SEASONALITY OF SOME ARCTIC ALASKAN CHIRONOMIDS

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Shane Dennis Braegelman

In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Program:
Environmental and Conservation Sciences

December 2015

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Seasonality of Some Arctic Alaskan Chironomids

By

Shane Dennis Braegelman

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

DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Malcolm Butler
Chair

Kendra Greenlee

Jason Harmon

Daniel McEwen

Approved:

June 1 2016
Date

Wendy Reed
Department Chair

ABSTRACT

Arthropods, especially dipteran insects in the family Chironomidae (non-biting midges), are a primary prey resource for many vertebrate species on Alaska's Arctic Coastal Plain. Midge-producing ponds on the ACP are experiencing climate warming that may alter insect seasonal availability. Chironomids display highly synchronous adult emergence, with most populations emerging from a given pond within a 3-5 day span and the bulk of the overall midge community emerging over a 3-4 week period. The podonomid midge *Trichotanypus alaskensis* Brundin is an abundant, univoltine, species in tundra ponds near Barrow, Alaska, with adults appearing early in the annual emergence sequence. To better understand regulation of chironomid emergence phenology, we conducted experiments on pre-emergence development of *T. alaskensis* at different temperatures, and monitored pre-emergence development of this species under field conditions. We compared chironomid community emergence from ponds at Barrow, Alaska in the 1970s with similar data from 2009-2013 to assess changes in emergence phenology. Overwintering larvae of *T. alaskensis* increased in larval size, dry weight, and head capsule size between pond thaw and pupation, indicating substantial larval growth as well as development preceding pupation in the year of emergence. Pupal development showed a consistent degree hour requirement independent of mean daily temperature. We detected a significant advancement of overall midge emergence by about one week in Barrow tundra ponds since the late-1970s. Chironomid midge development clearly is regulated by temperature, but at least some species require substantial feeding and growth during the post-thaw period, raising the possibility of nutritional influences on emergence phenology. Under a warming climate, altered adult emergence timing may result from earlier thaw, warmer temperatures, and possible changes in food availability.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

THP.....Thermal hysteresis proteins

LEA.....Late embryogenesis abundant protein

RCH .....Rapid cold hardening

HSP .....Heat shock protein

PTTH.....Prothoracicotropic hormone

SBASC.....Small pond south of BASC building

DH.....Degree hours

FP .....Development in the field beginning at pupation.

LP.....Development in the lab beginning at pupation.

FT.....Development in the field beginning at thaw.

AbSS .....Absolute spring species

BEO.....Barrow Environmental Observatory land

IBP .....International Biological Program land

CPH.....Cox proportional hazards

HR.....Hazard ratio

1. INTRODUCTION

Insects at high latitudes must be prepared for the long freeze of winter, and short cold snaps during the summer. During the winter, two fundamental strategies are generally recognized: freeze tolerance and freeze avoidance (Duman, 2015). These two strategies are often accomplished with the same cryoprotectants, ice nucleators, and ice-binding factors – but differ in the timing of up/down regulation and the need to either synthesize, or purge, a given compound (Lee, 2010). Freeze-tolerant animals are particularly well represented at higher latitudes, but the vast majority of ectotherms are intolerant of freezing (Chown and Sinclair, 2010). As a general pattern for high latitude species, freeze avoidance seems to be the predominant strategy among aquatic species, whereas most terrestrial species are thought to be freeze tolerant (Lencioni, 2004). During the short arctic summer, many species are capable of rapid cold-hardening as a means to enhance cold tolerance on a short time scale. This process requires only hours to minutes for preparation, and limits cell and tissue damage during sudden cold snaps (Teets and Denlinger, 2013). The physiological mechanisms of low temperature biology, overwintering biology, and rapid cold-hardening are the subjects of this review.

1.1. The Low Temperature Environment

Low temperatures are a considerable constraint on ectotherm life cycles, but the high latent heat properties of water can make aquatic environments particularly advantageous in cool climates. As well as sheltering animals from harsh winds, water serves as a thermal buffer, limiting variation in both daily and seasonal temperatures. An extended season allows insects to exploit aquatic environments later into the year, when air temperatures dip below minimum developmental temperatures for terrestrial species (Lencioni, 2004). Habitats that remain partially unfrozen during winter are often associated with higher densities of macroinvertebrates

than habitats that freeze completely (Irons et al., 1993). Unfrozen habitats may prove favorable because animals living there do not incur physiological costs of preventing internal ice formation, and a greater proportion of nutrients may be allocated to development. Some animals living in habitats that do freeze completely may avoid bodily freezing by dehydrating up to 90% of their normal body weight. Lencioni (2004) reported that larvae of *Diamesa zernyi* removed from solid blocks of ice were physically wrinkled from dehydration, but recovered fully in the lab after 45 minutes in 4°C water. Developmental limitations due to hypoxia may still exist, however.

Ice formation presents a considerable threat because it damages the cell membrane's stability and selective permeability. The resulting thermotropic damage causes a metabolic imbalance, as the cell can no longer control solute concentrations (Lee, 2010). Greater damage may result from the ineffectiveness of ion homeostasis, resulting in disproportionate increases in potassium, while sodium and magnesium levels either remain relatively constant or decrease slightly (Koštál et al., 2007). When concentrations of these ions are altered in the hemolymph there is a reduction of electrochemical potentials across cell membranes (Lee, 2010). Experiments conducted by Koštál and colleagues (2007) provided evidence for using fluctuating thermal regimes rather than constant temperatures when rearing insects. They found increased concentrations of potassium ions in the hemolymph for animals maintained at constant low temperatures, indicating that larvae need a short relief period to maintain a normal ion balance. Also, mechanical damages may result from ice formation, as expanding ice crystals exert physical force on cells. This stress can stretch and tear delicate membranes and vulnerable tissues. Recrystallization, or the reformation of ice after spring thaw, can also severely damage

tissues. At high subzero temperatures, smaller ice crystals may shrink as larger crystals continue to grow, increasing pressure at points where it was already greatest (Lee, 2010).

Occasionally, damages caused by cold exposure can be repaired. If a brief period of warming occurs, an organism may be able to restore ion gradients (Koštál et al., 2007), up-regulate metabolic proteins (e.g., for glycolysis and synthesis and conversion of ATP molecules) and protein chaperones (e.g., heat shock proteins), or repair various cytoskeletal components (Colinet et al., 2007). Short warming periods may also facilitate the removal of diapause-accumulated biotoxins, and allow the organism to recharge its depleted energy reserves (Andersen et al., 2015; Lee, 2010).

1.2. Seasonal Cold Hardening

1.2.1. Freeze Intolerance

Freeze intolerant (or freeze avoidant) species exhibit the dominant strategy for aquatic insects (Chown and Sinclair, 2010; Irons et al., 1993), and this strategy is viable in environments with temperatures as low as -60°C (Danks, 2004). As temperature approaches 0°C, ice does not immediately form in the body fluid as ions, sugars, amino acids, and proteins colligatively depress the freezing point of hemolymph by 1.86°C per osmole of solute (Lee, 2010). Many insects naturally freeze at about -10 °C, but can readily supercool down to -35°C (Lencioni, 2004). Depressing the freezing point of internal fluids can be done by removing ice nucleators, dehydrating cells, or producing antifreeze proteins and other cryoprotectants (Duman, 2015). Often some combination of these adaptations act in concert (Walters et al., 2009). Species experiencing extreme temperatures must be able to control internal solute concentrations to avoid ice formation. Additionally, the ability to supercool is often inversely related to water volume, with larger animals unable to supercool to the same extent as smaller animals (Lee, 2010).

Supercooling ability varies substantially across terrestrial and aquatic insects, and freezing may occur at -2°C, or, in extreme cases, fluids may remain unfrozen at more than -100°C (Duman et al., 2010). Insects must first dehydrate cells in preparation for winter, and then begin converting glycogen to various carbohydrate cryoprotectants. Common cryoprotectants are alcohols (glycol, sorbitol, mannitol, ribitol, erythritol, threitol, ethylene), sugars (trehalose and glucose), and amino acids (proline and alanine) (Lee, 2010). Cryoprotectants depress the freezing point of body fluids, as well as stabilizing various enzymes and cell membranes (Klowden, 2007). These molecules can represent a considerable proportion of body mass, reaching concentrations of 25% total dry mass of the organism (Klowden, 2007) and are often small, stable molecules that are non-toxic in high concentrations. As ice increases in the hemolymph the solutes continually concentrate, which further depresses the freezing point and reduces the absolute amount of freezing during the coldest part of winter. Insects appear to survive freezing 64-66% of their body water (Lee, 1991). When solutes become increasingly freeze-concentrated, glycerol may serve as a solvent. This reduces osmotic stresses because of glycerol's ability to readily cross the cell membrane (Lee, 2010). Cryoprotectants also represent a reserve of amino acids, carbon and water, readily available after spring thaw. In some instances, the freeze-concentration of solutes may cause harm to the organism. This type of injury remains poorly studied mechanistically, but damages apparently occur to the cell membrane, leading to cytolysis (Lee, 2010).

Antifreeze proteins may be used to bind to ice crystals, preventing further crystal growth. Termed 'thermal hysteresis proteins' (THPs), these high molecular mass molecules occur in ectothermic animals as well as in plants (Duman, 2015). These proteins lower the freezing point of water, but have no effect on the melting point, and the subsequent difference between the

normal freezing point and the THPs depression of freezing point is the hysteresis. THPs are found in a variety of insects, and are synthesized by both freeze-tolerant and intolerant species. These proteins circulate through the hemolymph and bind to ice nucleators that would otherwise seed ice formation (Klowden, 2007). THPs appear essential to the overwintering biology of many insects, but also play a role during the active season. In temperate environments that freeze and thaw during the winter, THPs can prevent ice formation when water temperature drops (Duman, 2015). In Arctic and alpine environments that undergo freeze thaw cycles during the summer, THPs prevent ice formation and recrystallization when an animal has already metabolized its winter cryoprotectants.

1.2.2. Freeze Tolerance

A second strategy for seasonal cold-hardening is to allow the freezing of extracellular body fluids, while keeping cells and tissues healthy. Many species living at high latitudes have evolved ways to tolerate freezing, and this strategy appears to be more prevalent in the Arctic than in the Antarctic (Chown and Sinclair, 2010; Klowden, 2007). The degree of freeze tolerance varies considerably, and often a substantial nutrient allocation (as high as 25% of dry mass) must be made to prepare for winter (Danks, 1971). Freeze-tolerant species typically freeze at high subzero temperatures, after preparation by dehydrating cells, converting blood sugars and proteins to cryopreservation compounds, degrading mitochondria to limit metabolism, and remodeling cell membrane lipids to protect against the cold (Clark and Worland, 2008).

Freezing at high sub-zero temperatures is crucial for freeze-tolerant species as it significantly slows ice crystal formation. To assure that body fluids will begin to freeze at high sub-zero temperatures, many species synthesize their own ice nucleator compounds; the most efficient of which will determine the freezing point of the body fluid (Clark and Worland, 2008).

Once ice formation has been initiated, the heat of crystallization will raise the body temperature several degrees before temperature begins to decline (Lee, 1989). This rise in temperature assures that ice doesn't begin to form in other parts of the body, allowing ice-binding factors to better influence ice formation. In species that do not synthesize nucleating proteins, ice nucleation may occur on food particles or crystalloid compounds in the Malpighian tubules. When two bacteria species were fed to the freeze intolerant Colorado potato beetle, *Leptinotarsa decemlineata*, both bacteria triggered ice nucleation at high subzero temperatures, significantly reducing the survival of adult beetles relative to an unfed control group (Castrillo et al., 2001). Crystalloid compounds, such as ammonium, calcium carbonate, uric acid, and potassium phosphate, may also seed ice formation (Mugnano et al., 1996). The gall fly *Eurosta solidaginis* was found to have large crystalloid spheres within the Malpighian tubules of overwintering larvae (Mugnano et al., 1996). These structures may serve to nucleate ice in freeze tolerant animals that are unable to synthesize the more efficient protein nucleators.

Other proteins, like aquaporins, neither interact with the surface layer of ice nor lower the freezing point of body fluids. Rather, they assist appropriate freezing by helping cells regulate water volume between intra- and inter-cellular spaces (Teets and Denlinger, 2013). Because the rate of water flow across cell membranes is considerably faster when transported by aquaporins rather than by diffusion alone, water can flow much more readily in and out of cells, which prevents the accumulation of toxins inside cells. Blocking aquaporin sites with mercuric chloride significantly reduced survival of fat body and midgut cells in the gall fly *Eurosta solidaginis* (Philip et al., 2008), indicating the importance of aquaporins in keeping cells healthy when temperatures get cold. Many animals also accumulate trehalose, which stabilizes membranes by interacting with the phosphate of phospholipids (Clark and Worland, 2008). This extreme type of

cold tolerance may play a role in the extremely long life cycle of the arctic lymantriid moth *Gynaephora groenlandica*, which requires 3-4 years to complete development through instars III-VI (Kevan and Kukal, 1993).

A final type of freeze tolerance combines severe dehydration with completely suspended development. Severe dehydration, or anhydrobiosis, has been reported in the arctic moth *G. groenlandica* (Kevan and Kukal, 1993), the African sleeping midge *Polypedilum vanderplanki* (Cornette et al., 2010), some other small insects, and collembolans (Danks, 2006). Anhydrobiosis allows animals to tolerate extremely low temperatures, even immersion in liquid nitrogen (Danks, 2006). This is accomplished by degrading up to half of the mitochondria in preparation for desiccation, further limiting the accumulation of toxic metabolic end products (Convey, 2010). Mitochondrial degradation typically occurs over a prolonged period (>2 months in *Gynaephora grownlandica*), but reconstituting mitochondria the following spring is a considerably faster process, which can be completed in <1 week (Kukal et al., 1989). This quick restoration is accomplished with stable RNAs that are stored in fat body cells overwinter, which can readily produce mitochondrial proteins when temperatures rise (Levin et al., 2003). Other desiccation-associated proteins, such as dehydrins, were first discovered in plants, and have only recently been discovered in insects (Kikawada et al., 2006). Dehydrins are termed late embryogenesis abundant (LEA) proteins, which are highly hydrophilic; they are found in chironomids, such as *P. vanderplanki*, that are able to tolerate almost complete desiccation (Kikawada et al., 2006). LEA proteins are unstructured in water, assuming their native conformation upon drying (Hand et al., 2011). LEA-like proteins have now been identified in other animal groups, including brine shrimp (Menze et al., 2009), the arctic springtail *Onychiurus arcticus* (Clark et al., 2007), and nematodes (Gal et al., 2004). LEA proteins are

likely utilized by many other desiccation-tolerant animal groups, but little research has yet been conducted.

1.2.3. Rapid Cold Hardening

Rapidly responding to thermal changes in one's environment can be crucial for survival, especially given the great short-term thermal variability seen during summer at high latitudes and altitudes. Exposure to temperatures as much as 5°C above an insect's supercooling point may be low enough to cause damaging cold-shock or direct chilling injury (Lee and Denlinger, 2010). Rapid cold hardening (RCH) is one strategy for surviving such thermal fluctuations. When given a short period for preparation, RCH helps animals survive low temperature exposures close to their lower lethal limit. RCH stands in contrast to winter hardening by the amount of time required for preparation. Winter hardening may involve weeks to months of preparation, whereas rapid cold hardening can be accomplished in hours to minutes (Lee and Denlinger, 2010).

Rapid cold hardening may be accomplished by a number of different mechanisms, and is likely the result of increased levels of protective sugars, amino acids, heat-shock proteins, alteration of cell membranes, or some combination of the above (Lee and Denlinger, 2010). Glycerol and sorbitol are common polyols often associated with RCH responses. Sorbitol is unique in this instance though, as it decreases prior to diapause, but increases in preparation for RCH (Sim and Denlinger, 2013). Other sugars, like the dominant insect blood sugar trehalose, appear to respond differently depending on species. Trehalose levels in Drosophila melanogaster and *Heterorhabditis bacteriophora* increased during RCH, but decreased in *Sarcophaga crassipalpis* (Lee and Denlinger, 2010). Enzymes associated with trehalose levels also appear to fluctuate in a cold-shock environment. Jagadale et al. (2005) noted a 6 fold increase in trehalose after exposure to a cold-shock environment, as well as an increase in trehalose-6-phosphate

synthase, an enzyme involved with the biosynthesis of trehalose. Additionally, there was a decrease in the trehalose degrading enzyme, trehalase, which was restored to normal levels within 3 hours of returning to culturing temperature (Jagdale et al., 2005). Alanine, an amino acid with colligative properties similar to glycerol, is also associated to RCH. After exposure to low temperature, heat shock protein (HSP) synthesis increases to facilitate the recovery process (Stetina et al., 2015).

