the 12 instar II or III larvae that did molt to the next instar survived to another molt, possibly due to greater food requirements for these smaller larvae than was available in our small 2015 rearing wells.

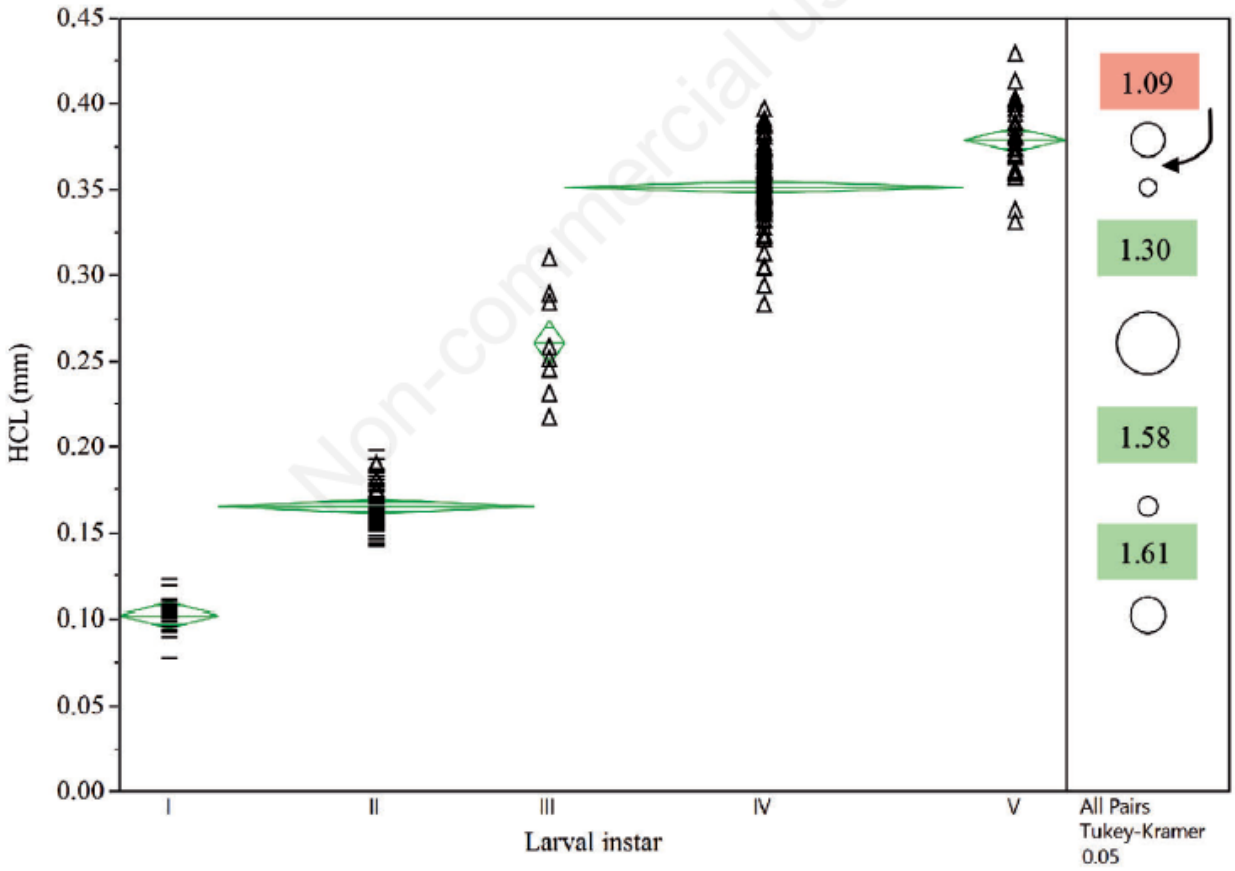

Figure 2.4. Head capsule length (HCL) measurements for the five larval instars of *T. alaskensis*. Open triangles represent data collected in 2015, dashes 2016. Non-overlapping circles on the right represent sizes of 95% confidence intervals for HCL, illustrating significant statistical differences between instars (all pairs Tukey-Kramer HSD test, alpha=.05). Numbers in green and red boxes show ratios of growth in HCL from one instar to the next. This molting increment follows the Brooks-Dyar Rule through instar IV, but is significantly smaller (1.09) for the molt to instar V.

In 2016 we began our Pond OH group rearing with 41 instar IV larvae. One larva died prior to any molt, 34 molted to the fifth instar, and 40 pupated. Thus we deduced that six larvae had already molted to instar V prior to collection, or that we failed to find their instar IV LEs in the rearing cup. Of the 40 pupae, 33 reached the imago (18 females, 15 males). Most of these

adults had been reared in isolation after their molt to instar V (see methods for details), and these adults were preserved immediately upon emergence.

We left some larvae together in the group rearing to encourage reproduction by emerging adults, and on 14 June 2016 we found a male and a female that had emerged together. The female was spent, and we found her egg rope on the bottom of the rearing cup. On 24 June 2016 94 larvulae hatched from these eggs. On 3 July 2016 these larvulae molted to instar II, where they remained until preservation at the end of our field season on 31 July 2016. In addition, two females and one male emerged together on 16 June 2016. One female was spent and we recovered another egg rope which produced 52 larvulae on 25 June 2016. These larvulae also molted on 3 July 2016 and they remained as second instars through the end of our field season. These two egg ropes were reared independently in cups that contained only pond water and detritus (i.e. no other chironomids were present). From these progeny, we measured a total of 26 instar I and 79 instar II *T. alaskensis* for HCL (Fig. 2.4).

In 2016 we also group reared 100 instar IV *T. alaskensis* larvae from Bear Pond (Fig. 2.3 [C]). As we recovered 94 shed penultimate LEs, we again deduced that six larvae had already molted to instar V prior to collection (or that we missed six LEs in the rearing cups). Eleven larvae died in the final instar, but 89 pupated and we collected their shed instar V LEs. Pupation failure was high (71%), as only 26 adults eclosed (15 females, 11 males). Such high pupal mortality (compared to only 17.5% for the 2016 Pond OH rearings), likely resulted from use of different trays for rearing individual instar IV larvae and pupae. *T. alaskensis* collected from Pond OH molted earlier in June than larvae reared from Bear Pond, and we isolated final-instar larvae from Pond OH in six-well trays with wells of 19 ml volume (3.7 cm dia x 1.8 cm). These six-well trays were all in use when the Bear Pond rearings escalated, necessitating the use of

twelve-well trays with wells of 7.5 ml volume (2.4 cm dia x 1.7 cm). These smaller wells held ~60% less water volume with a 58% reduction in surface area. The water surface in the smaller wells had a prominent meniscus that may have inhibited the pupae's ability to respire and/or emerge successfully. For future work rearing this species, we advise use of wells with a minimum surface area of 10 cm^2.

In all rearing experiments, ecdysis to the fifth larval instar was more synchronous than molts to pupation or eclosion. This is evident in the 95% confidence intervals around mean ecdysis dates for these final three molts (Fig. 2.3).

Our 2016 rearings indicated that feeding was unnecessary during instar V. Eight of nine instar V Pond OH larvae reared with food eclosed as adults (89%), as did nine of ten such larvae reared without food (90% eclosion). Nine of 43 instar V larvae from Bear Pond reared with food eclosed (21%), while eclosion success for larvae reared without food was 18% (8 of 45 emerging as adults). Despite much lower eclosion success among the Bear Pond larvae (discussed above), successful metamorphosis was comparable between treatments. The final (fifth) larval instar in *T. alaskensis* seems devoted to development, not growth. Nonetheless, Butler and Braegelman (2018) documented larval growth between pond thaw and emergence for *T. alaskensis* in another Utqiaġvik population, and concluded that the species did not meet Danks and Oliver's (1972) criteria for an "absolute spring species". The pre-pupation growth reported by Butler and Braegelman may occur largely during instar IV, and not the final instar.

We found the average increment in HCL during the instar IV-V molt to be only 1.09, based on measurement of individually-tracked *T. alaskensis* larvae (Fig. 2.4). This does not conform to the "Brooks-Dyar Rule" (Brooks, 1886; Dyar, 1890), which holds that linear molt increments in arthropods generally follow a growth ratio of about 1.3-1.7 times their previous

size. The Brooks-Dyar Rule may also be used to predict which instars (if any) are missing in a sampled population of arthropods (Crosby, 1973). Our documentation of this small molt increment in head-capsule size between penultimate and final instars indicates that coarse analyses of larval instar distribution in chironomids have potential to underestimate instar count in some taxa. Sexual dimorphism in head capsule dimensions can be significant in the final instar (Ford, 1959; Atchley, 1971), and can potentially confound recognition of instar distinctions if the molt ratio is low.

Comparing the magnitude of HC size variation due to sex, relative to variation due to instar difference, requires independent determination of both variables. *Trichotanypus alaskensis* larvae can be sexed easily during both larval instars IV and V because genital primordia are visible in the eighth and ninth abdominal segments, much as in *Chironomus* (Wülker and Götz, 1968; Ineichen *et al*. 1983). We also discovered that the molt to instar V occurs at a consistent stage of thoracic development, about midway through the Wülker and Götz (1968) scheme for final instar *Chironomus*. Comparing thoracic primordial development of 14 (5♀, 9♂) recently molted instar V larvae (with poorly-sclerotized, white head capsules) to developmental phases of larvae in instar IV and later instar V (with sclerotized HCs), it is evident that this molt occurs when the wing and leg primordia in thoracic segment II become confluent (Fig. 2 C-E). Lack of a sex-specific difference in this developmental event permits one to distinguish instar IV and V larvae based on their developmental stage.

Using thoracic primordial development as an indicator of larval instar, we measured head capsule dimensions of 91 instar IV and 92 instar V Kahle's-preserved *T. alaskensis* collected during June 2014 from Ponds OH and HB. As larvae from these ponds showed no significant differences in HC measurements (t-tests), we pooled the data from both ponds.

During larval instar IV, female *T. alaskensis* HCL and HCW are ~6-7% larger than males. During instar V, female head capsules are about 6-8% larger than males in HCW and HCL (Table 2.1). Between instars IV and V, HCL increases by only 9% on average (Fig. 2.4), and HCW by 14% (data not shown). For *T. alaskensis*, variation in HC size between sexes, coupled with smaller-than-expected growth increments during the molt to the final larval instar, obscured recognition of a fifth instar by traditional methods.

Table 2.1. *T. alaskensis* sexual dimorphism in HCW and HCL across larval instars IV and V.

Instar	Metric	Sex	Mean (mm)	L 95%	U 95%	N
IV	length	F	0.378	0.366	0.389	9
IV	length	M	0.356	0.344	0.368	8
V	length	F	0.416	0.407	0.425	13
V	length	M	0.386	0.373	0.399	6
IV	width	F	0.386	0.382	0.390	54
IV	width	M	0.361	0.356	0.365	54
V	width	F	0.449	0.443	0.454	38
V	width	M	0.423	0.417	0.428	37

2.5. Discussion

Our finding of five larval instars in the podonomine midge *Trichotanypus alaskensis* means we can no longer assume that all chironomids will have four larval instars. We hypothesize that more than four instars may be a plesiomorphic character state within the Chironomidae. This fifth instar appears obligatory in *T. alaskensis*, at least in the habitat we studied. Potential flexibility of this life history feature could be tested by rearing this species from egg to adult under ideal conditions (e.g. with *ad libitum* food and without an overwintering diapause). Although five larval instars in *Trichotanypus alaskensis* may be viewed as an

exception to the rule, further study of this and other related taxa may uncover additional surprises.

The synchrony of the molt to instar V is strong (Fig. 2.3). How these larvae physiologically regulate such a precisely-timed event is unclear. However, we do know that these larvae molt to the fifth instar at a consistent developmental stage (Fig 2.2D) permitting the distinction of instar IV and V larvae based on thoracic development. Once these larvae reach the fifth instar, our evidence suggests they no longer require feeding. Whether or not these larvae may feed during the fifth larval instar in natural conditions remains unknown, but could be tested by monitoring growth (e.g. dry weights) during the fifth larval instar.

Trichotanypus alaskensis deviates from the Brooks-Dyar rule in the last of its four larval molts, with a growth ratio so small that individual variation and sexual size dimorphism can easily confound distinction of the final two instars. Why *Trichotanypus alaskensis* has an "extra" larval instar is open to speculation. Head capsule shape in this species is quite cylindrical, relative to the more tapered-spherical head capsules typical of larvae in the Tanypodinae, Orthocladiinae, and Chironominae. Yet the apparent lack of growth in the brief final instar of *T. alaskensis* confounds arguments based on shape/volume considerations. Perhaps more plausible than any "adaptive" scenario is the possibility that this fifth larval instar is a phylogenetic legacy from an ancient time when instar number was not as rigidly constrained as we have heretofore believed. Much remains up to speculation as to why *T. alaskensis* has an extra larval instar.

2.6. Conclusion

In his discussion of nematoceran phylogeny, Edwards (1926) broadly stated that the Chironomidae seemed to have reduced the number of larval instars to four. Once additional

evidence had accumulated from the Chironominae and Orthocladiinae, Thienemann (1954) hypothesized that four larval instars likely would be found universally within the Family Chironomidae. Ford (1959) then tested Thienemann's prediction by studying instar numbers of six species within the Orthocladiinae, Tanypodinae, and Diamesinae. For all these species Ford found four larval instars, noting sexual dimorphism in HC dimensions within the final larval instar. He concluded that all the freshwater chironomid subfamilies conformed to Thienemann's generalization of four larval instars in the Chironomidae - although the Podonominae was not tested. McCauley (1974) studied 29 species across the Tanypodinae, Orthocladiinae, and Chironominae, finding all to have four larval instars based on HC sizes. Our discovery of five larval instars in the Podonominae, a phylogenetically basal subfamily (Cranston *et al*., 2012) breaks Thienemann's rule. Further life-history analysis of other basal taxa could improve our understanding of both chironomid and nematoceran evolution.

2.7. References

Armitage, P, Cranston, PS, & Pinder, LCV, (1995). The Chironomidae: The biology and ecology of non-biting midges. 1. Chapman and Hall, London: 572 pp.

Atchley, W, (1971). Components of sexual dimorphism in *Chironomus* larvae (Diptera: Chironomidae). *The American Naturalist* 105(945): 455-466.

Brooks, WK, (1886). Report on the Stomatopoda collected by HMS Challenger during the years 1873-1876. *Zoology*.

Butler, M G, (1980). Emergence phenologies of some arctic Alaskan Chironomidae. p. 307 314. In: D. A. Murray (ed.), Chironomidae: Ecology, Systematics, Cytology, and Physiology, Pergamon Press.

Butler, MG, & Braegelman, SD, (2018). *Journal of Limnology*.

Cranston, PS, Hardy, NB, Morse, GE, (2012). A dated molecular phylogeny for the Chironomidae (Diptera). *Systematic Entomology*. 37(1): 172-188.

Crosby, TK, (1973). Dyar's Rule predated by Brooks' Rule. *New Zealand Entomologist*, 5(2): 175-176.

Danks, HV, Oliver, DR, (1972). Seasonal emergence of some high arctic Chironomidae (Diptera). *The Canadian Entomologist*. 104(5): 661-686.

Dyar, HG, (1890). The number of molts of lepidopterous larvae. *Psyche: A Journal of Entomology*. 5(175-176): 420-422.

Edwards, FW, (1926). The phylogeny of nematocerous Diptera: a critical review of some recent suggestions. Verhandlungen III. Internationalen Entomologen-Kongres. 1: 111-130.

Ford, JB, (1959). A study of larval growth, the number of instars and sexual differentiation in the Chironomidae (Diptera). *Physiological Entomology*, 34(10-12): 151-160.

Ineichen, H, Meyer, B, Lezzi, M, (1983). Determination of the developmental stage of living fourth instar larvae of *Chironomus tentans*. *Developmental Biology*, 98: 278-286.

Kettisch, J, (1937). Zur Kenntnis der Morphologie und Oekologie der Larve von *Cricotopus trifasciatus*. Z. syst. *Insekt*, 15: 153-165.

McCauley, VJ, (1974). Instar differentiation in larval Chironomidae (Diptera). *The Canadian Entomologist*. 106(2): 179-200.Oliver, DR, 1971. Life history of the Chironomidae. Annual review of entomology. 16(1): 211-230.

Peckham V, (1971). Notes on the chironomid midge *Belgica antarctica* Jacobs at Anvers Island in the maritime Antarctic. Pac Insects Monograph. 25: 145-166.

Sæther, OA, (1968). Chironomids of Finse Area Norway with special reference to their distribution in a glacier brook. *Archiv fur Hydrobiologie*. 64(4): 426-483.

Styczynski, B, Rakusa-Susczczewski, S, (1963). Tendipedidae of selected water habitats, of Hornsund region (Spitzbergen). *Polskie Arch. Hydrobiol*. 2: 327-341.

Sugg, P, Edwards, JS, Baust, J, (1983). Phenology and life history of *Belgica antarctica*, an Antarctic midge (Diptera: Chironomidae). *Ecological Entomology*. 8(1): 105-113.

Thienemann, A, (1954). Chironomus. Leben, Verbreitung und wirtschaftliche Bedeutung der Chironomiden. Stuttgart: E.

Usher, MB, Edwards, M, (1984). A dipteran from south of the Antarctic Circle: *Belgica antarctica* (Chironomidae) with a description of its larva. *Biological Journal of the Linnean Society*, 23(1): 19-31.

Winterbourn, MJ, Gregson, KL, Dolphin, CH, (1989). Guide to the aquatic insects of New Zealand. Auckland: Entomological Society of New Zealand. 9: 80 pp.

Wülker, W, Götz, P, (1968). Die Verwendung der Imaginalscheiben zur Bestimmung des Entwicklungszustandes von *Chironomus*-Larven (Dipt.). *Zoomorphology*, 62(4): 363-388.

3. EVIDENCE OF PARTHENOGENETIC *PARATANYTARSUS LACCOPHILUS* (DIPTERA: CHIRONOMIDAE) POPULATIONS FROM THE ALASKAN ARCTIC

3.1. Abstract

Parthenogenesis, reproduction without fertilization, is not common in the Chironomidae (Diptera), a family of insects with more than 10,000 species. Nonetheless, parthenogenetic species and strains have been documented in at least three subfamilies (the Chironominae, Orthocladiinae, and Telmatogoninae), spanning 17 genera and ~30 species. One such species, *Paratanytarsus laccophilus* Edwards 1929, is known to be parthenogenetic in a small portion of its range in Finland, with most other European populations of this species showing evidence of sexual reproduction. We present evidence of parthenogenetic populations of this same species in the Nearctic, specifically a High Arctic site near Utqiaġvik (formerly Barrow), Alaska. During May-July of 2015 and 2016, we sampled emerging adult chironomids and pupal exuviae daily to document insect emergence phenologies. Across 15 local populations, all 623 *P. laccophilus* pupal exuviae collected were female. Larvae reared from two populations under controlled temperature treatments emerged as female adults (N=37). When isolated, these reared female adults oviposited, and eggs hatched successfully. These progeny were reared for another 12-13 days, reaching second instar larvae when they were preserved at the end of our field season. Taken together, this evidence strongly indicates parthenogenesis of *P. laccophilus* at this location. This species was not documented in the 1970s as a member of chironomid fauna of Utqiaġvik. Although a parthenogen, *P. laccophilus* emergence at this location was highly synchronized. In the harsh environment of arctic Alaska, the fitness rewards of parthenogenesis are likely great. Indeed, chironomid parthenogenesis in the northern hemisphere is most commonly documented from far-northern extremes. Investigation of genetic similarity between

parthenogenetic *P. laccophilus* populations from Finland and Alaska could provide valuable insight into the evolution of this reproductive mode in the Chironomidae.

3.2. Introduction

While sexual reproduction is dominant in the animal kingdom, parthenogenesis is a widespread form of asexual reproduction in which there is no fertilization of the egg (Suomalainen 1962; Lynch 1984). Parthenogenesis in animals evolves most commonly as an artifact of hybridization (Carew *et al*. 2013) and is most often thelytokous, *i.e.* where all asexually produced offspring are female. Because offspring are genetically monotonous with mutation as the only source of variation, (Lynch 1984), parthenogenesis is often considered an evolutionary dead end (Darlington 1946; Mayr 1970; Smith 1978). Nonetheless, patterns exist in the distribution of parthenogenetic forms that suggest this reproductive strategy could be evolutionarily advantageous in certain cases.

"Geographic parthenogenesis" describes the observation that parthenogenesis tends to evolve in habitats that are different from where their closely related, sexually-reproducing relatives reside (Vandel 1928). Indeed, parthenogenetic species are most commonly documented from extreme latitudes, especially in harsh habitat conditions (Lynch 1984). Proposals articulated to account for this phenomenon include the "biotic-uncertainty" hypothesis (Ghiselin 1974), and the "tangled bank" hypothesis (Bell 1982). The biotic-uncertainty hypothesis postulates that parthenogens tend to persist in harsh environments that are relatively barren biologically, and an environment with minimal biotic interaction favors the persistence of genetically monotonous lineages. The tangled bank hypothesis argues that harsh environments are less niche-specialized because they are frequently disturbed. This selects against genetically diverse progeny pre-adapted to fill a diverse array of niches, providing opportunities for clonal populations. These

rather intuitive hypotheses include many assumptions, remain untested, and are unsatisfactory in explaining geographic parthenogenesis (Lynch 1984).

Although parthenogenesis is rather widespread across some insect groups such as the Hymenoptera (Suomalainen 1962), it is relatively uncommon in the Chironomidae (Lindeberg 1951; Armitage *et al*. 1995). These non-biting midge flies are often the most common and species-rich macroinvertebrate taxon in freshwater habitats, with estimates of the total richness of this family surpassing 10,000 species (Armitage *et al*. 1995), with the tropics still poorly studied (Ferrington 2007).

Of the 6,434 described chironomid species (International Symposium on Chironomidae 2017), less than 0.5% (~30 species) are known as parthenogens (Table 3.1). These represent 3 of the 11 extant chironomid subfamilies (Cranston *et al*. 2012): the Chironominae, Orthocladiinae, and Telmatogoninae. In the Chironominae, there are 17 parthenogenetic species found among 7 genera (Grimm 1870; Thienemann 1954; Lindeberg 1958; Lindeberg 1971; Grodhaus 1971; Oliver and Danks 1972; Armitage *et al*. 1995; Langton 1998; Donato and Paggi 2008; Porter and Martin 2011); in the Orthocladiinae there are 12 species from 9 genera (Edwards 1919; Thienemann 1954; Edward and Colless 1968; Forsyth 1971; Armitage *et al*. 1995; Siri and Donato 2014; Andersen *et al*. 2016; Bartlett *et al*. 2018); and in the Telmatogetoninae there are two species from the type genus (Crafford 1986; Delettre *et al*. 2003).

Of all these parthenogenetic species (Table 3.1), the best represented genera are *Tanytarsus*, *Paratanytarsus*, *Micropsectra* and *Corynoneura*, while at the other extreme some genera show very limited evidence of parthenogenesis (e.g. *Chironomus*) (Oliver and Danks 1972; Armitage *et al*. 1995). Although most parthenogenetic chironomids are found in arctic and subarctic habitats (Armitage *et al.* 1995), others are reported from sub-Antarctic islands

(Crafford 1986; Delettre et al. 2003), phytotelmata in Argentina (Siri *et al*. 2014), and a cave in Croatia (Andersen *et al*. 2016). Considering the wide range of habitats and the taxonomic difficulty of identifying female midges, parthenogenesis in the Chironomidae may be more common than previously believed.

Table 3.1. Chironomid taxa with evidence of parthenogenesis as of 2019.

Subfamily	Taxon	Reference
Chironominae	*Tanytarsus norvegicus*	Lindeberg 1971
	Tanytarsus gregarius	Lindeberg 1971
	Tanytarsus sp. *(sylvaticus)*	Lindeberg 1971
	Tanytarsus sp. *(lestagei)*	Lindeberg 1971
	Tanytarsus heliomesonyctios	Langton 1998
	Paratanytarsus grimmii	Grimm 1870
	Paratanytarsus laccophilus	Lindeberg 1958
	Paratanytarsus sp. (*boiemicus*)	Lindeberg 1971
	Micropsectra silvesterae	Langton 1998
	Micropsectra sp. (*nigripila*)	Armitage et al 1995
	Micropsectra sedna	Porter et al 2011
	Chironomus atrella	Grodhaus 1971
	Chironomus attenuatus	Grodhaus 1971
	Chironomus stigmaterus	Grodhaus 1971
	Lauterborniella sp.	Oliver et al 1972
	Polypedilum parthenogeneticum	Donato et al 2008
	Zavreliella marmorata	Thienemann 1954
Orthocladiinae	*Corynoneura celeripes*	Edwards 1919
	Corynoneura donovani	Forsyth 1971
	Corynoneura scutellata	Edward et al 1968
	Limnophyes minimus	Armitage et al 1995
	Limnophyes vestitus	Forsyth 1971
	Abiskomyia virgo	Armitage et al 1995
	Bryophaenocladius furcatus	Armitage et al 1995
	Eretmoptera murphyi	Armitage et al 1995
	Metriocnemus abdominoflavatus	Thienemann 1954
	Phytotelmatocladius delarosai	Siri et al 2014
	Pseudosmittia baueri	Armitage et al 1995
	Troglocladius hajdi	Andersen et al 2016
Telmatogetoninae	*Telmatogeton amphibius*	Crafford 1986
	Telmatogeton sp.	Delettre et al 2003

Not only do parthenogenetic chironomids vary in habitat, but they also vary in the extent of their parthenogenesis. Some of the taxa in Table 3.1 are strictly parthenogenetic, like *Paratanytarsus grimmii* (Schneider 1885), the globally-distributed, notorious pest of water-supply systems (Carew *et al*. 2013). Other taxa exhibit population-specific parthenogenesis. This is the case for *Paratanytarsus laccophilus* Edwards 1929. Lindeberg (1958, 1971) documented parthenogenetic populations of *P. laccophilus* in rock pools on the isles of the Gulf of Finland, yet found bisexual reproduction in all other locations where he studied this species. Here we present evidence of additional parthenogenetic populations of *P. laccophilus*, found in the North American High Arctic near Utqiaġvik, Alaska.

3.3. Methods

The study site is located at Utqiaġvik (formerly Barrow), Alaska (71°17'27.5"N 156°47'18.5"W). We sampled a total of fourteen tundra ponds (Fig. 3.1) within an 8 km radius of the village for chironomid pupal exuviae (PEs) across the 2015 and 2016 emergence seasons (*i.e.* from thaw in mid-late May to late July, when chironomid emergence ended). We conducted this phenological sampling daily in both years, with Ponds A, C, E, J, G, and Kaleak sampled on even days, while Ponds Bear, OH, HB, Icy, and Sub-Bear sampled on odd days. Ponds Snowfence and Scuzzy were part of the odd day sampling in 2015 only, and Pond Infinity was sampled only twice in 2015.

Ponds A, C, E, J, G, OH, Bear, and Infinity are low-centered polygon ponds (Liljedahl *et al*. 2012) with a maximum depth of 0.5 m, surface areas largely consisting of open water, and ranging in size from ~175 m^2 to 900 m^2 total area. Ponds HB and Icy are degrading ice-wedge ponds (Liljedahl *et al*. 2012) with a maximum depth of 2 m, largely open water, and size ranging ~120 m^2 to 180 m^2 total area. Ponds Kaleak, Snowfence, Sub-Bear, and Scuzzy are the smallest

ponds (areas ~18 m^2 to 750 $m^{2)}$ and shallowest (maximum depth ~30 cm) and are thoroughly vegetated with *Carex* and *Arctophila.* Sub-Bear, and Scuzzy are so shallow they lost most standing water by late June).

