Anthropogenic change is affecting the seasonal biology of many organisms from plants and insects to birds and mammals (Scheffers et al. 2016, Pecl et al. 2017). These impacts on organisms are due to both long term-climatic changes, and changes in land use patterns ranging from intensification of agriculture to urbanization. While global change is often thought of colloquially as "warming" and is associated with higher summer temperature extremes, many facets of seasonality are affected (Vasseur et al. 2014, Williams et al. 2015). In temperate regions, the duration of the growing season is expanding and the winter cold period is shrinking with over-winter temperatures rising substantially, especially nighttime temperatures (Williams et al. 2015). Furthermore, temperatures on the seasonal shoulders in fall and spring are becoming overall warmer and more variable, with relatively warm periods interspersed with a greater frequency of extreme events ranging from severe storms to cold fronts (Easterling et al. 2000, Vasseur et al. 2014). These changes in climate are increasingly being recognized as major drivers of species redistribution, community assembly, and ecosystem function (Scheffers et al. 2016, Pecl et al. 2017).

One of the ways that increasing temperatures affect ectotherms, including insects, is by directly increasing their metabolic rates (Sinclair 2015). Increasing metabolic rates during the growing season has been associated with faster population growth rates and greater food consumption to support growth, reproduction, and body maintenance (Deutsch et al. 2018). These increases in ectotherm metabolic rates are expected to not only affect ectotherm population parameters including lifecycle timing, voltinism, and population sizes, but also to have cascading effects on communities and potentially whole-ecosystem function (Scheffers et al. 2016, Pecl et al. 2017). For example, it has been suggested that increased insect metabolic rates in the growing season as a result of projected climate change, combined with greater insect population sizes, will directly contribute to major increases in insect-driven losses in three critical human food crops: corn, rice, and wheat in coming decades (Deutsch et al. 2018).

In addition to increases in insect metabolism during the growing season, warmer winters are also expected to increase the metabolic rates of dormant insects (Sinclair 2015). Insects use a diversity of behavioral and physiological strategies to survive inclement periods that are unfavorable to growth and reproduction, but the two most common types of dormancy are environmentally induced quiescence and diapause (Denlinger 2002, Kostal 2006). During environmentally induced quiescence, an insect's growth and reproduction is halted directly by the environment, such as low temperature or lack of resources (Kostal 2006). For example, females of the spotted-wing *Drosophila*, *D. suzukii*, overwinter as non-reproducing adults (Toxopeus et al. 2016, Everman et al. 2018). However, these quiescent overwintering adults can begin reproduction as soon as environmental conditions are permissive for reproduction including permissive temperatures and adequate resources for adult feeding and oviposition (Rossi-Stacconi et al. 2016, Wallingford et al. 2018).

Insect diapause by contrast is a pre-programmed developmental pathway where insects will interrupt growth or reproduction before the onset of inclement conditions (Denlinger 2002, Kostal 2006). While in diapause, insects will be refractory to resuming growth or reproduction even if conditions become permissive. Diapause can either be an obligate part of an insect's lifecycle that is expressed every generation, or it can be induced flexibly in response to environmental cues that signal the onset of an inclement period. Univoltine insects typically use a form of obligate diapause that is constitutively expressed each generation to synchronize the short growing and reproductive portions of their lifecycle with seasonal resource availability (Nechols et al. 1999). In contrast, many multivoltine insects will develop continuously, fitting multiple generations into a single growing season. Then in response to environmental cues that predict the onset of an inclement period, such as photoperiod or host plant quality, facultative diapausing species will enter the diapause developmental trajectory (Nechols et al. 1999). The diapause developmental trajectory incudes a series of distinct developmental stages, including: the diapause preparatory phase, the diapause maintenance phase, and diapause termination (Kostal 2006, Kostal et al. 2017).