Much like winter cold-hardening, altered composition of the cell membrane has also been reported as a response to RCH. Phospholipid fatty acids (e.g. oleic and linoleic acid) have been reported to increase in response to RCH. Increases in both fatty and linoleic acids have been reported for D. melanogaster (Overgaard et al., 2005) and *S. crassipalpis* (Michaud and Denlinger, 2006). *S. crassipalpis* is considered much more cold-hardy species, and linoleic acid concentrations increased an order of magnitude (Lee and Denlinger, 2010). Increasing unsaturated fatty acid concentrations allows the insect to quickly adjust the fluidity of cell membranes at low temperatures (Lee and Denlinger, 2010). Additionally, short-chain fatty acids (which have a lower melting point than long-chain fatty acids) have been reported to increase in response to RCH (Michaud and Denlinger, 2006). These membrane changes allow a cell to maintain a liquid-crystalline state at low temperatures, a point that would otherwise cause the membrane to enter a gel state and lose its ability to regulate solutes (Michaud and Denlinger, 2006). Although it remains poorly understood, changes have also been noted in the protein structure of cells in response to RCH. In addition to lipid remodeling, Michaud and Denlinger (2006) reported an up-regulation of 36 protein transcripts in the adult cells of *D. melanogaster* following 2 hours of low temperature exposure. It is unknown if these proteins increased in

response to RCH, or if they were synthesized predominantly while recovering from low temperature exposure.

Rapid cold-hardening can be crucial for survival. For example, *Belgica antarctica* nearly doubled its survival at -15 °C and had ~75% survival at -20 °C when exposed to otherwise lethal temperatures after a short cold stimulus (Lee et al., 2006). Nonetheless, RCH has some physiological cost. Losses in fecundity have been reported in the house fly *Musca domestica*, the flesh fly *Sarcophaga crassipalpis*, and the mite *Euseius finlandicus* after exhibiting the RCH response (Lee and Denlinger, 2010). Additionally, the effects of RCH may be lost as quickly as they were gained. House flies and some migratory locust nymphs lost their RCH protection within 2 hours, western flower thrips within 1 hour, and the olive fruit fly lost RCH protection within 15 minutes of a return to warm temperatures (Lee and Denlinger, 2010).

1.2.4. Summary

Periods of extreme low temperature exposure have required non-migratory ectotherms to either adapt or be extirpated from cold regions. Intra-cellular ice formation is an ever present danger for many of these animals, and for many species cryoprotectants have become a fundamental adaptation. By either synthesizing their own cryoprotectants, or acquiring them from their environment, animals ensure that their tissues will remain relatively stable and minimal damage will occur. Most of these cryoprotectants are synthesized at low physiological cost, but more substantial protection may require antifreeze protein concentrations of 10% dry mass (Walters et al., 2009), and glycerol concentrations of 25% dry mass (Lee, 2010). Additionally, rapid cold-hardening is a newly developing field of study. This response allows organisms to tolerate continued freeze-thaw cycles while preventing recrystallization. Thermal hysteresis proteins (THPs) are immensely beneficial in this regard; they are not nearly as

efficient as low molecular mass solutes at extremely cold temperatures, but THPs can be synthesized during the warm season, allowing animals to undergo rapid development at a time when they are otherwise unprotected.

The ability to avoid freezing in an environment surrounded in ice remains poorly studied, and the advantage of clearing ice nucleators and creating a barrier between the organism and the surrounding ice if the animal must remain freeze tolerant has yet to receive much attention (Danks, 2007). Many species of chironomids are thought to be freeze tolerant when they overwinter in salivary-silk cocoons, as aquatic insects are not thought to be able to supercool in frozen habitats (Danks et al., 1994). In preparation for winter, the animal will purge the gut, then fold mid abdomen and completely seal both ends of the cocoon, clearing all water between the body wall and the cocoon casing (Danks, 1971). This allows the animal to increase inner temperature to slightly above ambient. Evacuating the gut prevents the contents from nucleating ice at an unpredictable temperature, as many freeze tolerant animals have selected for ice nucleators that freeze at highly specific temperature (Frisbie and Lee, 1997). These are adaptations indicative of freeze avoidance, but many aquatic overwintering animals are thought to freeze along with their habitat (Lencioni, 2004), so perhaps these adaptations serve strictly to stabilize conditions.

Determining the physiological investment of seasonally cold-hardening animals at multiple stages of their life cycle may help biologists better identify how such efficient cryopreservation is accomplished, and how these strategies may be implemented for cold preservation of mammalian tissues. Expanding cryoprotectants to an industrial scale may prove beneficial to future generations as the amount of time tissues (animal and plant) may be cryopreserved increases for both medical and agricultural purposes.

1.3. References

Andersen, J.L., Manenti, T., Sorensen, J.G., Macmillan, H.A., Loeschcke, V., Overgaard, J., 2015. How to assess Drosophila cold tolerance: Chill coma temperature and lower lethal temperature are the best predictors of cold distribution limits. Funct. Ecol. 29, 55–65. doi:10.1111/1365-2435.12310

Castrillo, L., Lee, R.E., Wyman, J., Lee, M., Rutherford, S., 2001. Field persistence of ice-nucleating bacteria in overwintering Colorado potato beetles. Biol. Control 21, 11–18.

Chown, S.L., Sinclair, B.J., 2010. The macrophysiology of insect cold-hardiness, in: Denlinger, D.L. (Ed.), Low Temperature Biology of Insects. Cambridge University Press, Cambridge, NY, pp. 191–222.

Clark, M.S., Thorne, M.A., Purać, J., Grubor-Lajsić, G., Kube, M., Reinhardt, R., Worland, M.R., 2007. Surviving extreme polar winters by desiccation: clues from Arctic springtail (*Onychiurus arcticus*) EST libraries. BMC Genomics 8, 1–12. doi:10.1186/1471-2164-8-475

Clark, M.S., Worland, M.R., 2008. How insects survive the cold: molecular mechanisms-a review. J. Comp. Physiol. B 178, 917–33. doi:10.1007/s00360-008-0286-4

Colinet, H., Nguyen, T.T.A., Cloutier, C., Michaud, D., Hance, T., 2007. Proteomic profiling of a parasitic wasp exposed to constant and fluctuating cold exposure. Insect Biochem. Mol. Biol. 37, 1177–88. doi:10.1016/j.ibmb.2007.07.004

Convey, P., 2010. Life-history adaptations to polar and alpine environments, in: Denlinger, D.L. (Ed.), Low Temperature Biology of Insects. Cambridge University Press, Cambridge, NY, pp. 297–321.

Cornette, R., Kanamori, Y., Watanabe, M., Nakahara, Y., Gusev, O., Mitsumasu, K., Kadono-Okuda, K., Shimomura, M., Mita, K., Kikawada, T., Okuda, T., 2010. Identification of anhydrobiosis-related genes from an expressed sequence tag database in the cryptobiotic midge *Polypedilum vanderplanki* (Diptera; Chironomidae). J. Biol. Chem. 285, 35889–99. doi:10.1074/jbc.M110.150623

Danks, H.V., 2007. How aquatic insects live in cold climates. Can. Entomol. 139, 443–471. doi:10.4039/N06-100

Danks, H.V., 2006. Insect adaptations to cold and changing environments. Can. Entomol. 138, 1–23.

Danks, H. V., 2004. Seasonal Adaptations in Arctic Insects. Integr. Comp. Biol. 44, 85–94. doi:10.1093/icb/44.2.85

Danks, H. V., 1971. Overwintering of some north temperate and Arctic Chironomidae: II. Chironomid biology. Can. Entomol. 103, 1875–1910.

Danks, H. V., Kukal, O., Ring, R., 1994. Insect cold-hardiness: insights from the Arctic. Arctic 47, 391–404.

Duman, J.G., 2015. Animal ice-binding (antifreeze) proteins and glycolipids: an overview with emphasis on physiological function. J. Exp. Biol. 218, 1846–1855. doi:10.1242/jeb.116905

Duman, J.G., Walters, K.R., Sformo, T., Carrasco, M.A., Nickell, P.K., Lin, X., Barnes, B.M., 2010. Antifreeze and ice-nucleator proteins, in: Denlinger, D.L., Lee, R.E. (Eds.), Low Temperature Biology of Insects. Cambridge University Press, pp. 59–90.

Frisbie, M., Lee, R.E., 1997. Inoculative freezing and the problem of winter survival for freshwater macroinvertebrates. J. North Am. Benthol. Soc. 16, 635–650.

Gal, T.Z., Glazer, I., Koltai, H., 2004. An LEA group 3 family member is involved in survival of *C. elegans* during exposure to stress. FEBS Lett. 577, 21–6. doi:10.1016/j.febslet.2004.09.049

Hand, S.C., Menze, M. a, Toner, M., Boswell, L., Moore, D., 2011. LEA proteins during water stress: not just for plants anymore. Annu. Rev. Physiol. 73, 115–134. doi:10.1146/annurev-physiol-012110-142203

Irons, J.G.I., Miller, K.L., Oswood, M.W., 1993. Ecological adaptations of aquatic macroinvertebrates to overwintering in interior Alaska (U.S.A.) subarctic streams. Can. J. Zool. 71, 98–108.

Jagdale, G.B., Grewal, P.S., Salminen, S.O., 2005. Both heat-shock and cold-shock influence trehalose metabolism in an entomopathogenic nematode. J. Parasitol. 91, 988–994.

Kevan, P.G., Kukal, O., 1993. Corrigendum: A balanced life table for *Gynaephora groenlandica* (Lepidoptera: Lymantriidae), a long-lived high arctic insect, and implications for the stability of its populations. Can. J. Zool. 71, 1699–1701.

Kikawada, T., Nakahara, Y., Kanamori, Y., Iwata, K., Watanabe, M., McGee, B., Tunnacliffe, A., Okuda, T., 2006. Dehydration-induced expression of LEA proteins in an anhydrobiotic chironomid. Biochem. Biophys. Res. Commun. 348, 56–61. doi:10.1016/j.bbrc.2006.07.003

Klowden, M.J., 2007. Physiological Systems in Insects, Second Edi. ed. Academic Press.

Koštál, V., Renault, D., Mehrabianová, A., Bastl, J., 2007. Insect cold tolerance and repair of chill-injury at fluctuating thermal regimes: role of ion homeostasis. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 147, 231–8. doi:10.1016/j.cbpa.2006.12.033

Kukal, O., Duman, J.G., Serianni, A.S., 1989. Cold-induced mitochondrial degradation and cryoprotectant synthesis in freeze-tolerant caterpillars. J. Comp. Physiol. B. 158, 661–671.

Lee, R.E., 2010. A primer on insect cold-tolerance, in: Denlinger, D.L. (Ed.), Low Temperature Biology of Insects. Cambridge University Press, Cambridge, NY, pp. 3–34.

Lee, R.E., 1991. Principles of insect low temperature tolerance, in: Lee, R.E., Denlinger, D.L. (Eds.), Insects at Low Temperature. Chapman and Hall, New York and London, pp. 17–46.

Lee, R.E., 1989. Insect cold-hardiness: To freeze or not to freeze. Bioscience 39, 308–313.

Lee, R.E., Damodaran, K., Yi, S.-X., Lorigan, G. a, 2006. Rapid cold-hardening increases membrane fluidity and cold tolerance of insect cells. Cryobiology 52, 459–63. doi:10.1016/j.cryobiol.2006.03.003

Lee, R.E., Denlinger, D.L., 2010. Rapid cold-hardening: Ecological significance and underpinning mechanisms, in: Denlinger, D.L., Lee, R.E. (Eds.), Low Temperature Biology of Insects. Cambridge University Press, pp. 35–58.

Lencioni, V., 2004. Survival strategies of freshwater insects in cold environments. J. Limnol. 63, 45–55.

Levin, D.B., Danks, H. V., Barber, S.A., 2003. Variations in mitochondrial DNA and gene transcription in freezing-tolerant larvae of *Eurosta solidaginis* (Diptera: Tephritidae) and *Gynaephora groenlandica* (Lepidoptera: Lymantriidae). Insect Mol. Biol. 12, 281–9.

Menze, M. a, Boswell, L., Toner, M., Hand, S.C., 2009. Occurrence of mitochondria-targeted Late Embryogenesis Abundant (LEA) gene in animals increases organelle resistance to water stress. J. Biol. Chem. 284, 10714–9. doi:10.1074/jbc.C900001200

Michaud, M.R., Denlinger, D.L., 2006. Oleic acid is elevated in cell membranes during rapid cold-hardening and pupal diapause in the flesh fly, *Sarcophaga crassipalpis*. J. Insect Physiol. 52, 1073–82. doi:10.1016/j.jinsphys.2006.07.005

Mugnano, J., Lee, R.E., Taylor, R., 1996. Fat body cells and calcium phosphate spherules induce ice nucleation in the freeze-tolerant larvae of the gall fly *Eurosta solidaginis* (Diptera, Tephritidae). J. Exp. Biol. 199, 465–71.

Overgaard, J., Sørensen, J.G., Petersen, S.O., Loeschcke, V., Holmstrup, M., 2005. Changes in membrane lipid composition following rapid cold hardening in *Drosophila melanogaster*. J. Insect Physiol. 51, 1173–82. doi:10.1016/j.jinsphys.2005.06.007

Philip, B.N., Yi, S.-X., Elnitsky, M. a, Lee, R.E., 2008. Aquaporins play a role in desiccation and freeze tolerance in larvae of the goldenrod gall fly, *Eurosta solidaginis*. J. Exp. Biol. 211, 1114–9. doi:10.1242/jeb.016758

Sim, C., Denlinger, D.L., 2013. Insulin signaling and the regulation of insect diapause. Front. Physiol. 4 JUL, 1–10. doi:10.3389/fphys.2013.00189

Stetina, T., Kostal, V., Korbelova, J., 2015. The Role of Inducible Hsp70, and Other Heat Shock Proteins, in Adaptive Complex of Cold Tolerance of the Fruit Fly (*Drosophila melanogaster*). PLoS One 10, e0128976. doi:10.1371/journal.pone.0128976

Teets, N.M., Denlinger, D.L., 2013. Physiological mechanisms of seasonal and rapid cold-hardening in insects. Physiol. Entomol. 38, 105–116. doi:10.1111/phen.12019

Walters, K.R., Sformo, T., Barnes, B.M., Duman, J.G., 2009. Freeze tolerance in an arctic Alaska stonefly. J. Exp. Biol. 212, 305–12. doi:10.1242/jeb.020701

2. PUPAL DEVELOPMENT OF THE HIGH ARCTIC MIDGE *TRICHOTANYPUS ALASKENSIS* (DIPTERA: CHRIONOMIDAE) UNDER CONTROLLED AND NATURAL THERMAL REGIMES

2.1. Abstract

High-latitude chironomid populations often show highly synchronous emergence and consistent seasonal timing during the brief arctic summer. The podonomid midge *Trichotanypus alaskensis* Brundin is an abundant, univoltine, non-tubicolous species in tundra ponds near Barrow, Alaska, appearing early in the seasonal emergence sequence. Pupal development rate for *T. alaskensis* was evaluated under controlled and natural temperature regimes to determine thermal requirements leading to adult emergence by this species. In the lab, pupal development required 1747 degree hours for males and 1720 for females, with no degree-hour difference among four thermal treatments. In the field, pupal development required an average of 1579 degree hours for males and 1543 for females, and there were considerable differences among our 10 experimental field trials (normal distribution 1127-1850). We detected a strong year effect in our field experiments conducted during three summers, resulting from year-specific differences in our ability to generate a range of thermal variation among the treatments. The thermal-time required for pupation was not invariant of experimental conditions, as individuals reared in colder treatments completed pupal development in significantly fewer degree hours than animals in warmer treatments. Development did not differ for overwintering larvae collected from different natal source ponds, based on a test during one of three years. Our results demonstrate that pupal development of *T. alaskensis* responds in a predictable way to average rearing temperature, but exhibits considerable environmental variation under field conditions.

