Title: Autumn larval cold tolerance does not predict the northern range limit of a widespread butterfly species

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Running head: Cold tolerance at a northern range limit

Abstract

Climate change is driving range shifts, and a lack of cold tolerance is hypothesized to constrain insect range expansion at poleward latitudes. However, few, if any, studies have tested this hypothesis during autumn when organisms are subjected to sporadic low temperature exposure but may not have become cold tolerant yet. In this study, we integrated organismal thermal tolerance measures into species distribution models for larvae of the Giant Swallowtail butterfly, *Papilio cresphontes,* living at the northern edge of its actively expanding range. Cold hardiness of field-collected larvae was determined using three common metrics of cold-induced physiological thresholds: the supercooling point (SCP), critical thermal minimum (CT_{min}), and survival following cold exposure. *P. cresphontes* larvae in autumn have a CT_{min} of 2.14°C, and were determined to be tolerant of chilling. These larvae have a SCP of -6.6°C and can survive prolonged exposure to -2°C. They generally die, however, at temperatures below their SCP (-8°C), suggesting they are chill tolerant or modestly freeze avoidant. Using this information, we examined the importance of low temperatures at a broad scale, by comparing species distribution models of *P. cresphontes* based only on environmental data derived from other sources to models that also included the cold tolerance parameters generated experimentally. Our modelling revealed that growing degree-days and precipitation best predicted the distribution of *P. cresphontes*, while the cold tolerance variables did not explain much variation in habitat suitability. As such, the modelling results were consistent with our experimental results: low temperatures in autumn are unlikely to limit the distribution of *P. cresphontes*. Further investigation into the ecological relevance of the physiological thresholds determined here will help determine how climate limits the distribution of *P. cresphontes*. Understanding the factors that limit species distributions is key to predicting how climate change will drive species range shifts.

Keywords: mechanistic species distribution model, low-temperature survival assays, Maxent, global warming, cold distribution limits, insect, low temperature limit

Introduction

Over the past few decades, range shifts due to climate and land use changes have been reported in both plants and animals. Many are experiencing a poleward and/or upward shift in distribution, pushing their northern and upper range limit to higher latitudes and altitudes (Chen et al., 2011; VanDerWal et al., 2013; but see Kerr et al. 2015). However, there has been substantial variation in the degree and direction of range shift across taxonomic groups (Chen et al., 2011; Lenoir & Svenning 2015). For example, some insect taxa (i.e., dragonflies, spiders)

have shifted northwards to a much greater extent than mammals and birds, whereas other groups have expanded southwards. This variation in range shifts remains difficult to explain, and thus necessitates a better understanding of the factors influencing species' distributions (Buckley & Kingsolver, 2012; Sunday et al. 2012; Sgrò et al., 2016).

A climate change-driven range shift in insects is often attributed to the relaxation of a harsher poleward climate since temperature acts as a physiological limitation and as a phenological cue (Logan & Bentz, 1999; Root et al., 2003; Elkinton et al., 2008). For those species with northern range edges associated with temperature clines, climatic warming has frequently resulted in the colonization of suitable areas in more northern latitudes (Parmesan, 1996; Parmesan & Yohe, 2003). For example, the northern range expansion of the deer fly (*Lipoptena cervi*) in Finland is due to warmer temperatures during the summer (Härkönen et al., 2010). However, for many other species, it remains unclear how climate change has led to range shifts (Chen et al., 2011; Sunday et al., 2012).

Low temperatures throughout the year can constrain the ability of insects to persist at northern range limits as they can influence any life cycle stage (Ungerer et al., 1999). Overwinter survival has been shown to be a key factor limiting the ranges of insects (Crozier, 2003). For example, the northern range edges of the Southern pine beetle (*Dendroctonus frontalis*) and Sachem skipper butterfly (*Atalopedes campestris*) are limited by temperatures below -16°C (Lesk, Coffel, D'Amato, Dodds, & Horton, 2017) and the -4°C January isotherm (Crozier, 2003), respectively. Overwintering insects can adopt one of multiple physiological strategies for surviving winter (Williams et al., 2015); some insects can survive internal ice formation and are termed freeze tolerant, some physiologically supress the temperature at which their body fluids spontaneously freeze (the supercooling point; SCP) and are termed freeze avoiding, while others remain susceptible to chilling (termed chill susceptible), and seek

relatively warm microhabitats to overwinter (Sinclair, 1999; Denlinger & Lee, 2010; Overgaard & MacMillan, 2017).

While winter represents a considerable challenge (Robinet & Roques, 2010; Williams et al., 2015), insects are also likely to be particularly vulnerable to low temperatures during autumn. Autumn is when most insects are acquiring cold tolerance through physiological adjustments (acclimatization) and have therefore not reached their peak cold hardiness (i.e., the capacity to tolerate intensity and duration of cold exposure; Tauber et al., 1986). For example, monarch butterflies, *Danaus plexippus,* that were not yet acclimated had a mortality rate of 60% when exposed to low temperatures in September and October compared to 20% for acclimated ones (Larsen & Lee, 1994). There is also a much higher frequency of cold temperature events in autumn than in the summer (Danks, 1978). Yet, no study, to our knowledge, has considered low temperatures during autumn as a limiting factor on the geographic distributions of insects.