Most diapausing insects do not feed during the long dormant period. Thus, metabolic suppression is a near universal hallmark of entering into the diapause maintenance phase (Hahn and Denlinger 2007, Sinclair 2015). Despite this metabolic suppression, diapause can be long and insects must take sufficient nutrient reserves into diapause to both sustain metabolic demands during dormancy and to support post-diapause functions including development, dispersal, and acquisition of new resources after diapause has been terminated. Many facultative-diapausing insect species accumulate greater nutrient reserves compared to their non-diapausing counterparts as part of the diapause preparatory phase (Hahn and Denlinger 2007, Sinclair 2015), although not all species do so. This observation of accumulating greater nutrient reserves as part of the diapause preparatory program is consistent with the greater metabolic demands of surviving dormancy and the need to execute post-dormancy functions compared to individuals of the same species undergoing continuous development.

Given that most diapausing insects act as sealed systems, taking the resources they need for both maintenance during diapause and post-diapause functions with them into dormancy, increasing temperatures during dormancy are expected to put greater demand on stored metabolic fuels (Hahn and Denlinger 2011, Sinclair 2015, Xiao et al. 2017). If metabolic stores are limiting, increasing metabolism due to higher ambient temperatures either overwinter or during the seasonal shoulders in spring and fall is expected to affect diapausing insects negatively in two ways. First, insects may run out of metabolic reserves during dormancy, reducing survival. Second, even if insects survive dormancy, greater metabolism during dormancy could reduce the resources insects have available for investment into post-diapause functions including development, dispersal, resource acquisition, mate finding, and eventually reproductive output. For – example of ellers here, and another example of warming depleting stores.

Of course, dormant insects are not a uniform group and not all will experience equivalent changes in overwintering in the future. Climate warming may increase metabolism and fuel use dramatically in some species or populations, while others may experience no change in metabolic demand due to factors such as microhabitat buffering. Some insects may even experience reduced overwinter metabolic demand as the insulating effects of snow cover are removed they experience overall cooler temperatures during dormancy (Williams et al. 2015, Sinclair 2015). But, the general trend for insects in temperate regions is expected to be towards warmer, more metabolically demanding winters.

Insects may respond to increased metabolic demand during dormancy by adaptively further reducing metabolic rates and/or increasing nutrient stores (i.e., increasing fuel economy, increasing the quantity of fuel in the tank, or a combination of both). Climate change has already been shown to exert strong selective pressure on insect diapause, with clear examples of diapause timing rapidly evolving to match shifting phenologies (Bradshaw and Holzapfel 2001, Gomi et al. 2007). Thus, we expect that selection may also drive adaption to mitigate increasing metabolic demand in insect populations. Although to our knowledge there are no studies clearly showing heritable changes in metabolic rates or nutrient reserves in insect populations in response to climate change, there are clear examples of both metabolic rates during dormancy and nutrient reserves varying across geographic populations in patterns that are consistent for selection on metabolic demand. For example, individuals from a population of the hesperiid butterfly *Erynnis propertius* from a location with high thermal variation in the fall period of their dormancy showed less thermal sensitivity in their metabolic responses to increased temperatures than a population from a location that experienced less high temperature events in the fall (Williams et al. 2012). This observation is consistent with local adaptation in the sensitivity of metabolic rate to temperature to compensate for greater metabolic demand in the more thermally variable site.

If we are to expect populations will undergo selection for greater metabolic efficiency during diapause or greater metabolic fuel storage prior to diapause to compensate for the greater metabolic demands along with warmer dormancy conditions occurring as a product of climate change, there must be sufficient genetic variation for these metabolic traits segregating in natural populations. There is much evidence for heritable variation in the propensity to enter diapause or the duration of diapause segregating both within and among in insect populations (Feder and Filchak 1999, Bradshaw and Holzapfel 2001, Gomi et al. 2007, Ito 2007, Dopman 2010, Xiao et al. 2015, and reviewed by Denlinger et al 2017 for just a few examples). However, to our knowledge there are no studies demonstrating heritable variation in overwintering energy reserves or metabolic efficiency in diapause-destined individuals. One way to assess the extent to which there is heritable variation in overwintering fuel stores that could be selected upon during adaptation to warmer, more metabolically demanding dormancy periods is to test for associations between current, naturally segregating genetic variation in diapause length and the quantity of nutrient reserves stored a the onset of diapause and/or the degree of metabolic suppression in dormancy. Our expectation is that longer-diapausing genotypes will possess greater nutrient reserves at the onset of diapause to help sustain them through the longer non-feeding diapause period than shorter-diapausing genotypes.