2.2. Introduction

Alaska's Arctic Coastal Plain is predominantly composed of shallow lakes and ponds, which support an abundant invertebrate community (Butler et al. 1980). Aquatic habitats in this region are relatively low in insect diversity, hosting a few species of Plecoptera, Coleoptera, and Trichoptera but more than 30 species of Diptera (Lougheed et al. 2011). The family Chironomidae is the most species-rich taxon, and chironomid midges contribute the largest percentage to total invertebrate biomass in freshwater ponds of the Outer Arctic Coastal Plain near Barrow (Butler et al. 1980). These chironomid species experience a brief Arctic summer, with growth and development restricted to the 3-4 months when pond temperatures are above freezing. Adult emergence by all insect species generally occurs only from late June through July, when temperatures are less variable and thus adult insects are at lower risk of experiencing lethal temperatures (Myers and Pitelka 1979). Although the aquatic insect community may emerge over a span of 3-4 weeks in a given pond and year, emergence by individual species generally occurs in less than one week (Butler 1980). Air temperatures near Barrow have been increasing at nearly 0.7°C per decade (Hobbie et al. 1999), which has also resulted in warmer water temperatures (Lougheed et al. 2011). Climatic warming has been shown to have substantial influences on species' phenologies, with breeding and blooming events occurring earlier than historical average dates (Root et al. 2003, Visser and Both 2005). Although diet and population density may influence insect development (Lobinske et al. 2002), temperature is often a primary influence (Sweeney and Vannote 1986, Rempel and Carter 1987, Huryn 1990, Frouz et al. 2002, Régnière et al. 2012).

Within the family Chironomidae, midge species in the subfamily Podonominae are restricted to low-temperature environments, with greatest species richness in the southern

hemisphere (Eggermont and Heiri 2012); three podonomid genera have been reported from the Canadian Arctic, with at least one alpine species of *Trichotanypus* found in the U.S. Rocky Mountains (Mihuc and Toetz 1996), and *Trichotanypus alaskensis* on Alaska's Arctic Coastal Plain near Barrow, AK (Butler 1980, Oliver and Dillon 1997). *T. alaskensis* is abundant in many tundra ponds around Barrow, and has been collected at other sites along Alaska's Outer Coastal Plain from Peard Bay to Prudhoe Bay. This species appears to drop out of the tundra pond fauna a short distance inland, having not been observed in surveys near the Village of Atqasuk (70^0 29"N, 151^0 25"W) ~50km inland from the Chukchi Sea, nor at a second inland site SE of Teshekpuk Lake ~37km inland from the Beaufort Sea (70^0 26"N; 153^0 08"W). Larvae of *T. alaskensis* are non-tubicolous, moving freely about in the water column and foraging on sediment and macrophyte surfaces. Final instar larvae are readily collected shortly after pond thaw, and can be easily monitored for pupation and subsequent adult emergence. *T. alaskensis* has a univoltine life history in Barrow ponds, simplifying its population structure relative to longer-lived midge species in these habitats (Butler 1982, 2000). Overwintering populations of *T. alaskensis* become active as soon as ponds thaw, complete larval and pupal development, then emerge as adults in a highly-synchronous manner, roughly 20 days after pond thaw.

A changing climate can be expected to affect invertebrate ecology in many ways, given the well-established influences of temperature on a variety of life history traits for insects and other heterotherms (Ratte 1985, Callaghan et al. 2004). Many arctic species are specialized for low-temperature conditions, as evidenced by the arctic collembolan *Hypogastrura tullbergi*, which can develop faster at low temperatures than its southern conspecific (Birkemoe and Leinaas 2000). Extended life cycles are also common among arctic species. A 3-5 year life cycle has been reported for the collembolan *Hypogastrura tullbergi* (Birkemoe and Leinaas

2000), 7-year life cycle large chironomids (Butler 1982), and more than 14 year life cycle for the Lepidopteran *Gynaephora groenlandica* (Kukal and Kevan 1987). Low temperatures and a short growing season are known to produce wingless morphs, thought to be the result of shifting energy allocation to growth and reproduction rather than flight (Callaghan et al. 2004). Warming temperatures may influence development in a variety of ways. Hogg and Williams (1996) showed that a 2-3.5°C increase in stream water temperature was associated with lower total animal densities, earlier onset of insect emergence, increased growth rates and precocious breeding in an amphipod, smaller size at maturity for a plecopteran, and altered sex ratio for a trichopteran.

More time for an insect to accomplish a set amount of development (such as completing pupation) under cooler pond temperatures implies a lower developmental rate, while faster development is expected under warmer temperatures (Culler et al. 2015). This is the essence of the thermal time model (Trudgill et al. 2005), which holds that a "thermal constant" should characterize any given developmental process in poikilothermic invertebrates. Thermal time models assume a linear response of developmental rate to increasing temperature, above some positive base temperature where development rate = 0. The thermal time approach is convenient, given its assumption that time and temperature contribute symmetrically to the developmental requirement such that a species should require a constant degree-day (or degree-hour) product to accomplish a set developmental task. Although this approach has been challenged on both theoretical and empirical grounds (Worner 1992, Trudgill et al. 2005), when the development-temperature relationship is approximately linear a thermal-time requirement can be used to quantify the time requirement for development of a particular life-cycle stage (Charnov and Gillooly 2003, Trudgill et al. 2005).

In this study, we investigated the development of *T. alaskensis* under a variety of temperature conditions, and evaluated the thermal time model as a tool for explaining variation in emergence timing. With a known starting time (date of thaw), a consistent starting point for pre-emergence development (a species-specific overwintering stage), and a species-specific thermal constant, any pond-to-pond or year-to-year variation in time to emergence should reflect variation in the thermal environment. We've tested predictions of this model in two ways, using both controlled-temperature experiments and field experiments. We determined pupation rate as the reciprocal of pupal development time, the latter in this case being the time needed by an individual to develop from the larval-pupal molt to pupal-adult ecdysis. Degree-hours required for development will likely vary among species and life-cycle stages; i.e., the degree-hours needed for *T. alaskensis* to complete pupal development may differ from the pupation requirement of a closely related species, and development rates for various larval instars will differ from pupation rates. However, insect development rates may respond to temperature in predictable ways, either within or among taxa. Thus a better understanding of how developmental rates for model species respond to the thermal environment should help to characterize the responses we might expect to observe within an insect community experiencing a warmer climate.

2.3. Materials and Methods

2.3.1. Collection of Chironomid Larvae

T. alaskensis is easily collected along pond margins and within emergent vegetation (*Arctophila fulva* and *Carex aquatilius*) of Barrow ponds, where the overwintering larvae swim actively as soon as ponds thaw. Final instar *T. alaskensis* larvae were collected from two sites near Barrow, AK: Humpback Pond (HB; located in the Barrow Environmental Observatory -

71°16’38.55”N, 156°38’31.79”W), and the South BASC Pool complex (SBASC; located a few meters south of the NARL laboratory building operated by the Barrow Arctic Science Consortium - 71°19’27.27”N, 156°40’38.84”W). HB is a deeper pond (Zmax=2m) formed in a degrading ice wedge polygon trough (Webber et al. 1980), which thaws later and runs cooler than most of the neighboring low-center polygons. Thus HB was a good source of prepupal *T. alaskensis* larvae in mid-June, when pupation of this and some other early-emerging species had already begun in most ponds. We collected larvae from Humpback Pond in 2009, and from SBASC Pond in 2010. In 2011 we collected larvae from both Humpback and SBASC, rearing larvae from both sources simultaneously. The SBASC Pool complex provided a convenient rearing site close to the BASC laboratory facility, with sub-ponds that varied in thaw date and water temperatures.

2.3.2. General Rearing Procedure

Larvae were housed in 250ml polycarbonate rearing containers that floated within treatment pools or experimental water baths. Mesh-covered side windows in rearing containers allowed water flow but prevented larval escape. HOBO Pro© U22 temperature loggers were placed in all treatment baths, programmed to log water temperatures hourly. All rearings were checked daily for pupating individuals, and new pupae were segregated to produce daily cohorts of known pupal age. Upon emergence, adults and pupal exuviae were preserved in 70% ethanol.

2.3.2.1. Lab Pupation (LP)

In 2008, the effect of temperature on pupation time for *T. alaskensis* was tested in laboratory incubators. Larvae collected from Pond HB on 16 June were shipped on ice to Fargo, ND, arriving as prepupae on 19 June. Larvae were immediately divided into four groups of at least 75, and each group was placed into one of four incubators. Two incubators were set to

maintain constant temperatures (+/- 1°C): one at 8°C, the other at 11°C. Two programmable incubators were set to oscillate over a 24-hour period, varying either 5-11°C or 8-14°C (+/- 3°C relative to the constant-temperature treatments).

2.3.2.2. Field Pupation (FP)

On 12 June 2009, we collected 225 *T. alaskensis* larvae from Pond HB, and split them into three groups. Rearing containers for FP test I were placed in a container at the edge of a large snow drift near SBASC; melt water from the snow provided cooler conditions relative to the other treatment pools. FP tests II and III were incubated in 20L pails suspended in separate pools within the SBASC pool complex that were unaffected by melt water from the FP I snow drift.

In 2010 we replicated the 2009 experiment with some modifications: We collected *T. alaskensis* larvae from SBASC itself, where animals from Pond HB had been reared the previous year in FP II and III. About 150 larvae were split into three trial groups of ~50 larvae each: FP IV larvae were incubated in the main pool of SBASC, while FP V larvae were reared in a smaller pool nearby; FP VI larvae occupied a 20L pail of pond water placed on the tundra and exposed to ambient air temperatures.

In 2011 more than 300 larvae were collected from both Pond HB and SBASC. Water temperatures in SBASC are typically warmer than in HB, and *T. alaskensis* collected from the SBASC pools are considerably smaller and weigh less than HB larvae. Eight trials in total (4 treatments X 2 larval sources) were conducted in 2011 to test the constancy of the pupation DH requirement for larvae from different habitats, with different prior-year growth histories. FP VII, VIII, IX, and X each began with two rearing containers, containing larvae from each of the two

source ponds, which were incubated in four different treatment pools within the flooded SBASC pool mosaic.

2.3.3. Data Analysis

The thermal time model assumes that a developmental rate for ectothermic animals is linearly related to temperature, within a non-lethal temperature range (Charnov and Gillooly 2003). To test this model for pupation by *T. alaskensis*, we determined the degree-hours required for pupation by date-specific cohorts within each rearing treatment (individuals that pupated, then subsequently emerged, on the same dates). We summed hourly temperature exposure in each rearing treatment from 14:00 Alaska Daylight Time (AKDT) on the day of pupation until 14:00 AKDT on the day of emergence. These treatment-specific degree-hour (DH) values represent the 'physiological time' or 'thermal time' needed by individuals pupating under the treatment conditions during that time span. T-tests were used to determine if development of larvae from different source habitats occurred at significantly different rates. T-tests of DH values for males versus females in a treatment were also used to test for sex-dependent developmental rates.

A corollary to the thermal constant hypothesis is its inverse: the rate of development should show a positive linear relationship to the mean temperature experienced during development. Our response variable 'development rate' is the reciprocal of the number of days between pupation and the date of emergence. We averaged hourly treatment temperatures over the period that each individual underwent pupal development.

We estimated outliers from our normal distribution with multivariate distance measures described by Filzmoser et al. 2005, using the package mvoutliers. This has become a standard method for multivariate outlier detection, which involves robust estimation of parameters in the

Mahalanobis distance (MD) measure, and then compares MD with the critical value of X^2 distribution. Any values larger than the critical value are treated as outliers of the distribution (Filzmoser et al. 2005).

2.4. Results

A total of 658 *T. alaskensis* successfully emerged in all of our experiments. Developmental times for all lab trials are shown in Table 1 and field trials in Table 2. The normal distribution of thermal time values for pupal development ranged 1127 to 1850 degree hours, averaging 1492 (st. dev. = 136). Pupal development time generally decreased with increasing mean temperature, from 275 hours at 3.8°C to fewer than 200 hours when the mean temperature increased above 8°C. The only major outlier group was FP IV, where all adults emerged in roughly 4 days (100 hours) at low temperatures (average 3.8°C).

Table 2.1. Average degree hour accumulation for males and females reared in four controlled-temperature lab trials in 2008.

Treatment	n	Mean	St.Dev	n	Mean	St.Dev
	Females			Males		
8°C	11	1709	133.3	15	1708	150.0
5-11°C	28	1703	160.0	32	1771	192.2
11°C	17	1783	144.5	29	1743	141.3
8-14°C	12	1686	3.9	22	1746	122.1

Table 2.2. Results of all field and lab trials. Trials are labeled Field Pupation (FP I - FP X). Average degree hour requirements are presented along with standard deviations. Animals in FPIII were not sexed.

Treatment	Avg Temp	n	Mean	St.Dev	n	Mean	St.Dev
			Females			Males	
FPI	3.7	25	1204.46	56.67	27	1237.69	61.10
FPII	4.9	25	1465.16	94.05	39	1447.86	89.61
FPIII	5.4	47	1438.42	75.46			
FPIV	3.8	12	788.45	65.01	10	762.97	39.10
FPV	7.3	18	1487.81	64.66	16	1616.43	78.66
FPVI	5.5	16	1605.21	97.41	16	1561.08	136.50
FPVII	6.6	27	1599.34	149.85	21	1655.94	130.32
FPVIII	7.3	26	1662.05	166.21	27	1754.78	102.75
FPIX	7.7	30	1629.02	106.81	23	1672.74	94.29
FPX	7.7	26	1673.52	157.61	28	1781.14	110.95

2.4.1. Lab Pupation (LP)

2008. The two constant-temperature lab trials ran slightly cooler than our targets of 8°C and 11°C, averaging 6.7°C and 10.4°C respectively, and never deviated from these means by more than 0.5°C. Water temperatures in the two oscillating treatments of our lab trials ran slightly warmer than our targeted ranges. Actual temperatures in the targeted 5-11°C treatment ranged 6.2-10.6°C (mean = 8.2°C), and in the targeted 8-14°C treatment were 9.4-14.0°C (mean = 11.6°C). Thermal time requirements for *T. alaskensis* pupation in four different controlled-temperature regimes were nearly identical (Figure 1), and we detected no significant differences among treatments.

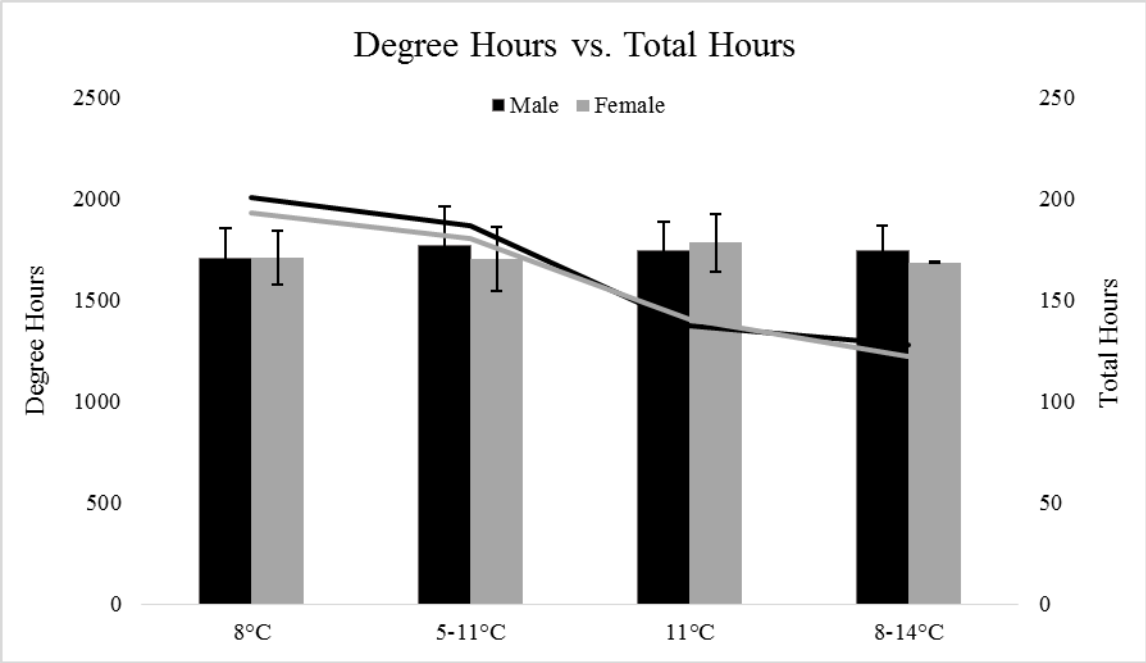

Figure 2.1. Degree hour and total hour requirements for all lab reared animals.
Error bars plot 1 standard error. Degree hour requirements were not statistically different among any lab reared trials, despite the large difference in total hours accrued during the experiment.