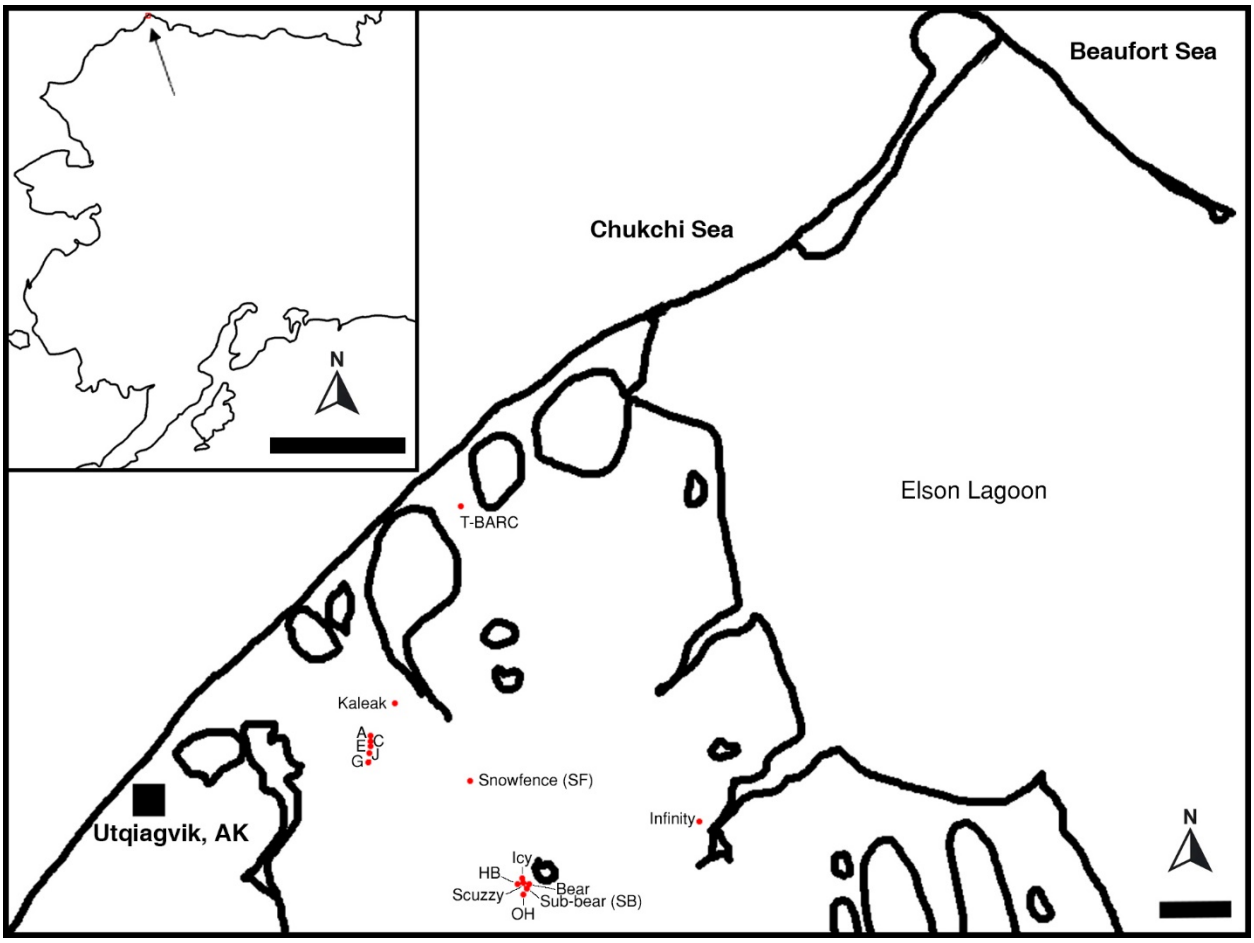

Figure 3.1. Map of the field site at Utqiaġvik, Alaska.
Paratanytarsus laccophilus were taken from the fifteen tundra ponds labeled (red dots). Scale bars = 500 km (inset), and 1 km.

Samples consisted of five 1 m dip net sweeps taken on the downwind side of the pond to collect surface-floating PEs and emerging or failed adults. Pooled net contents were stored in 35% ethanol in the field, and subsequently transferred to 70% ethanol. Sampled PEs were sorted, sexed, and tallied in the lab under a dissecting microscope, and identified to species according to Wiederholm (1986).

While processing 2015 field samples, it became evident that *P. laccophilus* might be parthenogenetic due to a lack of males. In 2016 we took larval samples for rearing under controlled temperature conditions in the lab. These larvae came from Kaleak Pond on 19 and 28 June, and from Pond T-BARC (Fig. 3.1) on 30 June. Small tanytarsine larvae hypothesized to include *P. laccophilus* were placed into rearing cups with detritus and pond water and incubated at various temperatures ranging from 5-25 °C. These lab incubations consisted of eight oxygenated aquaria controlled for water temperature (±1°C) with EcoPlus™ aquarium chillers and monitored with Onset® Hoboware temperature loggers. We monitored larvae daily for emergence of adult flies. For each emerged specimen, we recorded temperature treatment, source pond, species, sex (confirmed by examining of PE genital structures under a dissecting microscope), and date of eclosion. We immediately preserved emerged adults and their pupal exuviae (PEs) in 70% EtOH, except when an emerged *P. laccophilus* singleton was confirmed (see below). Adults were photographed laterally under a dissecting microscope (Fig. 3.2) in a thin film of 70% EtOH - just enough to submerse the specimen such that individuals would lie laterally.

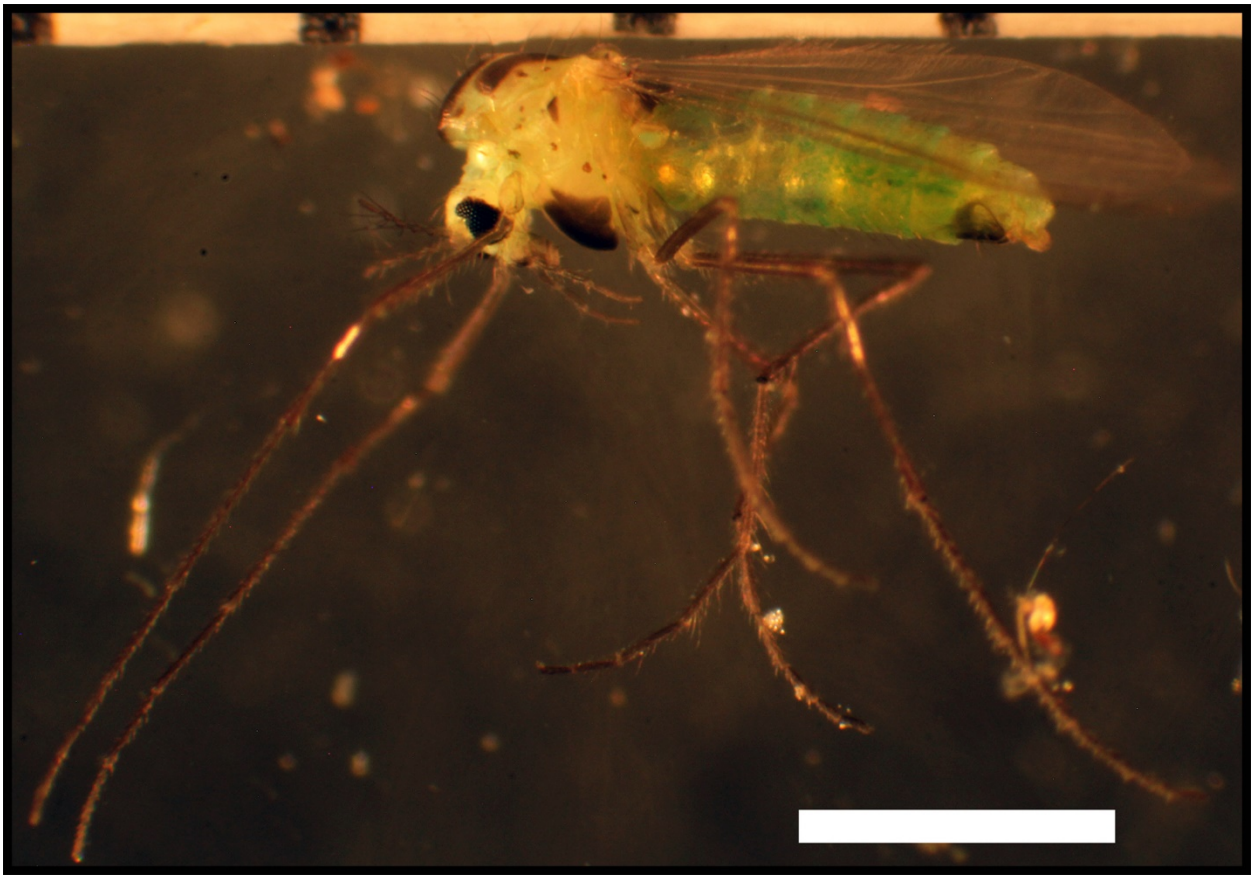

Figure 3.2. Lateral view of a recently emerged *Paratanytarsus laccophilus* female from Utqiaġvik, Alaska.
The specimen was submerged in a thin film of 70% EtOH immediately after it emerged from a rearing cup in the lab, and her natural coloration was captured via photomicroscopy. Scale bar = 1 mm.

We isolated reared *P. laccophilus* singletons that were confirmed as virgin (i.e. no other species or conspecifics had emerged in that daily cohort) by first locating the adult midge beneath the mesh of the rearing cup by shining a light through the base of the cup (Fig. 3.3A). When a potential specimen was located, we carefully coaxed her to the mesh of the lid (were she not already there). As adults of this species are agile fliers, we took care to prevent escapees. Once the female was on the mesh, (Fig. 3.3A), we carefully unscrewed the lid and quickly placed it on the table, trapping the fly. We then located and identified the lone PE within the opened rearing cup to confirm it was *P. laccophilus* (Fig. 3.3B). If confirmed, we prepared a new rearing

cup containing only pond water. The lid with the trapped *P. laccophilus* female clinging to the mesh was then quickly transferred to the new rearing cup. We monitored these isolated individuals for behavior, and ultimately, oviposition. If oviposition took place, we monitored and photographed the egg mass daily under a dissecting microscope, watching for egg development and hatching. Once hatched and larvulae had consumed the gelatinous mass surrounding the eggs, we added filtered (50μm) pond detritus (free of exotic chironomid larvae) to each microcosm.

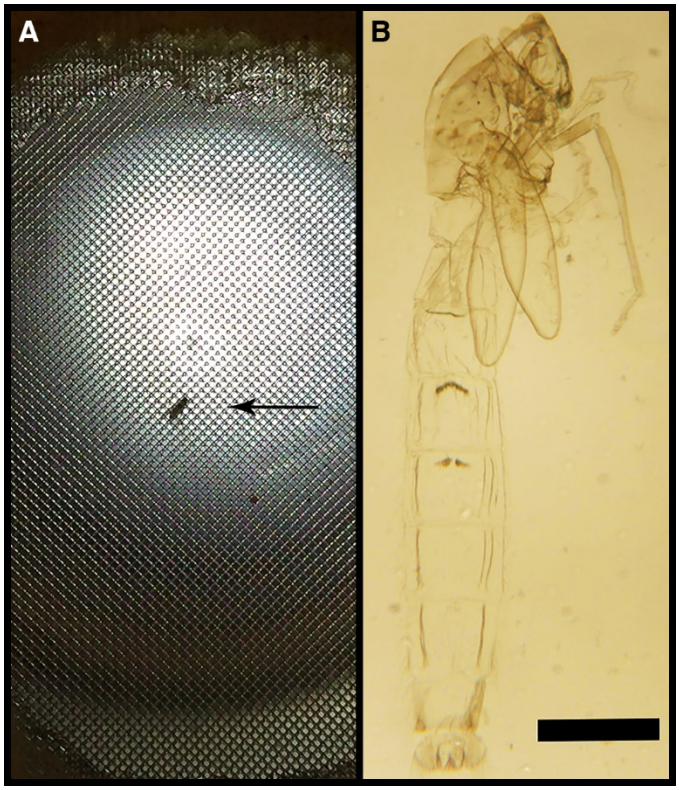

Figure 3.3. Adult *Paratanytarsus laccophilus* rearing.
A) A freshly emerged, virgin *Paratanytarsus laccophilus* (arrow) adult clinging to the mesh lid of the new rearing cup to which it was transferred. The isolated female was then monitored daily for signs of oviposition. B) The pupal exuviae (PE) collected from the larval rearing cup, used to confirm species identification. Note the diagnostic patterning on abdominal tergites III and IV (Wiederholm 1986). Scale bar in (B) = 0.5 mm.

We used JMP 14 Pro Statistical Discovery™ for statistical analysis and graphical output. Degree hours (DH) for each emerged specimen were calculated as mean temperature experienced*total hours for development.

3.4. Results

Over the 2015 and 2016 field seasons, we collected a total of 623 *P. laccophilus* PEs, identified according to Wiederholm (1986). All specimens were female, and came from fourteen tundra ponds (Fig. 3.1) representing a range of habitat types (see methods). We also reared 37 *P. laccophilus* (all female), from larvae, collected from two pond sources, to emergence under lab conditions (Table 3.2).

Table 3.2. Collection information for *Paratanytarsus laccophilus* in the present study.

N(2015, 2016)	N(total)	% ♀	Pond	Coordinates	Sample type
(183, 338)	521	100	Kaleak	71°17'54.36"N, 156°41'46.32"W	PE sweep
(36, NA)	36	100	Snowfence	71°17'23.52"N, 156°39'45.09"W	PE sweep
(1, 12)	13	100	Icy	71°16'39.46"N, 156°38'31.29"W	PE sweep
(4, 3)	7	100	J	71°17'36.75"N, 156°42'5.43"W	PE sweep
(2, 5)	7	100	G	71°17'32.84"N, 156°42'3.55"W	PE sweep
(2, 4)	6	100	Sub-bear	71°16'36.36"N, 156°38'24.19"W	PE sweep
(3, 2)	5	100	A	71°17'41.33"N, 156°42'9.39"W	PE sweep
(1, 4)	5	100	HB	71°16'38.42"N, 156°38'32.46"W	PE sweep
(5, NA)	5	100	Scuzzy	71°16'38.72"N, 156°38'31.49"W	PE sweep
(0, 5)	5	100	Bear	71°16'37.11"N, 156°38'22.99"W	PE sweep
(4, NA)	4	100	Infinity	71°17'4.26"N, 156°34'22.65"W	PE sweep
(1, 2)	3	100	C	71°17'40.21"N, 156°42'8.06"W	PE sweep
(1, 2)	3	100	E	71°17'38.85"N, 156°42'6.81"W	PE sweep
(1, 2)	3	100	OH	71°16'35.25"N, 156°38'27.91"W	PE sweep
(NA, 34)	34	100	Kaleak	71°17'54.36"N, 156°41'46.32"W	Lab rearing
(NA, 3)	3	100	T-BARC	71°19'25.18"N, 156°40'7.30"W	Lab rearing
N(All)	660	100	15	NA	

Kaleak Pond produced the most *P. laccophilus* of all habitats across both years, and the PE sweep data from this pond offers insight into the species' emergence phenology (Fig. 3.4). *P. laccophilus* showed highly synchronized emergence, even compared to other, sexually-

reproducing species at Utqiaġvik (Butler 1980a). In 2015, the peak emergence date for *P. laccophilus* (Julian date 170) was also the first day PEs of this species were collected from this pond, and these comprised 76% (139/183) of that season's total. Our 2015 sampling in Kaleak began 20 days earlier (on Julian date150: 30 May), and no *P. laccophilus* PEs were collected on any of the 13 day-specific samples prior to peak emergence on Julian date 170 (19 June). In 2016, 72% (242/338) of all collected *P. laccophilus* emerged in three consecutive samples from 4-8 July (Julian dates 186, 188, and 190) (Fig. 3.4A), again documenting a high degree of synchrony in the emergence of this species.

Despite this synchrony, *P. laccophilus* emergence is not predictable across years using DH. Thaw dates were nearly identical in Kaleak Pond between 2015 and 2016, with only a two day difference - 2015 having the later start (Fig. 3.4B). Yet peak emergence of *P. laccophilus* in 2015 was ~20 days earlier than in 2016 (Fig. 3.4A). This difference in phenological emergence pulse corresponds to a difference of more than 3,800 DH between the two years. In 2015, the median degree hour experience from thaw to emergence was 4,102 (mean: 5,339 ± 378 [95% CI]), while in 2016 it was 7,986 (mean: 8,026 ± 132 [95% CI]). Comparing median values, the DH required in 2015 was just 51% of that required in 2016 for emergence. These discrepancies between degree-hours to peak emergence in the two years may indicate that the response of *P. laccophilus* to temperature is non-linear, or that the larvae may overwinter at different stages of development from year-to-year.

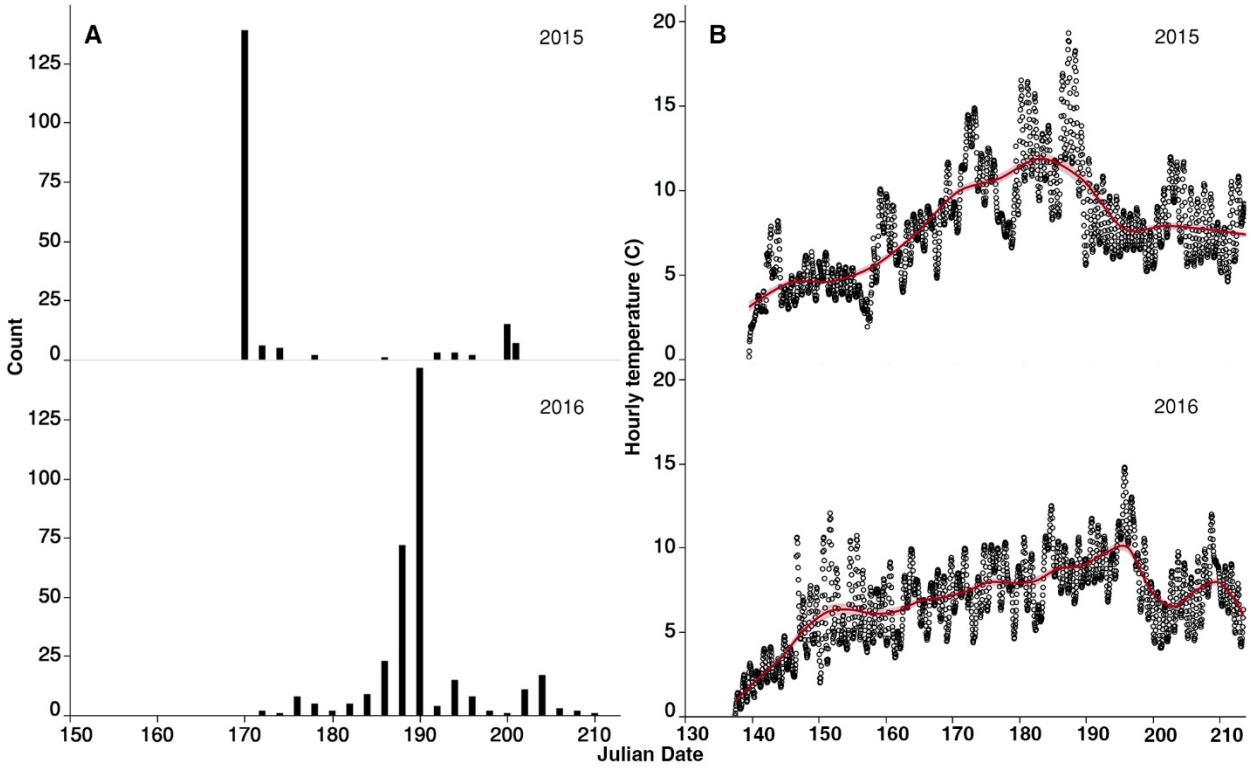

Figure 3.4. *Paratanytarsus laccophilus* phenology.
A) Emergence synchrony of *Paratanytarsus laccophilus* as evidenced by pupal exuviae count vs. sample date (Julian) in 2015 and 2016. In both years sampling began on Julian day 150 (30 May and 29 May, respectively). In 2015 sampling concluded on Julian date 201 (20 July), and in 2016 on day 213 (31 July). B) Hourly temperature (°C) vs. Julian date from a Hoboware data logger in Kaleak Pond. Thaw was defined according to McEwen and Butler (2018) using the pond-specific logger. In 2015 pond thaw occurred on day 139 (19 May), and in 2016 on day 137 (16 May). The red curve through the data points represents the cubic spline with a bootstrap confidence region at lambda = 0.05.

Of the 37 individual larvae reared to female adults in the lab, three emerged as singletons and were considered virgin. These were transferred into their own rearing cups and tracked for behavior and oviposition. These adult females were most often seen clinging to the mesh lid (Fig. 3.3A), but were sometimes observed skittering across, or resting on the water's surface in the rearing cup. We found one adult "belly-up" on the surface of the water three days after she eclosed and was moved to her isolated cup (Table 3.3). The adult was confirmed dead, and no signs of progeny were discovered in the rearing cup (*e.g.* no egg mass or larvae).

Table 3.3. Individual *Paratanytarsus laccophilus* adults reared in isolation and their subsequent life history events.

I	Trt. (°C)	Eclosion	Oviposit	Adult perished	# of eggs	Hatch	LEs present
1	18.3	2 July	NA	5 July	NA	NA	NA
2	15.3	8 July	11 July	11 July	109	16 July	28 July
3	21.0	11 July	13 July	13 July	87	15 July	28 July

All three individuals came from Kaleak Pond. Individual (I); Temperature treatment (Trt); Larval exuviae (LEs).

The other adults died 2-3 days after eclosion. Upon inspection, a single egg mass was immediately noticeable in the bottom of each cup (Fig. 3.5A-B). These egg masses consisted of a string of eggs in a spiral, within an outer, oval-shaped, gelatinous mass. After 2-5 days (depending on treatment) the eggs hatched (Fig. 3.5C-G). Larvulae remained within the spiral of the egg rope (*e.g.* Fig. 3.5G) in the hours immediately after hatch. By the next day they were moving within the outer gelatinous matrix of the egg mass or freely moving about the rearing cup. The egg mass morphology, time between eclosion and deposition of the egg mass, egg number, time to hatch, and larvulae behavior are all concordant with Lindeberg's (1958) pioneering work describing parthenogenetic populations of this species in southern Finland.

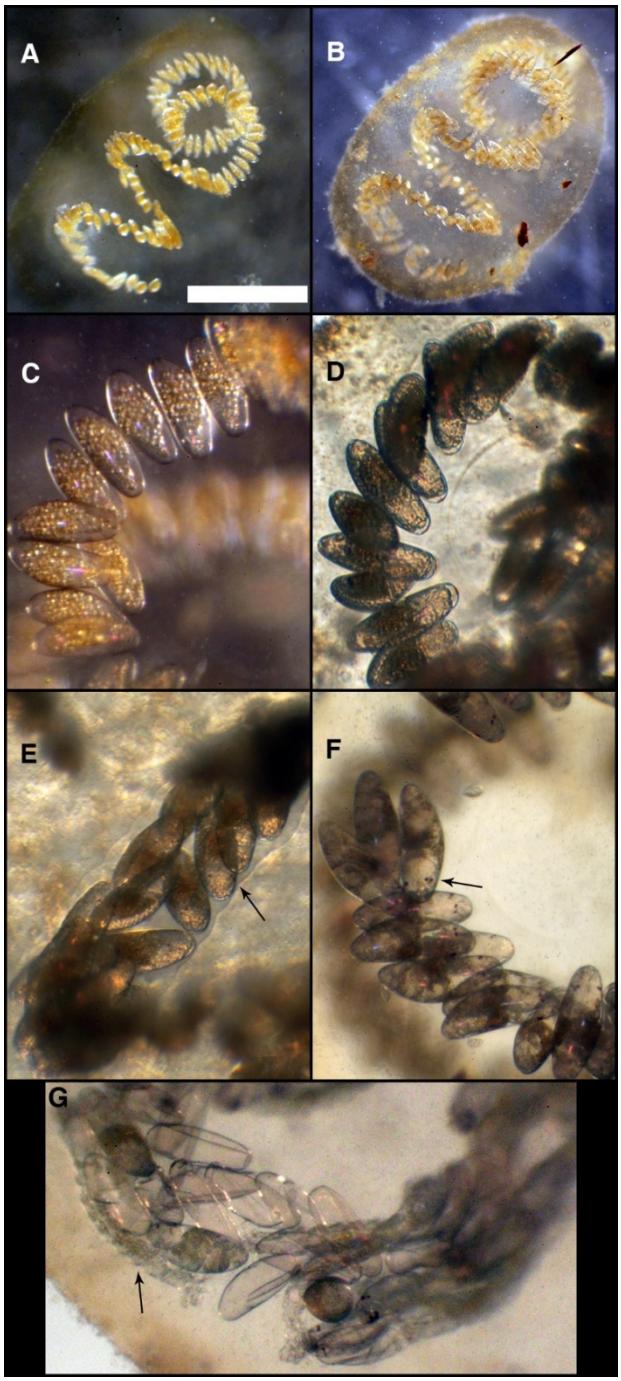

Figure 3.5. Development of parthenogenetic *Paratanytarsus laccophilus* progeny in the 15.3 °C treatment in 2016.
A) An egg mass freshly laid on 11 July. B) The same egg mass on 15 July, just before hatch. C-G) Daily progression of development. C) Eggs on 12 July 2016 at one day old. D) At two days old. E) At three days old, eye spots (arrow) and head capsules were evident inside the egg. F) At four days old, eye spots and head capsules (arrow) were well-defined within the egg. G) Larvulae (arrow) hatched on 16 July, five days after oviposition. Several vacated eggs are clearly notable. Scale bar = 1 mm for A and B, and 250 μm for C-G.

After we added pond detritus to the rearing cups, the newly hatched larvulae grew and developed in their respective temperature treatments for the remaining duration of the field season. As this species emerges rather late in the season (Fig. 3.4A) compared to known chironomid phenologies at Utqiaġvik (Butler 1980a; Butler 1982; Braegelman 2016; Lackmann and Butler 2018; Butler and Braegelman 2018), there was little time for additional larval development as our field season ended on 31 July. We preserved the thriving larvae on 28 July, and found numerous larval exuviae (LEs) in each rearing cup, indicating that these parthenogenetically-produced larvae had molted to second larval instar.

3.5. Discussion

In cases like *Paratanytarsus laccophilus*, where parthenogenesis appears population-specific, it remains unclear if populations are facultatively parthenogenetic in certain environments, or if obligatory bisexual and asexual strains have evolved independently and now coexist globally (Lindeberg 1971; Oliver and Danks 1972; Armitage *et al*. 1995). No matter how it evolved, parthenogenesis in a chironomid at Utqiaġvik, AK should not be surprising. The study site is located in the High Arctic where temperatures are low, winds are high, and the overwintering period encompasses most of the year (Butler 1980a). The fitness benefits of being parthenogenetic in such a habitat are likely great because every individual in the population is female and is theoretically capable of producing offspring herself. The risk involved in finding a mate is avoided, and only one individual is necessary to establish a population in a new habitat (Lynch 1984). Sexually reproducing species tend to require more individuals for population establishment to be successful, because future generations may be unlikely to find mates if founding numbers are low (Suomalainen 1962; Lynch 1984).

It is unknown when *Paratanytarsus laccophilus* evolved at (or colonized) Utqiaġvik. Similar to what has already been done for *Paratanytarsus grimmii* (Carew *et al*. 2013), genetic analyses on populations of *P. laccophilus* at a global scale (*e.g.* from Finland and Alaska) would be valuable in understanding this history. Such genetic information could provide insight on the dispersal of *P. laccophilus* over evolutionary time, and provide a basis for interpreting the species' present trajectory. However, it is interesting to note that this species was not documented in the Utqiaġvik chironomid fauna during the 1970s (Lougheed *et al*. 2011), and that the habitats where this species was most abundant in the present study (thoroughly-vegetated, shallow tundra ponds) have also increased substantially over this timeframe (Liljedahl *et al*. 2016).

The highly synchronous emergence of this Alaskan population is intriguing considering that males are apparently lacking. Thus selection for synchronized emergence for the purpose of mate-finding, as proposed by Butler (1980a), would seem unnecessary. Other factors (*e.g.* optimal temperatures) might maintain such eclosion synchrony. If optimal temperatures were a driver, perhaps it could help explain why DH requirements between the 2015-2016 seasons were so different. The growing season started early in 2015, had sustained periods of steep increases in temperature, and was very warm overall. The 2016 growing season began early and warm, but it did not have the rapid, sustained temperature increases seen in 2015 (Fig. 3.4B). In the current climate change era, arctic warming is amplified (IPCC 2014). If *P. laccophilus* populations are a very recent addition to the Alaskan Arctic, perhaps they are pre-adapted for this rapidly-evolving tundra landscape. Migratory shorebirds are also well-documented to feed on chironomids at this location (Braegelman 2016). If *P. laccophilus* populations do well here under such a warming climate, it might be a benefit for these avian insectivores.