Here we test for an association between the duration of overwintering diapause and the quantity of nutrient stores accumulated prior to diapause in two genetically distinct strains of the European corn borer, *Ostrinia nubilalis* (Hübner). While the European corn borer is not native to the North America, it has been established in North America for over 100 years through several introductions (Caffrey and Worthley, Mutuura and Munroe 1970). Although most noted by it's association with large scale agricultural production of corn, *O. nubialis* can feed on many different wild host plants (Ponsard et al. 2004), and there is some evidence for genetically determined host plant specialization segregating among some populations in Europe (Bethenod et al. 2005; Malausa et al. 2008). In North America, populations of *O. nubialis* can further be distinguished by the composition of female mating pheromones (Thomas et al. 2003, Tabata and Ishikawa 2011, Koutroumpa et al. 2016, Martin et al. 2016). Sex pheromone biosynthesis in European corn borer females involves the β-oxidation of palmitic acid into (E)-11-tetradecenoyl and (Z)-11-tetradecenoyl precursors that are reduced into their corresponding fatty alcohols, then acylated into a pheromone molecule (Lassance et al. 2010). The specific ratio of precursor molecules converted into pheromone molecules differs between two naturally segregating Z-chromosome genetic variants because of allelic variation in a critical enzyme responsible for pheromone synthesis (Lassance et al. 2010). The higher concentration of (Z)-11-tetradecenyl acetate in the Z-strain sex pheromone blend is due to the affinity of (Z)-11-tetradecenoyl precursors to the fatty acid reductase enzyme produced from the *pgFAR-Z* allele (Lassance et al. 2010). Alternatively, the high concentration of (E)-11-tetradecenyl acetate characteristic of the E-strain is due to the increased affinity of (E)-11-tetradecenoyl precursors to the fatty acid reductase produced from the *pgFAR-E* allele(Lassance et al. 2010).

Beyond pgFAR, a number of other loci that contribute to reproductive isolation of the Z and E-pheromone races are co-localized to a region of the Z-chromosome that shows reduced recombination indicative of inversion polymorphism (Dopman 2010, Wadsworth et al. 2015, Kozak et al. 2017). Loci residing in this Z-chromosome inversion also regulate male preference for Z or E pheromones, and seasonal adult flight timing (Dopman 2010, Wadsworth et al. 2015, Kozak et al. 2017). Although there are many places where one may find only one pheromone race or the other at locations throughout their range in North America, the E and Z-pheromone host races sometimes co-occur sympatrically (Klun and Cooperators 1975; Kochansky et al. 1975; Carde et al. 1978; Anglade and Stockel 1984). Where the two pheromone strains co-occur, adults of the E-strain appear earlier in the season than adults of the Z- strain (Levy et al. 2015). For example, in a region where both pheromone strains co-occur in upstate New York, the E-strain emerges as adults earlier in the summer than the Z-strain with the two strains having distinct, but slightly overlapping flight times wherein .

Allochronic isolation

stems from differences in the number of generations per

season (voltinism), in which bivoltine E-strain popula tions have one generation at the beginning of the season

(June) and a second generation at the end of the season

(August), whereas univoltine Z-strain insects have single

generation in the middle of the summer (July) (Eckenrode

et al., 1983; Roelofs et al., 1985; Dopman et al.,

2010) (Fig. 1a).

We predict that the longer-diapausing UZ-strain of European corn borer will accumulate greater fat reserves during the course of larval feeding when programmed for larval diapause than the shorter-diapausing BE-strain. We also predict that the greater fat reserves needed to make it through a longer larval diapause period in the UZ-strain will covary with greater lean mass and longer larval development times.

understanidn overwintering fuel use can have impacts for both understanind overwinter survial and crop liss/damage during the gowing season.