Regardless of season, cold exposure can cause insects to cross physiological thresholds leading to sublethal effects. At a species-specific low temperature, most insects lose the ability for coordinated movement (a critical thermal minimum; CT_{min}) after which they enter a state of complete neuromuscular silence termed chill coma (Gibert & Huey, 2001; MacMillan & Sinclair, 2011; Oyen & Dillon, 2018). Many insects can recover from this state with no immediate evidence of injury; but prolonged or severe cold exposure can cause behavioural defects, or slow or halt development (Asahina, 1970; Rojas & Leopold, 1996; Overgaard & MacMillan, 2017). Chill susceptible insects suffer from a loss of homeostasis at low temperatures well above their SCP, while those more tolerant to chilling survive such exposures (Overgaard & MacMillan, 2017). While freeze tolerant insects can survive freezing of the extracellular fluid at their SCP, they can suffer from ice-related injuries below this temperature. By contrast, freeze avoidant insects cannot survive at temperatures below their SCP. Thus, the relationship

101 between the SCP and injury/mortality is critical to determining the cold tolerance of these insects
102 (Sinclair, Coello Alvarado, & Ferguson, 2015).

103 Cold tolerance traits, like the CT_{min}, SCP, or survival following a cold stress frequently
104 correlate strongly with insect distribution (Bozinovic et al. 2011; Gouveia et al. 2014; Andersen
105 et al. 2015). For example, the CT_{min} and CT_{max} of *Drosophila* species have been used to
106 accurately predict their current distributions(Overgaard et al., 2014). Similarly, the SCPs and
107 potential for mortality at more mild temperatures were used to understand and predict the
108 potential invasion limits of pine beetles in North America (Ungerer et al., 1999; Bleiker et al.,
109 2019). However, given the limited number of taxa in which these relationships have been
110 explored, we have little to no ability to generalize predictions about how climate influences
111 species' distributions via low temperatures to other taxa (Ouimette 2018).

112 Species distribution modelling is a commonly used approach to evaluate recent range
113 shifts and forecast future shifts due to climate change. For example, they have been used to
114 predict invasive species' distributions (Jiménez-Valverde et al., 2011), define species' ranges
115 and predict impacts of climate change on available habitat (Araujo et al. 2005). Using
116 correlations between georeferenced occurrence records and a set of environmental variables
117 with geospatial data, these models predict a species' suitable habitat (Elith et al., 2011). While
118 the approach is useful for many applications, these models often violate key assumptions, such
119 as predicting habitat suitability in novel conditions and omitting key biotic variables known to
120 influence geographic distributions such as species interactions (Tingley et al. 2014; Briscoe et
121 al. 2019).

122 One way that has been demonstrated to improve the accuracy of these models is to
123 incorporate physiological variables or stress tolerance thresholds (e.g., heat tolerance,
124 metabolic needs, thermal limits; (Kearney & Porter, 2009; Overgaard et al. 2014). By using
125 variables derived directly from these physiological traits, the underlying processes explaining

126 the species' distribution are thought to be better incorporated into the model (Kearney & Porter,
127 2009). For example, Elith et al. (2010) built a distribution model for the cane toad (*Bufo marinus*)
128 based on the impact of topography on the above-ground activity of adults and the temperature
129 requirement of larval growth and survival. Models produced in such a way are called
130 mechanistic or process-based models. They calculate the likelihood of presence by eliminating
131 regions where the conditions are detrimental to species persistence (Buckley et al., 2010). In
132 some contexts, these models have been shown to be more accurate than (Peterson, 2011), or
133 complement weaknesses of (Martinez 2015), correlative models when modelling the
134 fundamental niche and can strengthen predictions about future distributions (Buckley et al.,
135 2011; Martinez 2015; Evans et al. 2016; Kotta et al. 2019). The main limitation with this
136 approach is that experimentally derived variables can be time-consuming to develop and thus
137 not feasible to do for large assemblages of species or over broad ranges (Peterson 2011; Evans
138 et al. 2016). It can also be difficult to find traits that are predictive and functional in the species'
139 environment (Kearney & Porter, 2009).

140 Here, we use a widespread butterfly, the Giant Swallowtail, *Papilio cresphontes*, as a case
141 study to test the hypothesis that low temperatures during autumn are the limiting factor of the
142 northern edges of insect species' distributions. Specifically, the two main objectives are to: i) find
143 relevant cold tolerance thresholds of *P. cresphontes* collected in autumn; and ii) determine the
144 relative importance of these thresholds on the distribution of *P. cresphontes* at the landscape
145 level. Simultaneously, we aim to determine the cold tolerance strategy of larvae at the northern
146 range limit. Determining the species' cold tolerance strategy improves our knowledge about the
147 importance of low temperatures on *P. cresphontes* survival and helps identify which low
148 temperature thresholds are likely to be most relevant at the species' northern range. In this
149 study, we focus on the pre-overwinter life stage (i.e., larval stage), which is in contrast to most
150 other studies which have determined the cold tolerance strategy of the life stage that

151 overwinters (Radchuk et al., 2013). To determine the importance of low temperatures at a broad
152 scale, we model the geographic distribution of *P. cresphontes* with the cold tolerance
153 parameters we generated experimentally.