2.4.2. Field Pupation

2009. The three field pupal (FP) rearing trials in 2009 essentially produced only two contrasting thermal environments. Animals from FP I reared near the snow field experienced the coolest average temperature (3.8°C) of all our experimental units. FP II & III, although conducted in different pools within the SBASC pool complex, had very similar thermal exposures, averaging 5.0°C for FP II and 5.4°C for FP III. Overall, thermal variation experienced by larvae in FP II and III was relatively low throughout the course of the 2009 experiment. Of the 163 individuals that completed pupation in 2009, similar numbers emerged from each treatment; both males and females were similarly represented in FP I and FP II; but sex of the 47 animals from FP III was not determined. The overall average thermal-time requirement for pupation was 1375 DH, with all three treatments producing mean thermal-time values <1500 DH (Table 2). Pupae in FP I required significantly fewer degree hours than those in FP II and III

(ANOVA, $n = 163$, $p < 0.001$), with no significant difference between FP II and III (Tukey's test, $p = 0.61$). Females did require slightly more thermal-time to complete pupal development than males, but this result was not significant (t-test, $t = -1.24$, $p = 0.22$ for FP I; $t = 0.73$, $p = 0.47$ for FP II, animals in FP III were not checked for sex).

2010. As happened in 2009, FP tests IV, V, and VI produced essentially two thermal environments. FP IV was the coolest, averaging 3.8°C, with FP V and VI both warmer at 7.4°C and 5.6°C, respectively. Only 23 T. alaskensis adults emerged from FP IV, completing pupal development with an average of only 781 DH – roughly half the thermal-time required for all other T. alaskensis pupae across all years. The FP IV animals all finished pupal development in 7-8 days, considerably faster than our overall average of 10 days, which is notable in light of the cool temperature of this trial. After removing FP IV (see Outliers section below), the overall average for FP V – FP VI was 1573 degree hours. Males tended to begin pupation earlier, but females required less thermal time to complete pupal development, which was highly significant in one treatment and not significant in another (t.test, FP V: $t = -5.22$, $p < 0.001$; FP VI: $t = 1.05$, $p = 0.3$).

2011. Trials FP VII, VIII, IX, and X included T. alaskensis animals from two different natal ponds. Pupal development requirements did not differ significantly between larvae collected from SBASC Pool and HB Pond (ANCOVA, $p = 0.163$). The average DH requirement across treatments for HB pupae was 1530 (std.dev = 162.4), versus 1513 (std.dev = 100.8) for SBASC Pool pupae. There was a small difference between the sexes, as males required significantly more DH (1566, st.dev = 119.8) than did females (1496; st.dev = 161.1; $p < 0.001$). This is consistent with some lab trials in 2013 (not reported here), which showed that females

required more degree hours to complete all pre-emergent development (i.e., both late-larval and pupal), while males required more degree hours than did females for pupal development alone.

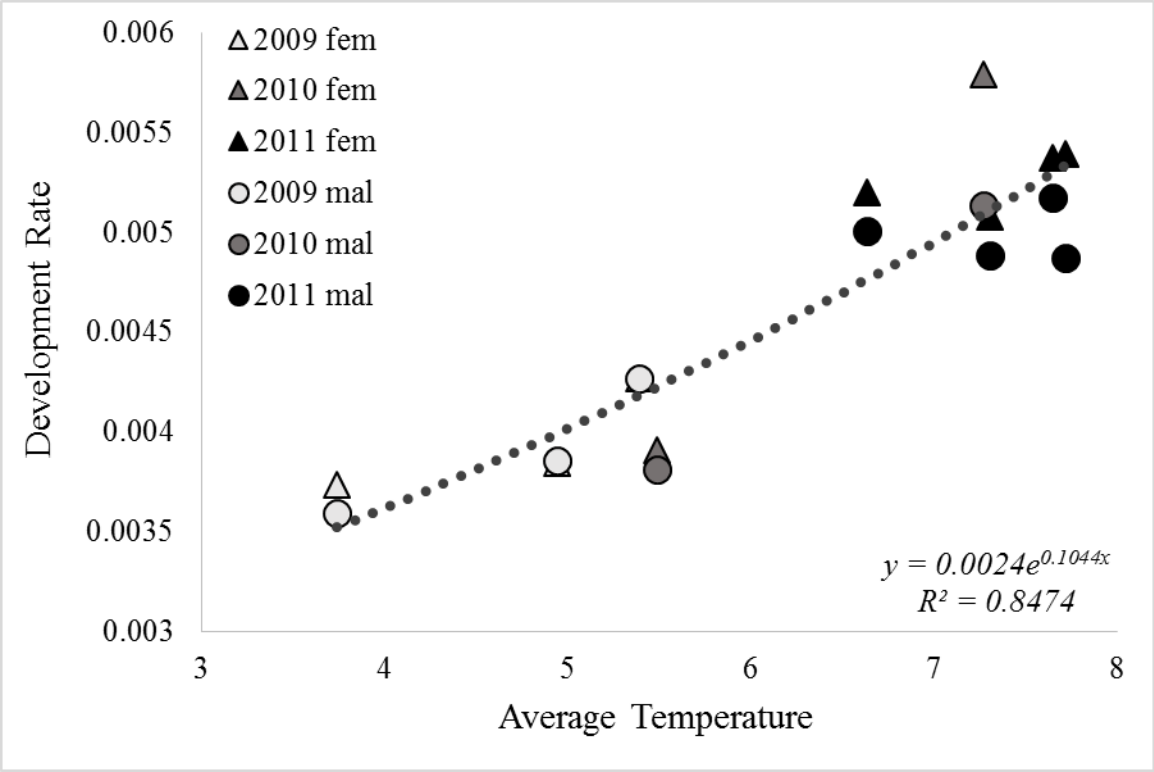

Figure 2.2. Developmental rate vs average temperature for all field treatments.

2.4.3. Outliers

We identified 31 outliers in our data set regarding pupation. Outliers were found in 3 of the 4 years of the study (No outliers detected in FP I-III). Pupal thermal time was overestimated 4 times, and underestimated in 27 cases. Pupation requirements of all 23 animals in FP IV (ranging 661-892 degree hours) appear to have been substantially underestimated. Most of the lab-reared animals fell within the range of our overall normal distribution.

2.5. Discussion

Temperature is one of the most important factors affecting insect life history, and degree-day models are useful for predicting rates of development in many species (Stevens 1998, Vogt

et al. 2007). *T. alaskensis* was selected as a model insect in the tundra pond community because of its favorable life history characteristics (abundant and easily collected, non-tubicolous pupation, highly synchronous emergence), and our results may be applicable to other species adapted to the environmental conditions in these arctic ponds.

A mean of 7-8°C seems to be the lowest constant temperature at which *T. alaskensis* can complete pupal development. Larvae and pupae appear to utilize peaks in the daily temperature cycle, as larvae kept at a constant 4°C (data not shown here) in the lab failed to pupate while others successfully emerged as adults from field trials where temperatures oscillated around an average of 4°C (Table 2). The physiological processes of freeze tolerant insects are complex, and development at temperatures close to freezing may be constrained for species that must metabolize and convert freeze-tolerance molecules. These metabolic processes may thus be restricted to the warmest temperatures of the day (Storey and Storey 2013).

The DH requirement for pupation by *T. alaskensis* was broadly consistent across 13 of 14 lab and field trials. Pupation by this chironomid takes more than 13 days in cool water averaging 4°C daily, but can be completed in around 5 days in warmer water with a mean of 11°C. Pupal development time was shorter for females, which suggests that protandrous emergence observed for this species (Butler 1980). *Chironomus tepperi* females also required significantly more time than males to finish their final (4th) instar, and the females of that species then completed pupation faster than males (Stevens 1998). The additional time females spend in the final larval instar may be used to acquire additional nutrients for maximizing fecundity (Butler and Walker 1992). After pupation, *T. alaskensis* lose functional mouthparts for the remainder of their life cycle, thus feeding is possible only during the larval stage. Thus females may pupate more

quickly to ‘catch-up’ to males, as the majority of a population typically emerges over a 2-6 day period.

Similar experiments could be attempted with other midge species, but many other abundant chironomid larvae in these tundra ponds are tubicolous (tube-dwelling) and thus date of pupation is very difficult to monitor. For such chironomids one can measure the response to varying temperature of all pre-emergence development - the time needed, from pond thaw to adult emergence - for all remaining larval development plus pupation. This is in fact the relevant variable if we are interested in developing a model of adult emergence timing in response to climate. Emergence dates for early-summer species might be controlled largely by the influence of temperature on pupal development, while larvae of later-emerging species may overwinter further from pupation and could thus be regulated more by thermal effects on larval development. If both larval and pupal development respond similarly to temperature, we might hope to treat both processes as one event when modeling the response of emergence phenology to temperature.

Our work on *T. alaskensis* suggests that differences among species in the seasonal timing of adult emergence observed for chironomids in Barrow tundra ponds are not simply explained by variations in life-cycle scheduling. With its early and highly-synchronous emergence, we had expected *T. alaskensis* to illustrate the “absolute spring species” life-history strategy described for arctic chironomids by (Danks and Oliver 1972). In the course of our rearing experiments with this species, we observed *T. alaskensis* to exhibit a life history pattern very similar to that described by Butler (1980) for the late-emerging *Tanytarsus nearcticus* (as Tanytarsus sp. 2). Both of these species overwinter in the late third or early fourth instar, then grow and complete all remaining larval development plus pupation prior to emergence. In both species, individual

larvae more than double in size after spring thaw, reaching asymptotic larval mass as late final-instar larvae before pupation (Braegelman and Butler, In prep). Despite their similarity in overwintering stage, these two species show very different patterns of subsequent pre-emergence development and emergence timing.

There appear to be considerable differences in how these arctic chironomid species grow and develop with respect to temperature, but aspects of other species' life cycles make them difficult to study in the detail described here. More work remains to be done on midges emerging at intermediate times in the annual sequence, and these species will likely show a range of developmental patterns. Differing modes of thermal adaptation among species, coupled with different overwintering strategies, may explain the regular sequence of species' emergence pulses that collectively make up chironomid (and other insect) emergence phenologies in Barrow ponds each summer.

2.6. References

Birkemoe, T., and H. P. Leinaas. 2000. Effects of temperature on the development of an arctic Collembola (*Hypogastrura tullbergi*). Funct. Ecol. 14: 693–700.

Braegelman, S. D., and M. G. Butler. (*in prep*). Seasonal growth and development of an arctic Alaskan midge *Trichotanypus alaskensis* (Diptera: Chironomidae).

Butler, M. G. 1980. Emergence phenologies of some arctic Alaskan Chironomidae, pp. 307–314. In Murray, D.A. (ed.), Chironomidae Ecol. Syst. Cytol. Physiol. Oxford: Pergamon Press, New York.

Butler, M. G. 1982. A 7-year life cycle for two Chironomus species in arctic Alaskan tundra ponds (Diptera: Chironomidae). Can. J. Zool. 60: 58–70.

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., M. C. Miller, and S. Mozley. 1980. Macrobenthos, pp. 297–339. In Hobbie, J.E. (ed.), Limnol. Tundra Ponds, Barrow, Alaska. Dowden, Hutchinson & Ross, Stroudsburg, PA.

Butler, M. G., and L. Walker. 1992. Fecundity, reproductive effort, and pupal size in the profundal midge *Chironomus cuccini* (Diptera: Chironomidae). Netherlands J. Aquat. Ecol. 26: 263–267.

Callaghan, T. V., L. O. Björn, Y. Chernov, T. Chapin, T. R. Christensen, B. Huntley, R. A. Ims, M. Johansson, D. Jolly, S. Jonasson, N. Matveyeva, N. Panikov, W. Oechel, G. Shaver, J. Elster, H. Henttonen, K. Laine, K. Taulavuori, E. Taulavuori, and C. Zöckler. 2004. Biodiversity, distributions and adaptations of Arctic species in the context of environmental change. Ambio. 33: 404–417.

Charnov, E. L., and J. F. Gillooly. 2003. Thermal time: body size, food quality and the 10°C rule. Evol. Ecol. Res. 5: 43–51.

Culler, L. E., M. P. Ayres, and R. A. Virginia. 2015. In a warmer Arctic, mosquitoes avoid increased mortality from predators by growing faster. Proc. R. Soc. B Biol. Sci. 282.

Danks, H. V., and D. R. Oliver. 1972. Seasonal emergence of some High Arctic Chironomidae (Diptera). Can. Entomol. 104: 661–686.

Eggermont, H., and O. Heiri. 2012. The chironomid-temperature relationship: expression in nature and palaeoenvironmental implications. Biol. Rev. Camb. Philos. Soc. 87: 430–56.

Filzmoser, P., R. G. Garrett, and C. Reimann. 2005. Multivariate outlier detection in exploration geochemistry. Comput. Geosci. 31: 579–587.

Frouz, J., A. Ali, and R. J. Lobinske. 2002. Influence of Temperature on Developmental Rate, Wing Length, and Larval Head Capsule Size of Pestiferous Midge *Chironomus crassicaudatus* (Diptera: Chironomidae). J. Econ. Entomol. 95: 699–705.

Hobbie, J. E., B. J. Peterson, N. D. Bettez, L. Deegan, W. J. O'Brien, G. W. Kling, G. W. Kipphut, W. B. Bowden, and A. E. Hershey. 1999. Impact of global change on the biogeochemistry and ecology of an Arctic freshwater system. Polar Res. 18: 207–214.

Hogg, I. D., and D. Williams. 1996. Response of stream invertebrates to a global-warming thermal regime: an ecosystem-level manipulation. Ecology. 77: 395–407.

Huryn, A. D. 1990. Growth and voltinism of lotic midge larvae: Patterns across an Appalachian Mountain basin. Limnol. Oceanogr. 35: 339–351.

Kukal, O., and P. G. Kevan. 1987. The influence of parasitism on the life history of a high arctic insect, *Gynaephora groenlandica* (Wocke) (Lepidoptera: Lymantriidae). Can. J. Zool. 65: 156–163.

Lobinske, R. J., A. Ali, and J. Frouz. 2002. Laboratory estimation of degree-day developmental requirements of *Glyptotendipes paripes* (Diptera: Chironomidae). Environ. Entomol. 31: 608–611.

Lougheed, V. L., M. G. Butler, D. C. McEwen, and J. E. Hobbie. 2011. Changes in Tundra Pond Limnology: Re-sampling Alaskan Ponds After 40 Years. Ambio. 40: 589–599.

Mihuc, T. B., and D. W. Toetz. 1996. Phenology of aquatic macroinvertebrates in an alpine wetland. 131–136.

Myers, J. P., and F. A. Pitelka. 1979. Variations in summer temperature patterns near Barrow, Alaska: analysis and ecological interpretation. Arct. Alp. Res. 11: 131–144.

Oliver, D. R., and M. E. Dillon. 1997. Chironomids (Diptera: Chironomidae) of the Yukon Arctic North Slope and Herschel Island. Insects Yukon. Biol. Surv. Canada (Terrestrial Arthropods), Ottawa. 615 – 635.

Ratte, H. T. 1985. Temperature and insect development, pp. 33–65. In Environ. Physiol. Biochem. Insects.

Régnière, J., J. Powell, B. Bentz, and V. Nealis. 2012. Effects of temperature on development, survival and reproduction of insects: Experimental design, data analysis and modeling. J. Insect Physiol. 58: 634–647.

Rempel, R. S., and J. C. H. Carter. 1987. Temperature influences on adult size, development, and reproductive potential of aquatic Diptera. Can. J. Fish. Aquat. Sci. 44: 1743–1752.

Root, T. L., J. T. Price, K. R. Hall, S. H. Schneider, C. Rosenzeig, and J. A. Pounds. 2003. Fingerprints of global warming on wild animals and plants. Nature. 421: 57–60.

Stevens, M. M. 1998. Development and Survival of *Chironomus tepperi* Skuse (Diptera: Chironomidae) at a Range of Constant Temperatures. Aquat. Insects. 20: 181–188.

Storey, K. B., and J. M. Storey. 2013. Molecular biology of freezing tolerance. Compr. Physiol. 3: 1283–308.

Sweeney, B. W., and R. L. Vannote. 1986. Growth and production of stream stonefly: influences of diet and temperature. Ecology. 67: 1396–1410.

Trudgill, D. L., A. Honěk, D. Li, and N. M. Van Straalen. 2005. Thermal time–concepts and utility. Ann. Appl. Biol. 146: 1–14.

Visser, M. E., and C. Both. 2005. Shifts in phenology due to global climate change: the need for a yardstick. Proc. Biol. Sci. 272: 2561–2569.

Vogt, C., A. Pupp, C. Nowak, L. S. Jagodzinski, J. Baumann, D. Jost, M. Oetken, and J. Oehlmann. 2007. Interaction between genetic diversity and temperature stress on life-cycle parameters and genetic variability in midge *Chironomus riparius* populations. Clim. Res. 33: 207–214.