Climate change, energetic demand and nutreint reserves.

Background on the UZ and BE strains of ECB The longer-diapausing UZ and shorter-diapausing BE strains of the European corn borer, *Ostrinia nubilalis*

(Hübner) offer an excellent opportunity to test the prediction that more energetically demanding overwintering periods will require greater energy reserves.

Methods:

Insect rearing:

*Ostrinia nubilalis* eggs were taken from colonies at Tufts University. The two genetically distinct strains used for this work were initially collected as a mixture of larvae, pupae, and adults from New York State prior to 2015 and kept as separate colonies (Wadsworth et al., 2015). Strain identity was determined genotypically using the *pgFAR* (Lassance et al., 2010), an autosomal locus coding for a critical enzyme for determining the female sex pheromone blends that play an important role along with seasonal timing in reproductively isolating these two strains from each other in the field (Dopman et al. 2010). For the duration of the experiment, colonies of each genotype were reared at the University of Florida at 26°C under a 16L:8D photoperiod to promote continuous development.

Comparing the timing of diapause initiation between strains:

Larval diapause in European corn borer begins after the termination of larval feeding, during the so-called wandering stage, and diapause ends when larvae undergo larval-pupal metamorphosis (Gelman and Hayes 1982). To determine whether the timing of larval feeding, pupation, and diapause initiation differed between the two strains, we observed feeding in last-instar larvae of both continuously developing and diapause-programmed larvae of each strain. To assay when feeding ended, synchronized cohorts of individuals were taken from eggs laid by multiple females from each strain on a single day. Upon hatching, each cohort was divided and reared in either a diapause-inducing treatment (12L:12D photoperiod, 23°C, and 65% rH) or a non-diapause treatment that promoted continuous development of larvae into pupae (16L:8D photoperiod, 23°C, and 65% rH). Larvae from each biological cohort were reared together en masse in groups of 50-100 individuals and provided artificial European corn borer diet ad libitum (Frontier Agricultural Sciences, Newark, DE, Product F9478B). When larvae from each biological cohort within each treatment reached the end of the fourth instar (penultimate instar), they were separated and reared individually in 32-well bioassay trays for the last larval instar (Frontier Agricultural Sciences, Newark DE, Product RT32W). Each well of the bioassay tray was provisioned with diet ad libitum and returned to either diapause-inducing or non-diapause treatment conditions until larvae were sampled. To assess whether an individual caterpillar had completed larval feeding and was transitioning into the wandering stage, each individual larva was removed from their rearing tray and placed into a similar tray that contained no artificial diet. Preliminary experiments revealed that actively feeding larvae always produced feces within 30 minutes of being isolated from food, thus each individual was scored as feeding or not feeding/wandering after 30 minutes.

Comparing lipid and lean mass between strains:

We estimated both fat mass and lean mass for each strain and each life-history trajectory, diapause or non-diapause, on the first day of the last larval instar and on the first day of wandering. We chose these time points to determine the extent to which strain or diapause trajectory affected lean or fat mass 1) at the onset of the last instar because it is the period of greatest mass accumulation in the lepidopteran life history, and 2) on the first day of wandering just after larvae stop feeding because fat and lean mass peak in larval insects at this time.

At the time of sampling, larvae were frozen, weighed, and stored at -80C in pre-weighed 1.5mL microcentrifuge tubes (USA Scientific, Ocala, FL., 34478) until further analysis. Larvae from each of the 4 treatments (strain by photoperiod) and developmental stage (day 1 of the final instar or day of wandering) were randomly assigned into sub-blocks of 16 larvae each for lipid extraction, such that each extraction block contained equal numbers of larvae from each comparison to be made (4 larvae from each of the 4 possible photoperiod by strain combinations). Lyophilized larvae were homogenized () in a 3:1 solvent mixture of hexanes and methanol then centrifuged () to produce layer separation. The hexanes layer containing lipids was siphoned away from the methanol layer and collected in pre-weighed 15-mL glass vials (Milipore-Sigma, St. Louis, MO., 27347) and both layers were saved. Lean mass was estimated by drying away the methanol from the solubilized insect tissue using a SpeedVac Concentrator (Thermo Scientific, Waltham, MA., 02451) and weighing the dry tissue powder. To estimate lipid mass, the hexanes were dried away from the lipids using a SpeecVac Concentrator and the dry lipids were weighed.