3.6. Conclusion

We present evidence of parthenogenesis in the arctic Alaskan chironomid *Paratanytarsus laccophilus*. This evidence suggests a reevaluation of geographic parthenogenesis in *P. laccophilus* is necessary. It may turn out that Lindeberg's original hypothesis of geographic parthenogenesis in this species is correct (1958), even though he later discarded it (1971). Although parthenogenesis in the Chironomidae is generally considered uncommon (Armitage *et al*. 1995), population-specific incidences such as *P. laccophilus* suggest other sexually-reproducing species may have parthenogenetic strains that are not yet known.

3.7. References

Andersen, T., Baranov, V., Hagenlund, L. K., Ivković, M., Kvifte, G. M., & Pavlek, M. (2016). Blind flight? A new troglobiotic Orthoclad (Diptera, Chironomidae) from the Lukina Jama–Trojama Cave in Croatia. *PloS one*, 11(4), e0152884.

Armitage, P, Cranston, PS, & Pinder, LCV, (1995). The Chironomidae: The biology and ecology of non-biting midges. 1. Chapman and Hall, London: 572 pp.

Baek, M. J., Yoon, T. J., & Bae, Y. J. (2012). Development of Glyptotendipes tokunagai (Diptera: Chironomidae) under different temperature conditions. *Environmental entomology*, 41(4), 950-958.

Bartlett, J. C., Convey, P., & Hayward, S. A. (2018). Life cycle and phenology of an Antarctic invader: the flightless chironomid midge, Eretmoptera murphyi. *Polar Biology*, 1-16.

Bell, G. (1982). The Masterpiece of Nature: The Evolution and Genetics of Sexuality. Univ. Calif. Press, Berkeley.

Butler, M. G. (1980a). Emergence phenologies of some arctic Alaskan Chironomidae. In Chironomidae (pp. 307-314).

Butler, M. G. (1982). A 7-year life cycle for two Chironomus species in arctic Alaskan tundra ponds (Diptera: Chironomidae). *Canadian Journal of Zoology*, 60(1), 58-70.

Butler, M. G., & Braegelman, S. D. (2018). Pre-emergence growth and development in the arctic midge Trichotanypus alaskensis Brundin. *Journal of Limnology*.

Braegelman, SD, (2016). Seasonality of some arctic Alaskan chironomids (Order No. 10147072). Available from Dissertations & Theses @ North Dakota State University; ProQuest Dissertations & Theses Global. (1830464132).

Carew, M., Gagliardi, B., & Hoffmann, A. A. (2013). Mitochondrial DNA suggests a single maternal origin for the widespread triploid parthenogenetic pest species, Paratanytarsus grimmii, but microsatellite variation shows local endemism. *Insect Science*, 20(3), 345-357.

Crafford, J. E. (1986). A case study of an alien invertebrate (Limnophyes pusillus, Diptera, Chironomidae) introduced on Marion Island: selective advantages. Vol 4.

Cranston, P. S., Hardy, N. B., & Morse, G. E. (2012). A dated molecular phylogeny for the Chironomidae (Diptera). *Systematic Entomology*, *37*(1), 172-188.

Darlington, C. D. (1946). Evolution of genetic systems. Cambridge University Press: London.

Delettre, Y. R., Frenot, Y., Vernon, P., & Chown, S. L. (2003). First record of Telmatogeton sp. (Diptera: Chironomidae) at Heard Island. *Polar Biology*, 26(6), 423-426.

Donato, M., & Paggi, A. C. (2008). Polypedilum parthenogeneticum (Diptera: Chironomidae): a new parthenogenetic species from Eryngium L.(Apiaceae) phytotelmata. *Aquatic Insects*, 30(1), 51-60.

Edward, D. H., & Colless, D. H. (1968). Some Australian parthenogenetic Chironomidae (Diptera). *Australian Journal of Entomology*, 7(2), 158-162.

Edwards, F. W. (1919). XX.—Some parthenogenetic Chironomidæ. *Journal of Natural History*, 3(14), 222-228.

Ferrington, L. C. (2007). Global diversity of non-biting midges (Chironomidae; Insecta-Diptera) in freshwater. In Freshwater Animal Diversity Assessment (pp. 447-455). Springer, Dordrecht.

Forsyth, D. J. (1971). Some New Zealand Chironomidae (Diptera). *Journal of the Royal Society of New Zealand*, 1(2), 113-144.

Grimm, O. (1870). Die ungeschlechtliche Fortpflanzung einer Chironomus-Art und deren Entwickelung aus dem unbefruchteten Ei. Akad. Wiss.

Grodhaus, G. (1971). Sporadic parthenogenesis in three species of Chironomus (Diptera). *The Canadian Entomologist*, 103(3), 338-340.

Ghiselin, M. T. (1974). The Economy of Nature and the Evolution of Sex. Univ. Calif. Press, Berkeley.

Intergovernmental Panel on Climate Change (IPCC). Fifth Assessment Report (2014).

International Symposium on Chironomidae (ISC) (2017). Trento, Italy.

Lackmann, A. R., & Butler, M. G. (2018). Breaking the rule: Five larval instars in the podonomine midge Trichotanypus alaskensis Brundin from Barrow, Alaska. *Journal of Limnology*.

Langton, P. H. (1998). Micropsectra silvesterae n. sp. and Tanytarsus heliomesonyctios n. sp. (Diptera: Chironomidae), two parthenogenetic species from Ellesmere Island, Arctic Canada. *Journal of the Kansas Entomological Society*, 208-215.

Liljedahl, A. K., Hinzman, L. D., & Schulla, J. (2012). Ice-wedge polygon type controls low-gradient watershed-scale hydrology. In *Proceedings of the Tenth International Conference on Permafrost* (Vol. 1, pp. 231-236). The Northern Publisher: Salekhard, Russia.

Liljedahl, A. K., Boike, J., Daanen, R. P., Fedorov, A. N., Frost, G. V., Grosse, G., Hinzman, L. D., Iijma, Y., Jorgenson, J. C., Matveyeva, N., Necsoiu, M., Raynolds, M. K., Romanovsky, V. E., Schulla, J., Tape, K. D., Walker, D. A., Wilson, C. J., Yabuki H., and Zona, D., (2016). Pan-Arctic ice-wedge degradation in warming permafrost and its influence on tundra hydrology. *Nature Geoscience*, 9(4), 312-318.

Lindeberg, B. (1958). A parthenogenetic race of Monotanytarsus boreoalpinus Th. (Dipt., Chironomidae) from Finland. *Ann. Entomol. Fennici*, 24, 35-38.

Lindeberg, B. (1971). Parthenogenetic strains and unbalanced sex ratios in Tanytarsini (Diptera, Chironomidae). In *Annales Zoologici Fennici* (pp. 310-317).

Lougheed, V. L., Butler, M. G., McEwen, D. C., & Hobbie, J. E. (2011). Changes in tundra pond limnology: Re-sampling Alaskan ponds after 40 years. *Ambio*, 40(6), 589-599.

Lynch, M. (1984). Destabilizing hybridization, general-purpose genotypes and geographic parthenogenesis. *The Quarterly Review of Biology*, 59(3), 257-290.

Mayr, E. (1970). Populations, Species, and Evolution. Belknap Press, Cambridge.

McEwen, D. C., & Butler, M. G. (2018). Growing-Season Temperature Change across Four Decades in an Arctic Tundra Pond. *Arctic*, 71(3), 281-291.

Oliver, D. R., & Danks, H. V. (1972). Sex ratios of some high arctic Chironomidae (Diptera). *The Canadian Entomologist*, 104(9), 1413-1417.

Porter, D. L., & Martin, J. (2011). Cytogenetics of a parthenogenetic Arctic species of Micropsectra (Diptera, Chironomidae). *Comparative cytogenetics*, 5(4), 315-328.

Siri, A., & Donato, M. (2014). Monopelopia caraguata (Chironomidae: Tanypodinae: Pentaneurini) and Phytotelmatocladius delarosai (Chironomidae: Orthocladiinae): two phytotelmatous chironomids distributed from Florida to Argentina. *Florida Entomologist*, 1226-1231.

Smith, J. M. (1978). The evolution of sex (No. 574.1 S5). Cambridge: Cambridge University Press.

Suomalainen, E. (1962). Significance of parthenogenesis in the evolution of insects. *Annual Review of Entomology*, 7(1), 349-366.

Thienemann, A. 1954. Chironomus. Leben, Verbreitung und Wirtschaftliche Bedeutung der Chironomiden. *Binnengewiisser*, 20: 1-834.

Vandel, A. (1928). La parthénogenèse géographique. Contribution à l'étude biologique et cytologique de la parthénogenèse naturelle. *Bull. Biol. France Belg*. 62, pp. 164-281.

Wiederholm, T. (1986). Chironomidae of the Holarctic region Part 2. Pupae. *Entomologica Scandinavica Supplement*, 28, 1-482.

4. TEMPERATURE-SIZE RULE VARIATION IN CHIRONOMIDAE FROM UTQIAĠVIK, ALASKA: IMPLICATIONS FOR A WARMING ARCTIC

4.1. Abstract

The Temperature-Size Rule (TSR) states that as temperatures rise, ectothermic organisms decrease in size. Although causal mechanisms are unclear, this phenomenon is documented across a wide range of taxa. Along with shifts in distributional range and seasonal phenology, changes in body size are one of three universal biological responses to global warming, with evidence of selection favoring smaller body sizes at higher temperatures. The Arctic is warming faster than any other region on earth, more than two times faster than the global average. Over the past 15-20 years the Arctic has been consistently > 4 °C warmer than during the 20th Century. Such accelerated rates of warming affect ecosystems. On the High Arctic tundra near Utqiaġvik (formerly Barrow), Alaska, numerous species of migratory shorebirds have evolved to nest in early summer so their young hatch during the time of peak insect activity. Foremost among these insect prey are chironomids, non-biting midge flies in the dipteran family Chironomidae. Chironomid emergence phenologies are shifting as this arctic climate warms, reducing fitness for some shorebird species. Optimal foraging theory would suggest that insectivorous birds may also be negatively impacted if their prey is shrinking in size. We present laboratory evidence of an overall TSR trend in Chironomidae collected from tundra ponds at Utqiaġvik. As response of adult body size to rearing temperature is not uniform across species and sexes, this phenomenon may be more complicated than previously considered. We reared larvae of 12 chironomid species (N=1,290 with at least N>31 for each sex) under 8 controlled temperature treatments, ranging from 5-25ºC from their overwintering developmental state, through pupation to emergence of the adult fly. We found that 7 of 12 species showed a significant adult size

decrease (reduction of thorax length) in accordance with the Temperature-Size Rule, while five species showed no trend. Whether these species-specific patterns will be evident in the wild as the arctic growing season becomes longer and warmer is unknown.

4.2. Introduction

In addition to changes in distribution (Davis and Shaw 2001; Walther *et al*. 2002; Chen *et al*. 2011) and phenology (Visser and Both 2005; Durant *et al*. 2007) of species, declining body size is considered one of the three universal responses to anthropogenic climate change (Daufresne *et al*. 2009; Garner *et al*. 2011). One example of this response, dubbed the Temperature-Size Rule (TSR), is the widespread phenomenon of ectothermic organisms decreasing in size with increasing temperature (Kingsolver and Huey 2008; Sheridan and Bickford 2011). This response has been documented in a variety of ectothermic organisms, from bacteria (Angilletta and Dunham 2003) to butterflies (Bowden *et al*. 2015). Although the TSR appears to be generalizable to most ectotherms, underlying mechanisms may not be as general (Angilletta and Dunham 2003). Even so, exceptions to the TSR have even been reported, even for arthropods (Angilletta and Dunham 2003). In addition, the effects of temperature on growth can vary through an organism's ontogeny. For example, an ostracod was found to show a reverse-TSR trend in early instars, but supported the TSR in its final two instars (Aguilar-Alberola and Mesquita-Joanes 2014).

Chironomids, non-biting midge flies, are often the most abundant and speciose macroinvertebrates in freshwater. These insects play crucial roles in both freshwater and terrestrial ecosystems, by cycling nutrients and producing forage for predators (Armitage *et al*. 1995). On the arctic tundra around Utqiaġvik, Alaska (formerly known as Barrow) chironomids are the vast majority of the insect fauna in both terrestrial and aquatic habitats (Butler 1982b;

Lougheed *et al*. 2011; Holmes and Pitelka 1968; MacLean and Pitelka 1971; Saalfeld *et al*. 2019). For migratory shorebirds, the highly synchronous pulse of emerging insect biomass that chironomids provide is crucial to reproductive success (Holmes 1966; Holmes and Pitelka 1968; MacLean and Pitelka 1971). Shorebird chicks in the High Arctic most often feed on intermediate-sized chironomid adults they find on tundra vegetation because these are the most abundant and readily available insects present, and chicks' beaks have yet to harden so probing substrate is not yet in their behavioral repertoire (Holmes 1966). Thus, it is very important to shorebird breeding success that chironomid biomass is reliably available from brood hatch to fledging (Holmes 1966).

Climate change is having significant impacts on this ecosystem, potentially disrupting the food web synchrony between these trophic levels. Fitness declined in nesting High Arctic shorebird species whose hatch-to-fledge period was later than the pulse of arthropodan biomass (McKinnon *et al*. 2012). The phenological emergence pulse of Chironomidae at Utqiaġvik has shifted earlier from the mid-1970s to the early 2010s by about one week on average (Braegelman 2016). Others have found that two species of migratory shorebird chicks monitored at Utqiaġvik showed decreased growth rates under timeframes that had minimal arthropodan biomass available (Saalfeld *et al*. 2019).

The effect of rising temperatures on the sizes of arctic chironomid prey items should also be considered in a shorebird-focused analysis of this food web, because changes in size could affect biomass availability. The TSR is an established, and well-studied phenomenon, has been documented in a chironomid (Baek *et al*. 2012), and size-shifts may already be underway in the warming Arctic. We test whether there is an experimental basis for the TSR in Utqiaġvik Chironomidae by laboratory rearings of 12 species from the range of chironomid diversity

present in this ecosystem (four subfamilies), across eight controlled temperature treatments (5-25 °C). We also conducted standardized sweep net samples in tundra vegetation across the growing seasons of 2015 and 2016 to document the relative abundances of chironomid taxa in this habitat. Since the TSR may affect female chironomids differently than males, we also conducted pupal exuviae (PE) sweeps in tundra ponds across the 2015-2016 seasons and determined the sex ratios of the abundant taxa.

4.3. Methods

4.3.1. Survey of Invertebrates in Tundra Vegetation

Over each emergence season in 2015-2016, we used an insect sweep net (0.5 m diameter, <100 μm mesh) to collect insects from the tundra habitat used by foraging shorebird chicks (vegetated tundra without standing water), taking standardized daily samples along three non-overlapping 20 m transects. One sweep of the net through terrestrial vegetation was taken for every meter traveled. All collected insects were flushed from the net using 30% ethanol, stored in WhirlPak® bags, and later identified to family (whenever possible) in the lab.

4.3.2. Lab Rearings

HOBO The temperature-treatment setup consisted of ten oxygenated aquaria (75 liters each) controlled for water temperature (±1 °C) using seven EcoPlus™ aquarium chillers, three 300W aquarium heaters, ten 1,514 liters/hour water pumps, a 100W aeration pump (149.9 liters/minute), plastic tubing, ten digital aquarium thermometers (for monitoring temperatures in real-time), and ten Onset® Hoboware temperature loggers (for logging hourly temperatures). The treatment tubs were randomized throughout the room to limit the effects of experimental bias (Hurlbert 1984). The aquaria, black plastic tubs with open tops, contained a mixture of pond water (~75%) and de-ionized water (~25%) and a layer (< 1 cm) of pond detritus. Each aquarium

was fitted with air hosing from the central bubbler to provide consistent aeration. Each tub had its own water pump for circulation, maintaining uniform temperature and dissolved oxygen levels within each treatment. Temperatures treatments in 2015 were targeted at 5, 8, 10, 12, 15, 18, 21, 24, 26 and 29 ºC, with the three warmest treatments maintained by heaters. In 2015 we found that the two warmest treatments were too thermally stressful for arctic chironomids (no emergence), and in 2016 those treatments were excluded.

We collected chironomid larvae from tundra ponds within a 5 km radius of Utqiaġvik, Alaska (71°17'27.5"N 156°47'18.5"W) shortly after thaw in mid-late May in both 2015 and 2016. Epiphytic and surface-dwelling taxa were collected with a 100 μm mesh dip net, while infaunal larvae were sieved from sediment using a 1 mm mesh. Samples were transported to the lab site where they were sorted, on ice, to species under a dissecting microscope. Chironomids were typically placed into the treatments on the same day the sample was collected, but in some cases samples in progress were stored overnight in a refrigerator at 3 °C and finished the following day. Sorted larvae of a given taxon and source pond were distributed evenly among rearing cups with detritus and pond water. Only chironomid larvae that were hypothesized to emerge during that growing season were used, that is, third-fifth larval instars depending on the species (Butler 1980a; Lackmann and Butler 2018). These insects only spent a portion of their final growing season under our experimental treatments. As many of these species have multiyear life cycles (Butler 1980a; Butler 1982) these laboratory incubations represent a variable, but very small, percentage of each larva's thermal experience, but includes their entire period of pupal development.

Rearing cups (~400 ml) isolated taxon, pond, and date-specific cohorts of larvae within each temperature treatment. Cups were buoyed by strips of closed-cell foam fastened above four

(2.5 cm diameter) openings in the side walls. These openings were covered with 150 μm mesh, allowing water exchange with the aquarium. We also manually exchanged water between each cup and its aquarium during daily inspections. A large hole in the lid of each rearing-cup was covered with ~300 μm mesh screen, coarse enough to observe contents within, but small enough to prevent escape of emerged adults.

Rearing cups were monitored at least once per day for emergence, being first checked through the screen, then more thoroughly after the lid was removed. If chironomid adults were noticed through the screen lid, they were lightly sprayed with 30% EtOH mist prior to opening to inhibit escape by flight. Emerged adults were collected along with their pupal exuviae (PEs - used to confirm taxonomic identity), which were immediately preserved in 70% EtOH. Information on the temperature treatment, pond of origin, species and emergence date were logged for each emerged specimen. Adults were photographed in a thin film of 70% EtOH, positioned so that individuals would lie laterally. The freeware program ImageJ was used to measure the adult insect's thorax length to the nearest micrometer (Fig. 4.1).

Figure 4.1. Thorax length ("A") of a *Chironomus riparius* male.

4.3.3. Phenology Sweeps

Daily pupal exuviae (PE) sweep samples were collected from several tundra ponds at Utqiaġvik in both 2015 and 2016, from late May to the end of July, spanning the entire chironomid emergence season. PE sweeps consisted of five, non-overlapping, 1 m sweeps along the downwind side of each pond. The sweep net contents were flushed into WhirlPak® bags in the field with 35% ethanol and a funnel. PEs were later sorted, sexed, and tallied by species, based on morphological characteristics (Wiederholm 1986), under a dissecting microscope.

4.3.4. Statistical Analysis

As linear relationship between body size and rearing temperature was hypothesized, we used a general linear model (GLM) fit by standard least squares to model effects, with adult thorax length as the dependent variable. The main explanatory variables were the temperature experience for a given individual in a treatment, and sex. Other variables were also tested for effects (*i.e.* sample date, pond, and year). Statistical analysis of the overall standard least squares model preceded more thorough and species-specific analyses (see Results).

For sex ratio analysis of the PE sweeps, a binomial proportion test was used to assess the probability that a species-specific sex ratio deviated significantly from 1:1. The number of successes was the number of female PEs. The total trials was the number of female and male PEs combined. The probability of success (=deviant from 1:1) was evaluated using the threshold of $p=0.05$ (i.e. a value <0.05 indicated a significantly deviant ratio).

We used JMP 14 Pro Statistical Discovery™ for statistical analysis and graphical output. The binomial proportion test was executed in R.

4.4. Results

4.4.1. Survey of Invertebrates in Tundra Vegetation

We collected a total of 3,708 individual invertebrates in our terrestrial sweep samples, belonging to 23 families. The Chironomidae comprised 86.2% of the total abundance **(**Fig. 4.2).

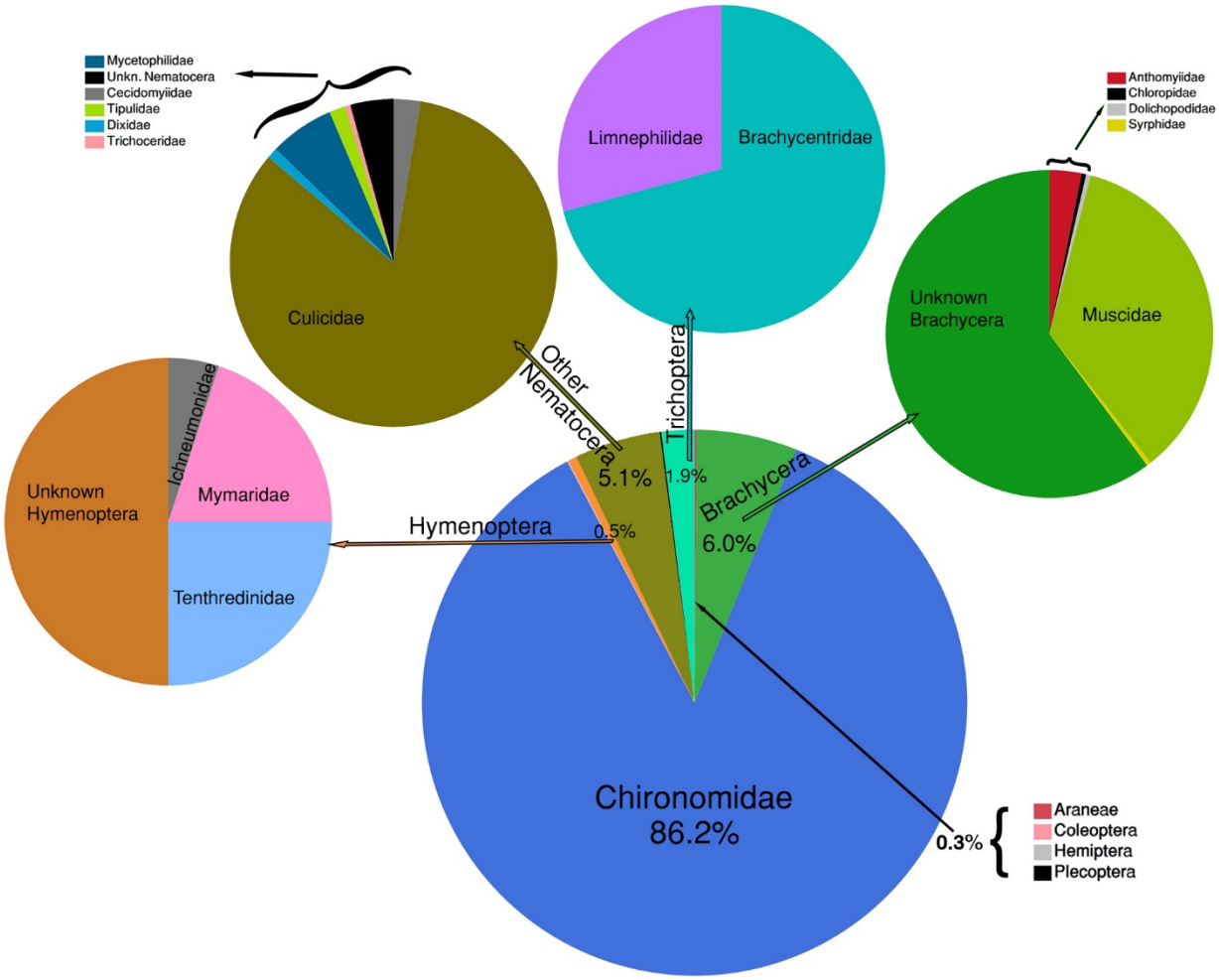

Figure 4.2. A composite pie chart of invertebrates collected in tundra vegetation across the growing seasons of 2015-2016.
Of 3,708 total individuals collected in this habitat (86.2%) were chironomids. The next most abundant family was the Culicidae (mosquitos) at ~4% of the total.

4.4.2. Temperature-Size Rule

Initially, all 1,700 measured adult thoraces spanning 27 lab-reared chironomid species were analyzed in the overall GLM for effects on thorax length. The results are shown in Table 4.1. Model effects included Taxon, Sex, Pond, Temperature, Year, Sample Date, and the interaction of Temperature and Sex (Sex*Temperature). Taxon, Sex, Pond, Temperature, Year, and Sample Date were all significant effects ($p<0.01$) while Sex*Temperature was non-significant ($p>0.05$). Overall, Thorax Length was negatively correlated with Temperature ($p<0.0001$) warranting more thorough analysis.

Table 4.1. Effect Summary for the General Linear Model fit by standard least squares of the entire dataset (N=1,700) with adult thorax length (mm) as the dependent variable.

Model Effect	LogWorth	p	Model Estimate	SE
Taxon	925.596	<0.0001	Varies	Varies
Sex	65.6	<0.0001	0.0612 [Female]	0.0034
Pond	11.321	<0.0001	Varies	Varies
Temperature	10.454	<0.0001	-0.0045	0.0007
Year	2.192	0.006	-0.8977	0.3290
Sample Date	2.160	0.007	2.7245e-8	1.007e-8
Sex*Temperature	0.822	0.151	Not significant	NA

Since a general decrease in size with increasing temperature was detected in the overall model, we conducted taxon-specific analyses on the most robust datasets. For this subsequent analysis, we excluded all species with $N<32$, leaving only the most abundantly reared taxa to be analyzed individually by sex. As was clear from the overall model, pond source was the third most influential variable for adult size (e.g. a species from one pond may be inherently larger than that same species from a different pond). Since pond sources were not equally represented across the range of temperatures for each taxon, data that contributed to a pond effect were excluded from the taxon-specific analysis. By excluding such data, the effects of increasing

temperature on adult size would be most transparent (e.g. the largest sample of a given species coming from a single pond source). After the overall dataset was vetted a total of 1,290 measured adults remained, spanning the 12 most successfully reared species (Table 4.2) from the most abundantly sampled ponds. Seven of these taxa displayed at least partial evidence in accordance with the TSR, and five showed no effect of temperature on size (Fig. 4.3). Interestingly, size-shifts in response to temperature are sex-specific. Furthermore, adult size of all investigated Tanytarsini (i.e. *Paratanytarsus* and *Tanytarsus*), was unaffected by rearing temperature. In addition, evidence suggests that in general, chironomid females are more affected than males (Table 4.2 and Fig. 4.3), not only in number of statistically significant tests, but also in the magnitude of size change per unit body mass (Fig. 4.3) (although this latter point does not hold in the overall GLM as Sex*Temperature was insignificant).

Table 4.2. Chironomid taxa reared under controlled temperature regimes in 2015-2016 for which >31 individuals completed emergence in at least eight treatments.

Subfamily	Taxon	N (♀, ♂)	p (♀, ♂)	Slope (♀, ♂)
Chironominae	*Ch. riparius*	(174, 130)	(0.162, **0.027**)	(“—”, —)
	Ch. sp (s-type)	(34, 55)	(0.636, 0.867)	(0, 0)
	Ch. tardus	(44, 39)	(**0.0002**, **0.012**)	(—, —)
	Ch. sp (t-type L)	(23, 53)	(**0.041**, 0.205)	(—,“—”)
	Ch. sp (t-type)	(82, 79)	(0.136, 0.909)	(“—”, 0)
	Par. laccophilus	(32, 0)	(0.875)	(0, NA)
	Tan. nearcticus	(40, 32)	(0.617, 0.156)	(0, “—”)
Orthocladiinae	*Corynoneura* sp	(43, 37)	(**0.016**, **0.022**)	(—, —)
	Psectrocladius sp1	(44, 35)	(**0.004**, 0.385)	(—, 0)
	Psectrocladius sp2	(41, 39)	(0.882, 0.574)	(0, 0)
Podonominae	*Tricho. alaskensis*	(59, 47)	(**0.0094**, **0.0492**)	(—, —)
Tanypodinae	*Dero. alaskensis*	(69, 59)	(**0.008**, **0.043**)	(—, —)

Significant p-values for a linear relationship between thorax length and rearing temperature are bolded. All four chironomid subfamilies present at Utqiaġvik show evidence of the TSR, with all non-significant ($0.05<p<0.21$) trends (“—”) also exhibiting a negative correlation between thorax length and rearing temperature.