154 *P. cresphontes* has undergone a rapid expansion over the past decade and now occurs
155 as far north as Ottawa, Ontario, Canada. Finkbeiner (2011) hypothesized that the range
156 expansion into New York state from 2000 to 2010 was due to the disappearance of frost (as
157 defined meteorologically) in September. In spite of this, they still showed frost resistance in
158 larvae and that individuals could survive exposure to -3.9°C. However, these observations were
159 based on field observations of only a few specimens and the cold tolerance strategy was not
160 determined. Therefore, a more in-depth study of the impact of low temperature on
161 *P. cresphontes* is warranted.

162 To establish the larval cold tolerance strategy, we measured three common cold-induced
163 physiological thresholds: SCP, CT_{min}, and survival following cold exposure. To gain a better
164 estimate of the cold tolerance strategy, thresholds (SCP and low temperature survival) were
165 measured for two generations and multiple sites across a latitudinal gradient at the northern
166 range limit. Latitude has the potential to affect cold tolerance due to its correlation with climate
167 (e.g., photoperiod, temperature; Sømme, 1982; Tanaka, 1996) but see Yoshio & Ishii, 2001).
168 Therefore, latitude could affect when and to what extent organisms are cold hardy. Combined,
169 these experiments were used to identify a potentially relevant low temperature threshold of
170 larvae collected at the northern range edge. To evaluate the importance of the cold tolerance
171 strategy at the northern range limit, we modelled the geographic distribution with Maxent, a
172 commonly used species distribution modelling technique for presence-only data (Merow et al.,
173 2013). We examined the importance of low temperatures at a broad scale by comparing species
174 distribution models of *P. cresphontes* based only on environmental data derived from other
175 sources to models that also included the cold tolerance parameters generated experimentally.

176

177 **Methods**

178 1. Study system

179 The Giant Swallowtail (*Papilio cresphontes*) butterfly is a member of the Papilionidae
180 family. The species' range extends from Costa Rica throughout the North American continent.
181 The bulk of the population is concentrated in the Eastern United States. It ranges as far north as
182 the Ottawa region, Ontario, Canada. (Figure 1)

183 *P. cresphontes* larvae undergo five instars before entering the chrysalis stage (Bullock,
184 1991). In Ontario, there are two generations with flights occurring from May to July and again
185 from late July to late September (Layberry, Hall & Lafontaine, 1998). The larval stage lasts from
186 3-4 weeks and pupae formed in the summer emerge after 10-12 days, but those that develop in
187 autumn will remain as pupae until spring. The overwintering pupae undergoes winter diapause,
188 a state of lowered metabolism, with reduced respiration, and no feeding or growth occurs (Scott,
189 1997). Once eclosed, adults live for 6-14 days (Layberry, Hall & Lafontaine, 1998).

190 *P. cresphontes* is known to use plants of the Rutaceae family as their primary food
191 source in the larval stage. In Ontario, those plants consist mainly of Northern Prickly Ash
192 (*Zanthoxylum americanum*) and Hoptree (*Ptelea trifoliata*). The adults are generalists and will
193 gather nectar from most flowering plants, for example, goldenrod (*Solidago*), swamp milkweed
194 (*Asclepias incarnata*) and azalea (*Rhododendron azaleastrum*)(McAuslane, 2004).

195

196 2. Cold tolerance experiments

197 *i) Experimental overview*

198 To determine the impact of low temperatures on *P. cresphontes* larval survival and
199 developmental success, three experiments were conducted (Figure 2; Figure S1). The first
200 experiment was done to determine the cold tolerance strategy of the larvae by measuring the

201 SCP in relation to cold survival. Additionally, since the results from this initial experiment were
202 not enough to clearly define the cold tolerance strategy, further low-temperature survival assays
203 (i.e. the ambient exposure of larvae to low temperatures for an extended period of time) were
204 conducted to better determine the impacts of prolonged low temperature exposure on survival
205 and developmental success. A third experiment was conducted to measure the CT_{min}.
206 The SCP and low-temperature survival assays were measured for both generations and
207 multiple sites (n=4) across a latitudinal gradient at the northern range limit (Figure 1, 2).
208
209 *(ii) Field sampling*
210 To gain a better understanding of the cold tolerance strategy, larval sampling occurred
211 during the two *P. cresphontes* generations: July and August of 2018. A total of 117 larvae were
212 collected from four sites around Ottawa, Ontario, Canada (Mud Lake, Shirley's Bay, Brockville
213 and Queens University Biological Station (QUBS)) spanning a 100 km latitudinal gradient at the
214 northern range limit of *P. cresphontes* (Figure 1). These four sites were accessible and had a
215 high abundance of larvae.
216 Until the start of the experiments, captured individuals in July were provided with a fresh
217 supply of *Z. americanum* and kept in an LTCP-19 Biochamber at the University of Ottawa,
218 Canada (45.423325, -75.683177) on a 21°C/25°C 15h: 9h L:D cycle with light intensity peaking
219 at noon. These conditions were chosen to match the average environmental conditions in July
220 for the Ottawa region. To replicate the conditions required for larvae to prepare to overwinter,
221 the chamber parameters were modified weekly for the August generation to match the
222 conditions from August to October (meteomedia.ca; Figure S1). Over the time spent in the
223 chamber, peak daily temperatures reached a maximum 25 °C and a minimum of 15°C overnight
224 at the beginning and by the end, daytime temperatures only reached 10°C and a minimum of
225 6°C overnight (Figure S1). Likewise, photoperiod fell from 14.5h to 11h. To validate our