Webber, P. J., P. C. Miller, F. S. Chapin III, and B. H. McCown. 1980. The vegetation: pattern and succession, pp. 186–218. In Brown, J., Miller, P.C., Tieszen, L.L., Bunnell, F.L. (eds.), An Arct. Ecosyst. Coast. Tundra Barrow, Alaska. Dowden, Hutchinson & Ross, Stroudsburg, PA.

Worner, S. P. 1992. Performance of Phenological Models Under Variable Temperature Regimes: Consequences of the Kaufmann or Rate Summation Effect. Environ. Entomol. 21: 689–699.

3. THE EARLY, SYNCHRONOUSLY EMERGING ARCTIC ALASKAN MIDGE *TRICHOTANYPUS ALASKENSIS* (DIPTERA: CHIRONOMIDAE) IS NOT AN ABSOLUTE SPRING SPECIES

3.1. Abstract

A brief, synchronous emergence of adults in early summer is common for many arctic insect species. Chironomids emerging from some Canadian High Arctic ponds have been termed "absolute spring species" (AbSS), with early and synchronous emergence hypothesized to result from larvae overwintering in a fully mature, pre-pupal phase. Under the AbSS mechanism, winter diapause of fully grown, developmentally mature larvae serve to synchronize pupation and emergence of the population shortly after pond thaw. Highly synchronous adult eclosion is well-illustrated by the podonomid midge *Trichotanypus alaskensis*, an abundant member of the chironomid fauna in freshwater ponds near Barrow, Alaska, and one of the earliest aquatic insects to emerge there each summer. *T. alaskensis* shows a univoltine life cycle in these shallow tundra ponds, with adults emerging over a period of a few days shortly after pond thaw in late May to early June. To determine if *T. alaskensis* uses the AbSS mechanism to synchronize emergence, we sampled individuals of this species before winter freeze, and from pond thaw to pupation and adult emergence the following spring. We found that most larvae overwintered as early fourth instars, with a few late third-instar larvae found shortly before the fall freeze. Only fourth instars were found immediately following pond thaw in early June, and these overwintering larvae showed no change in mass or developmental phase during the first four days post-thaw, when daily average pond temperatures ranged 0.4°C-3°C. Over the next nine days, mean weight increased by 85% and larvae developed rapidly to a mature prepupal phase. Pupae first appeared within two weeks of pond thaw, with virtually all larvae pupating by the

end of the third week. The larvae in our population required substantial feeding and growth during the post-thaw period. Less-mature overwintering larvae appeared to 'catch-up' developmentally to earlier-maturing larvae during the final 3-4 days before pupation. Overwintering stage appears to be less significant as a means of developmental synchronization in this species than for other chironomids that may use the AbSS mechanism.

3.2. Introduction

Developmental synchronization is important for the reproduction of many species that live in highly seasonal environments (Danks 2007). The Arctic Coastal Plain of northern Alaska is highly seasonal, with many small to medium sized water bodies on this tundra landscape. These lakes and ponds are mostly fishless, and provide exceptional habitat for invertebrate communities (Butler et al. 1980). Aquatic insects emerge in great abundance over a few weeks in early summer, providing abundant prey for insectivorous tundra-nesting birds (Yohannes et al. 2010). The Arctic is also a harsh environment; shallower water bodies at risk of freezing temperatures throughout the year prevent colonization by many aquatic species found at lower latitudes (Boumans and Brittain 2012). Low species diversity in arctic ponds has also been attributed to a limited nutrient supply, shorter growing season, poorer quality soils, reduced primary production, and low rates of adaptation to other harsh conditions (Strathdee and Bale 1998). The insect community in tundra ponds near Barrow, Alaska is composed predominantly of chironomid midges, with the 10 most abundant species contributing over 90% of emerging insect numbers in two surveys separated by three decades (Butler et al. 1980, Lougheed et al. 2011). One such abundant insect, the podonomid midge *Trichotanypus alaskensis*, is one of the earliest-maturing species in this community, emerging with high population synchrony only 3-4

weeks after pond thaw. *T. alaskensis* is a good model insect for life-history analysis because of its abundance and ease of collection shortly after thaw.

Categorizing aquatic insect life history strategies, Corbet (1964) defined "spring species" as having highly synchronized emergence, a short flight season, and "all, or a majority, of a population spend[ing] the winter before emergence in the final instar". This was in contrast to 'summer species' which likely required varying amounts of additional larval development before emergence. Corbet's spring species hypothesis was later modified by Danks and Oliver (1972) to only include "absolute spring species" (AbSS), where "Emergence … is synchronized because only larvae which are ready to pupate without further feeding in spring emerge in any year". They proposed the AbSS hypothesis as a mechanism to explain the high degree of emergence synchrony they observed in populations of chironomids from Canada's High Arctic (81°N). In this study, we tested the absolute spring species concept as a synchronizing mechanism for adult emergence of the arctic midge *Trichotanypus alaskensis* Brundin. We collected larvae pre- and post-winter over a 12mo period to determine the seasonal pattern of population-average weight change, along with patterns of late-larval development. Larvae collected pre- and post-winter did not differ in weight, but – in contrast to the AbSS model - shortly after pond thaw *T. alaskensis* larvae gained significant weight, pupating at nearly twice their weight at thaw.

3.3. Materials and Methods

3.3.1. Sites and Collection

T. alaskensis were collected for this study from an ice-wedge pool near the former Barrow Arctic Science Consortium (BASC) we called South-BASC (hereafter SBASC, 71°19'27.27"N and 156°40'38.84"W). The surface area of SBASC varies greatly across the summer, ranging from >72m2 at thaw to ~60m2 in late summer; emergent *Carex aquatilis* and

Arctophila fulva ring the pond margins, which drop abruptly to ~50cm depth. We collected chironomids and other epifauna by sweeping through the emergent vegetation with a 15cm diameter dip net (100µm mesh). We collected before pond freeze on two dates: 29 Sept 2010 and 30 Sept 2011, and larvae were sampled every 2-3 days after thaw in 2011. We attempted to collect larvae in mid-October 2012, but were unable to find *T. alaskensis* at any of our study sites under 10cm of ice cover.

All larvae were dried for more than 24 h at 60°C and weighed to the nearest 1µg. Larvae were photographed with a digital camera mounted on a dissecting microscope, and images were measured for body area (in mm2) and head capsule width (in mm) with the software ImageJ.

Hourly pond temperatures were recorded using Hobo data loggers. In 2011 we began recording temperatures in the SBASC pool on June 18th, placing four loggers at ~10-15cm depth at four different locations within the flooded pool. Lacking data from thaw in early June, we averaged temperatures from these four locations and compared values to two recordings in two similar ponds at the IBP site, for which we have multi-year temperature records including pond thaw. As hourly temperatures in these IBP ponds differed from our four SBASC loggers by only 0.16°C ± 0.8, and temperatures in the two sites were not statistically different ($t = -0.32$, $p = 0.38$). Thus we used average hourly temperature data from the two IBP ponds as surrogates for temperature values in SBASC during early June 2011.

3.3.2. Description of Developmental Stages

Instar and developmental phase were determined for preserved larvae in the series of samples collected immediately after pond thaw in June and prior to pond freeze in September. To determine instar, digital photographs of larval head capsules were measured using the software ImageJ. Head width was measured at the widest part of the head in a dorsal or ventral view.

Larval development was scored based on photographs of thoracic wing and leg primordia visible in fourth instar larvae, using developmental phases similar those established for *Chironomus* spp. (Wülker and Götz 1968, Ineichen et al. 1983, Goddeeris et al. 2001).

We established sequential developmental phases within instar IV, based on progressive changes in the size and shape of imaginal leg and wing primordia. These "developmental phases" were assigned integer values from 1 to 4, scores that represent the maximal degree of primordial development within each interval. As development is a continuous process, we scored development of fourth-instar larvae with fractional values reflecting the relative maturity within each developmental phase (e.g., phase 1.0, 1.5, or 1. 8, etc.). When pupating or emerging individuals appeared in the population, we scored the population for the relative proportions of larvae, pupae, and eclosed adults.

3.3.2.1. Phase 1

Begins immediately after the instar III/IV molt; the head capsule is initially transparent but soon becomes sclerotized. Leg primordia appear as small circular discs in all thoracic segments, but without well-defined leg sheaths. Wing and haltere primordia are present in segments 2 and 3, and are similar in size and appearance to the leg discs. Primordia occupy at most 50% of the vertical width of segments 2 and 3 in lateral view. Respiratory organ becoming evident as small disc in posterior-dorsal corner of segment 1, <1/3 the diameter of leg disc.

3.3.2.2. Phase 2

Head capsule completely sclerotized (darkened). Leg and leg sheath definition begin, most discernably in segment 2. Wing disk in segment 2 becomes roughly triangular (less circular than in phase 1). Haltere in segment 3 quadrate to oval, tip descending posterior to leg disc. Wing & leg primordia occupy >70% of the vertical width of segment 2, and >50% the width of

segment 3. Pupal respiratory organ clearly evident in segment 1 as a circular disc with diameter >1/3 that of leg disc.

3.3.2.3. Phase 3

Wing definition continues in segment 2, with wing disk becoming more polygonal, less triangular. Haltere in segment 3 becomes narrowly triangular, with acute tip extending posterior to leg disc. Leg sheaths well defined in segments 1, 2 and 3. Leg sheaths in segments 2 and 3 well separated. Primordia occupy >80% the vertical width of segment 2 and >70% in segment 3. Pupal respiratory organ a well-defined oval disc in segment 1, dorsal and posterior to leg disc. Segments 2 and 3 begin to swell and fuse.

3.3.2.4. Phase 4

Segments 2 and 3 completely fused and swollen. Imaginal discs occupy nearly the entire cavity, and leg sheaths of segments 2 and 3 nearly meeting; leg sheaths meeting wing disc ventrally (seg. 2) and posteriorly (seg. 3). Front leg sheath and pupal respiratory organ well defined.

3.3.3. Preservation

Two preservatives were used in this study: a 2.5% formaldehyde solution and Kahle's fluid. Formaldehyde is useful when preserving an entire sample, including additional pond water and detritus. When larvae can be separated from pond detritus, preservation in Kahle's fluid (15 parts ethanol, 5 parts concentrated formaldehyde, 1 part glacial acetic acid and 30 parts distilled water) is useful for preserving imaginal discs in late-instar larvae.

Previous studies report that fixation in formalin solution and Kahle's fluid have minimal effects on dry weight (Gotceitas and Clifford 1983, Leuven et al. 1985). However, a significant decrease in dry and wet weight of formalin-preserved worms and midge larvae has been reported

after several weeks in solution (Howmiller 1972). To test for differences in dry weight, we collected *T. alaskensis* on 10 & 19 June 2011 and fixed half the larvae with either Kahle's fluid or with 2.5% formalin. Dry weights and larval areas (based on lateral-view photographs) were then measured after several weeks of preservation.

3.3.4. Data Analysis

T-tests were used to compare differences between average larval size (as lateral area) or larval dry weight between pairs of samples; analysis of variance (ANOVA) was used when more than two groups were analyzed (e.g., comparing June 7 Kahle's-preserved larvae to June 10 Kahle's- and June 10 formalin-preserved larvae). Tukey's test was used to identify significantly different groups detected by ANOVA.

3.4. Results

A total of 293 T. alaskensis larvae was collected during 8 collection periods from late 2010 through late 2011. Of the 46 larvae collected in late September 2010, 5 (~10%) were third-instar. The first collection of larvae the following spring (June 7 in 2011) contained no third-instar larvae despite having over 3-fold as many individuals as the sample prior to the fall freeze. In late September 2011, 8 of the 27 larvae collected (~30%) were third-instar. All fourth-instar larvae collected before and after winter 2011-12 were less developed than phase 3. In our population level analyses detailed below, we've included sex-specific changes in larval size and development whenever larval sex could be determined.

Table 3.1. Weights and areas of all *T. alaskensis* collections.

Date	Pres.	n	x̄ Phase	x̄ Weight	x̄ Area	%Pupae	♂ : ♀
29IX10	L	43	-	0.111 (0.005)	2.042 (0.060)	0	-
7VI11	K	157	0.9	0.118 (0.005)	2.711 (0.038)	0	75 : 82
10VI11	F	56	-	0.121 (0.006)	2.927 (0.074)	0	-
10VI11	K	58	0.8	0.115 (0.006)	3.007 (0.084)	0	21 : 37
13VI11	F	31	-	0.141 (0.008)	2.809 (0.093)	0	-
16VI11	K	69	2.4	0.175 (0.007)	3.468 (0.080)	0	43 : 26
19VI11	F	14	-	0.145 (0.012)	3.426 (0.163)	27	-
19VI11	K	13	3.6	0.213 (0.020)	3.466 (0.146)	27	5 : 8
23VI11	K	8	3.3	0.180 (0.022)	3.183 (0.311)	87	2 : 6
25VI11	K	1	4.0	0.189 (0.000)	-	96	1 : 0
30IX11	K	19	0.8	0.088 (0.004)	2.362 (0.090)	0	11 : 8

Summary of all *T. alaskensis* collections from Sept 2010 to Sept 2011. Larvae were either dried while still live (L), or after preservation in either Kahle's fluid (K) or 2.5% formalin solution (F). Mean values (one standard error) are presented for dry weight and for larval area in lateral view, along with mean developmental stage index for instar IV larvae (see text), % pupae, and sex ratio when available.

3.4.1. Weight

Body mass did not change significantly during the period between our fall 2010 sample (30 Sept) and our first post-winter sample (7 June 2011). We did not directly observe pond thaw in 2011, but snow/ice conditions in the pond vicinity on 7 June suggest that larvae could have been active a maximum of 1-2 days prior to our sample. The mean larval weight of 0.119 on that date did not differ significantly from the pre-winter weight of 0.103 ($p = 0.07$, t.test). This indicates little to no feeding or growth by *Trichotanypus* larvae before freezing in late October, or in the short time following thaw. Larvae gained no additional weight during the first 3-4 days after 7 June (Figure 3.1). We found no significant differences between weights of larvae collected on 7 or 10 June 2011, or between the two preservation methods on June 10 ($p = 0.92$, ANOVA). Mean dry weight increased by 20% (0.03mg) from June 10 to June 13, a significant increase over just three days ($p = 0.01$, t.test). From June 13 to June 16, mean larval dry weight

gained another 25% (0.035mg), also a significant increase in weight ($p = 0.001$, t.test). From June 16 to June 19, a further weight gain of 0.031mg was recorded, with the population reaching its peak mean larval weight of 0.213mg. Given fewer individuals in the June 19 sample and greater variation in body weight, growth during 16-19 June was non-significant ($p = 0.17$, t.test). The peak weight of 0.213 was nearly double the average larval weight at pond thaw. Remaining larvae sampled on June 23 were lighter than on June 19, as average body weight declined by 0.023mg ($p = 0.48$, t.test). This non-significant decline may have been the result of a small sample size (n=12 and 9, on June 19 and 23, respectively), or the product of no real decline in size at this phase of the life cycle. We collected only 1 larva on 25 June, our final sampling date, as the remaining population having either pupated or died by this date.

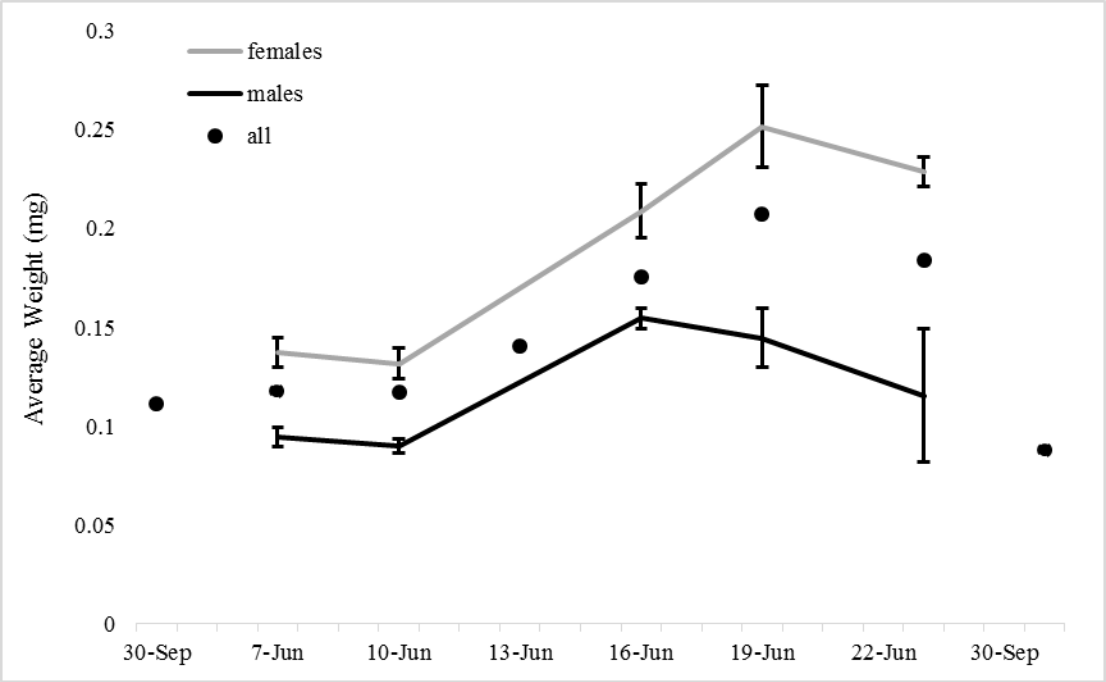

Figure 3.1. Average *T. alaskensis* dry mass on seven dates from Sept 2010 to Sept 2011 for male and female larvae, and both sexes combined (error bars represent ± 1 standard error). Solid lines connect mean dry mass values on six dates during June 2011, between pond thaw and adult emergence. Samples collected on 30 September of both 2010 and 2011 represent overwintering mass for two annual cohorts. Sex was not determined in samples collected 30 September 2010 or 13 June 2011. Larval mass of instar IV increased significantly from pond thaw to June 16 in 2011.