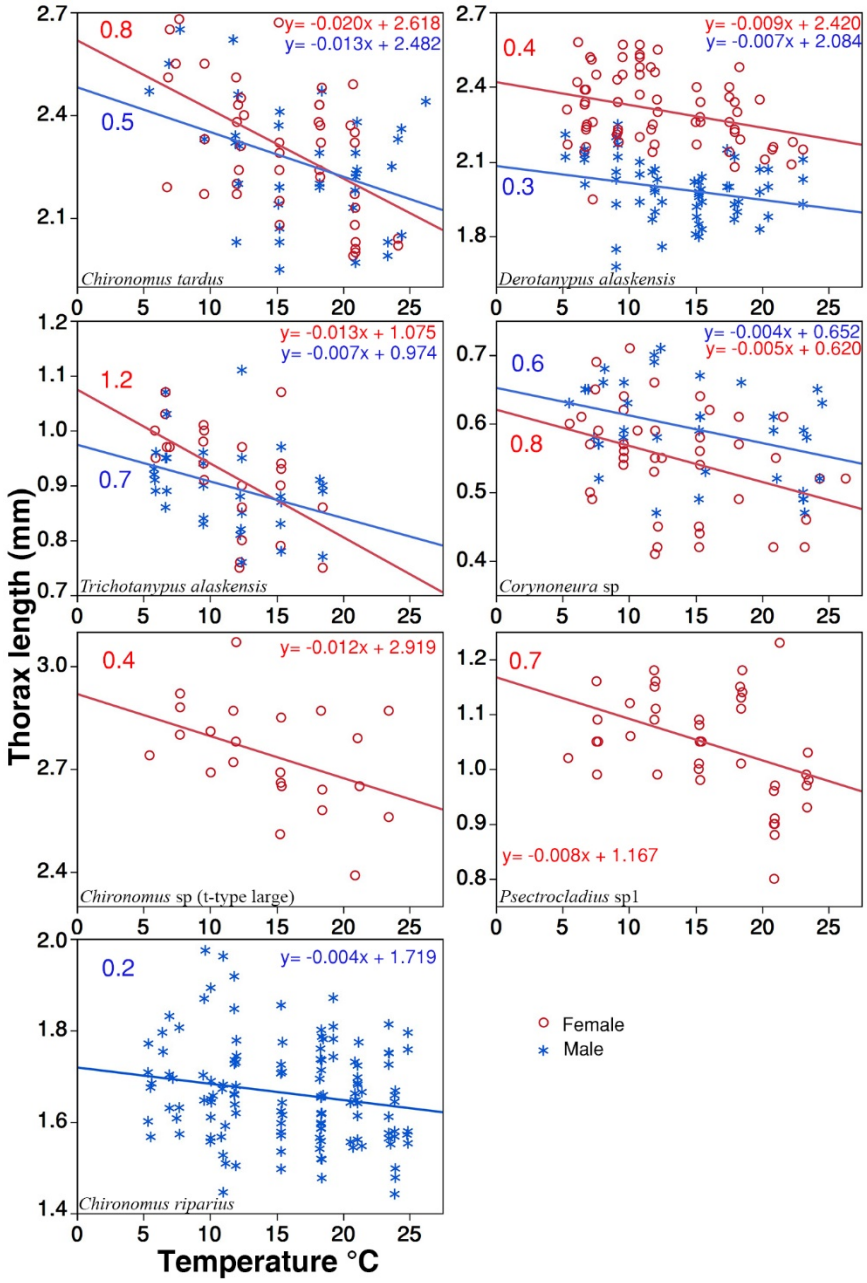

Figure 4.3. Thorax length vs. rearing temperature for the seven chironomid taxa showing significant ($p<0.05$) linear correlations.
Data points represent individuals. Numbers in the upper left indicate the percent change in thorax length per unit °C rise derived from the linear equations. Other taxa with N>31 specimens reared across ≥8 treatments showed no significant change in size with temperature.

4.4.3. Sex Ratios

Not only was female body size more affected by changes in temperature than males, Utqiaġvik chironomids tend to be comprised of more females. Of 23 taxa collected from the PE sweeps in 2015-2016, eight were significantly skewed female, whereas only three were skewed male (Fig. 4.4, Table 4.3). In addition, nine of 12 taxa reared in abundance (>31 specimens) in the lab, were also collected in abundance in PE sweeps for the sex ratio analysis, 5 of which showed significant TSR effects (Table 4.3). Only for *Corynoneura* sp. and *Trichotanypus alaskensis* was the null hypothesis rejected in both TSR and sex ratio tests.

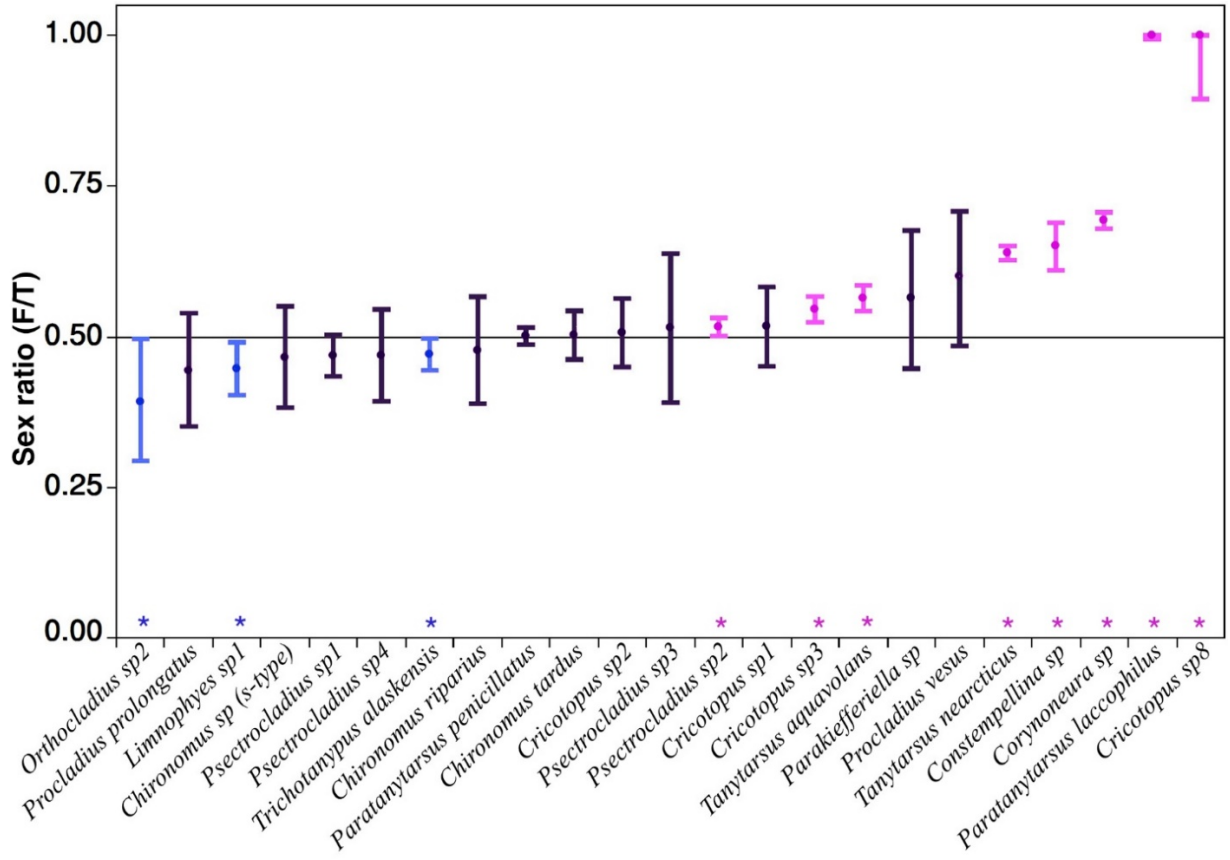

Figure 4.4. Sex ratios and probability.
Ratios (= females/total), ±95% confidence intervals surrounding the probability that a given sex ratio is deviant from the null hypothesis (binomial proportion test, assumed 1:1 ratio, $p<0.05$), of the 23 most abundant chironomid taxa (N>31 for each collected sex) in 2015-2016 combined. Asterisks denote taxa that were significantly deviant (blue = skewed toward males; pink = skewed toward females).

Table 4.3. Sex ratios (female N/total N) of the 23 most abundant chironomid taxa in 2015-2016 (years combined; N>31 for each collected sex).

Taxon	N	N♀	N♂	Ratio	L95%	U95%	p
Orthocladius sp2	97	38	59	0.392	0.294	0.496	**0.04173**
Procladius prolongatus	115	51	64	0.443	0.351	0.539	0.2631
Limnophyes sp1	515	230	285	0.447	0.403	0.491	**0.01725**
Chironomus sp (s-type)*	144	67	77	0.465	0.382	0.550	0.4534
Psectrocladius sp1**	837	392	445	0.468	0.434	0.503	0.07221
Psectrocladius sp4	175	82	93	0.469	0.393	0.545	0.4498
*Trichotanypus alaskensis***	1409	663	746	0.471	0.444	0.497	**0.02889**
*Chironomus riparius***	130	62	68	0.477	0.389	0.566	0.6612
Paratanytarsus penicillatus	4965	2487	2478	0.501	0.487	0.515	0.9096
*Chironomus tardus***	617	310	307	0.502	0.462	0.543	0.9358
Cricotopus sp2	312	158	154	0.506	0.449	0.563	0.8652
Psectrocladius sp3	68	35	33	0.515	0.390	0.638	0.9036
Psectrocladius sp2*	4355	2247	2108	0.516	0.501	0.531	**0.0365**
Cricotopus sp1	234	121	113	0.517	0.451	0.583	0.6473
Cricotopus sp3	2085	1137	948	0.545	0.524	0.567	**3.79E-05**
Tanytarsus aquavolans	2127	1199	928	0.564	0.542	0.585	**4.57E-09**
Parakiefferiella sp	78	44	34	0.564	0.447	0.676	0.3082
Procladius vesus	80	48	32	0.600	0.484	0.708	0.09291
*Tanytarsus nearcticus**	6534	4175	2359	0.639	0.627	0.651	**< 2.2e-16**
Constempellina sp	584	380	204	0.651	0.610	0.689	**2.95E-13**
Corynoneura sp**	4553	3155	1398	0.693	0.679	0.706	**< 2.2e-16**
*Paratanytarsus laccophilus**	623	623	0	1.000	0.994	1.000	**2.20E-16**
Cricotopus sp8	33	33	0	1.000	0.894	1.000	**2.33E-10**

A binomial proportion test was used to test the hypothesis that the ratio was deviant from 1:1, and the confidence limits and p values are presented. Significant p values are bolded. *taxon reared in abundance in lab but no TSR effect, **taxon reared in abundance in lab with TSR effect.

4.5. Discussion

Chironomids are likely the overwhelming majority (86%) of the invertebrate prey found in tundra vegetation at Utqiaġvik across the entire season (Fig. 4.2). As a dominant prey resource, this group is crucial to the reproductive success of migratory shorebirds (Holmes 1966; Saalfeld *et al*. 2019). Optimal foraging theory suggests that small changes in size to adult chironomids could have impacts on these birds' fitness, if it became more energetically costly to locate and consume sufficient prey. Our measures of insect thorax length can be converted to biomass using published regressions (Welch *et al*. 1988). The log-log relationship between body

mass and thorax size clearly demonstrates the potential for significant reductions in adult chironomid biomass for the species shown in Fig. 4.3. These smaller insect prey would theoretically become more calorically costly to consume (Cowie 1977).

Rearing temperature was shown to correlate negatively with adult size in seven of 12 chironomid taxa, a potentially intriguing result for three reasons. First, it is somewhat counterintuitive to see such a similar magnitude, and sex-specific pattern, in the TSR effect (Fig. 4.3) across "a continuum of life history types" (Butler 1980a). Take for example *Trichotanypus alaskensis* Brundin, among the earliest-emerging chironomids in these tundra ponds and one of the shortest-lived, with a one-year life cycle (Lackmann and Butler 2018; Butler and Braegelman 2018). In this case larvae were in lab conditions for 1-4 weeks before emerging, yet both sexes decreased in size (females more than males) as temperatures increased. A similar response was documented for the longest-lived chironomid known. *Chironomus tardus* Butler, one of two sibling species at Utqiaġvik reported to have 7-year life cycles (Butler 1982). Yet *C. tardus* spent only its final 4-8 weeks in experimental conditions. In this case both sexes also show evidence of a TSR pattern (females more strongly than males). Likely intermediate to these species in life cycle duration is *Derotanypus alaskensis* Malloch (Butler *pers. comm.* 2013), which spent only 1-3 weeks in lab conditions, yet showed a similar pattern of decreasing size in response to rearing temperature (Fig. 4.3). Taken together, our findings indicate that these insects are similarly sensitive to increases in temperature in the final growing season of their life cycle, no matter the duration of their total life cycle. We hypothesize that pupation, a time of extreme metamorphosis, and a developmental period that all midges reared in this study spent under experimental conditions, is the stage when most of these size changes occurred. This hypothesis could be tested through controlled rearings, in which midges are raised at different temperatures

as larvae, and then combined into one temperature as soon as they pupate, and vice versa. In addition, temperature effects could be tested across the entire ontogeny of the chironomid life cycle, as these effects have been shown to vary across development in other systems (Aguilar-Alberola and Mesquita-Joanes 2014).

TSR effects do not appear to be phylogenetically constrained at the subfamily level. The three species mentioned in the previous paragraph all belong to different chironomid subfamilies (Podonominae, Chironominae, and Tanypodinae) (Cranston *et al*. 2012). *Corynoneura* sp. belongs to the fourth and final chironomid subfamily present at Utqiaġvik (the Orthocladiinae) (Table 4.2). The four species in which similar TSR patterns were documented in both sexes (Table 4.2 and Fig. 4.3) all belong to different subfamilies, suggesting these responses to temperature are a phylogenetically-general response of the Family Chironomidae.

Finally, it is intriguing that these size reductions have been documented via the insect's thorax, one of the most rigid parts of the adult insect bauplan, especially so for insects that fly as the thorax provides the scaffolding for the flight muscles. Furthermore, the thoracic region of the adult begins rapid development during the fourth larval instar, with the appearance of imaginal discs (Wülker and Götz 1968; Ineichen *et al*. 1983; Butler and Braegelman 2018). In the case of *Chironomus tardus*, a species that spends three years as a fourth instar larva (Butler 1982), thorax length decreased significantly in higher temperatures despite spending only the final weeks of life under lab conditions. This suggests once again that the size-reductions documented in these taxa likely results from a resizing of the insect's body very late in development, likely during pupation.

It is also interesting that female chironomids appear to be more impacted than males in percent change in thorax length per unit rise in temperature. This could have a disproportionate

effect on reproductive fitness, since smaller chironomid females of the same species have been found to be less fecund (Butler and Walker 1992).

Although we have documented at least partial evidence in accordance with the TSR in 7 of 12 thoroughly-reared species in lab conditions, it remains to be investigated whether these patterns will become evident in nature as the arctic climate warms. The experimental temperatures these chironomids experienced were relatively constant (±1°C around a target temperature) relative to the oscillatory temperatures experienced in the wild - as much as 10°C in daily fluctuations on a sunny day (Butler 1980a). The ecological role that the TSR may play as this arctic ecosystem warms remains to be seen.

4.6. Conclusion

We present laboratory evidence of the TSR in Chironomidae from the Alaskan Arctic. Although not all species were in accordance, the general trend is for adult chironomids to get smaller in size as rearing temperatures rise late in the life cycle. This impact of rising temperatures appears capable of affecting adult chironomid size broadly (*i.e.* across subfamilies and life history types), as long as warmer temperatures are experienced throughout the final growing season of their life cycle. We know that chironomid phenologies are shifting earlier compared to historic data collected in the 1970s (Braegelman 2016), which may partially contribute to some population declines among migratory shorebirds. Thus tundra-nesting birds may be negatively affected by climate change not only because of a general trophic mismatch, but also because their individual prey items become smaller in size and thus less calorically valuable.

4.7. References

Aguilar-Alberola, J. A., & Mesquita-Joanes, F. (2014). Breaking the temperature-size rule: Thermal effects on growth, development and fecundity of a crustacean from temporary waters. *Journal of Thermal Biology*, *42*, 15-24.

Angilletta, Jr, M. J., & Dunham, A. E. (2003). The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. *The American Naturalist*, *162*(3), 332-342.

Armitage, P, Cranston, PS, & Pinder, LCV, (1995). The Chironomidae: The biology and ecology of non-biting midges. 1. Chapman and Hall, London: 572 pp.

Baek, M. J., Yoon, T. J., & Bae, Y. J. (2012). Development of Glyptotendipes tokunagai (Diptera: Chironomidae) under different temperature conditions. *Environmental entomology*, *41*(4), 950-958.

Bowden, J. J., Eskildsen, A., Hansen, R. R., Olsen, K., Kurle, C. M., & Høye, T. T. (2015). High-Arctic butterflies become smaller with rising temperatures. *Biology Letters*, *11*(10), 20150574.

Butler, M. G. (1980a). Emergence phenologies of some arctic Alaskan Chironomidae. In *Chironomidae* (pp. 307-314).

Butler, M. G. (1982). A 7-year life cycle for two Chironomus species in arctic Alaskan tundra ponds (Diptera: Chironomidae). *Canadian Journal of Zoology*, *60*(1), 58-70.

Butler, M. G. (1982b). Production dynamics of some arctic Chironomus larvae. *Limnology and Oceanography*, 27(4), 728-736.

Butler, M. G., & Braegelman, S. D. (2018). Pre-emergence growth and development in the arctic midge Trichotanypus alaskensis Brundin. *Journal of Limnology*.

Butler, M. G., & Walker, L. (1992). Fecundity, reproductive effort, and pupal size in the profundal midge Chironomus cuccini (Diptera: Chironomidae). *Netherland Journal of Aquatic Ecology*, 26(2-4), 263-267.

Braegelman, SD, (2016). Seasonality of some arctic Alaskan chironomids (Order No. 10147072). Available from Dissertations & Theses @ North Dakota State University; ProQuest Dissertations & Theses Global. (1830464132).

Chen, I. C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, *333*(6045), 1024-1026.

Cowie, R. J. (1977). Optimal foraging in great tits (Parus major). *Nature*, *268*(5616), 137.

Cranston, P. S., Hardy, N. B., & Morse, G. E. (2012). A dated molecular phylogeny for the Chironomidae (Diptera). *Systematic Entomology*, *37*(1), 172-188.

Daufresne, M., Lengfellner, K., & Sommer, U. (2009). Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences*, *106*(31), 12788-12793.

Davis, M. B., & Shaw, R. G. (2001). Range shifts and adaptive responses to Quaternary climate change. *Science*, *292*(5517), 673-679.

Durant, J. M., Hjermann, D. Ø., Ottersen, G., & Stenseth, N. C. (2007). Climate and the match or mismatch between predator requirements and resource availability. *Climate research*, *33*(3), 271-283.

Gardner, J. L., Peters, A., Kearney, M. R., Joseph, L., & Heinsohn, R. (2011). Declining body size: a third universal response to warming?. *Trends in ecology & evolution*, *26*(6), 285-291.

Holmes, R. T. (1966). Feeding ecology of the red-backed sandpiper (Calidris alpina) in Arctic Alaska. *Ecology*, 47(1), 32-45.

Holmes, R. T., & Pitelka, F. A. (1968). Food overlap among coexisting sandpipers on northern Alaskan tundra. *Systematic Zoology*, 17(3), 305-318.

Ineichen, H, Meyer, B, Lezzi, M, (1983). Determination of the developmental stage of living fourth instar larvae of *Chironomus tentans*. *Developmental Biology*, 98: 278-286.

Intergovernmental Panel on Climate Change (IPCC). Fifth Assessment Report (2014).

Kingsolver, J. G., & Huey, R. B. (2008). Size, temperature, and fitness: three rules. *Evolutionary Ecology Research*, *10*(2), 251-268.

Lackmann, A. R., & Butler, M. G. (2018). Breaking the rule: Five larval instars in the podonomine midge Trichotanypus alaskensis Brundin from Utqiaġvik, Alaska. *Journal of Limnology*.

Lougheed, V. L., Butler, M. G., McEwen, D. C., & Hobbie, J. E. (2011). Changes in tundra pond limnology: Re-sampling Alaskan ponds after 40 years. *Ambio*, 40(6), 589-599.

MacLean Jr, S. F., & Pitelka, F. A. (1971). Seasonal patterns of abundance of tundra arthropods near Utqiaġvik. *Arctic*, 19-40.

McKinnon, L., Picotin, M., Bolduc, E., Juillet, C., & Bêty, J. (2012). Timing of breeding, peak food availability, and effects of mismatch on chick growth in birds nesting in the High Arctic. *Canadian Journal of Zoology*, 90(8), 961-971.

Saalfeld, S. T., McEwen, D. C., Kesler, D. C., et al. (2019). Phenological mismatch in Arctic-breeding shorebirds: Impact of snowmelt and unpredictable weather conditions on food availability and chick growth. *Ecology and Evolution*, 9, 6693–6707.

Sheridan, J. A., & Bickford, D. (2011). Shrinking body size as an ecological response to climate change. *Nature Climate Change*, *1*(8), 401.

Visser, M. E., & Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society of London B: Biological Sciences*, *272*(1581), 2561-2569.

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J., Fromentin, J.M., Hoegh-Guldberg, O. and Bairlein, F., (2002). Ecological responses to recent climate change. *Nature*, *416*(6879), p.389.

Wiederholm, T. (1986). Chironomidae of the Holarctic region Part 2. Pupae. *Entomologica Scandinavica Supplement*, 28, 1-482.

Welch, H. E., Jorgenson, J. K., & Curtis, M. F. (1988). Emergence of Chironomidae (Diptera) in fertilized and natural lakes at Saqvaqjuac, NWT. *Canadian Journal of Fisheries and Aquatic Sciences*, *45*(4), 731-737.

Wülker, W, Götz, P, 1968. Die Verwendung der Imaginalscheiben zur Bestimmung des Entwicklungszustandes von *Chironomus*-Larven (Dipt.). *Zoomorphology*, 62(4): 363-388.

5. DEVELOPMENTAL RATES AND PHENOLOGICAL SHIFTS OF CHIRONOMIDAE EMERGING IN THE ALASKAN HIGH ARCTIC OVER FOUR DECADES

5.1. Abstract

Via modified landscapes, altered food webs, and mass extinctions, climate change has significantly altered the course of history on earth. Anthropogenic climate change is most accentuated in the Arctic, where mean annual temperatures are now consistently over 4 °C warmer than long-term norms. The immediate effects on ecosystems of such rapid changes in climate are poorly understood. At Utqiaġvik (formerly Barrow), Alaska, the predator-prey relationship between migratory shorebirds and flies in the dipteran Family Chironomidae is of interest because these trophic levels may decouple, potentially reducing breeding success for this already-declining group of tundra-nesting bird species. As ectotherms, chironomids may experience significant life-history effects from even slight changes in local climate. We tested the hypothesis that development rates of these Utqiaġvik Chironomidae respond uniformly (*i.e.* no species-level differences) to changes in temperature by rearing a set of highly-abundant chironomid species under eight controlled thermal regimes in the lab, with target mean temperature treatments ranging from 5-25 °C. We also used field rearings of three species in their natural pond habitat to test whether lab-derived equations for the thermal dependence of pre-emergence developmental rates can accurately predict natural emergence phenomena. During 2014-2016 we reared 16 species from their overwintering state to emergence of the adult fly, in sufficient abundance to generate sex-specific developmental rate responses. We also measured rates of pupal development alone for three of these species in both the lab and field. We documented the natural emergence phenologies of chironomids across each growing season in tundra ponds near Utqiaġvik during 1972, 1975-1977, 2009-2011, 2013, and 2015-2016 by

collecting emerging adults and/or pupal exuviae (N=76,330 emergence events), with pond temperature data available primarily after 2009. We found that Utqiaġvik chironomids do not respond uniformly to changes in temperature, that lab-derived equations can be used to predict field emergence, and that the natural chironomid emergence phenology has shifted about 8-10 days earlier in the more recent era. Our findings combine theoretical and empirical evidence to provide a detailed understanding of climate change effects on this aspect of the arctic food web.

5.2. Introduction

Over the past century climate change has caused global ecological changes (Walther *et al*. 2002; Visser and Both 2005; Chen *et al*. 2011; Bowden *et al*. 2015), with many species predicted to go extinct because of insufficient adaptation rates, habitat destruction, and other anthropogenic effects on the environment (Davis and Shaw 2001). Recently, the Arctic has experienced longer growing seasons, higher average temperatures, and increased temperature variation (IPCC 2014; Osborne *et al*. 2018). In fact, the Arctic is the fastest warming region on earth, consistently > 4ºC warmer than long term norms (1981-2010) (IPCC 2014; Osborne *et al*. 2018). Annual mean temperature anomalies in the Arctic are more than two times greater than the global average, and for the past five consecutive years (2014-2018) have exceeded any other year since records began in 1900 (Osborne *et al*. 2018).

At Utqiaġvik, Alaska these arctic trends are locally apparent. On the Barrow Peninsula, mean air temperatures since the 1960s have been increasing at a rate of 0.7ºC decade^{-1} (Hobbie *et al*. 1999). Climatic changes have also been documented in the tundra pond habitat. In a well-studied low-centered polygon pond, mean water temperature during the growing season warmed at a rate of 0.5ºC decade^{-1} from 1971 to 2012 (McEwen and Butler 2018). Also, the heat sum during the first month of the growing season increased significantly, and the length of the

growing season increased by ~2 weeks. Other low-centered polygon ponds exhibit similar temperature patterns based on contemporary measurements alone, suggesting that similar climatic changes have taken place across the tundra pond ecosystem in Alaska's Arctic Coastal Plain (ACP).

The ACP is well known as a hotspot of avian abundance, hosting tens of millions of migratory birds during the spring and summer (Andres *et al*. 2012; Bart *et al*. 2012a) with high species diversity (Custer and Pitelka 1978; Tulp and Schekkerman 2008; Lanctot *et al*. 2015; Weiser *et al*. 2018). In Alaska's North Slope alone, the avian community is comprised of at least 185 species, with 151 species in the Utqiaġvik region (Pitelka 1974). Approximately 95% of the avifauna is migratory (Johnson and Herter 1990), including at least 33 species of shorebirds (Charadriiformes) (Pitelka 1974). In fact, shorebirds are the dominant avian taxon in the Arctic, and ~50 % of North American's breeding shorebird diversity is found in Northern Alaska (Boyd and Madsen 1997; Johnson and Herter 1989; Morrison et al. 2006; Andres *et al*. 2012; Andres, Smith *et al*. 2012). These species migrate for thousands, even tens of thousands of kilometers to rear their young in this region (Conklin *et al*. 2010).