226 treatments, we recorded the realized climate regime in the environmental chamber using a
227 temperature logger (HOBO Pendant, Onset Computer Corporation, Bourne, MA).
228
229 *(iii) Experimental details*
230 Experiment 1: Supercooling point (SCP) test
231 To identify the cold tolerance strategy of *P. cresphontes* larvae, we quantified the SCP
232 and survival following a freezing event in the first generation (i.e. the July generation; n=27 from
233 QUBS; Figure 2; Figure S1). The SCP test was done following the recommended methodology
234 of Sinclair (2015). Before being cooled, the larvae were weighed with an ultra-micro Sartorius
235 scale and placed in individual 15 ml centrifuge tubes. Body temperatures were measured using
236 type K thermocouples in direct contact with the larvae and linked to a Pico-Tech TC-08 data
237 logger. The vials were then suspended in a refrigerated circulator (21L with advanced controller,
238 VWR) filled with a mixture of ethylene glycol and water, and cooled down at 0.1°C per minute
239 from 21°C. The test lasted between 200 and 300 min until half the specimens had reached SCP.
240 The vials containing the July individuals were removed all at once when ~50% of the submerged
241 larvae had shown an exothermic spike for their respective body temperature (i.e., the SCP).
242 Larvae were then moved to individual containers inside the LTCP-19 Biochamber at
243 temperature and photoperiod described above (section ii). Larvae were monitored daily until
244 death or emergence. Individuals were considered dead if they did not react to physical stimuli:
245 larvae were poked with a stick, sprayed with water and then shaken.
246 To get a more accurate estimate of SCP, another SCP test was done on the second
247 generation (i.e. the August generation; n=29; Figure 2, Table S1; Figure S1). The individuals
248 from the August generation were subjected to the same cooling protocol as described above but
249 were kept submerged until all of the larvae had frozen. This was done to obtain the full
250 distribution of SCP values.

251

252 Experiment 2: Low-temperature survival assays

253 To identify the consequences of chronic low temperature likely to be experienced by the
254 larvae before pupation, three low-temperature survival assays were conducted (Figure 2; Figure
255 S1). The duration and temperature of the assays were chosen based on the SCP determined in
256 the first experiment and on meteorological data in the Ottawa area representing the
257 developmental period before pupation. In the Ottawa region, this period corresponds to the
258 month of October. We considered temperatures from 2012 to 2017, which correspond with the
259 timing of the recent range expansion of *P. cresphontes* into the Ottawa area (Ontario Butterfly
260 Atlas: www.ontarioinsects.org/atlas_online.htm) and used the 'OTTAWA CDA RCS' weather
261 station (45.38, -75.71). For this timeframe, the mean and lowest temperatures recorded during
262 frost events (as determined meteorologically: the span of time in which the temperature is below
263 0°C for more than one hour) were -1.33°C and -6.3°C, respectively, and the average frost time
264 was 6.24 hours. Consequently, and in conjunction with the measured SCP of -5.6°C determined
265 in the first experiment, trials were run at -2°C, -6°C, -8°C for 7 hours each.

266 To determine whether larvae were chill susceptible, the first assay was done at -2°C
267 (Figure 2). Mortality at this temperature (i.e., above the SCP; see results) would indicate this
268 species is chill susceptible whereas survival would confirm the ability of larvae to tolerate
269 exposure to low temperatures above their SCP, thus making them chill tolerant or freeze
270 avoidant. The second test was done at -6°C to distinguish between freeze avoidant and freeze
271 tolerant strategies (Figure 2). Considerable mortality near the mean SCP would mean larvae are
272 freeze avoidant whereas high survival would mean they are more likely freeze tolerant. Finally,
273 to find a potentially ecologically relevant low temperature threshold causing high mortality (i.e. a
274 potential lower lethal limit), we also tested larval survival at the lowest recorded temperature for
275 October at this weather station from 2012-2017 (i.e. -8°C). Exposure at this temperature tests