3.4.2. Body Area

Larval body area, while sometimes difficult to measure consistently due to variable effects of formaldehyde on body shape (noted above), yet proved a useful metric for tracking larval growth. Our pre- and post-winter (29 Sept 2010 to 7 June 2011) samples showed an average body size increase of more than 20% (2.04 mm2 to 2.61 mm2, $t = -6.88$, $p < 0.001$), during a time when body weight was stable. Shortly after thaw, larvae began to grow larger body sizes, as both June 10 samples showed an increase in body size over the average size documented on June 7 (ANOVA, $p = 0.001$; Tukey's comparing June 10 Kahle and June 10 Form, $p = 0.579$), with no subsequent differences between preservation method. Larvae

continued to grow until reaching their peak of 3.47mm2 by June 19, 12 days after thaw, which was a 28% increase in body size before pupation (increase from 2.7mm2 to 3.5mm2). Larvae then decreased marginally in size before pupation, although not significantly (area, $p = 0.43$).

3.4.3. Developmental Phase Distribution

Figure 3.2 shows the developmental phase distribution of T. alaskensis after thaw through adult emergence. Fourth instar larvae collected on June 7 (about one day after thaw) were all relatively immature, and were either developmental phase 1 or between phases 1-2. Larvae matured little during the following 3 days, and there was no statistically significant change in developmental phase scores (t.test, $p = 0.9$). Unfortunately, on June 13 no larvae were preserved in Kahle's fluid, and those in formalin were poorly-preserved and could not be scored for developmental phase. During the next 6 days (from June 10 to June 16), larvae matured rapidly and individuals were found across all developmental phases 1-4. This desynchronization of larval development may have resulted from the population passing a developmental threshold into a period of rapid maturation. By the next sample on June 19, nearly all individuals in the population had either reached phase 4, or had pupated. A few larvae were still found in earlier phases of development, possibly representing parasitized individuals or larvae that overwintered as third instars. By June 23, 87% of the population had undergone pupation and first emergence was documented. On June 25 the majority of the population had either pupated (35%) or emerged as adults (61%).

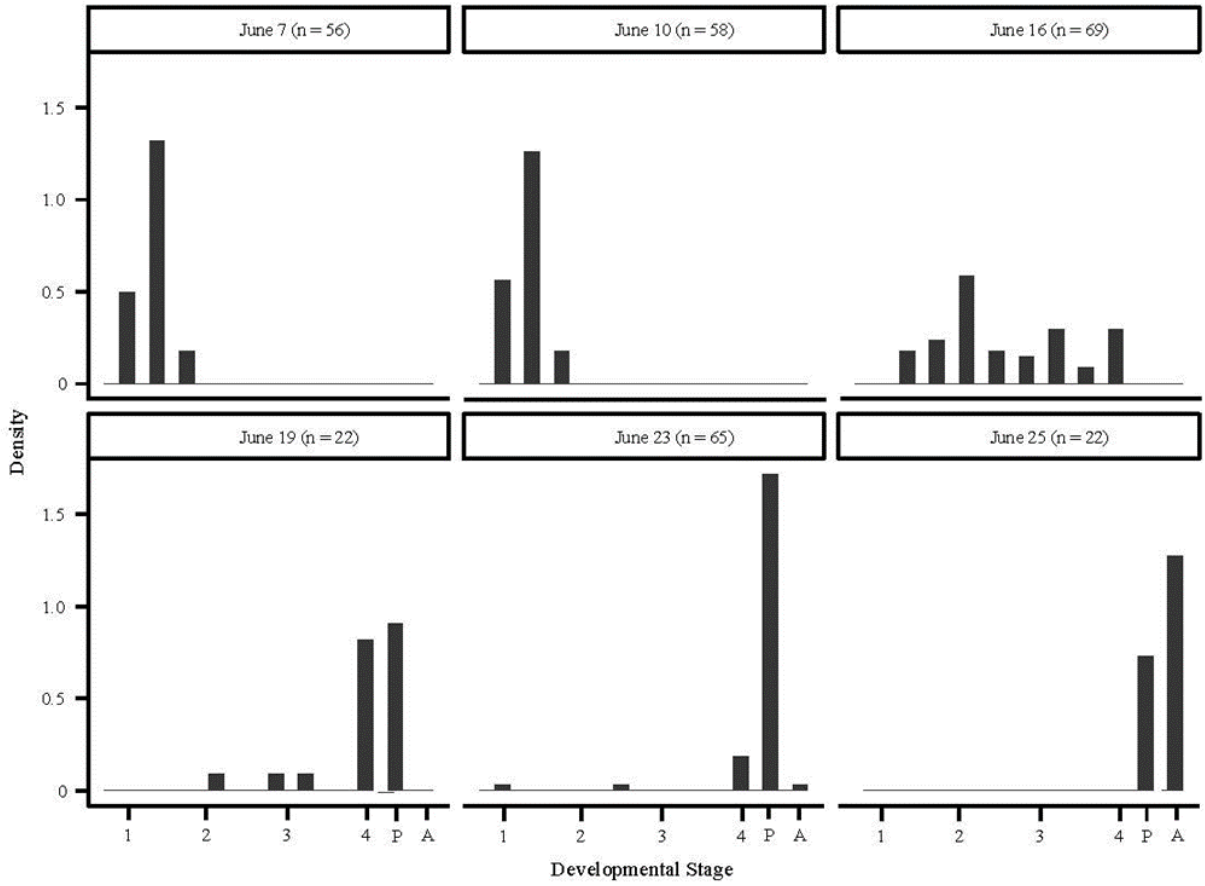

Figure 3.2. Developmental distribution of instar IV larvae, pupae, and adult *T. alaskensis* from Pond SBASC.
Pond thaw occurred on approximately 6-7th June, when daily average air temperatures ranged from -1.7-1.1°C and pond temperatures were slightly higher at 0.4-3.0°C. Stages 1-4 represent the full range of IV instar larval maturation, from the III-IV larval molt until pupation (P), and adult eclosion (A).

3.4.4. Head Capsule

Third instar larvae were found in both of our pre-winter (late Sept) samples, but not in the earliest post-winter sample. It is likely that most, but not all, larvae reach the fourth instar before winter freezing, although we may have failed to collect any third instar larvae in our 7 June sample. Mean head capsule widths increased from 0.235 mm (n = 5) to 0.349 (n = 41) in the sample collected in late September 2010. The following year, mean head capsule width increased from 0.198 mm (n = 8) to 0.328 (n = 19) for larvae molting from the third to the fourth instar.

Thus mean head capsule width was 148% and 166% greater following the molt in these two samples where both third and fourth instars were present. These values are consistent with Dyar's rule, which states that head capsules increase in a regular linear progression by a ratio of 30% to 70% (Dyar and Rhinebeck 1890).

Sex-specific head capsule size also increased shortly after pond thaw. On June 7, mean head capsule size for male larvae was 0.312 mm (n = 26), comparable to 0.309 mm (n = 23) on June 10. The next sex-specific size measurements (on June 16) showed male head capsule size to have increased by 18% to 0.366 mm (n = 43), similar to 0.363 mm (n = 5) on June 19 (Tukeys, $p < 0.001$ for all comparisons between early-late groups and $p = 0.987$ and $p = 0.993$ separating early-early and late-late groups, respectively). Female larvae showed a similar pattern of increasing head capsule size shortly after pond thaw, but with a slight time delay. Mean head capsule width of female larvae was similar in the first two post-thaw samples (0.338 mm, n = 30 on June 7; 0.335 mm, n = 34 on June 10), but then increased by 14% in the next two samples (to 0.382 mm, n = 26 on June 16; 0.391, n = 7 on June 19: Tukeys, $p < 0.001$ for all comparisons between early-late groups and $p = 0.976$ and $p = 0.888$ separating early-early and late-late groups, respectively). These head capsule size increases of 18% and 14% are insufficient to meet the criteria of Dyar's ratio for ecdysis between instars (Dyar and Rhinebeck 1890).

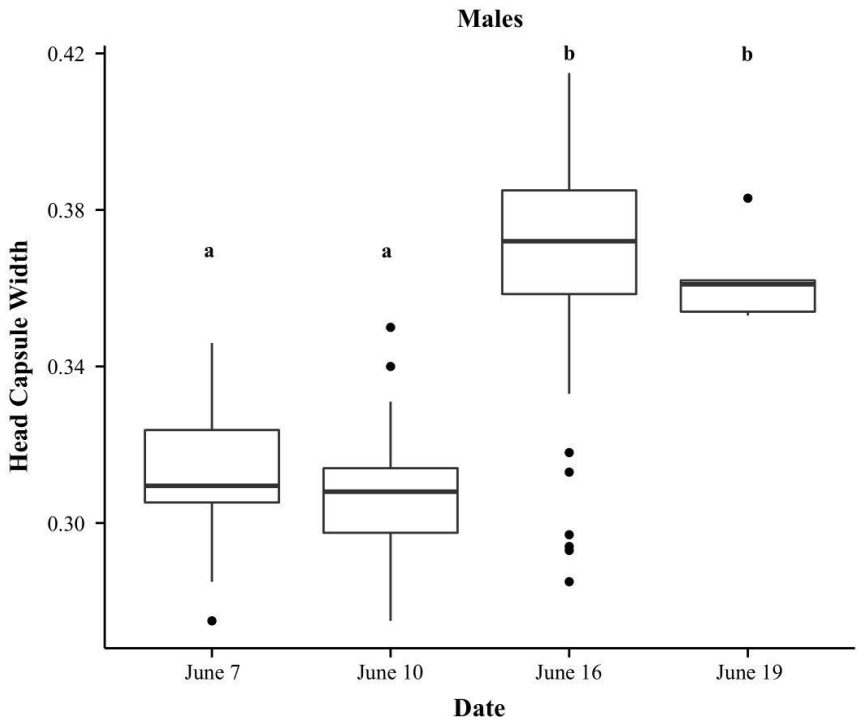

Figure 3.4. Post thaw head capsule size increase for males.

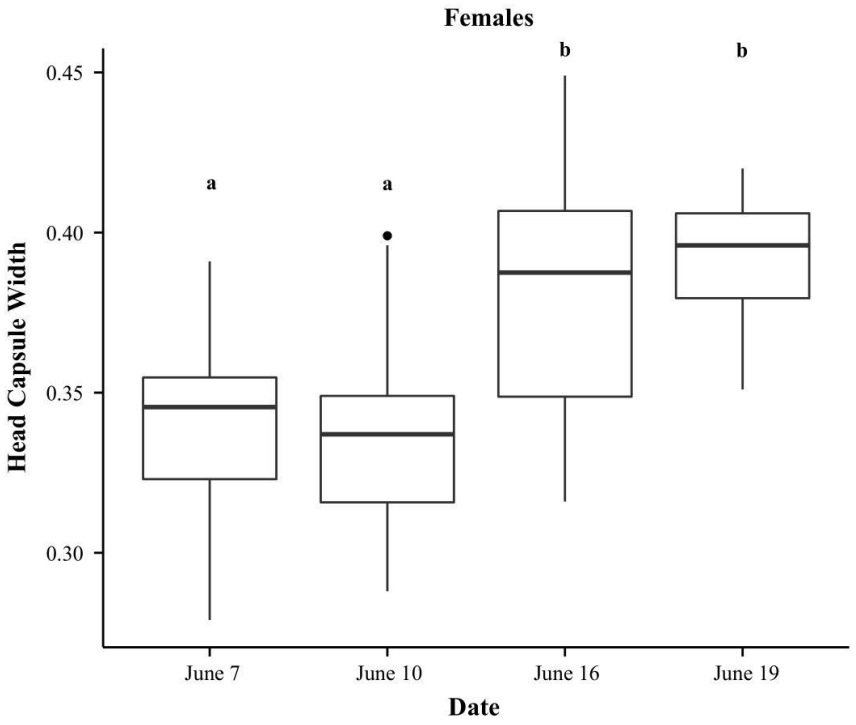

Figure 3.5. Post thaw head capsule size increase for females.
Letters denote significantly different groups (Tukey's test), which larvae appeared to molt between June 10 and June 16 in 2011. HCW is in mm.

3.5. Discussion

Larval feeding, growth, and development are all required early in the final summer before *T. alaskensis* can pupate and emerge. Most larvae overwinter in the fourth-instar, with most third-instar larvae apparently molting to the fourth-instar before the fall freeze. Absolute spring species, as defined by Danks & Oliver (1972), require no additional feeding after their habitat freezes, thus arresting development of mature, prepupal larvae is halted at a specific point for the entire population before pupation. Development is arrested during winter, and we documented no resumption through the first week after pond thaw. The second week after thaw, larvae grew and developed rapidly. About 10 days after thaw, all final-instar phases of maturation were observed in the population, but developmental synchronization increased markedly 13 days post-thaw, with adult emergence peaking 15-16 days after pond thaw. Thus nearly all final instar

development for *T. alaskensis* occurred between June 10 (4 days post-thaw) and June 19 (13 days post-thaw).

Larvae nearly doubled their overwintering mass during a period of ~9 days in their final summer. Such growth must require considerable feeding, as larvae grow faster during this period than at any other time of their one-year life cycle. Larvae failed to gain in weight in the first few days post-thaw when pond temperatures remained cool, but they did increase in size (as reflected by body area). This may have been the result of cuticle expansion in freshly-molted final instar larvae. Larvae decreased in weight in the final days before pupation, which seems consistent among chironomids that must arrest feeding to clear the gut in preparation for pupation (Servia et al. 2006). *T. alaskensis* doesn't achieve its characteristic emergence synchrony at winter diapause; rather population synchrony occurs very late in larval development. Butler (1980, 2000) documented a similar developmental pattern for the late-emerging midge *Tanytarsus nearcticus* (as *Tanytarsus inequalis*), where a similar fraction of larval growth and development occurred in the final year – but over a more extended period of 4-6 weeks.

An increase in head capsule width of 48-66% during the molt from the third to the fourth instar is consistent with Dyar's prediction for the size ration between two instars (Dyar and Rhinebeck 1890). The subsequent 14-18% increase we observed in sex-specific head capsule size might suggest yet a fifth instar, but this smaller size increase is not in keeping with Dyar's ratio. Supernumerary larval instars have been reported for other insect species, with size increases that often do not conform to Dyar's rule (Garcia-Barros 2006, Esperk et al. 2007). We are not presently aware of any studies reporting a fifth larval instar in the family Chironomidae.

Overwintering in freezing environments requires species to employ a suite of adaptive strategies. As ponds were beginning to freeze in mid-October of 2012, we searched but were

unable to find any *T. alaskensis* larvae in unfrozen sediments beneath a 10cm ice cover. The overwintering habitat of *Trichotanypus alaskensis* is unknown, but there seem two possibilities: Larvae may burrow deeply into the sediment as a strategy to limit some of the physical stresses of ice formation – although eventual winter freezing is inevitable in these ponds. Alternatively, larvae may migrate to the pond margins prior to freezing, overwintering in a narrow band where they can thaw with the earliest melt waters that pool around the pond circumference during spring thaw.