The prey for these shorebirds is almost entirely insectan (Holmes and Pitelka 1968; Pitelka 1974; Høye and Forchhammer 2008), and at Utqiaġvik almost all insects (~95% of the total abundance) belong to the Diptera (Holmes and Pitelka 1968; MacLean and Pitelka 1971; Pitelka 1974). Two families in this order, the Tipulidae (3 species) and Chironomidae (~40 species), make up 80% of the food available to shorebirds (Holmes 1966; Holmes and Pitelka 1968). The Family Chironomidae predominates among these dipterans, both in total abundance and species diversity (Butler 1982b; Lougheed *et al*. 2011). Within tundra ponds at Utqiaġvik there are at least 31 chironomid species (Lougheed *et al*. 2011), yet about 12 of these taxa are

estimated to comprise 90% of emerging chironomid biomass on an annual basis (Lougheed *et al*. 2011). That adult Chironomidae dominate arthropod biomass on the terrestrial tundra at Utqiaġvik is also impressive (Holmes and Pitelka 1968; MacLean and Pitelka 1971; Saalfeld *et al*. 2019), given the predominantly aquatic existence of most chironomid species as larvae (Armitage *et al*. 1995). While larger-bodied crane fly (tipulid) species are also significant shorebird prey at Utqiaġvik (Holmes 1966; MacLean and Pitelka 1971), the smaller but highly abundant chironomids dominate total insect biomass on the tundra (Saalfeld et al. 2019). Consequently, chironomids are crucial components of both aquatic and terrestrial portions of the tundra ecosystem at Utqiaġvik.

Chironomid species at Utqiaġvik have highly synchronized emergence during the already brief (~3 month) growing season (Butler 1980a; Butler 1982a; Braegelman 2016). Reported life cycles range from 1-7 years, depending on the species (Butler 1980, Butler 1982a; Lackmann and Butler 2018). Most chironomids spend almost their entire existence as aquatic larvae, then complete their life cycle by pupating and emerging as adults with a brief terrestrial existence during which reproduction occurs. In short, the pupal and adult stages are essentially their final week alive. With generally low temperatures, frequent winds, and a relatively shelterless landscape (there are no trees, nor even shrubs over 0.3 m tall), the tundra environment at Utqiaġvik is inhospitable for an insect that uses flight for dispersal and to find a mate for sexual reproduction. Thus, selection has acted strongly to maintain emergence synchrony in these populations (Danks and Oliver 1972; Butler 1982a; Hodkinson *et al*. 1996; Høye and Forchhammer 2008). Indeed, most emergence of any one species occurs within about a week, and the entire chironomid phenology is largely completed in 3-4 weeks in any given year (Butler 1980a; Braegelman 2016).

For migratory shorebirds, this highly synchronous pulse of insect biomass to the terrestrial tundra is crucial to reproductive success (Holmes 1966; Holmes and Pitelka 1968; MacLean and Pitelka 1971). Shorebird young are precocial, and in the High Arctic predominantly feed on chironomid adults found on tundra vegetation. Chicks rarely probe substrate because their immature beaks are small and not hardened (Holmes 1966). Thus, it is important that sufficient chironomid adults are available during the 2-3 week period from brood hatch until fledging (Holmes 1966).

The ability to predict the effects of thermal change on chironomid prey phenology would be useful in understanding broader trophic-level linkages within this rapidly evolving ecosystem. Through experiment and empirical observation, we test the validity of developmental responses seen in lab-reared chironomids under controlled thermal regimes. We also test whether chironomids respond uniformly to changes in temperature, both among species, and within different stages of their life cycle. Using phenology data collected from different time periods (1970s vs. 2010s), we ask whether a community-level shift in the chironomid emergence phenology is already apparent.

5.3. Methods

5.3.1. Field Site

We conducted our study on arctic tundra east of the village of Utqiaġvik, Alaska (71°17'27.5"N 156°47'18.5"W). Fig. 5.1 shows locations of all ponds used for field monitoring of chironomid emergence, field rearings, or for collection of larvae used in lab rearing experiments (see details in specific sections below).

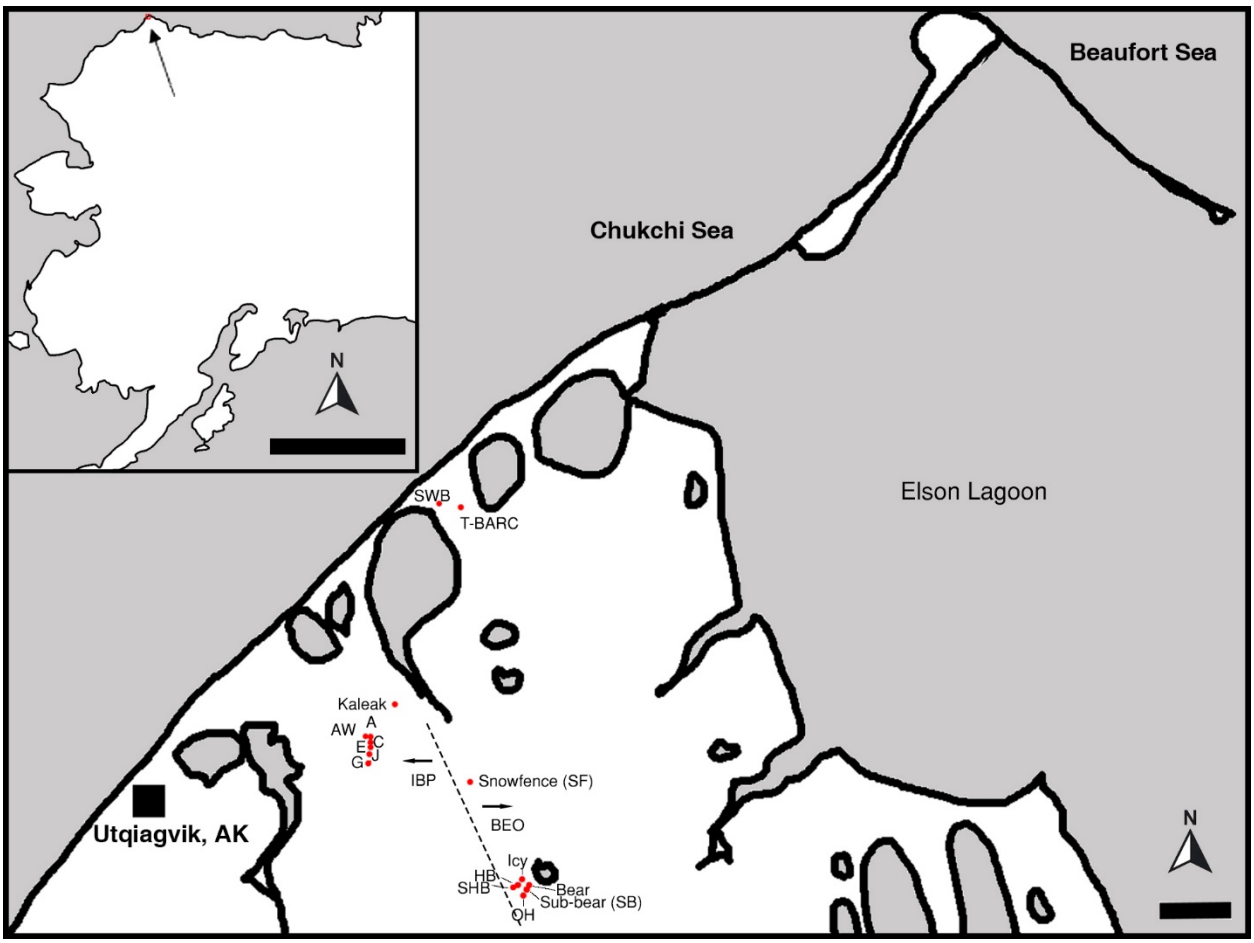

Figure 5.1. Map of the study site at Utqiaġvik, Alaska.
Ponds sampled for chironomid larvae used in lab treatments (2015-16), or sampled for pupal exuviae (PEs; phenology samples) are marked with red dots and labeled. Ponds J, E, and G were sampled for PEs in the 1970s. All ponds except SWB, T-BARC, and SHB were sampled for PEs in the 2010s. Ponds range in size from ~175 m^2 to 900 m^2. Scale bars = 500 km (inset), and 1 km. IBP (International Biological Program) and BEO (Barrow Environmental Observatory) denote sites. Dotted line = road.

5.3.2. Assessing Chironomid Developmental Response to Temperature

In 2015-16 we collected late-instar chironomid larvae from ten ponds, to be reared under various temperature treatments in the lab. The temperature-treatment setup consisted of ten 75-liter aerated aquaria (plastic tubs - Fig. 5.2). Ten water temperature treatments were produced with seven EcoPlus™ aquarium chillers, and three 300 W aquarium heaters. Water was circulated within each aquarium with a 1,514 l/hr water pump, and all ten were aerated via

plastic tubing from a 100W air pump (150 l/min). Water temperature in each treatment was monitored in real-time with a digital aquarium thermometer and logged hourly with an Onset® Hoboware temperature logger. Treatment tubs were randomized throughout the room to limit experimental bias (Hurlbert 1984). All aquaria were filled with a mixture of pond water (~75%) and de-ionized water (~25%), and a layer (< 1 cm) of pond detritus was added to the bottom of each tub. Temperature treatments in 2015 were controlled at 6, 8, 10, 12, 15, 18, 21, 24, 26 and 29 ºC (±1 °C), the three warmest treatments utilizing the heaters. In 2015 we found that the two warmest treatments were too thermally stressful for these arctic chironomids (no emergence), and in 2016 those treatments were excluded, leaving eight treatments.

Figure 5.2. Rearing set-up in 2015 with treatments ranging from 6-29 °C.
The ten black tubs (aquaria) containing floating rearing cups represent the treatments. Note the black aquarium chillers above seven of the treatments; three others were heated. The aerator is the silver pump in the center, with airline tubing running to each tub. Larger diameter hosing and a pump circulate water between each tub and its chiller in the seven cooler treatments.

Larvae were collected from source ponds within 2-3 days following pond thaw with a 100 μm mesh dip net. For infaunal benthic taxa such as *Chironomus* spp., larvae were sieved from sediment using a 1 mm mesh. Pond-specific samples were transported to the lab where larvae were sorted (on ice) to species under a dissecting microscope. Chironomids were usually placed into the treatments on the same day the sample was collected, but in some cases samples were stored at 3 °C overnight and finished the following day. Larvae of each taxon from a given

source pond were divided evenly among the treatments, and placed in rearing cups with detritus and pond water. We used only chironomid larvae expected to emerge during that growing season, ranging from third-fifth larval instars depending on the species (Butler 1980a; Lackmann and Butler 2018).

The rearing cups (~400 ml each) floated in the treatment tubs (see Fig. 5.2), and had four (2.5 cm) holes punched around the sides of each cup that were covered with 150 μm mesh, allowing water exchange between cup and aquarium. Strips of closed-cell foam fastened around the top edge of the cup, above the aeration holes, provided flotation. We also manually introduced aquarium water into each cup during daily inspections. A large hole cut in each rearing-cup lid and covered with 300 μm mesh permitted observation of cup contents, while preventing the escape of emerged adult midges.

Rearing cups were monitored at least once per day for emergence or pupation. If chironomid adults were noticed, a light mist of 30% EtOH sprayed through the screened lid was generally effective in preventing escapees. Emerged adults were collected along with their pupal exuviae (PEs), and immediately preserved in 70% EtOH. PEs were used to assist in taxonomic identity of reared chironomids. For species with free-swimming larvae and pupae (i.e. non-burrowing or tube-making taxa), the transition from larva to pupa could be observed. Upon pupal discovery, daily cohorts of pupae were transferred into new rearing cups, also monitored for subsequent emergence. Thus, pupal development times were also obtained for these species.

In 2014 and 2016 we reared chironomids in the field at Utqiaġvik. In 2014 we collected two species of free-swimming chironomid larvae, *Psectrocladius* sp2 and *Trichotanypus alaskensis*, at ice-out in Pond C (14 June) with a 100 μm dip net. After sorting larvae in the field, we placed approximately 1/3 of the total of each species into a rearing arena in Pond C, and the

remaining thirds into Pond J and Pond G in an effort to broaden the temperature gradient among treatments. These three ponds were at different stages of thaw: Pond C - at thaw, Pond J - fully thawed a few days before Pond C, and Pond G - yet to fully-thaw. These larvae were placed in a large ventilated rearing cup within a larger ventilated arena (a 19 liter bucket with side vents, and the bottom removed) that was anchored to the pond with rebar. An Onset® Hoboware temperature logger was placed in each arena to log hourly temperature. This setup (Fig. 5.3) was replicated in each of the three ponds.

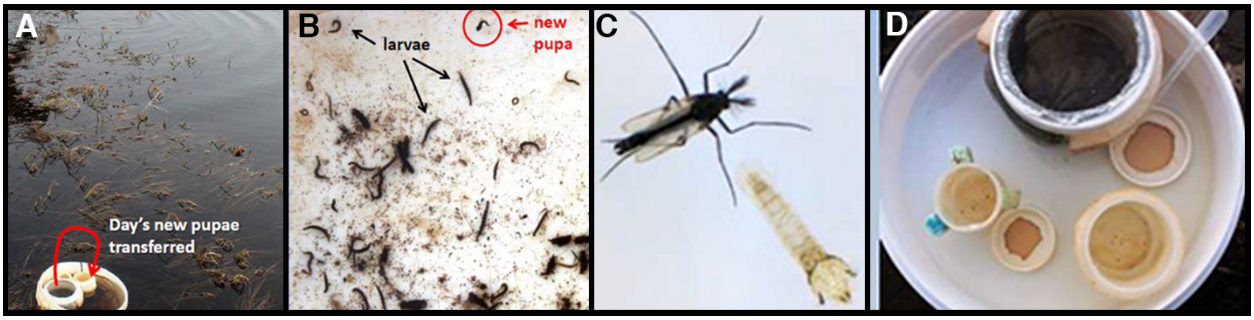

Figure 5.3. Experimental procedure for field-reared individuals in 2014 at IBP.
A) Rearing cups in the arena in Pond C and general procedure. B) *Trichotanypus alaskensis* and *Psectrocladius* sp2 were monitored and sorted daily in the field, pupae being morphologically distinct from larvae. C) Upon emergence both the adult and the pupal exuviae were preserved to confirm taxonomic identity. D) A closer view of the rearing cups.

The larvae were monitored daily for morphological change into pupae and emergence of adults (Fig. 5.3). Each day's new pupae were transferred into a smaller rearing cup, yet retained in the same arena. After pupae were transferred to their new cup, they were checked daily for emergence. Emerged individuals were sexed and identified to species.

In addition to the *Psectrocladius* sp2 and *T. alaskensis* field rearings at IBP in 2014, we also conducted rearings of these same species at BEO. At thaw on 19 June 2014, we collected larvae from HB Pond, separating the two species. We then divided each collection into fourths for distribution into four temperature treatments in the field. In this case the treatments were not ponds, but light vs. dark, and insulated vs. non-insulated, plastic tubs (Fig. 5.4) in an attempt to

establish a wider temperature gradient in the field. Each tub contained its own temperature logger, and we followed the daily procedure described above.

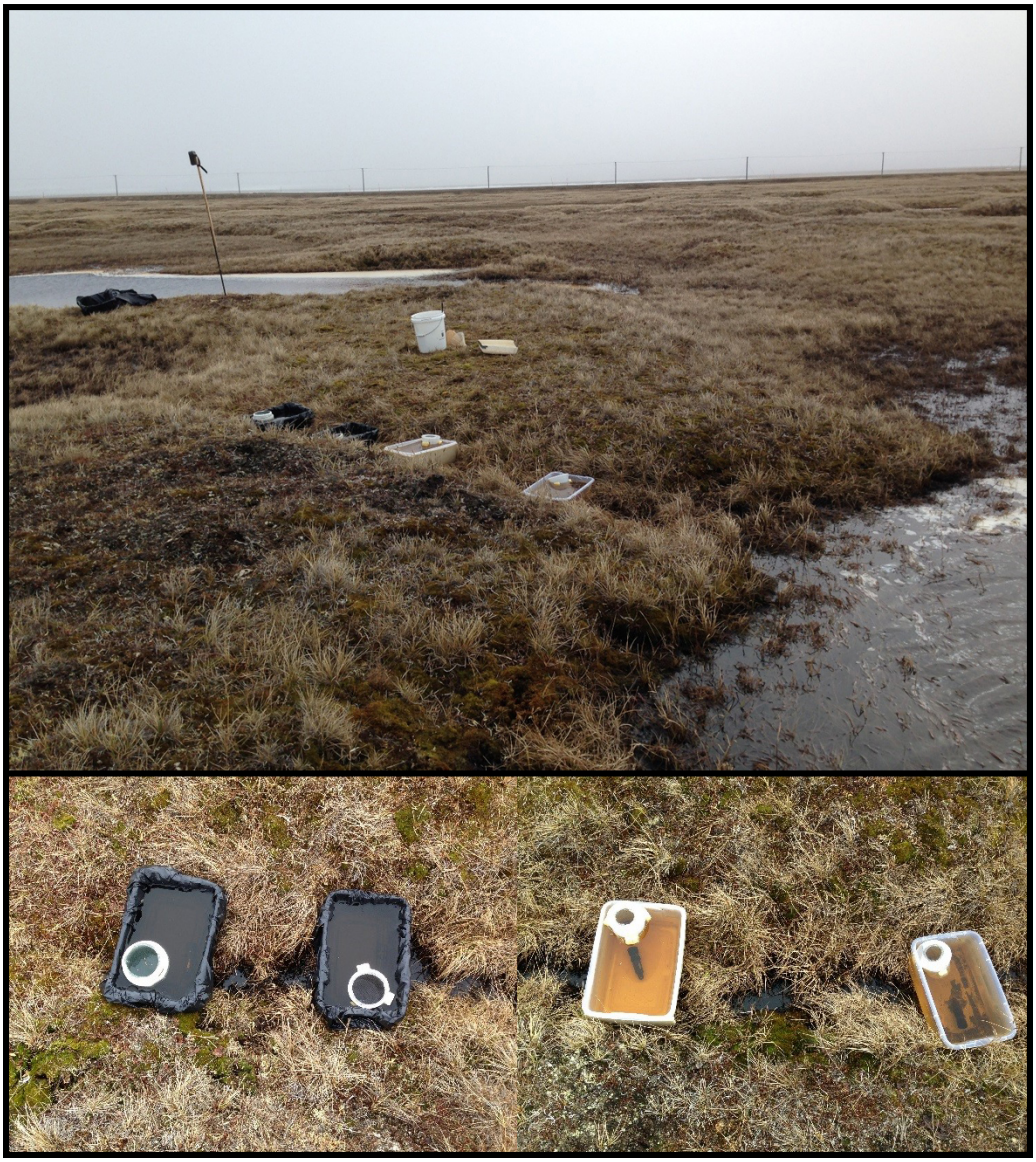

Figure 5.4. Experimental setup of the BEO four-tub experiment.
Top panel) Upper left pond is HB, from which *Psectrocladius* sp2 and *T. alaskensis* larvae were collected and distributed into the four treatments. At the lower right, the southwest edge of Icy Pond is visible. Bottom panel) The four treatments (from left to right) were dark-insulated, dark, light-insulated, and light.

In 2016 we conducted a similar field-rearing experiment with prepupal *Derotanypus alaskensis* larvae collected from Pond AW, which we reared to emergence in a Hobo logger-

equipped arena within Pond C. As in our 2014 field rearings, we tracked both pupation and adult emergence of this species.

5.3.3. Emergence Phenology Monitoring

We sought to determine if recent climatic warming in northern Alaska has led to detectable change in the seasonal timing of chironomid emergence. Emergence phenologies were monitored in tundra ponds at Utqiaġvik in two different eras, in the mid-1970s and again in the second decade of the 21st Century). Species-level data on insect emergence timing are available throughout the annual emergence season from these tundra ponds in 1972, 1975-1977, 2009-11, 2013, and 2015-16. Some data also exist from 2012 and 2014, (Table 5.1), but were excluded from this analysis because the sampling effort was truncated and does not span the entire seasonal phenology. In the 1970s, ponds in the IBP site were sampled with emergence traps in 1972 as part of the International Biological Program's Tundra Biome Project (Hobbie 1980), and again in 1975-77 by Butler (1980a, 1980b). More recently, chironomid emergence in ponds within both the IBP and the Barrow Environmental Observatory (BEO; Fig 5.1) was monitored by collection of pupal exuviae (PE sweeps). (Table 5.1). The emergence traps used by Butler in 1975-77 collected both adults and their pupal exuviae. In 2013, these traps were used alongside PE sweeps (Braegelman 2016), with both methods producing similar results regarding the overall seasonal phenology.

Table 5.1. Phenology sampling information for emerging Chironomidae at Utqiaġvik.

Year	Sampling frequency by pond	IBP ponds	BEO ponds	EM span (JD)	Total PEs	Total species	Note
1972	Daily	J		185-202	773	13	Trapped adults
1975	2-3 day intervals	E, J, Ω		184-226	2,029	16	Trapped adults, PEs
1976	Every other day	E, J, G		182-224	2,072	17	Trapped adults, PEs
1977	Daily (Pd J) or every other day	D, E, J, G		173-204	1,531	17	Trapped adults, PEs
2009	2-3 day intervals staggered by site	C, J, G	Bear, HB	167-197	9,227	20	PE sweeps
2010	Every 3 days	C, J	Bear, HB	176-212	7,383	20	PE sweeps
2011	2-3 day intervals staggered by site	C, J, G	Bear, HB	169-196	8,455	20	PE sweeps
2012*	Every other day	C, J		173-184	2,132	16	Sampling truncated
2013	Daily	C, J, G	Bear, HB	166-201	13,747	28	Traps & PE sweeps
2014*	Daily^	A, C, E, J, G	Bear, HB OH, Icy	168-206	18,184	33	Sample gap JDs 180-190
2015	Daily but staggered by site	A, C, E, J, G, Kaleak	Bear, HB OH, Icy, SF, SB	150-201	9,599	36	PE sweeps
2016	Daily but staggered by site	A, C, E, J, G, Kaleak	Bear, HB, OH Icy, SB	150-213	19,226	44	PE sweeps

Data sorted by year of emergence. EM = emergence, JD = Julian Date; *years excluded from analyses, ^excluding gap.

PE sweeps consist of five, non-overlapping, 1 m-long sweeps with a 15 cm diameter, 100 μm mesh net along the downwind side of each pond. The sweep net contents were then flushed into WhirlPaks® in-field using a 35% ethanol solution and a funnel. PEs were then sorted, sexed, and tallied by species based on morphological characteristics (Wiederholm 1986) under a dissecting microscope. PEs that were missing large portions of their anterior or posterior, although rare, were not tallied because they were 1) difficult to identify and 2) likely did not reflect the most recent emergence (*i.e*. they were likely older than 2-3 days).

Sampling effort differed between the two eras for which we have emergence data, but data serve to reflect the seasonal timing of chironomid emergence at the species level. We estimated adult biomass by measuring the average size of the adult thorax for male and female chironomids for 30 species (N=1,717, see Chapter 4 for methods), then we converted thorax measurements to dry mass using existing sex-specific regressions for chironomids of northern Canada (Welch *et al*. 1988).

5.3.4. Statistical Analysis

The exponential relationship between rates of biological activity and environmental temperature is well established (Gillooly *et al*. 2002, Brown *et al*. 2004) and forms the basis for our analyses of thermal effects on chironomid developmental rates. Initially we conducted an overall analysis of pre-emergence developmental rate vs. temperature for the entire dataset of lab-reared chironomids. Given this expected exponential relationship, we used a generalized linear model (GLIM) to test for the effects of explanatory variables on the response variable developmental rate (the reciprocal of the time, in days, from experimental onset to adult emergence). The standard GLIM components (random, link, and systematic) used were exponential, reciprocal, and mixed, respectively (McCullagh and Nelder 1989). The main explanatory variable was each chironomid's mean temperature experience during its pre-emergence incubation (excluding any potential development between pond thaw and larval placement in a lab incubator). We also tested other variables for significance (*i.e.* taxon, sample date, sex, and pond). We then conducted analyses at the species level. Samples that were unevenly distributed across temperature treatments, or were very sparse (fewer than 10 emergence events), were excluded from these detailed analyses. The species-specific datasets analyzed were generally large, single-pond source samples collected once, or on narrow range of dates. As our goal was to understand the nature of each species' developmental rate response to temperature, we graph (Fig. 5.5) resulting models for the largest and most robust data set for each species. These data typically reflect larvae from a single collection date and pond. As there was never a significant sex effect at the species level, we pooled sexes within each species. We first analyzed the exponential relationship between developmental rate and temperature using each emerged adult as its own data point, and then compared this result to the mean response per

temperature treatment by binning individuals within a given temperature treatment, similar to Baek *et al*. (2012). We present these latter relationships graphically for visual clarity.

We performed similar analyses of pre-emergence development rates for three species of field-reared chironomids, *T. alaskensis* and *Psectrocladius* sp2 in 2014, and *D. alaskensis* in 2016. Here, temperature treatments in the field comprised the thermal history of emerging individuals, binned within each 1 ºC mean temperature experienced during the experiment. There were no significant pond or tub effects in these field rearings.

We used this same approach to quantify pre-emergence development times (and their reciprocal development rates) with our emergence phenology data, based on PE sweeps in 2015-16. For each of 15 abundant species, we defined "EM_{50}" as the date in each pond and year, when 50% of that species' total emergence was reached. We used pond-specific thaw date each year as the developmental starting point, and calculated mean daily temperature, from pond thaw to each species' EM_{50}, from hourly temperature data from one or more overwintering, pond-specific loggers.

For analyses in which the underlying data were hypothesized to be normally distributed (*e.g.* field phenology data), we used a general linear model (GLM) fit by standard least squares to model treatment effects, as described in Chapter 4. For graphs with uni-multimodal continuous probability distribution overlays, we selected the best model based on the corrected Akaike's Information Criterion (AICc), the Bayesian Information Criterion (BIC), and parsimony (Burnham and Anderson 2004). We used JMP 14 Pro Statistical Discovery™ for statistical analysis and graphical output.

5.4. Results

5.4.1. Chironomid Developmental Rate Responses

Our initial, overall analysis of development rates for lab-reared chironomids included 1,700 emerging individuals from 27 species (Table 5.2) and identified temperature as the strongest explanatory variable explaining pre-emergence development rate. Taxonomic identity also had a highly significant effect. A lesser effect of sex was significant, but varied among taxa.

Table 5.2. Effect Summary for the Generalized Linear Model (distribution: exponential, link function: reciprocal) for all 27 chironomid species (N=1,700 adults) with developmental rate (1/days) as the response variable.

Model Effect	LogWorth	p	Model Estimate	SE
Temperature	63.545	<0.0001	-0.5105	0.0306
Taxon	26.115	<0.0001	Varies	Varies
Sex	2.438	0.0036	0.4097 [Female]	0.1403
Pond	10.454	0.2132	Not significant	NA
Sample Date	2.192	0.9840	Not significant	NA

Our subsequent, species-specific analyses focused on 16 chironomid species, with a total of 1016 individuals reared from late instar larvae to emergence. Additionally, we modeled pupal development rate alone for three species, *T. alaskensis*, *Psectrocladius* sp2, and *Derotanypus alaskensis* both in lab and field rearings (Fig. 5.5; Tables 5.3-5.4). In all but one case, a statistically significant ($p<0.05$) exponential relationship ($y = ae^{bx}$) described the insect's developmental response to temperature, with some species-level differences found in the developmental response parameter ***b*** (Fig. 5.6). The only exception was for *Derotanypus alaskensis* pupae reared in the field, with insufficient data points (N=14) from a narrow temperature range. Nonetheless, these field pupation rates corresponded closely to the well supported (N=85) lab-derived equation for pupation by this species (Figs. 5.5, 5.7). For most species, males tended to have slightly higher rates of development at a given temperature, but as

there was never a significant sex effect (GLIM: $p>0.05$ for each species) we pooled sexes in these analyses.

We compared the lab-derived larva-to-adult development rate equations (presented in Fig. 5.5, pooling data by temperature treatment) to development in the field by overlaying the field emergence data onto each species-specific panel. As thermal gradients within these field data sets were insufficiently broad to produce significant correlations with developmental rates, we were unable to explicitly test equation parameters for larva-to-adult development (although this was possible with pupation-only data. In general, the field data (red circles) were consistent with lab predictions. For 14 of 15 taxa, field data fell on, or straddled, the lab-generated equation, reflecting emergence dates within a few days of those predicted by the model. *Procladius prolongatus* and *Chironomus* sp (t-type large) were the greatest outliers, in that all development rates from field data fell below the curve. For these species, field emergence was about 5-10 days later than predicted by the lab equation for the same temperature experience. Clusters of field data for some other species also tended to fall below, not above, the curve (see Discussion).