Statistical Analyses

All statistical analyses were performed using R studio software (version 1.1.383). Days until wandering was calculated as the total number of days between molting into the final larval instar and the day frass production ended for each sampled larva. Wandering was measured in 48 individuals, 12 in each strain x photoperiod combination, and analyzed using a generalized linear mixed effects model. The statistical model to explain differences in wandering day included: diapause genotype and photoperiod as fixed effects, diapause genotype and photoperiod as interacting effects, and cohort as a random factor with a Poisson distribution and z-tests to estimate effects of each model term. Lipid stores were measured in 266 individuals and analyzed using a generalized linear mixed effects model. The statistical model to explain lipid mass included: diapause genotype and photoperiod as fixed effects, diapause genotype and photoperiod as interacting fixed effects, and lean mass as a covariate. Lean mass was measured in 338 individuals. The statistical model to test for differences among groups in lean mass at wandering included: diapause genotype and photoperiod as fixed effects, diapause genotype and photoperiod as interacting fixed effects, and extraction block as a random effect with a normal distribution and Satterthwaite's method to estimate t-values for each parameter. Cohort was also included in each generalized linear model as nested within extraction block, and extraction block was always used as a random factor.

Results:

Diapause-destined larvae of both strains spent longer feeding than non-diapause larvae. When considering only larvae programmed for diapause, the long-diapausing UZ strain larvae took longer to finish larval feeding than larvae of the short-diapausing BE strain, but there was no difference in the time spent feeding between the two strains when they were programmed for continuous development (Fig 2, GLM, strain x photoperiod z=-8.76, p<0.001). Larvae of both strains programmed for either diapause or non-diapause development were monitored for pupation for up to 40 days after molting into the last instar. All diapause-programmed larvae from the long-diapausing UZ strain remained in larval diapause until the end of the 40-day observation period (Fig. 3), showing a strong diapause initiation and maintenance response. In contrast, a few individuals of the short-diapausing BE strain began to pupate just a few days after wandering, considering that diapause-programmed BE individuals wandered 9 days after entering the last instar on average (Fig. 3). A few diapausing BE larvae terminated diapause each day, such that less than 40 % of the larvae in this treatment remained in diapause at the end of the 40-day observation period. Long-diapausing UZ strain expresses a "deeper diapausing" dormancy phenotype where they are refractory to terminating diapause; whereas the short-diapausing BE strain expresses a "shallower" diapause such that individuals are more likely to terminate diapause and initiate development. XXX Here we should talk about tests you did to show that body mass at wandering had nothing to do with diapause length in BE XXX.

When considering wet mass of live caterpillars, the long-diapausing UZ strain achieved greater body size than the shorter-diapausing BE strain in both continuously developing and diapause-programmed individuals (Fig 4). XXX I need stats for wet mass and also figures changed to show the stats with letters of symbols to denote points in time where the two strains differ within photoperiodic treatment XXX then we need an analysis of lean mass data and maybe changes to the figure, I expect it will match fat mass looking at the current figure XXX lipid mass, surprised the 3-way interaction between time\*strain\*photoperiod was not significant, also surprised that photoperiod\*strain interaction term was not significant XXX looks like from the other two interaction terms that 1) the photoperiodic differences got larger with time (photoperiod\*time), that is to say that there was no difference between the diapause-programmed and non-diapause individuals between the two strains on the first day of the last instar, but that UZ got progressively fatter the BE through time when programmed for diapause, and 2) the strain\*time interaction also showed that in both photoperiods the UZ strain gained more fat mass than the BE strain between the onset of the last larval instar and wandering. If I am interpreting this correctly, there is no difference between the two strains or two photoperiodic treatments at the onset of the last larval instar - i.e., did they all start at the same point with respect to wet mass, lean mass, or fat mass?