276 the maximum larval cold tolerance and their resistance to freezing since it is well below the
277 mean SCP obtained in the previous experiment (i.e., -5.77 °C; Figure 2).
278 The low-temperature assays were done in an environmental growth chamber
279 (Biochambers model LTCB-19) for both larval generations and across multiple sites (Figure 2;
280 Table S1). The -2°C test was not repeated in August because i) there was high survival with the
281 July individuals (see results); ii) a low number of specimens were collected in the field, and iii)
282 this temperature was unlikely to be a limiting factor for larval survival and thus was not a priority
283 to test. Larvae were weighed and measured before being placed in individual containers inside
284 the chamber. The chamber temperature was brought down from 21°C to the predetermined test
285 temperature at a rate of 0.1°C per minute. Once the desired minimum temperature was
286 reached, the temperature remained constant for seven hours before being increased back to
287 21°C at the same rate. Larvae were then left to recuperate. They were allowed to feed on
288 freshly harvested *Z. americanum* leaves during the trial and were checked daily for mortality.
289 Death was determined when larvae did not react to physical stimuli: larvae were poked with a
290 stick, sprayed with water and then shaken. All larvae that survived to pupation were kept until
291 emergence.
292
293 Experiment 3: Critical thermal minimum (CT_{min})
294 To identify the temperature at which larvae lose coordination, a CT_{min} test was done
295 following the method of Andersen et al (2014). Twenty larvae were caught in July from the
296 QUBS site, weighed and measured before being placed in 50 ml Eppendorf vials. These vials
297 were then mounted on an aluminum rack, which was submerged in a refrigerated circulator (21L
298 with advanced controller, VWR) containing a mixture ethylene glycol and water. The bath was
299 cooled from 21°C to -20°C at a rate of 0.2°C per minute. The tubes were periodically (i.e.,
300 approximately every 2 min) prodded using a plastic spoon during the cooling process to

301 stimulate movement. The CT_{min} was determined when individuals fell on their sides and were no
302 longer able to stand. Larvae were kept in the bath until the final larva fell, at which point the
303 whole rack was removed from the solution and allowed to warm back up. Larvae were then left
304 to recuperate in a growth chamber (Biochambers model LTCB-19) at the average conditions in
305 July, as described above. Larvae were checked daily for mortality. The CT_{min} test was only done
306 on July individuals since it required a higher number of specimens than the other tests and our
307 sample size was limited.
308
309 *iv) Statistical analysis*
310 To compare survival rates of larvae of the July generation that froze to those that did not
311 freeze during the SCP experiment, a $\chi 2$ test was used. The rate above and below the SCP for
312 the August generation could not be compared since all larvae were exposed to low
313 temperatures until SCP was reached (i.e. no survival rate was calculated above SCP). To test
314 whether mean SCP differed across sites for the August generation, an ANOVA was run. Since
315 there was no significant difference in mean SCP across sites (df=3, F-value=0.94 $p = 0.44$), site-
316 level data were pooled. The assumptions of normality and homogeneity were evaluated using
317 the Shapiro-Wilk test and the Brown-Forsythe for homogeneity of variance test, respectively.
318 To compare the larval survival rate and the rates of pupation and adult emergence
319 between generations and sites for the low-temperature assays, a $\chi 2$ test was used. There was
320 no impact of site on survival rate or developmental success in any assay (Table S2), so site-
321 level data were pooled. All analyses were conducted in R (version 3.4.1).
322
323 2. Distribution modelling
324 *i) Overall approach*

To determine the limiting factors of the current geographic distribution of *P. cresphontes*, we modelled its range at two different spatial extents: northern range and the entire distribution (Figure 1); and using two different modelling approaches. We wanted to determine whether the relative importance of variables differs between the northern range and the entire distribution. We defined the northern range as the area encompassing the upper 50th percentile of occurrences based on both latitude and longitude in the northeastern part of the range (Figure 1). The 50th percentile represented a clear threshold in the latitudinal distribution of occurrences. To assess if the physiological-based cold tolerance metrics were significant predictors of *P. cresphontes'* distribution, the modelling was done using two approaches: correlative and mechanistic. The correlative approach was based on environmental data derived from other sources (e.g., remote sensing, weather stations) whereas the mechanistic approach additionally included parameters derived from the cold tolerance experiments.

For both approaches, we tested the importance of the cold tolerance strategy parameters by comparing i) model fit with and without them and ii) the proportion of suitability explained among a number of climate-related factors hypothesized to constrain the range of *P. cresphontes* at the landscape scale.

ii) Data

Models were built using all available occurrence records for North America from e-butterfly (www.e-butterfly.org, accessed July 2018, 2255 records), the Global Biodiversity Information Facility (GBIF; https://www.gbif.org, accessed September 2018, 3420 records) and Moths and Butterflies of North America (www.butterfliesandmoth.org, accessed October 2018, 1206 records) from 1980 to 2018. This timeframe was chosen to match the timeframe of the available environmental variables. Records that were obviously inaccurate (e.g., in the middle of the ocean) or had no geographical coordinates were removed. To reduce the likelihood of an

occurrence being based on the same individual being observed on multiple occasions, duplicate records across data sources were also removed. We used a low-end estimate of dispersal ability (1 km) and randomly selected a single occurrence across data sources per 1 km^2. This action serves. In total, 3510 and 1457 occurrences were used for the North American and the northern range extent, respectively. Occurrences were then mapped onto a North America Lambert Conical Conic projection.