Table 5.3. Parameter estimates for the exponential equation ($y = ae^{bx}$) used to model the dependence of pre-emergence development rate on temperature (°C) for lab-reared chironomids from ponds at Utqiaġvik, AK.

Taxon	Sex	St	a (95%CI)	b (95%CI)	R^2	p	N
C. tardus	♀,♂	la	0.027 (0.018, 0.039)	0.058 (0.036, 0.081)	.847	<.001	9
C. tardus	♀,♂	**la**	**0.024 (0.017, 0.035)**	**0.059 (0.035, 0.083)**	**.351**	**<.0001**	**49**
Tricho. alaskensis	♀,♂	la	0.047 (0.036, 0.061)	0.076 (0.055, 0.097)	.963	<.001	6
Tricho. alaskensis	♀,♂	**la**	**0.046 (0.040, 0.053)**	**0.078 (0.066, 0.090)**	**.775**	**<.0001**	**55**
Ps. sp2	♀,♂	la	0.061 (0.036, 0.105)	0.078 (0.043, 0.112)	.833	.0015	8
***Ps.* sp2**	♀,♂	**la**	**0.046 (0.040, 0.054)**	**0.101 (0.087, 0.114)**	**.626**	**<.0001**	**141**
Tan. nearcticus	♀,♂	la	0.015 (0.010, 0.021)	0.097 (0.073, 0.121)	.957	<.0001	7
Tan. nearcticus	♀,♂	**la**	**0.015 (0.011, 0.019)**	**0.094 (0.077, 0.111)**	**.713**	**<.0001**	**52**
Par. laccophilus	♀	la	0.015 (0.004, 0.055)	0.098 (0.020, 0.176)	.675	.024	7
Par. laccophilus	♀	**la**	**0.023 (0.010, 0.050)**	**0.062 (0.015, 0.109)**	**.195**	**.011**	**32**
Dero. aclines	♀,♂	la	0.048 (0.031, 0.074)	0.099 (0.068, 0.130)	.931	<.001	7
Dero. aclines	♀,♂	**la**	**0.052 (0.038, 0.072)**	**0.095 (0.075, 0.114)**	**.712**	**<.0001**	**41**
Proc. prolongatus	♀,♂	la	0.041 (0.021, 0.081)	0.110 (0.066, 0.154)	.954	.0042	5
Proc. prolongatus	♀,♂	**la**	**0.042 (0.024, 0.071)**	**0.104 (0.068, 0.140)**	**.787**	**<.0001**	**13**
Corynoneura sp.	♀,♂	la	0.018 (0.009, 0.038)	0.112 (0.066, 0.159)	.918	<.001	6
***Corynoneura* sp.**	♀,♂	**la**	**0.014 (0.008, 0.025)**	**0.129 (0.088, 0.171)**	**.656**	**<.0001**	**24**
C. sp. (t-type D)	♀,♂	la	0.024 (0.016, 0.037)	0.113 (0.085, 0.141)	.943	<.0001	8
***C.* sp. (t-type D)**	♀,♂	**la**	**0.023 (0.019, 0.029)**	**0.115 (0.100, 0.129)**	**.907**	**<.0001**	**30**
C. sp. (s-type)	♀,♂	la	0.028 (0.017, 0.046)	0.118 (0.082, 0.154)	.935	<.001	7
***C.* sp. (s-type)**	♀,♂	**la**	**0.031 (0.026, 0.036)**	**0.116 (0.104, 0.128)**	**.809**	**<.0001**	**89**
C. sp. (t-type)	♀,♂	la	0.021 (0.014, 0.031)	0.117 (0.090, 0.144)	.950	<.0001	8
***C.* sp. (t-type)**	♀,♂	**la**	**0.023 (0.020, 0.028)**	**0.107 (0.096, 0.117)**	**.712**	**<.0001**	**161**
C. riparius	♀,♂	la	0.021 (0.015, 0.029)	0.119 (0.098, 0.140)	.970	<.0001	8
C. riparius	♀,♂	**la**	**0.022 (0.019, 0.026)**	**0.114 (0.105, 0.123)**	**.799**	**<.0001**	**156**
Dero. alaskensis	♀,♂	la	0.033 (0.024, 0.045)	0.123 (0.102, 0.145)	.971	<.0001	8
Dero. alaskensis	♀,♂	**la**	**0.032 (0.028, 0.037)**	**0.124 (0.115, 0.134)**	**.881**	**<.0001**	**95**
C. sp. (t-type L)	♀,♂	la	0.024 (0.016, 0.036)	0.126 (0.100, 0.152)	.960	<.0001	8
***C.* sp. (t-type L)**	♀,♂	**la**	**0.027 (0.022, 0.034)**	**0.119 (0.106, 0.132)**	**.936**	**<.0001**	**26**
Ps. sp1	♀,♂	la	0.027 (0.018, 0.043)	0.127 (0.098, 0.156)	.951	<.0001	8
***Ps.* sp1**	♀,♂	**la**	**0.028 (0.019, 0.041)**	**0.122 (0.096, 0.147)**	**.703**	**<.0001**	**42**
Par. penicillatus	♀,♂	la	0.020 (0.013, 0.032)	0.132 (0.101, 0.163)	.984	<.001	5
Par. penicillatus	♀,♂	**la**	**0.023 (0.017, 0.032)**	**0.121 (0.101, 0.141)**	**.961**	**<.0001**	**10**
Ps. sp2	♀,♂	p	0.104 (0.067, 0.162)	0.108 (0.073, 0.144)	.924	<.001	7
***Ps.* sp2**	♀,♂	**p**	**0.114 (0.088, 0.147)**	**0.101 (0.082, 0.121)**	**.703**	**<.0001**	**48**
Tricho. alaskensis	♀,♂	p	0.062 (0.039, 0.100)	0.109 (0.056, 0.161)	.975	.012	4
Tricho. alaskensis	♀,♂	**p**	**0.065 (0.055, 0.078)**	**0.103 (0.086, 0.121)**	**.820**	**<.0001**	**33**
Dero. alaskensis	♀,♂	p	0.045 (0.035, 0.058)	0.132 (0.116, 0.149)	.985	<.0001	8
Dero. alaskensis	♀,♂	**p**	**0.046 (0.041, 0.051)**	**0.132 (0.126, 0.139)**	**.951**	**<.0001**	**85**
Ps. sp2	♀,♂	pf	0.100 (0.088, 0.113)	0.136 (0.118, 0.154)	.995	<.001	5
***Ps.* sp2**	♀,♂	**pf**	**0.113 (0.097, 0.133)**	**0.112 (0.088, 0.136)**	**.179**	**<.0001**	**392**
Tricho. alaskensis	♀,♂	pf	0.055 (0.051, 0.062)	0.106 (0.093, 0.118)	.998	<.001	4
Tricho. alaskensis	♀,♂	**pf**	**0.055 (0.051, 0.059)**	**0.108 (0.099, 0.118)**	**.610**	**<.0001**	**309**
Dero. alaskensis	♀,♂	pf	N/A	N/A	N/A	N/A	2
Dero. alaskensis	♀,♂	**pf**	**0.050 (0.008, 0.299)**	**0.114 (-0.098, 0.327)**	**.103**	**.263**	**14**

Taxa are ranked from slowest to fastest by the developmental response parameter (***b***), based on the first entry for each species, using binned temperatures. **Bolded entries show results using all individual data**. St=stage; la=larva-to-adult; p=pupation alone; pf=pupation in field rearing.

Table 5.4. Taxon and treatment-specific developmental rates (DR) (and Table 5.3 & Fig. 5.5).

Taxon	Pond	Date in Treatment	°C	N(♀, ♂)	DR	SD
Dero. alaskensis	AW	4.VI.16	5	(2, 2)	0.057	0.013
			7	(9, 4)	0.066	0.012
			9	(7, 9)	0.103	0.019
			12	(6, 6)	0.169	0.038
			15	(6, 10)	0.266	0.056
			18	(10, 8)	0.298	0.049
			20	(4, 6)	0.350	0.045
			23	(3, 3)	0.504	0.138
pupae			5	(1, 2)	0.087	0.005
			7	(7, 4)	0.101	0.009
			9	(4, 6)	0.147	0.011
			12	(6, 6)	0.239	0.021
			16	(5, 10)	0.443	0.062
			18	(10, 8)	0.541	0.084
			22	(4, 6)	0.698	0.206
			24	(3, 3)	1.072	0.083
C. riparius	SB; SF	8.VI.16; 27.VI.16	5	(5, 4)	0.032	0.006
			7	(6, 6)	0.048	0.007
			10	(8, 9)	0.080	0.011
			12	(18, 13)	0.096	0.020
			15	(7, 10)	0.135	0.029
			19	(12, 12)	0.219	0.061
			21	(16, 13)	0.251	0.043
			24	(7, 10)	0.286	0.067
C. sp. (s-type)	SB	8-9.VI.16	5	(1, 5)	0.043	0.005
			7	(2, 1)	0.057	0.005
			9	(6, 10)	0.094	0.012
			12	(7, 14)	0.142	0.011
			15	(5, 14)	0.207	0.030
			19	(12, 10)	0.262	0.054
			21	(1, 1)	0.26	0.000
C. tardus	Bear, C; AW	5-11.VI.15; 6.VI.16	7	(3, 2)	0.032	0.004
			8	(2, 1)	0.037	0.005
			10	(3, 1)	0.063	0.018
			12	(9, 7)	0.051	0.027
			15	(2, 4)	0.085	0.018
			18	(4, 0)	0.078	0.022
			21	(5, 1)	0.078	0.025
			24	(2, 2)	0.098	0.032
			26	(0, 1)	0.130	0.000
C. sp. (t-type D)	SB	8-10.VI.2016	5	(1, 2)	0.040	0.000
			7	(5, 2)	0.046	0.009
			10	(0, 2)	0.070	0.000
			12	(2, 3)	0.120	0.022
			15	(0, 3)	0.173	0.021
			19	(3, 0)	0.237	0.026
			21	(1, 2)	0.21	0.000
			24	(2, 2)	0.300	0.040
C. sp. (t-type L)	SF	27.VI.16	5	(0, 2)	0.040	0.000
			8	(0, 2)	0.055	0.005

Table 5.4. Taxon and treatment-specific developmental rates (DR) (and Table 5.3 & Fig. 5.5) (continued).

Taxon	Pond	Date in Treatment	°C	N(♀, ♂)	DR	SD
C. sp. (t-type L) (cont.)	SF (continued)	27.VI.16 (continued)	10	(2, 2)	0.095	0.005
			12	(1, 3)	0.133	0.011
			15	(1, 2)	0.200	0.000
			18	(0, 3)	0.287	0.038
			21	(1, 3)	0.320	0.035
			24	(2, 2)	0.383	0.074
C. sp. (t-type)	SB	8-10.VI.16	6	(7, 6)	0.033	0.002
			7	(9, 7)	0.041	0.004
			10	(12, 9)	0.066	0.007
			12	(11, 16)	0.101	0.009
			15	(11, 6)	0.162	0.046
			19	(11, 11)	0.218	0.026
			21	(14, 12)	0.220	0.072
			24	(7, 12)	0.262	0.085
Corynoneura sp.	Kaleak; SF	28.VI.16; 1.VII.16	8	(1, 2)	0.038	0.001
			10	(5, 2)	0.046	0.004
			12	(5, 1)	0.088	0.060
			15	(3, 3)	0.122	0.032
			18	(0, 1)	0.171	0.000
			23	(0, 1)	0.204	0.000
Dero. aclines	Bear, SF, OH, HB; AW, SB, SF	5-14.VI.15; 4-23.VI.16	6	(1, 1)	0.076	0.003
			7	(1, 1)	0.089	0.025
			10	(1, 3)	0.125	0.014
			12	(3, 0)	0.187	0.030
			15	(7, 4)	0.274	0.073
			18	(8, 7)	0.301	0.061
			21	(4, 0)	0.300	0.048
Par. laccophilus	Kaleak&T-BARC	20-28.VI.16	8	(1, 0)	0.026	0.000
			11	(1, 0)	0.059	0.000
			12	(4, 0)	0.060	0.032
			15	(6, 0)	0.068	0.015
			18	(17, 0)	0.068	0.029
			21	(2, 0)	0.067	0.012
			24	(1, 0)	0.270	0.000
Par. penicillatus	Bear, SF; AW, SHB	6-9.VI.2015; 4-17.VI.2016	8	(1, 0)	0.053	0.000
			10	(0, 3)	0.079	0.006
			12	(0, 1)	0.102	0.000
			18	(4, 0)	0.205	0.016
			21	(1, 0)	0.355	0.000
Ps. sp1	Kaleak	28-29.VI.16	5	(0, 2)	0.050	0.016
			8	(3, 5)	0.074	0.025
			10	(2, 1)	0.077	0.007
			12	(3, 4)	0.189	0.169
			15	(4, 6)	0.195	0.077
			18	(6, 0)	0.285	0.077
			21	(1, 0)	0.375	0.000

Table 5.4. Taxon and treatment-specific developmental rates (DR) (and Table 5.3 & Fig. 5.5) (continued).

Taxon	Pond	Date in Treatment	°C	N(♀, ♂)	DR	SD
Ps. sp1 (continued)	Kaleak (continued)	28-29.VI.16 (cont.)	24	(1, 4)	0.564	0.433
Ps. sp2	OH&SF	6-28.VI.15, 1.VII.16	6	(13, 9)	0.082	0.024
			8	(13, 19)	0.100	0.028
			10	(14, 16)	0.154	0.054
			12	(16, 10)	0.159	0.045
			15	(6, 7)	0.217	0.063
			19	(7, 6)	0.375	0.158
			22	(2, 2)	0.364	0.010
			24	(1, 0)	0.253	0.000
pupae			6	(1, 1)	0.180	0.021
			8	(1, 2)	0.231	0.021
			9	(3, 8)	0.277	0.044
			10	(7, 3)	0.424	0.176
			12	(2, 5)	0.367	0.065
			16	(3, 4)	0.506	0.170
			19	(4, 4)	0.892	0.180
Tan. nearcticus	AW	4.VI.16	7	(3, 4)	0.025	0.003
			10	(1, 0)	0.041	0.000
			12	(5, 0)	0.044	0.010
			15	(3, 3)	0.073	0.009
			18	(10, 8)	0.083	0.017
			20	(3, 0)	0.094	0.033
			22	(2, 10)	0.112	0.031
Tricho. alaskensis	OH	7.VI.15	6	(2, 5)	0.067	0.004
			7	(4, 6)	0.082	0.007
			10	(5, 5)	0.096	0.011
			12	(6, 7)	0.126	0.029
			15	(5, 5)	0.163	0.014
			18	(2, 3)	0.175	0.000
pupae			6	(2, 2)	0.118	0.011
			7	(2, 5)	0.136	0.009
			10	(3, 8)	0.187	0.026
			12	(5, 6)	0.227	0.021
Proc. prolongatus	Bear, C, SWB; AW, OH	5-12.VI.15; 11-12.VI.16	7	(0, 1)	0.092	0.000
			10	(1, 3)	0.127	0.048
			15	(3, 1)	0.190	0.012
			19	(1, 2)	0.285	0.016
			21	(0, 1)	0.486	0.000

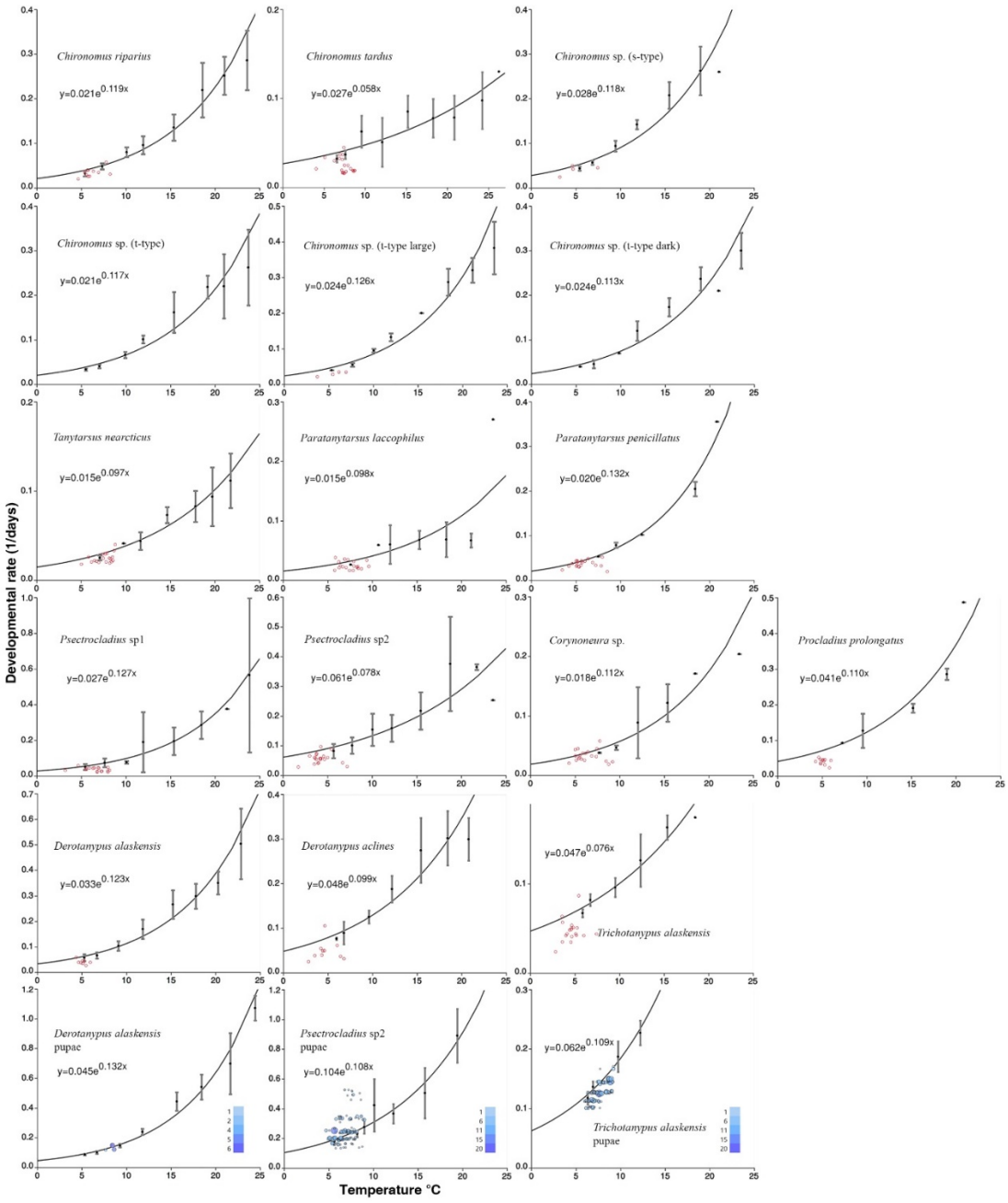

Figure 5.5. Mean pre-emergence developmental rate (1/days, ± SD) vs. rearing temperature (°C) for 16 chironomid taxa collected near thaw as late-instar larvae and reared with daily monitoring in controlled temperature regimes until emergence.
Curves show the exponential equation used to model development rate in lab rearings, using binned temperature data (Table 5.2). Red points are an overlay of field data, as each is based on time to cumulative emergence of 50% of a species in each pond monitored in 2015 and 2016. An additional three plots (bottom row) illustrate pupation rate alone in lab rearings, with bubble plot overlays representing cohort-specific data for field-reared pupae (data also shown in Fig. 5.7). Bubble size reflects the number of individuals in each cohort, in units of 1 from 1 to 23, with color gradients in the lower right for reference. See Tables 5.3 & 5.4 for more details.

Ten of the 16 species reared in the lab had developmental response coefficients (***b*** in the equation $y = ae^{bx}$) between 0.11-0.13 (Fig. 5.6, Table 5.3), none of which differed significantly. However this developmental response parameter did vary significantly for several species comparisons (95% confidence intervals non-overlapping). We found notably slow responses to temperature for *Chironomus tardus* and *Trichotanypus alaskensis*, compared to a fast response for *Derotanypus alaskensis*.

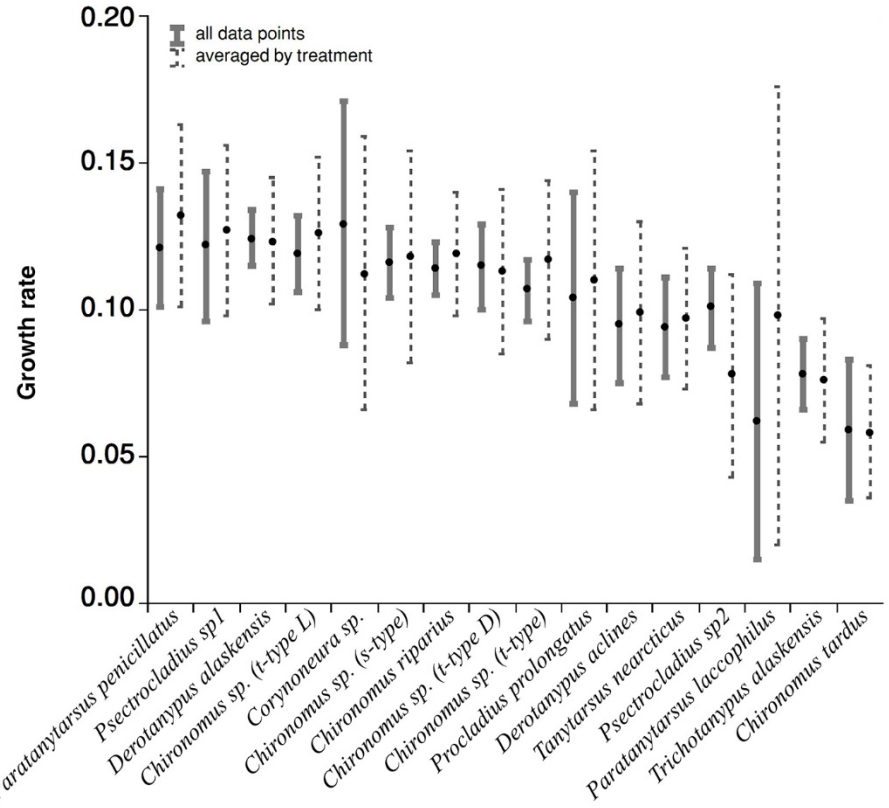

Figure 5.6. Larva-to-adult developmental response coefficients (i.e. ***b*** in equation $y = ae^{bx}$) with 95% CI for the top 16 species in Fig. 5.5.
Solid grey intervals indicate parameter estimates derived from all data points (bolded rows in Table 5.3); dashed intervals indicate estimates based on averaged temperature treatments (Fig. 5.5 equations; non-bolded rows in Table 5.3).

We found no treatment (tub/pond) or site (IBP vs. BEO) effects in our field rearings of pupae, so we combined all data for each species in these field experiments (Table 5.3). Field and lab equations for rates of pupation by each chironomid did not differ statistically (Fig. 5.7). The

temperature range at which all 14 *D. alaskensis* pupated in the 2016 field rearing was insufficient to generate a significant exponential relationship, but these field data conform well to the equation for lab-reared *D. alaskensis* pupae (dotted line in Fig. 5.7).

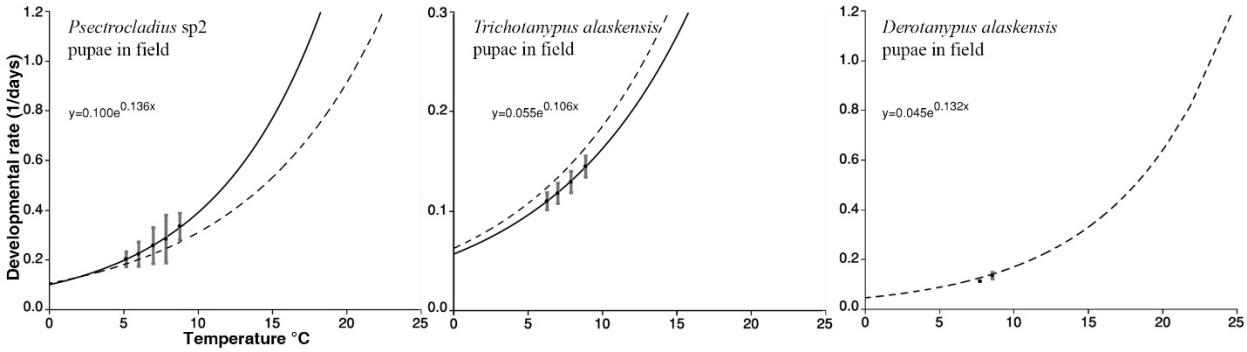

Figure 5.7. Mean developmental rate (1/days) ± SD vs. mean pond temperature (binned into 1 °C intervals) for pupae reared to emergence in the field.
During 2014: Left panel: *Psectrocladius* sp2 (N=392), & center panel: *Trichotanypus alaskensis* (N=309), with solid curves showing fitted exponential equations used to model pupal development of these species and dashed curves showing pupation model from lab rearings (Fig. 5.5, Table 5.3). Right panel shows *Derotanypus alaskensis* (N=14) reared in 2016, with dashed curve showing pupation model from lab rearing (Fig 5.5, Table 5.3).

We compared our lab-derived pupation responses to responses for pupae reared in the field, by comparing ***b*** coefficients for three species (Fig. 5.8). In all cases, parameter estimates and their 95% CIs overlapped (compare green to red in Fig. 5.8). We also compared late larva-to-adult development to just pupal development in the lab rearings, we also found that ***b*** coefficients did not differ statistically within any of these three taxa based on the life stages included in pre-emergence development.

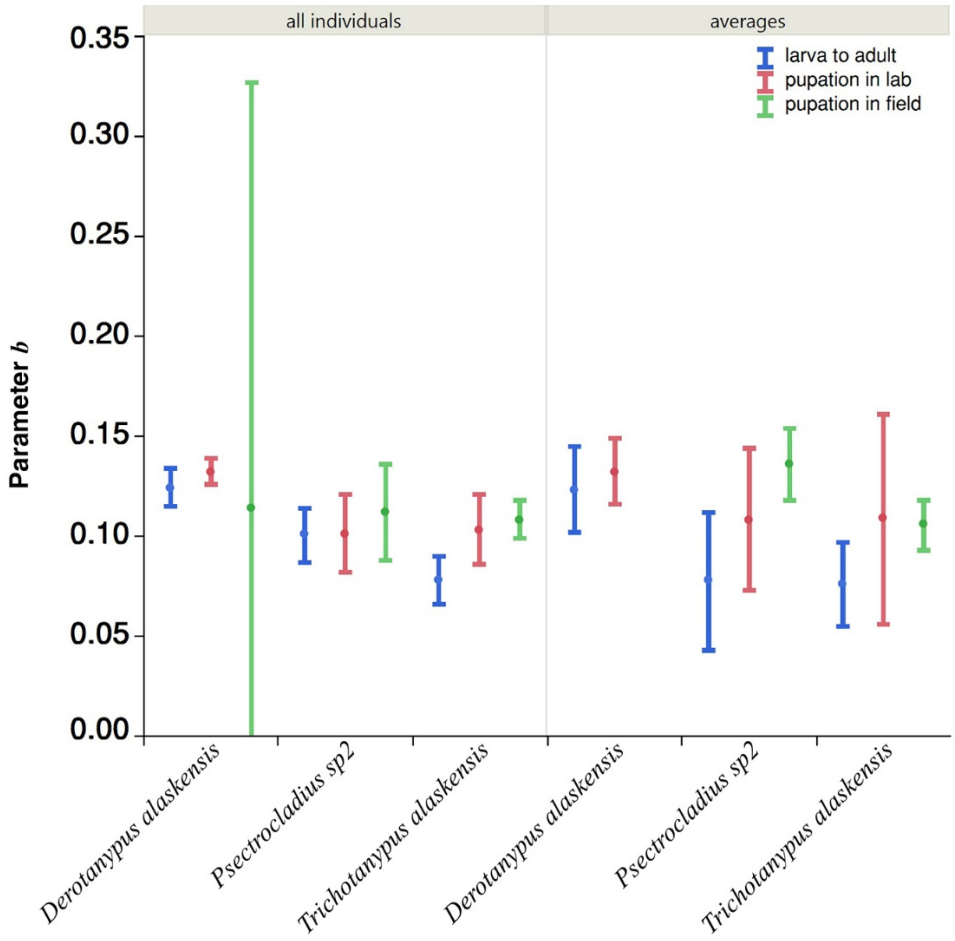

Figure 5.8. Parameter ***b*** coefficients in equation $y = ae^{bx}$ with 95% CI for taxa in which pupation rate response to temperature was measured in both the lab (red) and field (green). Also shown (in blue) are parameters for each species' total pre-emergence development, from overwintering larva to adult eclosion, in the lab rearings. Data on left ("all individuals") correspond to bolded rows in Table 5.3; data on right ("averages") are non-bolded rows in Table 5.3.