Discussion: To come

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# A combination of sexual and ecological divergence contributes to rearrangement spread during initial stages of speciation.

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# A recombination suppressor contributes to ecological speciation in OSTRINIA moths.

[Wadsworth CB](https://www.ncbi.nlm.nih.gov/pubmed/?term=Wadsworth%20CB%5BAuthor%5D&cauthor=true&cauthor_uid=25626887)1, [Li X](https://www.ncbi.nlm.nih.gov/pubmed/?term=Li%20X%5BAuthor%5D&cauthor=true&cauthor_uid=25626887)1, [Dopman EB](https://www.ncbi.nlm.nih.gov/pubmed/?term=Dopman%20EB%5BAuthor%5D&cauthor=true&cauthor_uid=25626887)1.

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# Explaining the sawtooth: latitudinal periodicity in a circadian gene correlates with shifts in generation number.

[Levy RC](https://www.ncbi.nlm.nih.gov/pubmed/?term=Levy%20RC%5BAuthor%5D&cauthor=true&cauthor_uid=25430782)1, [Kozak GM](https://www.ncbi.nlm.nih.gov/pubmed/?term=Kozak%20GM%5BAuthor%5D&cauthor=true&cauthor_uid=25430782), [Wadsworth CB](https://www.ncbi.nlm.nih.gov/pubmed/?term=Wadsworth%20CB%5BAuthor%5D&cauthor=true&cauthor_uid=25430782), [Coates BS](https://www.ncbi.nlm.nih.gov/pubmed/?term=Coates%20BS%5BAuthor%5D&cauthor=true&cauthor_uid=25430782), [Dopman EB](https://www.ncbi.nlm.nih.gov/pubmed/?term=Dopman%20EB%5BAuthor%5D&cauthor=true&cauthor_uid=25430782).

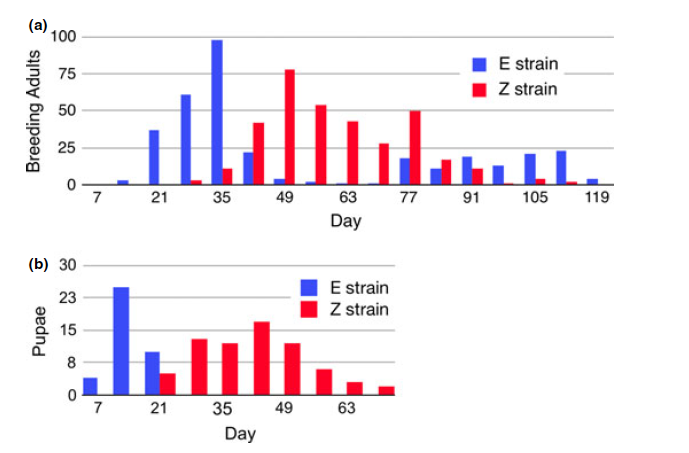


Figure 1. Differences in the duration of larval diapause generate distinct adult flight timing. (a) Differences in the timing of trap captures of adults in the field in Farmington, NY where blue bars represent the E-strain and red bars represent the Z-strain illustrating seasonal temporal isolation (adapted from Dopman et al. 2010). (b) Timing of the termination of larval diapause in the early-emerging E-strain (blue) and the later-emerging Z-strain (red) when reared in a laboratory common garden (adapted from Glover et al. 1992).



Figure 2. Diapause-programmed larvae of both strains spent longer feeding than non-diapause larvae, but there was no difference in the number of days from molting into the last instar until wandering in either strain within a photoperiodic treatment.



Figure 3. All larvae of the long-diapausing Z-strain (blue) remained stably in diapause for at least 40 days after stopping larval feeding when maintained in short-day conditions (23oC, 12L:12D), where as a few individual larvae of the short-diapausing E-strain were apparently in a more shallow state of diapause with individual larvae beginning to terminate larval diapause and initiate larval-pupal metamorphosis as soon as 11 days after stopping larval feeding with a few terminating diapause more or less continuously throughout the 40 day observation period.

Figure 4.