Trichotanypus alaskensis is adapted for growth and development under cold arctic conditions, and is one of the earliest to emerge in this cold-adapted community. Pre-emergent maturation of synchronously emerging insects in the tundra pond community may be regulated by different threshold temperatures. After the pond thaws, the water may warm sufficiently to meet demands for growth to resume, and development likely resumes a few °C above that. These threshold temperatures could keep the population at a common age, as no individuals would be maturing below the developmental zero threshold temperature. When the water does warm, larvae begin to grow rapidly, nearly doubling weight by the time they're mature enough for pupation. Most larvae are also relatively immature in the fourth-instar at thaw, which progresses rapidly once water temperature warms sufficiently for development to resume. The mechanism that triggers rapid development in the final summer, and the possibility of a fifth-instar, which would be unique to this species among chironomids, warrants further study.

3.6. References

Baek, M. J., T. J. Yoon, and Y. J. Bae. 2012. Development of *Glyptotendipes tokunagai* (Diptera: Chironomidae) Under Different Temperature Conditions. Environ. Entomol. 41: 950–958.

Boumans, L., and J. E. Brittain. 2012. Faunistics of stonelies (Plecoptera) in Finnmark, northern Norway, including DNA barcoding of Nemouridae. Nor. J. Entomol. 59: 196–215.

Butler, M. G., M. C. Miller, and S. Mozley. 1980. Macrobenthos, pp. 297–339. In Hobbie, J.E. (ed.), Limnol. Tundra Ponds, Barrow, Alaska. Dowden, Hutchinson & Ross, Stroudsburg, PA.

Corbet, P. S. 1964. Temporal patterns of emergence in aquatic insects. Can. Entomol. 96: 264–279.

Danks, H. V. 2007. The elements of seasonal adaptations in insects. Can. Entomol. 139: 1–44.

Danks, H. V., and D. R. Oliver. 1972. Seasonal emergence of some High Arctic Chironomidae (Diptera). Can. Entomol. 104: 661–686.

Dyar, H. G., and N. Y. Rhinebeck. 1890. The number of molts of lepidopterous larvae. Psyche (Stuttg). 420–422.

Esperk, T., T. Tammaru, and S. Nylin. 2007. Intraspecific variability in number of larval instars in insects. J. Econ. Entomol. 100: 627–45.

Forster, J., A. G. Hirst, and G. Woodward. 2011. Growth and development rates have different thermal responses. Am. Nat. 178: 668–78.

Garcia-Barros, E. 2006. Number of larval instars and sex-specific plasticity in the development of the small heath butterfly, *Coenonympha pamphilus* (Lepidoptera : Nymphalidae). Eur. J. Entomol. 103: 47–53.

Goddeeris, B. R., A. C. Vermeulen, E. De Geest, H. Jacobs, B. Baert, F. Ollevier, and E. D. Geest. 2001. Diapause induction in the third and fourth instar of *Chironomus riparius* (Diptera) from Belgian lowland brooks. Arch. für Hydrobiol. 150: 307–327.

Gotceitas, V., and H. F. Clifford. 1983. The life history of *Dicosmoecus atripes* (Hagen) (Limnephilidae: Trichoptera) in a rocky mountain stream of Alberta, Canada. Can. J. Zool. 61: 586–596.

Howmiller, R. P. 1972. Effects of preservatives on weights of some common macrobenthic invertebrates. Trans. Am. Fish. Soc. 101: 743–746.

Ineichen, H., B. Meyer, and M. Lezzi. 1983. Determination of the developmental stage of living fourth instar larvae of *Chironomus tentans*. Dev. Biol. 98: 278–86.

Jarosík, V., L. Kratochvíl, A. Honěk, and A. F. G. Dixon. 2004. A general rule for the dependence of developmental rate on temperature in ectothermic animals. Proc. Biol. Sci. 271 Suppl : S219–21.

Leuven, R. S. E. W., T. C. M. Brock, and H. A. M. van Druten. 1985. Effects of preservation on dry-and ash-free dry weight biomass of some common aquatic macro-invertebrates. Hydrobiologia. 127: 151–159.

Lobinske, R. J., A. Ali, and J. Frouz. 2002. Laboratory estimation of degree-day developmental requirements of *Glyptotendipes paripes* (Diptera: Chironomidae). Environ. Entomol. 31: 608–611.

Lougheed, V. L., M. G. Butler, D. C. McEwen, and J. E. Hobbie. 2011. Changes in Tundra Pond Limnology: Re-sampling Alaskan Ponds After 40 Years. Ambio. 40: 589–599.

Mackey, A. P. 1977. Growth and development of larval Chironomidae. Oikos. 28: 270.

Mochizuki, S., Y. Kayaba, and K. Tanida. 2006. Larval growth and development in the caddisfly *Cheumatopsyche brevilineata* under natural thermal regimes. Entomol. Sci. 9: 129–136.

Rempel, R. S., and J. C. H. Carter. 1987. Temperature influences on adult size, development, and reproductive potential of aquatic Diptera. Can. J. Fish. Aquat. Sci. 44: 1743–1752.

Servia, M., M. Heydorff, A. Péry, J. Garric, and L. Lagadic. 2006. Sex-and Developmental Stage-Related Changes in Energy Reserves in Fourth-instar Larvae of the Midge *Chironomus riparius* Meigen (Diptera: Chironomidae): Physiol. Ecol. 35: 865–874.

Strathdee, A. T., and J. S. Bale. 1998. Life on the edge: insect ecology in arctic environments. Annu. Rev. Entomol. 43: 85–106.

Wülker, W., and P. Götz. 1968. Die Verwendung der Imaginalscheiben zur Bestimmung des Entwicklungszustandes von Chironomus-larven (Dipt.). Zeitschrift für Morphol. der Tiere. 62: 363–388.

Yohannes, E., M. Valcu, R. W. Lee, and B. Kempenaers. 2010. Resource use for reproduction depends on spring arrival time and wintering area in an arctic breeding shorebird. J. Avian Biol. 41: 580–590.

4. CLIMATE-RELATED SHIFT IN EMERGENCE PHENOLOGY FOR ARCTIC CHIRONOMIDS OVER FOUR DECADES

4.1. Abstract

Climate warming has led to significant changes in species' phenology. Warming in the Arctic is predicted to be greater than in any other place on the planet. The dipteran family Chironomidae is the most species-rich arthropod taxon in tundra thaw ponds on Alaska's Arctic Coastal Plain. Chironomids in ponds near Barrow, Alaska display highly synchronous adult emergence, with most populations emerging from a given pond within a 3-5 day span and the bulk of the overall midge community emerging over a 3-4 week period. Using emergence phenology data collected in the 1970s and in the 2000s, we compared seasonal timing of chironomid emergence from freshwater ponds near Barrow, between data sets separated by nearly four decades. For a single, intensively studied pond, emergence of 14 most-abundant chironomid species advanced by an average of 8 days. Proportional composition of the chironomid community has changed somewhat in these ponds, but not the relative sequence of species' emergence peaks. Our analyses of phenology data from five ponds over four recent years indicate that 83% of the variance in chironomid emergence date could be explained by just three factors: species identity (44%), year (30%), and pond (9%). These 'year' and 'pond' effects likely were driven by varying dates of pond thaw, and differential accumulated degree-days in different year-pond combinations. We suggest that continued warming at this and other Arctic tundra sites may lead to further advancements in chironomid (and other insect) emergence timing. Coupled with potential shifts in aquatic community structure, avian consumers dependent on emerging tundra pond insects may experience changes in resource availability in a warmer Arctic.

4.2. Introduction

Climate warming has induced phenological shifts across a broad range of plant and animal taxa (Forrest and Miller-Rushing 2010, Bjorkman et al. 2015). Arctic environments are and will continue to experience the greatest degree of warming (Høye et al. 2007), and species are responding rapidly to these changes (Richter et al. 2008, Wheeler et al. 2015). Different species will likely respond differently to warming (Jensen 2003), yet most long-term arthropod studies from the Arctic take a broad taxonomic approach (Hodkinson et al. 1996, Meltofte et al. 2008, Tulp and Schekkerman 2008). We propose that a more precise taxonomic approach may reveal valuable information about phenological responses of individual species to rapid climate warming that is not discernable at broader taxonomic levels (Forrest and Miller-Rushing 2010).

Arctic arthropods are a seasonally abundant nutrient source for many vertebrates (Pearce-Higgins et al. 2005). Many arthropod taxa have shifted their phenology in response to a changing climate (Pearce-Higgins et al. 2005, Tulp and Schekkerman 2008), altering the period during which they're available to predators. Documenting changes shown by arthropods at higher taxonomic levels has proved useful for quantifying a baseline of phenological change (Pearce-Higgins et al. 2005, McKinnon et al. 2012, Bolduc et al. 2013), but is limited when investigating species' changes. The timing of peak arthropod abundance at an arctic site on Siberia's Taimyr Peninsula was best predicted by date of snow cover loss (Høye and Forchhammer 2008). At Barrow, Alaska, snow cover has been both decreasing in amount, and melting earlier, over the past several decades (Stone et al. 2005).

Species-level studies on arctic arthropods are rare, as family-level data are often sufficient to test many climate-related hypotheses (Høye and Forchhammer 2008). This is despite the fact that species-level studies allow investigation into the drivers of variation in

phenology, and may elucidate potential mechanisms of phenological synchronization (Høye et al. 2014). Phenological synchronization can be important for maximizing reproductive encounters (Maclean and Pitelka 1971), for parasitizing a specific host (Yukawa et al. 2013) or some other predator-prey-pollinator interaction (Visser et al. 2010, Yukawa et al. 2013). Arctic midge species have adult lives lasting only a few days, requiring species to emerge synchronously to reproduce successfully (Danks and Oliver 1972, Welch 1973, Butler 1980a).

The insect community in tundra ponds near Barrow, Alaska is typically composed of a dozen or more relatively abundant chironomid midge species, with non-chironomid insects like beetles, stoneflies, and caddis flies being quantitatively rare (Butler 1980b, Butler et al. 1980, Lougheed et al. 2011). Here we use a long-term (1975-1977; 2009-2013) dataset on species-level chironomid emergence to address two objectives. First, we test the hypothesis that at the family level, chironomids are emerging earlier in the 2000's than they were during the 1970's. Second, we investigate which environmental variables are most important in explaining emergence variability by comparing detailed emergence patterns in five ponds across four recent years (2009-2011 and 2013). We hypothesize that pond-specific variation in emergence phenology may result from differences in pond morphology and landscape setting that determine both the timing of a pond's thaw and its subsequent thermal behavior; year-specific differences will reflect variable thaw dates and heat inputs based on stochastic annual weather characteristics. We expect taxonomic identity to be the most important explanatory variable influencing individual midge emergence timing, because of species-specific life-history patterns.

4.3. Materials and Methods

4.3.1. Invertebrate Collection

In 1975, 1976, and 1977, quantitative emergence traps were used to collect emerging adult insects (and associated pupal exuviae) in Pond J (71°17'37.23"N, 156°42'5.99"W) and two adjacent ponds near Barrow, AK. A full description of methods can be found in Butler (1980a, 1980b). Traps were placed in both the open water and the emergent-*Carex* zone of the three ponds. In the summers of 2009-2013, we expanded the number of ponds surveyed and instead of using emergence traps, used a standardized sweep-net procedure similar to that described by Corbet & Danks (1973). Sampling was conducted with a circular dipnet with area roughly 177cm2 and mesh size <100 µm. Five pooled, 1-meter sweeps were taken at the water's surface along the leeward shore, based on the recent predominant wind direction. Sweeps were collected with the net half submerged ensuring collection at the net's maximum diameter, and targeted evident traces of accumulated surface flotsam. Samples were collected at 2-day (occasionally 3-day) intervals. A comparison of both trap- and sweep-net sampling techniques was conducted in 2011, by re-deploying the emergence traps used by Butler in the 1970s in three ponds where pupal exuviae were sampled simultaneously.

Samples during 2009-2013 were collected from five ponds, with three (Ponds C, G, and J) located on the historic International Biological Program (IBP) study site (Hobbie 1980). Two other ponds (Bear and Humpback) were located approximately 2 km southeast of the IBP site, at the SW corner of the Barrow Environmental Observatory ((BEO); 71°16'38.50"N; 156°38'32.68"W Table 1). Pond J was the only site sampled in all years (1975-1977; 2009-2013).

Monitoring of insect emergence usually began less than a week after pond thaw, with the first emergence typically occurring more than two weeks after thaw. Emergence timing was monitored by collection of chironomid pupal exuviae (shed pupal skins) that indicate recent (past 2-3 days) emergence of individual species (Bouchard and Ferrington 2011). Sampling these exuviae is preferred over sampling adults for several reasons. Unlike adult insects that differ in their propensity to fly away, pupal exuviae are left behind by all species when adult midges emerge at the water surface. Exuviae from all microhabitats in a pond are integrated by wind drift and remain intact in leeward flotsam until bacteria begin to decompose the outer waxy layer of the pupal cuticle (Ferrington et al. 1991). Large numbers of exuviae representing all recently emerging species can be collected easily. Species identification using pupal exuviae is often easier and faster than any other character (Coffman & de La Rosa 1998; Siqueira et al. 2008).

4.3.2. Temperature Data

HOBO brand underwater temperature loggers (Model U-22) were weighted and placed on the sediment surface in all study sites, and set to record temperatures on an hourly basis. Most loggers remained in the pond overwinter, and recorded temperatures throughout the year. There was a noticeable shading effect in some logger locations, and surrogate data were used from thermally similar ponds in some instances. We placed loggers in open-water areas, away from emergent *Carex aquatilis* and *Arctophila fulva* stands (where we observed reduced water mixing). Given such microhabitat effects on local temperatures, our temperature data best reflect the thermal exposure of chironomid taxa found in the surface sediments of the open water zone (Butler et al. 1980).

Despite having recorded hourly temperature values, we used daily average temperature values to represent environmental conditions due to our daily sampling interval. Samples were

typically collected early to mid-afternoon, and our daily average temperature reflected logged values during the prior 24hr time period.

4.3.3. Data Analysis

Phenological changes between two decades - We used a paired t-test (paired by species) for the average date of emergence during 1975-1977 (the "early decade") relative to the average date of emergence during 2009-2013 (the "late decade"). In essence, the between-decade difference in average emergence date was computed by averaging, for each species, mean emergence date for all years in the early decade, and again for all years in the late decade. A t-test was then used to test the null hypothesis that the mean difference for all species between the early- and late-decade is zero (implying no shift in overall midge emergence phenology). Given that Pond J was the only pond surveyed both in the early and late decades, this test for a phenological shift was restricted to Pond J only.

Using these same data, we ranked species by order of emergence separately for the early and late decades, and tested the null hypothesis that the order of species' emergence was the same in the two decades using Spearman's rank correlation test. Finally, we analyzed for a shift in community structure in Pond J between the two decades by summing the total captures for a species separately in the early and late decades, then used a chi-square analysis to test the null hypothesis that the count frequencies by species were the same in both decades. Analyses were performed using the R 3.2.2 base package.

Variance partitioning for emergence phenology - We used a random effects model to partition variance in emergence date among three factors: 'taxon' (29 species), 'pond' (5 ponds), and 'year' (5 years). For these analyses, we used only the late decade data (2009-2013). The model was run twice, once using the actual Julian date for emergence for each animal collected,

and next using the difference between the Julian date for emergence and the Julian date for thaw during a given year. We used published snowmelt records from NOAA as our date of 'thaw'. We hypothesized that the majority of variance attributable to the factor 'year' results from variation in the thaw date at Barrow for that year. By running the model both ways, we can estimate how much of the 'year' variance can be attributed to thaw date. The random effects model was run using the lme4 package in R 3.2.2.

We expected species identity (the 'taxon' effect) to be an important determinant of chironomid emergence timing, given the predictable annual sequence of species' emergence peaks within each decade. Species presence and abundance varies among ponds and years; to visualize this variation we created a heat map based on log transformed total counts collected for each species by year and pond. The heat map was created using the base package in R 3.2.2.

4.4. Results

4.4.1. Chironomid Community Compositional Differences

Overall community composition of emerging chironomids was generally similar between the 1970s and the 2000s, with some differences in detection of rare taxa and the relative abundances of some of the most common taxa. We collected over twice as many individuals annually in our late decade samples (2,251/yr) as in the early decade (886/yr). Nine taxa (*Cryptochironomus* sp., *Conchapelopia* sp., *C. riparius*, *Cricotopus* sp. 3, *Tanytarsus* sp. 2, *Psectrocladius* sp. 2, *Parakiefferiella* sp., *Lapposmittia* sp., and *Limnophyes* sp.) were present in Pond J in the late decade but absent during all three years of the early decade, and a single taxon (*Orthocladius* sp.) was present in the early decade but absent from our Pond J samples in the late decade. After eliminating these species to generate a set of common taxa between the decades, we were left with 14 taxa of appreciable abundance for which we are confident of consistent

taxonomic identification across all years (Table 1). A χ^2 test indicated that frequency of capture for taxa differed between the late and early decades ($\chi^2_{16} = 2218, p < 0.001$). Driving this difference in proportional abundances between decades were the early-emerging *T. aquavolans*, which represented 39.9% of animals captured in the late period but only 15.3% of the animals captured in the early period, and the early-emerging *P. penicillatus*, which represented 35.3% of the animals in the early period but only 25.3% of the animals in the late period. All other species had proportional differences of less than 10% (Table 4.1).