We also compared other development rate coefficients such as larvation. We define "larvation" as completion of remaining larval development by overwintering larvae, from pond thaw until pupal formation. Using data from our 2014 field experiments at the IBP site, we compared the temperature response of larvation alone, and the temperature response of total pre-emergence development (larvation plus pupation), for *Psectrocladius* sp2 (N=293) and *Trichotanypus alaskensis* (N=337) (Fig. 5.9A). Comparing the variability in developmental rates,

pupal initiation spanned 12 days for *Psectrocladius* sp2 and 12.5 days for *Trichotanypus alaskensis*, while emergence of the same cohorts of each species was reduced to 10 and 8 days respectively (Fig. 5.9B). This suggests that species become more synchronous during pupation, perhaps due to rising temperatures, concordant with Butler and Braegelman (2018). Nonetheless, the significant negative correlations between developmental rate and mean temperature spanning larval to adult development (Fig. 5.9B), suggest variable amounts of larval development outweigh any thermal responses at these temperatures, even as pond temperatures rise as the season progresses.

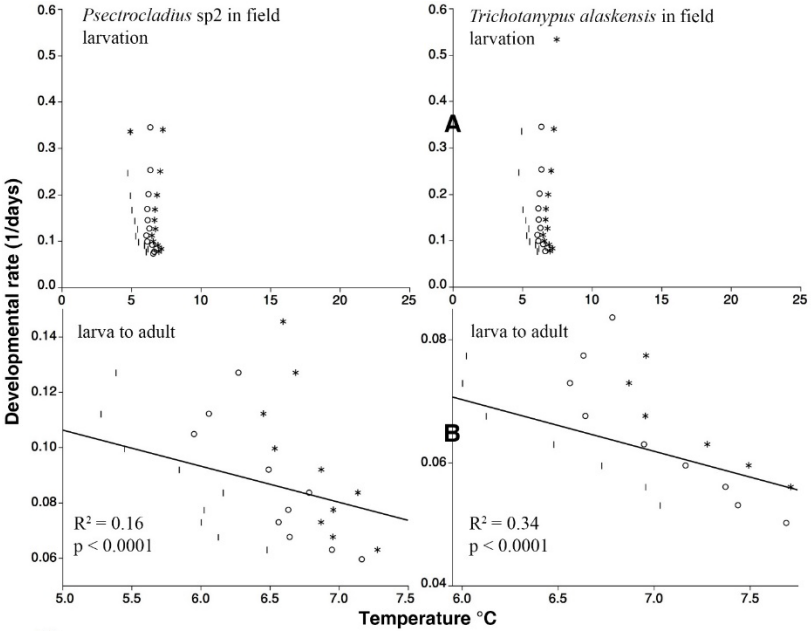

Figure 5.9. Additional development periods for the field-reared *Psectrocladius* sp2 (N=293) and *Trichotanypus alaskensis* (N=337) at IBP.
A) Larval to pupal developmental rate was varied and not correlated with mean temperature. B) Larval to adult developmental rates of the same larvation cohorts (*P*. sp2 N=211, and *T. alaskensis* N=221) do not correlate with temperature as expected, even though developmental rates fall into the expected ranges for these taxa (Fig. 5.5), and pupation exemplified the predicted thermal response (Fig. 5.7). A pond effect was not significant but pond-specific symbols are shown to portray the minimal differences in thermal regimes of tundra ponds. Circles = Pond C, stars = Pd J, vertical dashes = Pd G.

5.4.2. Chironomid Emergence Phenologies

To test for temporal changes in chironomid emergence over a four-decade time span from the 1970s to the 2010s, we compared emergence data from three ponds (J, E, G) sampled at Utqiaġvik in both eras. For all comparisons, we analyzed both numerical abundance and estimated chironomid biomass to test whether abundance can serve as a consistent proxy for chironomid biomass at the community level. In Pond J we found a 10 day shift toward earlier emergence in the more recent era for both emerging chironomid numbers and biomass (Fig. 5.10). We found similar shifts in Pond E, of 10 days for abundance and 13 days for emerging biomass (Fig. 5.11), while Pond G had a 12 day shift in both variables (Fig. 5.12). Clearly, chironomid emergence has, on average, shifted earlier over the past 40 years in these IBP ponds at Utqiaġvik.

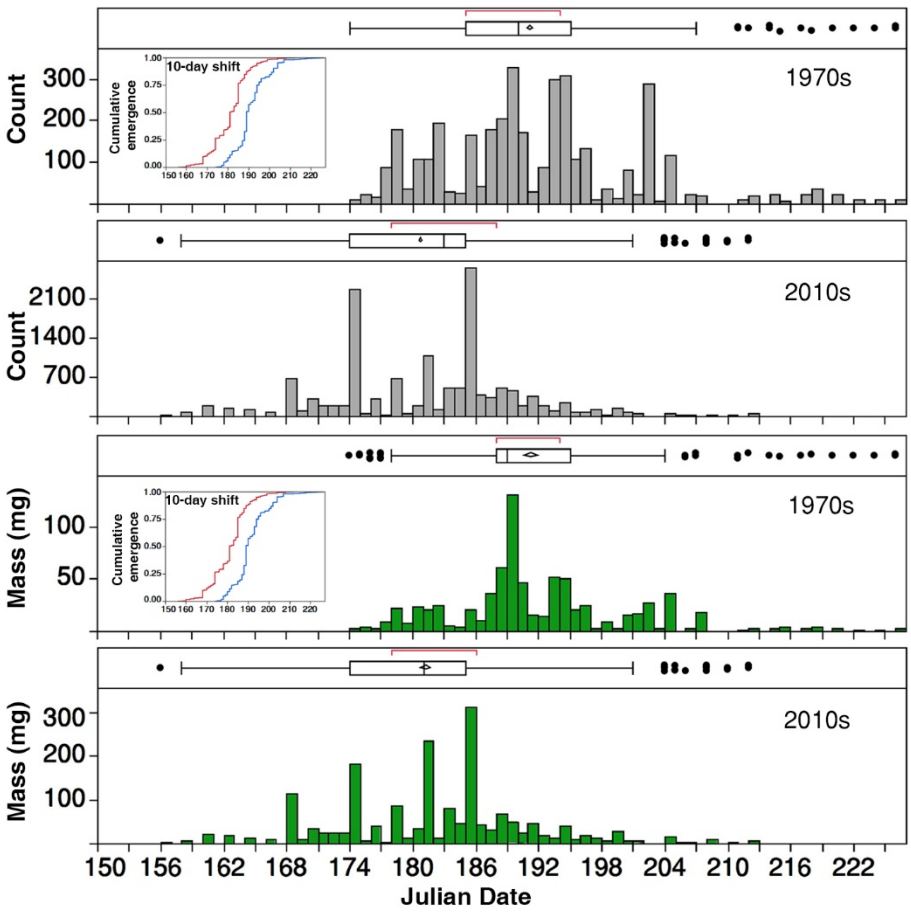

Figure 5.10. Chironomid emergence phenologies from Pond J in two eras.
Histograms show distributions of chironomid abundance (grey) and estimated adult biomass (green) over Julian dates for the 1970s (1972, 1975-1977; N=3,522 specimens) and the 2010s (2009-2011, 2013, 2015-2016; N=16,752 specimens). Insets show cumulative emergence patterns for each variable in the 1970s (blue) vs. the 2010s (red). Average emerging numbers and biomass are shifted 10-11 days earlier in the 2010s relative to the 1970s. Box plots: diamond= mean ± 95% CI, vertical line = median, box = interquartile range, whisker bounds = 1st quartile – 1.5*interquartile range to 3rd quartile + 1.5*interquartile range, bracket = shortest half.

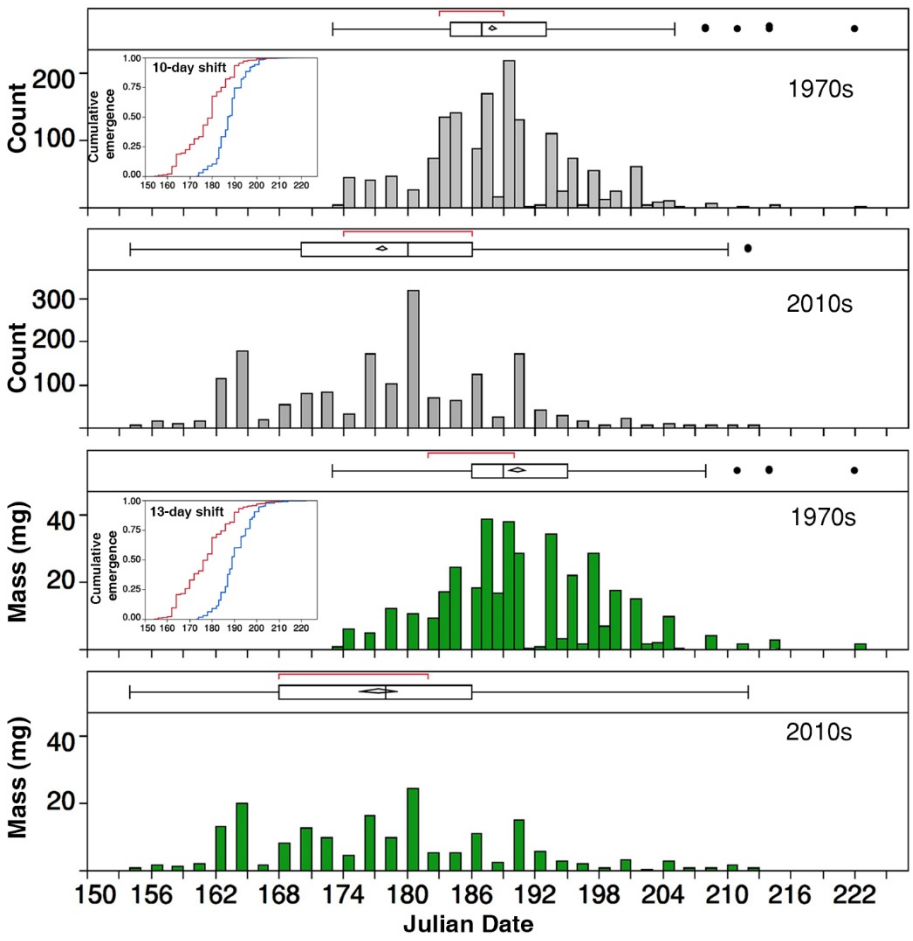

Figure 5.11. Chironomid emergence phenologies from Pond E in two eras.
Chironomid abundance (grey) and predicted adult biomass (green) vs. Julian Date during the 1970s (1975-1977; N=1,495 specimens) and 2010s (2015-2016; N=1,706 specimens). Insets show cumulative emergence patterns for each variable in the 1970s (blue) vs. the 2010s (red). Abundance and biomass phenologies are on average 10-11 and 13-14 days earlier in the 2010s than in the 1970s. Box plots: diamond= mean ± 95% CI, vertical line = median, box = interquartile range, whisker bounds = 1st quartile – 1.5*interquartile range to 3rd quartile + 1.5*interquartile range, bracket = shortest half.

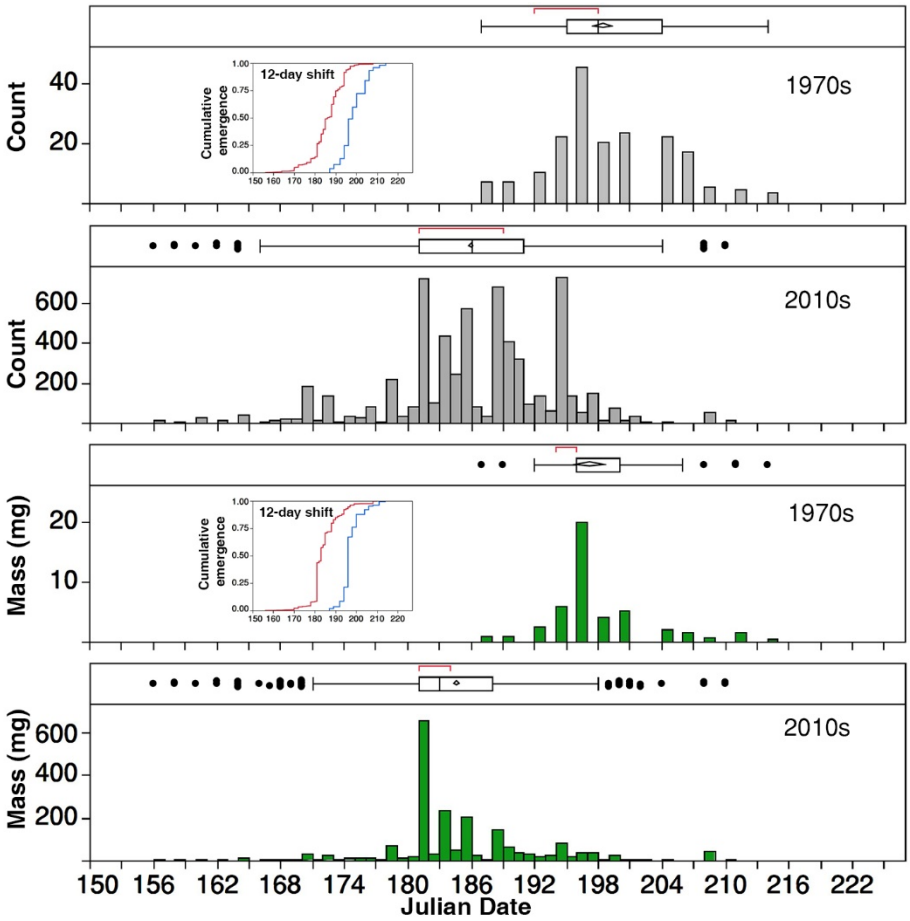

Figure 5.12. Chironomid emergence phenologies from Pond G in two eras. Chironomid abundance (grey) and predicted adult biomass (green) vs. Julian Date during the 1970s (1976; N=185 specimens) and 2010s (2009, 2011, 2013, 2015-2016; N=5,879 specimens). Insets show cumulative emergence patterns for each variable in the 1970s (blue) vs. the 2010s (red). Both abundance and biomass phenologies are on average 12-13 days earlier in the 2010s than in the 1970s. Box plots: diamond= mean ± 95% CI, vertical line = median, box = interquartile range, whisker bounds = 1st quartile – 1.5*interquartile range to 3rd quartile + 1.5*interquartile range, bracket = shortest half.

Since BEO ponds were not sampled in the 1970s, we could not compare that site directly across both eras. We can compare chironomid emergence patterns among era- and site-specific groupings (1970s IBP ponds, 2010s IBP ponds, and 2010s BEO ponds) by combining all ponds, years, and species into three composite datasets (Fig. 5.13). On this scale, we detect an 8-day shift toward earlier emergence, for both abundance and biomass, during the 2010s in the IBP ponds compared to the 1970s. Yet we see no difference between the different sites (IBP vs.

BEO) in the 2010s for either variable. We could have combined all 2010s data (GLM, response variable = Julian Date, site effect = non-significant), but kept the two sites separate for comparison with subsequent figures.

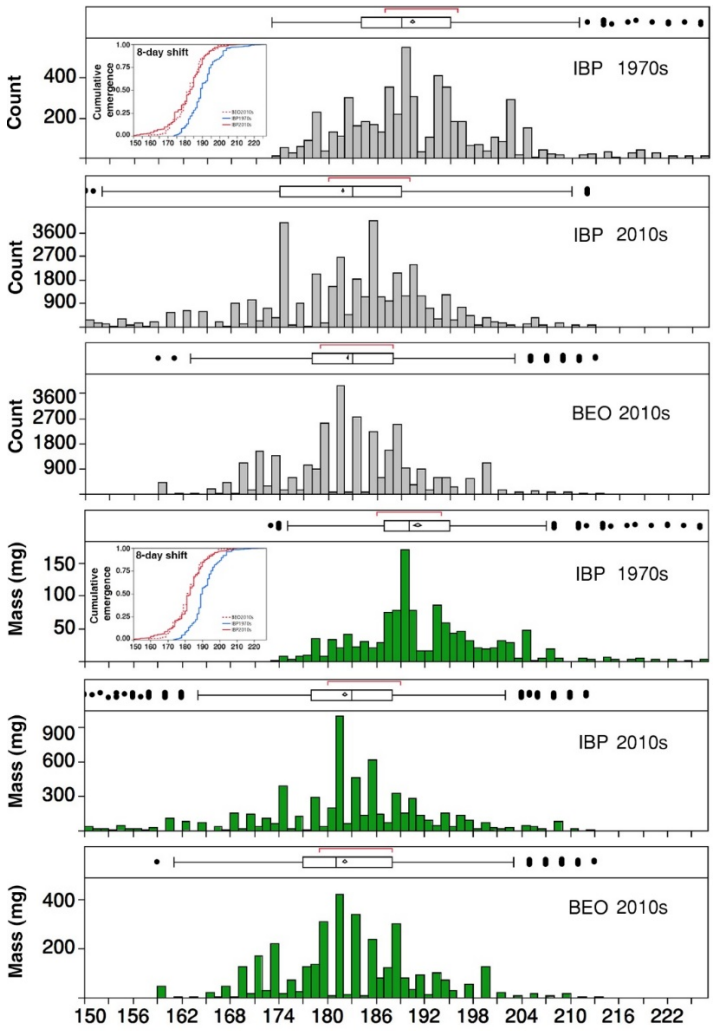

Figure 5.13. Chironomid emergence phenologies grouped by site (IBP or BEO) and era (1970s:1972, 1975-77, or 2010s: 2009-11, 2013, 2015-16).
Chironomid abundance (grey: IBP1970s (Ponds J, E, and G) N=5,256; IBP2010s (Ponds A, C, E, J, G, and Kaleak) N=38,155; and BEO2010s (Ponds OH, Bear, HB, Icy, and Snowfence) N=29,171 specimens) and predicted adult biomass (green) vs. Julian Date. Insets show cumulative emergence patterns for each variable in the 1970s (blue) vs. the 2010s (red). Both abundance and biomass phenologies are on average 8-9 days earlier in the 2010s than in the 1970s. In the 2010s, central tendencies of site-specific phenologies do not differ significantly. Box plots: diamond= mean ± 95% CI, vertical line = median, box = interquartile range, whisker bounds = 1st quartile – 1.5*interquartile range to 3rd quartile + 1.5*interquartile range, bracket = shortest half.

To look for site-specific effects within these community-level results, we analyzed species-specific chironomid phenologies in detail for the 2016 emergence (Figs. 5.14-5.16, Tables 5.5-5.6). We focus on 2016 data because they were the most robust in almost all criteria (Table 5.1). That year we sampled 19 species in sufficient abundance (N>30 specimens). We detected significant site shifts in EM50s for 13 of 19 taxa, only one of which (*Psectrocladius* sp1) was shifted towards earlier emergence at BEO (a 9 day difference). Thus for 12 of 19 taxa, EM50s occurred earlier at IBP, ranging from 6-25 days in magnitude (Table 5.5). For the remaining 6 taxa, there was no significant site effect, and thus data were pooled. In addition to site effects, some taxa exhibited minor pond or sex effects within a given site (or pooled sites). For taxa with significant pond shifts, EM50s typically varied by about 1-6 days around the central, site-specific tendency. For taxa with significant sex effects, protandry was documented at a level of 0.5-3 days depending on the taxon, except for one species in which protogyny is evident (Table 5.5). Nonetheless, only site effects are displayed in Fig. 5.14.

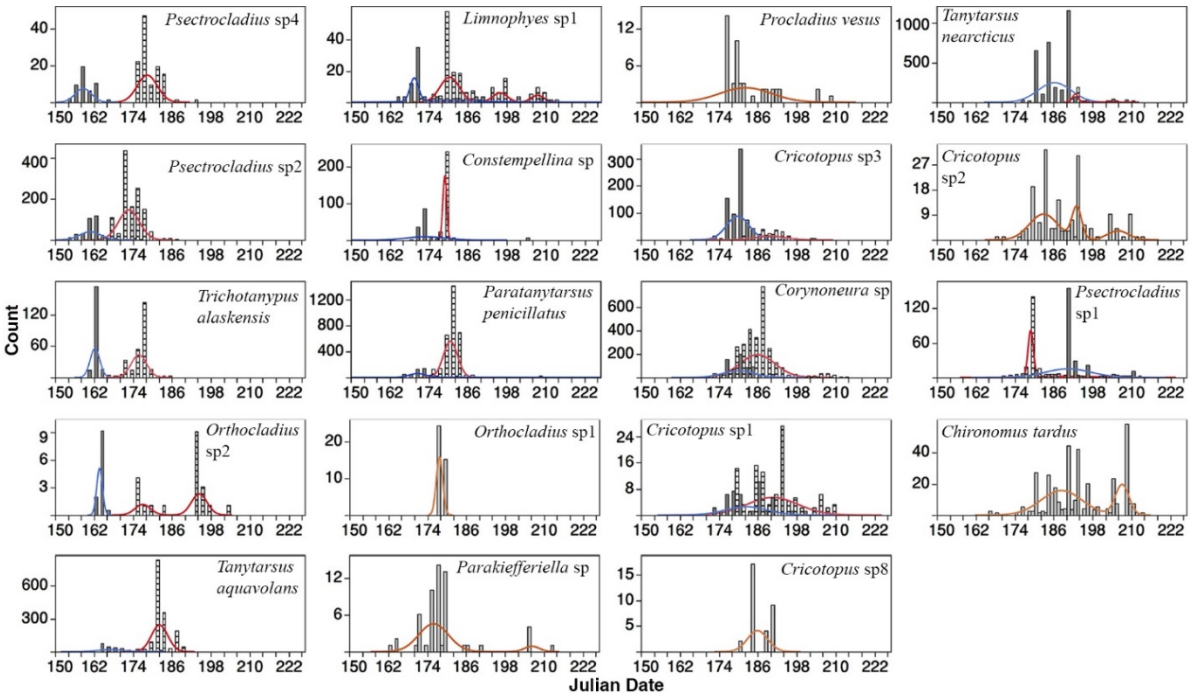

Figure 5.14. Species-specific chironomid emergence phenologies in 2016, comparing IBP Site (Ponds A, C, E, J, G) to BEO Site (Ponds OH, Bear, HB, and Icy) for taxa with N>30 collected specimens (Table 5.5).
Species are listed in phenological order of 50% emergence at IBP (Fig. 5.15) from top to bottom and left to right. If there was not a significant site effect ($p>0.05$), then data were pooled. Normal distribution curves were fit to the data. Model selection (e.g. unimodal vs. multimodal) was executed via AICc, BIC, and parsimony (Methods). Blue curves and dark grey bars = IBP; red curves and hashed bars = BEO; light grey bars and orange curves = sites pooled.

Cumulative emergence curves for these 19 taxa were plotted in Fig. 5.15 which shows that the rank order of species (defined by EM50s) does vary substantially between ponds at IBP versus BEO, but some generalities prevail. Most striking is the difference between sites in the breadth of the emergence season. Emergence at IBP (again defined by species' EM50s) becomes established on Julian Date 158, but not until Julian date 172 at BEO (Table 5.6). As both emergence seasons reach their last EM50 on Julian Date 193, these 19 species show a 5-week emergence span at IBP, but a 40% shorter 3-week span at BEO(). We repeated this analysis with only the top 12 most-abundant taxa (N>73 within a single site), and the same general patterns are evident (Fig. 5.16, Table 5.6).

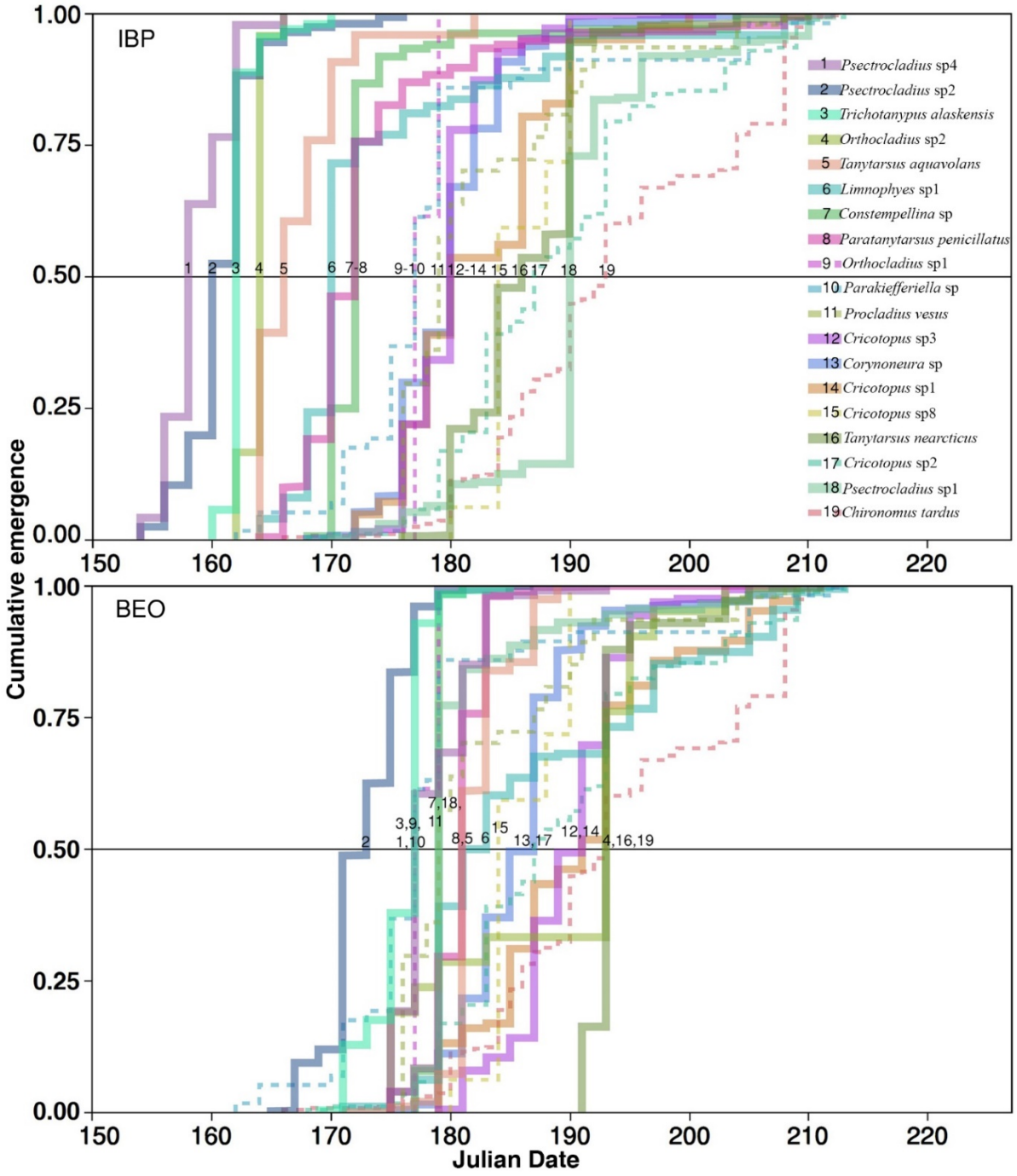

Figure 5.15. Cumulative emergence of 19 common Utqiaġvik chironomid taxa (N>30 in total) in 2016 plotted by site.
Taxa are ranked by order of appearance (EM50s) at IBP. Dashed lines indicate taxa for which a site effect was not significant (p>0.05), and thus all specimens were combined.