Sixteen environmental variables (Table 2) were initially considered for the model building based on previous work done on butterflies (Araújo & Luoto, 2007; Finkbeiner et al., 2011; Roland & Matter, 2016). The variables included represented both the direct and indirect effects (i.e. via a species' interaction) climate can have on a species' range. Variables included low and high temperature requirements (i.e., mean temperature of the coldest month and extreme maximum temperature), precipitation, measures of heat accumulation (i.e., growing degree-days), vegetation (i.e., NDVI) and frost (i.e., temperatures below 0°C for at least one hour) (Sykes, Prentice, & Cramer, 1974). As a measure of heat accumulation, GDD measures the length of the growing season, which determines the amount of time there are favourable conditions for growth and development in plants and insects (Chuine, 2010; Régnière, St-Amant, & Duval, 2012). Other variables are related to primary production and resource availability such as NDVI and precipitation. The impact of frost events were assessed since they were previously hypothesized to be a contributing factor for the range expansion of *P. cresphontes* (Finkbeiner et al., 2011). Specifically, we tested different frequencies of frost events: the number of frost-free days and duration of the frost-free period. The effect of the intensity of frost events (i.e., how cold it was during the frost event) could not be tested since the necessary climatic variables were not available.

All climatic data were downloaded at a 1 km resolution and restricted to 1980-2010. All data was downloaded at a North American extent, with the exception of Normalized Difference

375 Vegetation Index (NDVI) which was global in extent. Variables were created for both range
376 extents (Table 2). Growing degree-days (GDD) were modelled using a 10°C base (see Table 3
377 for calculation), which is a commonly used threshold among butterflies and other insects (Nufio
378 et al. 2010; Cayton et al. 2015).

379 For the mechanistic models, we created variables for both range extents based on the
380 results from the cold tolerance experiments and minimum daily temperature data from Daymet
381 (Table 2). Three variables were built for each extent by summing the number of days below the
382 following thresholds: 2.14°C (i.e., CT_{min}), -6.6°C (i.e. the SCP from the August generation)
383 and -8°C (i.e. potential lower lethal limit) for each year of the study period 1980-2010 (Table 2).
384 The SCP from the August generation was chosen since it is the one most likely to match when
385 larvae are more likely to experience low temperatures.

386 To reduce collinearity among variables, a variance inflation factor (VIF) analysis ('car'
387 package (John Fox)) with a threshold of 10 was conducted. This threshold was chosen instead
388 of a more restrictive one (e.g., 3 or 5) as it provides a compromise between collinearity and
389 model usefulness (O'brien, 2007). The test was repeated for each of the model extents and
390 approaches (n=4). With the VIF test, the initial list of 16 variables was reduced to 8 for the
391 northern range and six for the full range model (Table 3). The final list of variables used for the
392 correlative modelling approach was: extreme maximum temperature, precipitation as snow,
393 precipitation, GDD, NDVI, and mean temperature of the coldest month. Additionally, for the
394 mechanistic models, CT_{min} and the potential lower lethal limit temperature were also included.
395

396 *iii) Modelling*

397 Models were built using Maxent (version 3.4.1, Philipps et al. 2006) with the BIOMOD2
398 package (Damien Georges) in R (version 3.41.1). Maxent uses a maximum-entropy approach to
399 model species distributions. It relates the species' presences and pseudo-absences to the

environmental variables to predict where the habitat is suitable across the mapped geographical space. Maxent is one of the more common species distribution modelling software (Elith et al., 2011) and it performs very well compared to other modelling techniques when dealing with presence-only data (Hernandez, Graham, Master, & Albert, 2006).

To model the range, we included hinge features, which allow non-linear relationships, in the model and allowed the model the possibility of clamping (i.e., the default). Clamping serves to limit how much leniency there is in the range of values projected compared to what was observed. This action is important when predicting into novel geographic space (Stohlgren et al. 2011). However, since the training data and the projection extent were the same, no clamping was done. For each model extent and approach, a background extent was created using the minimum convex polygon function in R (Package adehabitatHR v0.4.16; Calenge,2006). Pseudo-absences were randomly generated within the minimum convex polygons (n=10000; default value).

The models were calibrated using five-fold cross-validation (i.e., the observations were divided into k groups, or folds, of equal size). In cross-validation, the first fold is treated as a validation set, and the model is fit on the remaining k – 1 folds (James et al., 2013). Iterations were limited to 5000 to leave enough time for model convergence. All other parameters were left at default values.

Models were evaluated using three metrics that assess various aspects of accuracy and discrimination (i.e., the ability of the model to distinguish between suitable and non-suitable habitat): Area Under the Curve of the receiver operating characteristics (AUC), Kappa, and True Skills Statistic (TSS). AUC characterizes the model's ability to correctly predict if a presence or absence is a true presence or absence. A value below 0.5 means that the model is no better at predicting occurrences than random, whereas a value of 1 would mean the model predicts all presences/absences perfectly (Yackulic et al., 2013). Although it is the most commonly used

metric for assessing model accuracy of species distribution models (Yackulic et al., 2013), it has been criticized for being unreliable when sample sizes are small and due to its reliance on the number of background points. As such, in these contexts, AUC should mainly be used to compare models built with the same variables and occurrences.