Table 4.1. Chironomid composition and mean emergence date near Barrow, AK.

			Days	Mean Emergence		Total Counts	
Tribe	Genus	Taxon	Shifted	1975-1977	2009-2013	1975-1977	2009-2013
Chironomini	*Chironomus*	*C. prior*	-9.7	7/10	6/30	37	144
		C. tardus	-3.8	7/14	7/11	124	70
	Stictochironomus	*Stictochironomus* sp.	-7.5	7/5	6/27	12	7
Tanytarsini	*Cladotanytarsus*	*Cladotanytarsus* sp.	-12.4	7/12	6/30	122	104
	Constempellina	*Constempellina* sp.	-3.9	7/6	7/2	173	507
	Paratanytarsus	*P. penicillatus*	-1.4	7/4	7/2	939	2732
	Tanytarsus	*T. aquavolans*	-9.6	7/4	6/25	406	4490
		T. nearcticus	-15.7	7/23	7/8	566	1456
Metriocnemini	*Corynoneura*	*Corynoneura* sp.	-9.5	7/17	7/8	78	732
Orthocladini	*Psectrocladius*	*Psectrocladius* sp. 1	-5.5	7/9	7/4	25	202
Macropelopini	*Derotanypus*	*D. aclines*	-10.6	7/9	6/28	1	15
		D. alaskensis	-8.0	7/10	7/2	22	50
	Procladius	*P. prolongatus*	-0.3	6/29	6/28	26	52
		P. vesus	-16.6	7/20	7/3	4	40
Boreochlini	*Trichotanypus*	*T. alaskensis*	-7.2	7/1	6/24	84	458

Fourteen chironomid species collected from Pond J near Barrow, AK that were tested for shifts in emergence phenology between 1975-1977 and 2009-2013. 'Days shifted' indicates the difference in Julian date for mean emergence of a species during 2009-2013 relative to 1975-1977. A negative shift indicates earlier emergence in the recent years; zero days indicates no phenological change. Mean emergence within each decade is given as calendar date (mo/day). Total counts are the total number of emerging specimens of each taxon collected during each decade, with percent contribution to the total emerging fauna in parentheses.

4.4.2. Phenological Changes Between Two Decades

We found that the average chironomid emerged 6.12 days earlier (e.g. a shift from July 8 to July 2) in the late decade relative to the early decade ($t16=3.447$, $p < 0.01$). The shift was most pronounced for *P. vesus* and *T. nearcticus* (mean emergence date over two weeks earlier), followed by *Cladotanytarsus* sp., *D. aclines*, *C. prior*, *T. aquavolans*, *Corynoneura* sp., *D. alaskensis*, *T. alaskensis* (one to two weeks earlier), and finally *Psectrocladius* sp. 1, *Constempellina* sp., *C. tardus*, *P. penicillatus*, and *P. prolongatus* (less than one week earlier). Such variable advances in mean emergence dates caused some shifts in the temporal sequence of species' emergence peaks. We detected significant differences in the emergence sequence for species (Fig. 4.1; Spearman $r = 0.726$, $t_{13}=3.835$, $p < 0.01$).

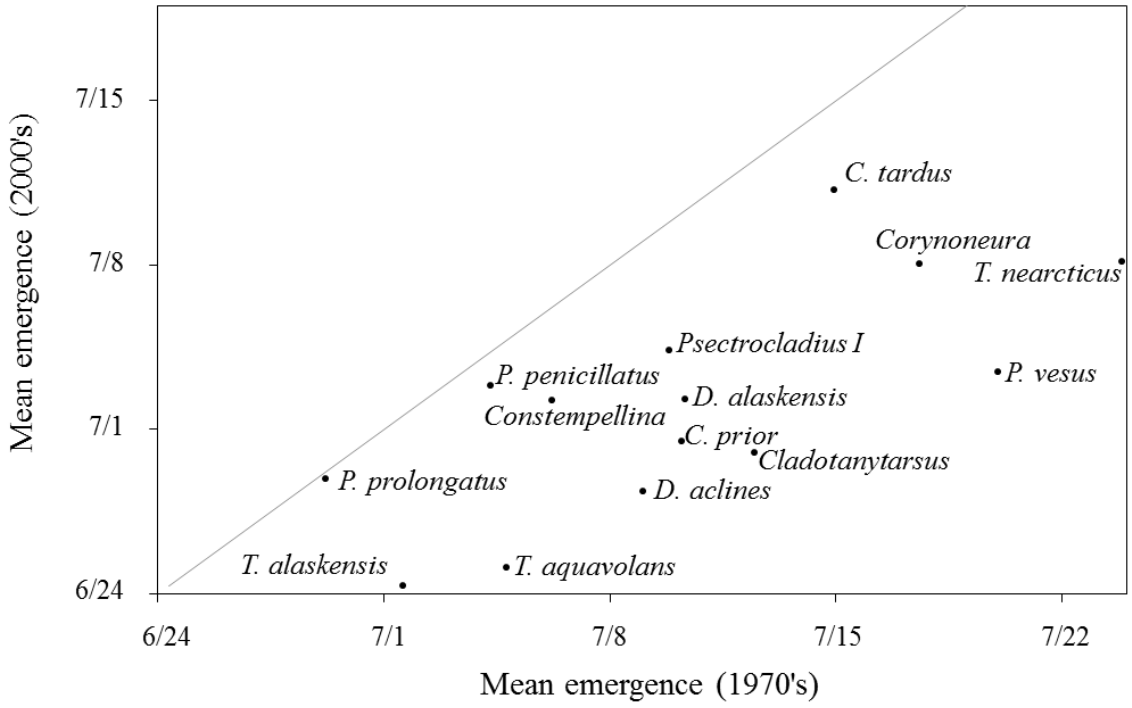

Figure 4.1. Biplot comparing emergence timing between 2000s and 1970s for 14 most-abundant midge species. The point closest to each species name indicates mean emergence dates in the two decades. As all points fall below the grey 1:1 line, all these species emerged earlier in 2009-2013 than in 1975-1977.

4.4.3. Explained Variation in Emergence Timing

The Julian date on which an individual emerged was regressed against the random effects 'year' (2009-2013), 'pond' (C, G, J, Bear or Humpback), and 'taxon'. This three-factor random effects model explained 83.3% of the variance in emergence timing. The factor 'taxon' was most important in predicting emergence timing (44.1% of explained variance), followed by 'year' (29.8%), and pond (16.7%). Rerunning the model to predict Julian date post thaw as the response variable (rather than the true Julian date), reduced the variance attributed to 'year' from 29.8% to 5.7%. This suggests that 80.1% of the 'year effect' in the first model can be attributed to annual differences in the date of thaw (as indicated by the NOAA-recorded date of snowmelt at Barrow; https://www.climate.gov/#dataServices).

4.5. Discussion

The objective of our study was to determine whether emergence of Barrow pond chironomids has changed during the past 40 years, and to identify the primary correlates of seasonal emergence timing. From 1975-1977 to 2009-2013, we found that the average date of adult midge emergence has advanced approximately 6 days for all taxa, with 13 of the 14 individually-analyzed taxa emerging in recent years ahead of their historic date from the 1970s. One species (*Procladius prolongatus*) showed no change in mean emergence date, and no species emerged later during the recent decade. We found taxonomic identification to be the most useful variable in explaining the variation in midge emergence timing, with year-to-year variation secondarily important, and pond-to-pond variation explaining a small, but significant, amount of the variation we observed.

Temperature should affect pre-emergent developmental rate similarly among these chironomids – with most species likely requiring a certain total degree day product that is

independent of daily average temperature. Thus species's developmental rates should respond similarly to changes in temperature. However, we did not observe a constant change in developmental response, rather some species appear to emerge earlier than their historical average, and others emerge later. These species-specific responses are likely the result of species-specific differences in the amount of growth and development required to reach pupation following pond thaw many nuances in pond thermal heterogeneity, species specific behaviors (e.g., microhabitat selection). Additionally, some species may have nutritional requirements after winter in their year of emergence, as shown for *Trichotanypus alaskensis* (Chapter 2). Thus the availability of food resources to support larval growth, in addition to thermal control of developmental processes, may influence emergence timing.

The effect of pond explained the least amount of variation but still accounted for nearly 10% of the variation in emergence timing. Ponds like G and Humpback are considerably deeper than the other sites, and tend to form a thermocline, which creates habitat heterogeneity, and likely accounts for the significant contribution of 'pond' to our model. Ponds C, J, and Bear, are all similar in fetch and depth, and thus have a similar composition of species and the variation in emergence timing is likely due to the date the pond thawed.

The strong phenological shift of chironomids in response to climate change over the last several decades, and the clear differences in the degree of shift among our most abundant species, suggest that species-specific responses should be a high priority in arctic arthropod studies. The overall chironomid community has now shifted its emergence about one week earlier, with a considerable variability in the magnitude of this shift among species. The three most abundant genera in the Barrow ponds we studied (*Tanytarsus aquavolans*, *T. nearcticus* and *Paratanytarsus penicillatus*) have all shifted toward earlier adult emergence, yet these shifts

range from a only one day for *P. penicillatus* to an advance of more than two weeks for *T. nearcticus*. Both long-term and more detailed studies of arctic arthropods will be particularly helpful in identifying the influence climate change may have on the seasonal abundance of arthropod populations in the future Arctic (Høye et al. 2014).

4.6. References

Bjorkman, A. D., S. C. Elmendorf, A. L. Beamish, M. Vellend, and G. H. R. Henry. 2015. Contrasting effects of warming and increased snowfall on Arctic tundra plant phenology over the past two decades. Glob. Chang. Biol. 21: 4651–4661.

Bolduc, E., N. Casajus, P. Legagneux, L. McKinnon, H. G. Gilchrist, M. Leung, R. I. G. Morrison, D. Reid, P. a. Smith, C. M. Buddle, and J. Bêty. 2013. Terrestrial arthropod abundance and phenology in the Canadian Arctic: modelling resource availability for Arctic-nesting insectivorous birds. Can. Entomol. 145: 155–170.

Bouchard, R. W., and L. C. Ferrington. 2011. The effects of subsampling and sampling frequency on the use of surface-floating pupal exuviae to measure Chironomidae (Diptera) communities in wadeable temperate streams. Environ. Monit. Assess. 181: 205–23.

Butler, M. G. 1980a. Emergence phenologies of some arctic Alaskan Chironomidae. Chironomidae Ecol. Syst. Cytol. Physiol.

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

Butler, M. G., M. C. Miller, and S. Mozley. 1980. Macrobenthos, pp. 297–339. In Hobbie, J.E. (ed.), Limnol. Tundra Ponds, Barrow, Alaska. Dowden, Hutchinson & Ross, Stroudsburg, PA.

Danks, H. V., and D. R. Oliver. 1972. Seasonal emergence of some High Arctic Chironomidae (Diptera). Can. Entomol. 104: 661–686.

Ferrington, L. C., M. A. Blackwood, C. A. Wright, N. H. Crisp, J. L. Kavanaugh, and F. J. Schmidt. 1991. A protocol for using surface-floating pupal exuviae of Chironomidae for rapid bioassessment of changing water quality. Sediment Stream Water Qual. a Chang. Environ. Trends Explan. 181–190.

Forrest, J., and A. J. Miller-Rushing. 2010. Toward a synthetic understanding of the role of phenology in ecology and evolution. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 365: 3101–12.

Hinzman, L. D., N. D. Bettez, W. R. Bolton, F. S. Chapin, M. B. Dyurgerov, C. L. Fastie, B. Griffith, R. D. Hollister, A. Hope, H. P. Huntington, A. M. Jensen, G. J. Jia, T. Jorgenson, D. L. Kane, D. R. Klein, G. Kofinas, A. H. Lynch, A. H. Lloyd, a. D. McGuire, F. E. Nelson, W. C. Oechel, T. E. Osterkamp, C. H. Racine, V. E. Romanovsky, R. S. Stone, D. a. Stow, M. Sturm, C. E. Tweedie, G. L. Vourlitis, M. D. Walker, D. a. Walker, P. J. Webber, J. M. Welker, K. S. Winker, and K. Yoshikawa. 2005. Evidence and Implications of Recent Climate Change in Northern Alaska and Other Arctic Regions. Clim. Change. 72: 251–298.

Hobbie, J. E. 1980. Limnology of tundra ponds, Barrow, Alaska. Dowden, Hutchinson & Ross, Stroudsburg, PA.

Hodkinson, I. D., S. J. Coulson, N. Webb, W. Block, A. Strathdee, J. S. Bale, and M. R. Worland. 1996. Temperature and the biomass of flying midges (Diptera: Chironomidae) in the high Arctic. Oikos. 75: 241–248.

Høye, T. T., A. Eskildsen, R. R. Hansen, J. J. Bowden, N. M. Schmidt, and W. D. Kissling. 2014. Phenology of high-arctic butterflies and their floral resources: Species-specific responses to climate change. Curr. Zool. 60: 243–251.

Høye, T. T., and M. Forchhammer. 2008. Phenology of high-arctic arthropods: Effects of climate on spatial, seasonal, and inter-annual variation. Adv. Ecol. Res. 40: 299–324.

Høye, T. T., E. Post, H. Meltofte, N. M. Schmidt, and M. C. Forchhammer. 2007. Rapid advancement of spring in the High Arctic. Curr. Biol. 17: R449–51.

Jensen, M. N. 2003. Consensus on Ecological Impacts Remains Elusive. Science (80-.). 299: 299.

Lougheed, V. L., M. G. Butler, D. C. McEwen, and J. E. Hobbie. 2011. Changes in Tundra Pond Limnology: Re-sampling Alaskan Ponds After 40 Years. Ambio. 40: 589–599.

Maclean, S. F., and F. A. Pitelka. 1971. Seasonal Patterns of Abundance of Tundra Arthropods near Barrow. Arctic 24:19-40

McKinnon, L., M. Picotin, E. Bolduc, and J. Bêty. 2012. Timing of breeding, peak food availability, and effects of mismatch on chick growth in birds nesting in the High Arctic. Can. J. Zool. 90: 961–971.

Meltofte, H., T. T. Høye, and N. M. Schmidt. 2008. Effects of food availability, snow and predation on breeding performance of waders at Zackenberg. Adv. Ecol. Res. 40: 325–343.

Pearce-Higgins, J. W., D. W. Yalden, and M. J. Whittingham. 2005. Warmer springs advance the breeding phenology of golden plovers Pluvialis apricaria and their prey (Tipulidae). Oecologia. 143: 470–6.

Richter, O., F. Suhling, O. Müller, and D. Kern. 2008. A model for predicting the emergence of dragonflies in a changing climate. Freshw. Biol. 53: 1868–1880.

Stone, R., D. Douglas, G. Belchansky, and S. Drobot. 2005. Correlated declines in Pacific Arctic snow and sea ice cover. Arct. Res. United States. 12: 18–25.

Tulp, I., and H. Schekkerman. 2008. Has Prey Availability for Arctic Birds Advanced with Climate Change? Hindcasting the Abundance of Tundra Arthropods Using Weather and Seasonal Variation. 61: 48–60.

Visser, M. E., S. P. Caro, K. van Oers, S. V Schaper, and B. Helm. 2010. Phenology, seasonal timing and circannual rhythms: towards a unified framework. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 365: 3113–27.

Welch, H. E. 1973. Emergence of Chironomidae (Diptera) from Char Lake, Resolute Northwest Territories. Can. J. Zool. 51: 1113–1123.

Wheeler, H. C., T. T. Høye, N. M. Schmidt, J.-C. Svenning, and M. C. Forchhammer. 2015. Phenological mismatch with abiotic conditions—implications for flowering in Arctic plants. Ecology. 96: 775–787.

Yukawa, J., K. Nakagawa, T. Saigou, T. Awa, T. Fukuda, and M. Higashi. 2013. Adult behavior of an ambrosia gall midge Illiciomyia Yukawai (Diptera: Cecidomyiidae) and synchronization between its emergence and host plant phenology. Entomol. Sci. 16: 400–412.