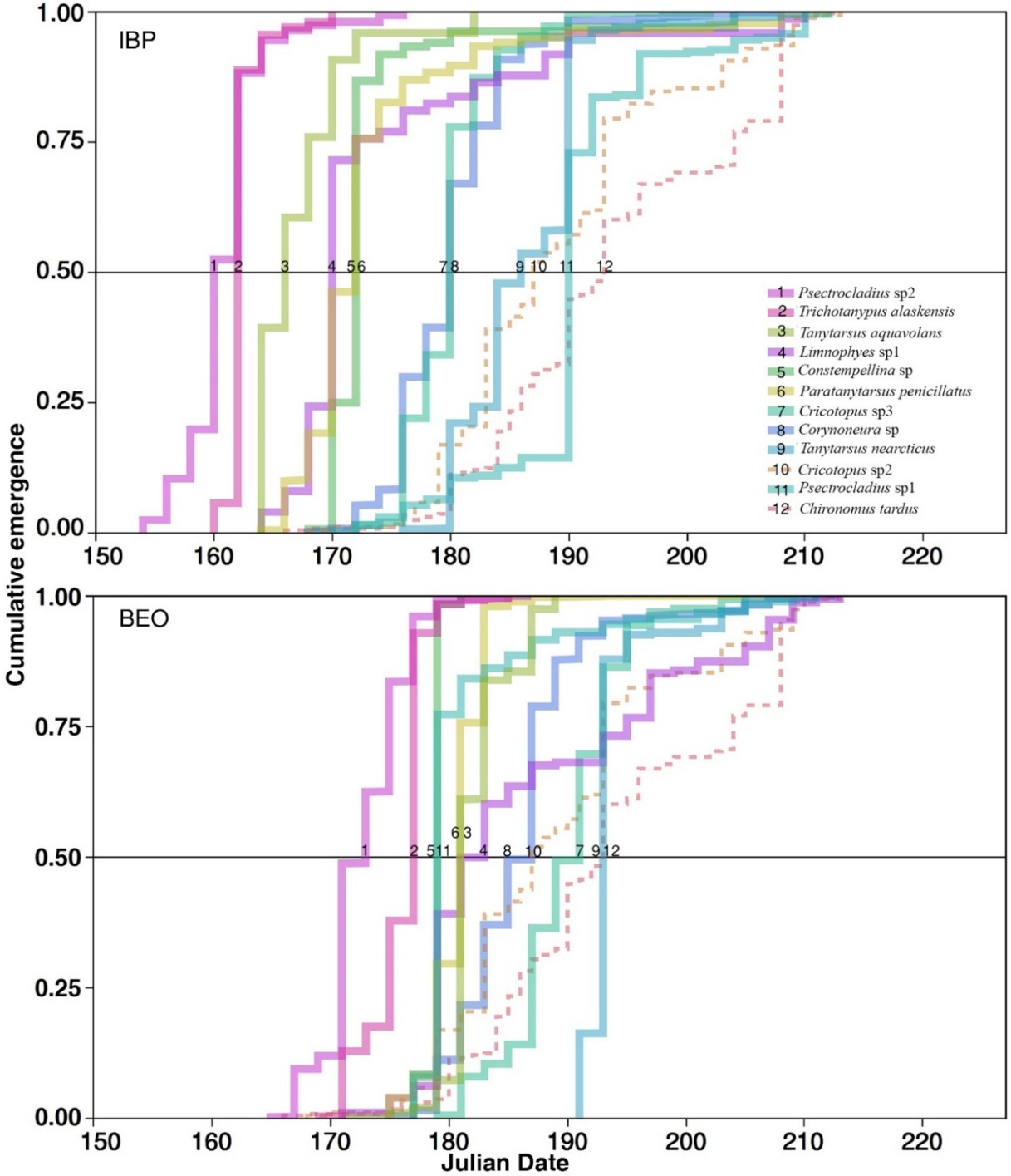

Figure 5.16. Cumulative emergence of the 12 most-abundant Utqiaġvik chironomid taxa (N>73, single site) in 2016 by site (Fig. 5.14; Table 5.5).
Taxa are ranked by order of appearance (EM50s) at IBP. Dashed lines indicate taxa for which a site effect was not significant (p>0.05), and thus all specimens were combined.

Table 5.5. Results of the general linear model - standard least squares analysis of mean date for Utqiaġvik chironomid emergence in 2016 (Figs. 5.14 and 5.15).

Taxon	N (i, b)	Site (Lshift)	Sex (Sshift)	Pond (Pshift)	Notes on sex or pond effect
Ch. tardus	(253, 101)	ns	ns	**(±5)	
Constempellina sp	(136, 264)	**(6)	ns	i*(±2)	
Corynoneura sp	(694, 2660)	**(6)	b**(1.5)	ib**(both ±2)	Protogyny
Cricotopus sp1	(41, 106)	**(8)	ns	b**(±6), i^(±5)	Pds with few data
Cricotopus sp2	(16, 155)	ns	ns	b**(±16)	Pds with few data
Cricotopus sp3	(762, 162)	**(10)	ib**(1.5, 3)	ib**(both ±2)	Protandry
Cricotopus sp8	(32, 0)			ns	All collected PEs female
Limnophyes sp1	(74, 176)	**(13)	ns	ns	
Orthocladius sp1	(0, 39)		ns	ns	
Orthocladius sp2	(24, 21)	**(25)	ns	ns	
Parakiefferiella	(8, 49)	ns	ns	^(±6)	marginal effect
Par. penicillatus	(435, 3063)	**(7)	ib**(3, 0.5)	b**(±2), i*(±2)	Protandry
Procladius vesus	(30, 17)	ns	ns	ns	
Psectrocladius sp1	(263, 203)	**(-9)	ns	i**(±7), b**(±6)	
Psectrocladius sp2	(316, 1176)	**(12)	ib**(2, 2)	i**(±1.5), b**(±3)	Protandry
Psectrocladius sp4	(47, 114)	**(20)	ns	i**(±3), b**(±1.5)	
Tan. aquavolans	(175, 1540)	**(15)	ns	b**(±4), i^*(±2.5)	
Tan. nearcticus	(3162, 257)	**(7)	i**(1)	i**(±1), b**(±5)	Protandry
Tricho. alaskensis	(207, 256)	**(13)	i*^(0.5)	i**(±2), b**(±2.5)	Protandry

For taxa with fewer than 17 specimens from a given pond site (e.g. *Cricotopus* sp2), site effects (if any) were deemed non-significant *a priori* because of small sample size. All shifts in (days). Lshift = shifted later than IBP; i = IBP, b= BEO; **<0.0001, *<0.001,^*<0.01 ^<0.05; ns= not significant.

Table 5.6. Mean date of emergence by taxon and site (Figs. 5.14 and 5.15; Table 5.4).

Taxon	Site	N	Mean Julian Date	Std Error	Lower 95%	Upper 95%
Chironomus tardus	combined	354	193.90	0.30	193.30	194.49
Constempellina sp	BEO	264	178.87	0.28	178.33	179.42
Constempellina sp	IBP	136	172.94	0.49	171.98	173.90
Corynoneura sp	BEO	2660	185.90	0.09	185.73	186.08
Corynoneura sp	IBP	694	180.00	0.22	179.58	180.43
Cricotopus sp1	BEO	106	190.59	0.44	189.73	191.44
Cricotopus sp1	IBP	41	182.39	0.89	180.65	184.13
Cricotopus sp2	combined	171	189.09	0.44	188.24	189.95
Cricotopus sp3	BEO	162	189.78	0.35	189.08	190.47
Cricotopus sp3	IBP	762	179.84	0.21	179.44	180.25
Cricotopus sp8	combined	32	185.94	1.01	183.96	187.91
Limnophyes sp1	BEO	176	186.81	0.34	186.14	187.47
Limnophyes sp1	IBP	74	173.51	0.66	172.22	174.81
Orthocladius sp1	combined	39	177.77	0.91	175.98	179.56
Orthocladius sp2	BEO	21	188.62	0.98	186.69	190.55
Orthocladius sp2	IBP	24	163.75	1.16	161.47	166.03
Parakiefferiella sp	combined	57	178.68	0.75	177.21	180.16
Paratanytarsus penicillatus	BEO	3063	180.74	0.08	180.58	180.89
Paratanytarsus penicillatus	IBP	435	173.07	0.27	172.54	173.61
Procladius vesus	combined	47	182.28	0.83	180.65	183.91
Psectrocladius sp1	BEO	203	181.10	0.32	180.48	181.72
Psectrocladius sp1	IBP	263	190.50	0.35	189.81	191.19
Psectrocladius sp2	BEO	1176	172.79	0.13	172.53	173.05
Psectrocladius sp2	IBP	316	160.84	0.32	160.21	161.47
Psectrocladius sp4	BEO	114	178.46	0.42	177.63	179.28
Psectrocladius sp4	IBP	47	158.72	0.83	157.09	160.35
Tanytarsus aquavolans	BEO	1540	182.24	0.11	182.01	182.46
Tanytarsus aquavolans	IBP	175	167.06	0.43	166.22	167.91
Tanytarsus nearcticus	BEO	257	193.55	0.28	192.99	194.10
Tanytarsus nearcticus	IBP	3162	186.34	0.10	186.14	186.54
Trichotanypus alaskensis	BEO	256	175.82	0.28	175.27	176.37
Trichotanypus alaskensis	IBP	207	162.29	0.40	161.51	163.07

5.5. Discussion

Our findings from lab rearings across a wide thermal range indicate that we cannot assume a uniform developmental rate response to environmental temperature for Chironomidae at Utqiaġvik. Using all reared individuals as data points, two species (*Trichotanypus alaskensis* and *Chironomus tardus*) show significantly reduced developmental response coefficients compared to at least eight other taxa (Fig. 5.6, Table 5.3). The slowest species to mature, *Chironomus tardus*, is smaller in mass than its congener *Chironomus* sp (t-type L), but much

larger in mass than *Paratanytarsus penicillatus*, yet *C. tardus* has a significantly reduced developmental response to temperature compared to each of these other two species (Fig. 5.6). This suggests that the metabolic rate of *C. tardus*, adjusted for mass and temperature, differs significantly from at least one of these taxa, a result in conflict with the Metabolic Theory of Ecology (Brown *et al*. 2004). The fact that species have variable amounts of larval development remaining prior to pupation may confound inferences about metabolic rate. While development of all species ends with adult emergence, different taxa have variable post-thaw starting points. Yet this potential confounding variable seems unlikely, as comparisons of response rate coefficients for different amounts of development within single species (*i.e.* pupation alone vs. all overwintering larval-to-adult development) do not differ significantly across these broad temperature gradients in the lab, yet there is a trend for a higher pupation response rate (Fig. 5.8).

We consistently found significant exponential relationships for a temperature effect on chironomid development rate, from overwintering larva to adult eclosion. Development rates calculated from field-monitored emergence phenologies generally corresponded to these thermal response patterns (Fig. 5.5: top 16 panels). We could not detect a similar statistical relationship for field data alone however, for several possible reasons. First, the range in daily mean pond temperatures was much narrower than in our lab treatments (5-9 °C vs. 5-25 °C). Indeed, across narrow thermal gradients, a linear equation is likely a more suitable model for insect development (Charnov and Gillooly 2003, Kipyatkov and Lopatina 2010), yet we found no significant linear signal in these field data.

Second, nonlinear equations exhibit a mathematical property called Jensen's inequality (Hölder 1889; Jensen 1906), applicable to accelerating or decelerating functions that have variance > 0 (Ruel and Ayres 1999). The exponential equation $f(x) = ae^{bx}$ is an accelerating

function (second derivative is positive) and thus the average result of $f(x)$, $\overline{f(x)}$, will be greater than the result of the average x, $f(\bar{x})$, as long as the variance associated with x is positive. Thus, the lab-derived equation may over-predict actual developmental rates for a given mean temperature.

Third, the constant nature of the mean temperature experience in the lab was fundamentally different from the oscillatory daily thermal patterns in natural tundra ponds (Hobbie 1980, Butler 1980a). Discrepancies could thus arise from the rate summation effect (Worner 1992). The rate summation effect postulates that for nonlinear functions, development is accelerated at low oscillating temperatures, yet diminished at higher temperatures where fluctuations enter the realm of thermal stress, both relative to a constant temperature of the same mean. In our case, this rate summation effect seems to be outweighed by other factors, as field developmental rates (always at relatively low temperatures) were often slightly lower, not higher, than rates estimated for the same species reared under comparable, but relatively constant temperatures in the lab (Fig. 5.5).

A fourth point may be most influential: the developmental starting point differed somewhat between the field and lab chironomids. We used pond thaw as the starting point for pre-emergence development in the field, defined by our pond temperature loggers (McEwen and Butler 2018). That date was usually a few days earlier than the day when we placed newly-collected chironomid larvae into the treatments (the lab starting point). Thus, we should expect the developmental rates in the lab to be slightly elevated, on average. In addition, our calculation of the thermal regime actually experienced by chironomids in the field is likely less precise than this calculation in the lab cultures. Tundra ponds at Utqiaġvik certainly show temperature variations among microhabitats, especially on calm days, among open-water versus vegetated

regions, and at different depths in pond sediments. In contrast, we controlled temperature (±1 °C) in the lab. Although useful, a single logger at the sediment-water interface in each pond cannot accurately depict the thermal experience of every chironomid larva, as different species are well known to vary in their microhabitat preferences, both generally (Armitage *et al*. 1995) and within these tundra ponds (Butler 1980b). Despite these caveats, we find it encouraging that in general, our lab-derived equations correspond to observed field emergence times (EM50s) within a few days.

It is also intriguing, and perhaps encouraging, that we found no significant sex effect in the development response coefficient $\boldsymbol{b}$ in the exponential equation $y = ae^{bx}$. Had a sex effect existed, we might expect that as temperatures increase substantially, sexes would diverge in their emergence timing. In the long term, such divergence would decouple the sexes temporally, disrupting any natural protandry, protogyny, or a sex-unified synchronous emergence within a given species.

Our findings on the thermal dependence of pupal development alone raise confidence in the utility of our approach for predicting chironomid emergence in the field. Although adult emergence timing depends on post-winter development in both larval and pupal stages, the starting point for pupation alone is standardized (*i.e.* the molt from final instar larva to pupa). We could observe the start of pupation (within 1 day) for three species, and found statistically similar exponential relationships for pupal duration of these taxa when reared in both the lab and in the field, even over a limited temperature range in the field (Figs. 5.5, 5.7, Table 5.3). The theoretical caveats mentioned above regarding temperature conditions in lab vs. field still apply. Also, we likely monitored pupation in the lab more precisely. We monitored lab rearing cups

more frequently, often multiple times per day versus only once per day in the field, and visual distinction of newly-molted pupae from pre-pupal larvae was more challenging in the field.

Despite these significant patterns in the field pupation data for mean temperatures ranging from 5-9 °C, the same pattern was not detected for larvation and larva-to-adult development where data were available (IBP) from the same species cohorts (Fig. 5.9). However, the temperature gradient was even smaller for larvation (5-7 °C) and larva-to-adult development (5.5-7.5 °C). All larvae were collected from the same pond on the very date the experiment was initiated (Methods). Fig. 5.9A suggests that there is considerable variability in the amount of larval development left to complete for both species post-thaw. But it is important to consider that these effects may be amplified at these low, albeit natural, temperatures. Thus, Fig. 5.9B possibly suggests that larval-to-adult development is actually little affected by changes in temperature on such a narrow thermal gradient, because the variability in remaining larvation outweighs its effect. It is likely that the later emerging individuals emerged late because they were the ones that were developmentally behind from thaw, and since the season gets warmer as it progresses (McEwen and Butler 2018), the "tardy" individuals' mean temperature experience was slightly elevated.

Indeed, we know that Utqiaġvik has warmed. Mean air temperatures since the 1960s have been increasing at a rate of 0.7ºC decade^{-1} (Hobbie *et al*. 1999). Growing-season pond temperatures have also changed—at a rate of of 0.5ºC decade^{-1} from 1971 to 2012 (McEwen and Butler 2018). Here we provide more evidence that chironomid phenologies are shifting at Utqiaġvik (Braegelman 2016). Using three well-studied ponds from the 1970s and 2010s, broad-sweeping comparisons (Figs. 5.10-5.12) show that the overall pulse of emergence has shifted about 8-12 days earlier in the more recent era. In addition, ponds from another site (BEO) also

follow this trend. At the community level, over multiple years in each era, chironomid phenologies in the 2010s at both sites (BEO and IBP) have shifted earlier by ~8 days compared to IBP in the 1970s. At this broad level, the two sites do not differ in their emergence patterns in the 2010s. Yet, if one looks precisely, there is strong indication that a localized shift is also occurring.

In the most robust PE sample (2016), species-specific phenological comparisons between the two sites suggest IBP emergence typically occurs earlier, is more staggered, and has a slightly different rank order of species than at BEO, even though chironomid emergence at both sites ends at approximately the same time (Figs. 5.14-5.16). This localized effect may be caused largely by increased human activity near the IBP site in the 2010s compared to the 1970s. Although anecdotal and speculative, in the 2010s we have noticed the IBP ponds to thaw slightly earlier than those at the BEO because of road dust lowering the albedo. All roads at Utqiaġvik are gravel, and prevailing winds are from the east. The only inland road runs between the two sites (Fig. 5.1), and its dust is blown primarily toward the IBP, settles on the snow, and accelerates the spring melt relative to the BEO, even though the BEO ponds are physically closer to the road. This localized effect warrants further study.

5.6. Conclusion

We found models of pre-emergence development by chironomids reared under controlled temperatures in the lab to provide a solid basis for predicting emergence under natural conditions. While these exponential equations are useful, one must be cautious about broad generalizations because some species-level differences in thermal response do exist. As confidence limits are broad for many of our coefficients, it is also possible that additional species-specific differences exist, that we could not detect. We document that for two early-

emerging species, developmental synchrony increases in the field as the season warms, when pupal duration is increasingly sensitive to minor increases in pond temperature. We also show that as the arctic climate has warmed since the 1970s, phenologies of tundra pond chironomids in northern Alaska have shifted earlier by about 8-10 days. Site-specific differences in emergence phenology are evident between two study sites on the tundra near Utqiaġvik, with emergence duration for the same chironomid community differing by two weeks. Overall, our results indicate that arctic chironomids are quite sensitive to changes in temperature and climate, with implications for the nesting success of tundra-breeding birds.

5.7. References

Andres, B. A., Johnson, J. A., Brown, S. C., & Lanctot, R. B. (2012). Shorebirds breed in unusually high densities in the Teshekpuk Lake Special Area, Alaska. *Arctic*, 411-420.

Andres, B. A., Smith, P. A., Morrison, R. G., Gratto-Trevor, C. L., Brown, S. C., & Friis, C. A. (2012). Population estimates of North American shorebirds, (2012). *Wader Study Group Bull*, 119(3), 178-194.

Armitage, P, Cranston, PS, & Pinder, LCV, (1995). The Chironomidae: The biology and ecology of non-biting midges. 1. Chapman and Hall, London: 572 pp.

Baek, M. J., Yoon, T. J., & Bae, Y. J. (2012). Development of Glyptotendipes tokunagai (Diptera: Chironomidae) under different temperature conditions. *Environmental entomology*, *41*(4), 950-958.

Bart, J., Brown, S., Andres, B.A., Platte, R., and Manning, A. (2012a). North Slope of Alaska. Chapter 4. In: Bart, J.R., and Johnston, V., eds. Arctic shorebirds in North America: A decade of monitoring. *Studies in Avian Biology,* 44. Berkeley: Cooper Ornithological Society and University of California Press. 37– 96.

Bowden, J. J., Eskildsen, A., Hansen, R. R., Olsen, K., Kurle, C. M., & Høye, T. T. (2015). High-Arctic butterflies become smaller with rising temperatures. *Biology Letters*, *11*(10), 20150574.

Boyd, H., & Madsen, J. (1997). Impacts of global change on Arctic-breeding bird populations and migration. "Global change and Arctic terrestrial ecosystems" (pp. 201-217). Springer, New York, NY.

Braegelman, S. D. (2016). Seasonality of some arctic Alaskan chironomids (Order No. 10147072). Available from Dissertations & Theses @ North Dakota State University; ProQuest Dissertations & Theses Global. (1830464132).

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, *85*(7), 1771-1789.

Butler, M. G. (1980a). Emergence phenologies of some arctic Alaskan Chironomidae. In *Chironomidae* (pp. 307-314).

Butler, M. G. (1980b). Population ecology of some arctic Alaskan Chironomidae (Diptera). Ph.D. thesis, Univ. Michigan. 157p.

Butler, M. G. (1982a). A 7-year life cycle for two Chironomus species in arctic Alaskan tundra ponds (Diptera: Chironomidae). *Canadian Journal of Zoology*, *60*(1), 58-70.

Butler, M. G. (1982b). Production dynamics of some arctic Chironomus larvae. *Limnology and Oceanography*, 27(4), 728-736.

Butler, M. G., & Braegelman, S. D. (2018). Pre-emergence growth and development in the arctic midge Trichotanypus alaskensis Brundin. *Journal of Limnology*.

Burnham, K. P., and Anderson, D. R. (2004). "Multimodel Inference: Understanding AIC and BIC in Model Selection." *Sociological Methods and Research* 33:261–304.

Charnov, E. L., & Gillooly, J. F. (2003). Thermal time: body size, food quality and the 10 C rule. *Evolutionary Ecology Research*, *5*(1), 43-51.

Chen, I. C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, *333*(6045), 1024-1026.

Conklin, J. R., Battley, P. F., Potter, M. A., & Fox, J. W. (2010). Breeding latitude drives individual schedules in a trans-hemispheric migrant bird. *Nature Communications*, 1, 67.

Custer, T. W., & Pitelka, F. A. (1978). Seasonal trends in summer diet of the Lapland Longspur near Utqiaġvik, Alaska. *The Condor*, 80(3), 295-301.

Danks, H. V., & Oliver, D. R. (1972). Seasonal emergence of some high arctic Chironomidae (Diptera). *The Canadian Entomologist*, 104(5), 661-686.

Davis, M. B., & Shaw, R. G. (2001). Range shifts and adaptive responses to Quaternary climate change. *Science*, *292*(5517), 673-679.

Durant, J. M., Hjermann, D. Ø., Ottersen, G., & Stenseth, N. C. (2007). Climate and the match or mismatch between predator requirements and resource availability. *Climate research*, *33*(3), 271-283.

Gillooly, J. F., Charnov, E. L., West, G. B., Savage, V. M., & Brown, J. H. (2002). Effects of size and temperature on developmental time. *Nature*, *417*(6884), 70.

Hobbie, J.E. (1980). Limnology of tundra ponds, Barrow, Alaska. Stroudsburg, PA: Dowden, Hutchinson and Ross.

Hobbie, J. E., Peterson, B. J., Bettez, N., Deegan, L., O'Brien, W. J., Kling, G. W., ... & Hershey, A. E. (1999). Impact of global change on the biogeochemistry and ecology of an Arctic freshwater system. *Polar Research*, 18(2), 207-214.

Hodkinson, I. D., Coulson, S. J., Webb, N. R., Block, W., Strathdee, A. T., Bale, J. S., & Worland, M. R. (1996). Temperature and the biomass of flying midges (Diptera: Chironomidae) in the high Arctic. *Oikos*, 241-248.

Hölder, O. (1889) Ueber einen Mittelwertsatz, *Göttinger Nachr*. 38–47

Holmes, R. T. (1966). Feeding ecology of the red-backed sandpiper (Calidris alpina) in Arctic Alaska. *Ecology*, 47(1), 32-45.

Holmes, R. T., & Pitelka, F. A. (1968). Food overlap among coexisting sandpipers on northern Alaskan tundra. *Systematic Zoology*, 17(3), 305-318.

Høye, T. T., & Forchhammer, M. C. (2008). Phenology of high-arctic arthropods: effects of climate on spatial, seasonal, and inter-annual variation. *Advances in Ecological Research*, 40, 299-324.

Hurlbert, S. H. (1984). Pseudoreplication and the design of ecological field experiments. *Ecological monographs*, 54(2), 187-211.

Intergovernmental Panel on Climate Change (IPCC). Fifth Assessment Report (2014).

Jensen, J. L. W. V. (1906). Sur les fonctions convexes et les inégalités entre les valeurs moyennes. *Acta mathematica*, *30*, 175-193.

Johnson, S. R., & Herter, D. R. (1989). The birds of the Beaufort Sea. Anchorage: British Petroleum Exploration (Alaska).

Johnson, S. R., & Herter, D. R. (1990). Bird migration in the Arctic: a review. In *Bird Migration* (pp. 22-43). Springer, Berlin, Heidelberg.

Kipyatkov, V. E., & Lopatina, E. B. (2010). Intraspecific variation of thermal reaction norms for development in insects: new approaches and prospects. *Entomological Review*, *90*(2), 163-184.

Lackmann, A. R., & Butler, M. G. (2018). Breaking the rule: Five larval instars in the podonomine midge Trichotanypus alaskensis Brundin from Utqiaġvik, Alaska. *Journal of Limnology*.

Lanctot, R. B., Weiser, E. L., Sandercock, B. K., & Brown, S. C. (2015). 2010–2014 Final report: Using a Network of Sites to Evaluate How Climate-mediated Changes in the Arctic Ecosystem are Affecting Shorebird Distribution, Ecology and Demography.

Lougheed, V. L., Butler, M. G., McEwen, D. C., & Hobbie, J. E. (2011). Changes in tundra pond limnology: Re-sampling Alaskan ponds after 40 years. *Ambio*, 40(6), 589-599.

MacLean Jr, S. F., & Pitelka, F. A. (1971). Seasonal patterns of abundance of tundra arthropods near Utqiaġvik. *Arctic*, 19-40.

McCullagh, P., & Nelder, J. A. (1989). Generalized linear models., 2nd edn.(Chapman and Hall: London).

McEwen, D. C., & Butler, M. G. (2018). Growing-Season Temperature Change across Four Decades in an Arctic Tundra Pond. *Arctic*, 71(3), 281-291.

Morrison, R. G., McCaffery, B. J., Gill, R. E., Skagen, S. K., Jones, S. L., Page, G. W., ... & Andres, B. A. (2006). Population estimates of North American shorebirds. Bulletin-Wader Study Group, 111, 67.

Osborne, E., Richter-Menge J., Jeffries, M. O., (2018). Arctic report card 2018.

Pitelka, F. A. (1974). An avifaunal review for the Utqiaġvik region and North Slope of Arctic Alaska. *Arctic and Alpine Research*, 6(2), 161-184.

Ruel, J. J., & Ayres, M. P. (1999). Jensen's inequality predicts effects of environmental variation. *Trends in Ecology & Evolution*, *14*(9), 361-366.

Saalfeld, S. T., McEwen, D. C., Kesler, D. C., et al. (2019). Phenological mismatch in Arctic-breeding shorebirds: Impact of snowmelt and unpredictable weather conditions on food availability and chick growth. *Ecology and Evolution*, 9, 6693–6707.

Tulp, I., & Schekkerman, H. (2008). Has prey availability for arctic birds advanced with climate change? Hindcasting the abundance of tundra arthropods using weather and seasonal variation. *Arctic*, 48-60.

U.S. Census Bureau. Census of Population and Housing (2010).

Visser, M. E., & Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society of London B: Biological Sciences*, *272*(1581), 2561-2569.

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J., Fromentin, J.M., Hoegh-Guldberg, O. and Bairlein, F., (2002). Ecological responses to recent climate change. *Nature*, *416*(6879), p.389.

Wiederholm, T. (1986). Chironomidae of the Holarctic region Part 2. Pupae. *Entomologica Scandinavica Supplement*, 28, 1-482.

Weiser, E. L., Brown, S. C., Lanctot, R. B., Gates, H. R., Abraham, K. F., Bentzen, R. L., ... & English, W. B. (2018). Effects of environmental conditions on reproductive effort and nest success of Arctic-breeding shorebirds. *Ibis*, 160(3), 608-623.

Welch, H. E., Jorgenson, J. K., & Curtis, M. F. (1988). Emergence of Chironomidae (Diptera) in fertilized and natural lakes at Saqvaqjuac, NWT. *Canadian Journal of Fisheries and Aquatic Sciences*, *45*(4), 731-737.

Worner, S. P. (1992). Performance of phenological models under variable temperature regimes: consequences of the Kaufmann or rate summation effect. *Environmental Entomology, 21*(4), 689-699.