The discrimination of the models was further tested using Kappa and TSS. These two metrics use confusion matrices to compare models' abilities to predict occurrences correctly (Allouche et al. 2006). While Kappa has been shown to be more sensitive to prevalence (i.e. the proportion of locations that are occupied), TSS provides an alternate validation metric that is independent of prevalence (Allouche et al., 2006). Kappa scores between 0.4 to 0.6 indicate fair agreement, 0.6 to 0.8 indicate moderate agreement, and values greater than 0.8 indicate strong agreement. A TSS score between 0.40 and 0.75 indicates good predictive performance of the model while a TSS score above 0.75 indicates excellent performance (Allouche et al., 2006).

Models were run 100 times, and the sensitivity (i.e., the proportion of correctly predicted presences) and specificity (i.e., the proportion of correctly predicted absences) were extracted from each iteration and used to calculate mean and standard error of AUC, Kappa, and TSS(Cerasoli et al., 2017). The proportion of variation explained by the variables was also extracted from each iteration and averaged across all runs.

iv) Statistical analysis

To determine the role of low temperature in limiting the distribution of *P. cresphontes*, model accuracy was compared across the two extents and approaches using t-tests. Comparisons were based on AUC, Kappa and TSS.

449 **Results**

450 *1. Cold tolerance experiments*

451 Experiment 1: Supercooling point (SCP) test

452 The results from the July and August SCP experiments provide support for a chill
453 susceptible strategy in *P. cresphontes*. The mean SCP for July was -5.8°C (0.2SE; n=14). There
454 was no significant difference in survival for July larvae that froze compared those that did not
455 freeze during the SCP experiment (above: 77% (10/13) vs. below: 43% (6/14 larvae); $\chi 2$= 1.2, df
456 = 1, p-value = 0.2).

457 The mean SCP for all August larvae was -6.6°C (0.2SE; n=28) and for the upper 50% of
458 larvae (directly comparable to the July experiment), the mean SCP was -5.9°C (0.2SE; n=14).
459 The August larvae had a 3.5% (1/29) survival rate following freezing (i.e., exposure to
460 temperatures below the mean SCP). The low survival below SCP and apparent lack of change
461 in the SCP suggests that larvae are tolerant of moderate chilling, but intolerant of freezing, and
462 are not undergoing a change in SCP under simulated autumn conditions.

463

464 Experiment 2: Low-temperature survival assays

465 *i) Survival*

466 Our low-temperature survival assays further demonstrate that larvae are chill tolerant
467 or modestly freeze avoidant, as they are unable to survive exposure to temperatures below their
468 SCP. In the -2°C test, July larvae had a survival rate of 93% after 24 hours (Table 1). Therefore,
469 single ecologically-relevant exposures to temperatures above their SCP do not have a large
470 impact on larval survival. Moreover, while the survival rate of larvae in July was more affected
471 by exposure to -6°C than -2°C (Table 1), it was still rather high (70%). Therefore, larvae are able
472 to survive a 7 h cold exposure near their SCP and thus may be chill tolerant or freeze avoidant.

August larval survival was significantly higher than that of the July larvae following exposure to -6°C (100%, 17/17; χ^2 -squared = 5.1, df = 1, p-value = 0.024; Table 1). This finding provides further evidence in support of a modestly freeze avoidant strategy since the mean SCP for the August generation (-6.6°C) was lower than the test temperature (-6°C) and survival was still quite high.

In comparison, in the extreme low-temperature test (-8°C), larvae had marginal survival in both generations (July: 12.5% (1/8); August: 10% (1/10)) and there was no improvement in survival across generations (χ^2 = 3.03e-31, df = 1, p-value = 0.99; Table 1). This low survival further suggests larvae may use a freeze avoidant strategy since this temperature is below the mean SCP measured in the August generation.

ii) Developmental success

Overall, low temperature exposure impacted pupation and adult eclosion. The percentage of larvae that successfully pupated and eclosed generally decreased as the temperatures got lower (Table 1). The percentage of larvae that successfully pupated at -8°C was low (July: 12.5%; August: 10%). Therefore, although larval survival can be high after exposure to low temperatures, chilling also had effect on developmental success, which is indicative of sub-lethal chilling injury occurring, even in the absence of freezing.

Experiment 3: CT_{min}

The average CT_{min} of larvae was 2.14°C (0.26SE; n=20).

2. Distribution modelling

i) Model performance

Model predictive ability varied depending on the metric of accuracy used. All models were considered good based on TSS (i.e. between 0.4-0.75) and AUC (>0.8), whereas based on Kappa, the northern range models were weak (<0.4) and the full range models were fair (>0.5; Table S3).

The accuracy of the two modelling approaches were similar at both extents (AUC: northern range: t = 0.21, df = 999.81, p-value = 0.84; full range: t = 0.62, df = 999.56, p-value = 0.89; Figure 3). Note that these results are identical across accuracy metrics and only AUC is reported here (Table S3). Therefore, the inclusion of cold tolerance metrics does not improve the prediction of habitat suitability of *P. cresphontes*.

The accuracy of the full range models was significantly higher than the accuracy of the northern range models for all evaluation metrics and approaches (see Table S4 and S5). Therefore, the habitat suitability of *P. cresphontes* is predicted more accurately across its full range than its northern range.

ii) Variable importance

Growing degree-days was the most important factor explaining the habitat suitability of *P. cresphontes* across all model extents and approaches, explaining between 27-35% of the variation in habitat suitability (Table 3). Precipitation was the second most important factor across both extents and approaches. Across the full and northern range extents, GDD and precipitation combined explained the majority of the variation in habitat suitability (~50%; Table 3). The physiologically derived variables included in the final model (i.e., CT_{min} and the potential lower lethal limit) only explained a small part of variation in habitat suitability (~7.5%) and ranked 5th and 6th in model contribution.

521

522 **DISCUSSION**

523 Although insects are likely to be particularly vulnerable to low temperatures during
524 autumn, no study, to our knowledge, has considered low temperatures during autumn as a
525 limiting factor on the geographic distributions of insects. Here, we test this hypothesis at the
526 northern edge of the distribution of the widespread butterfly, the Giant Swallowtail,
527 *P. cresphontes*. Our study contributes three main findings that lead to a rejection of this
528 hypothesis. First, given the survival of larvae to prolonged exposures to temperatures close to
529 but not below their SCP, they are chill tolerant or modestly freeze avoidant at their northern
530 range limit at this time of year. Their low survival below the SCP provides support that this
531 species is unable to handle freezing before pupation and that they may use a freeze avoidant
532 strategy. However, further testing for seasonal plasticity in the SCP is required to confirm a
533 freeze avoidant strategy as we had limited power and were unable to directly compare the
534 change in SCP between the two generations. An active depression of the SCP over the season
535 would indicate that larvae are accumulating cryoprotectants and that the larval cold tolerance
536 could be increasing, thus supporting a freeze avoidant strategy.

537 Our results demonstrate that ecologically relevant exposures to temperatures above the
538 SCP are not lethal, but below the SCP are; thus providing a clear picture of the lower end of
539 thermal tolerance for this species. This means that for *P. cresphontes* larvae, a single overnight
540 exposure to temperatures near -8°C will impede their survival and could represent a potential
541 lower lethal limit. Consequently, areas with early autumn temperatures that reach -8°C should
542 be inhospitable for *P. cresphontes*. However, in the Ottawa region, which is at the northern
543 range limit, there are only 0-3 frost episodes on average from September to October. Therefore,
544 low temperatures (i.e. <-7°C) during early autumn are unlikely to be experienced by the larvae
545 there or anywhere in its current range. This suggests that a single exposure to low temperatures

546 in early autumn is unlikely to limit the current northern range of *P. cresphontes* through cold-
547 induced mortality. However, there may be sublethal effects of chilling (e.g., effects on
548 developmental success), which could still impact overall insect fitness.

549 Second, we found that exposure to normal frost temperatures for this time of year in this
550 area (i.e., -1°C to -2°C), which are above the SCP, did not affect larval survival. Therefore, it is
551 also unlikely that frost in early autumn is a limiting factor of *P. cresphontes'* northern range. This
552 is in concordance with the field observations from Finkbeiner (2011), which showed that
553 *P. cresphontes* larvae can survive frost events. Since larvae had no problem reaching pupation
554 after exposure to -2°C, the most common frost temperature, and the majority of larvae still
555 successfully pupated (60%) after the low frost temperature test (i.e., -6°C), the cold tolerance of
556 larvae at the current northern range limit seems sufficient to cope with the climate they
557 experience. However, further testing in ambient conditions and with a proper control is needed
558 to determine whether cold exposure on larvae have significant sub-lethal effects on eclosion as
559 rates of eclosion in this experiment were less than 30% but a control group was not included
560 due to sample size constraints. It has been demonstrated elsewhere that the success of each
561 life stage matters in the response of butterfly species to climate change (Radchuk et al. 2012).

562 Third, the species distribution modelling results were consistent with the experimental
563 results: cold related variables did not explain the distribution of *P. cresphontes* at a broad scale.
564 Together, these results provide strong support that exposure to temperatures above -6.6°C
565 during autumn does not limit the northern range of *P. cresphontes*. Instead, growing degree-
566 days and precipitation are the most important predictors, of those tested here, on the distribution
567 of *P. cresphontes* at a broad scale. While other factors were not tested here, for example, biotic
568 interactions (e.g., host plant occurrence), our results suggest that *P. cresphontes* depends on
569 specific heat accumulation and water availability to complete its life cycle. These factors have
570 also been identified as important in predicting the range of other butterflies (Luoto et al., 2006;

571 Eskildsen et al., 2013). Evidence suggests there is a strong relationship between the number of
572 growing degree-days and the growth rate of larvae, and the foraging activities of adults (Ritland
573 & Scriber, 1985; Kukal & Dawson, 1989; Schneider & Root, 2002). Precipitation can limit the
574 range of insects directly due to dehydration or indirectly by imposing limitations on primary
575 productivity, which in return transposes to higher resource availability.

576 The full range model was more accurate at predicting the distribution of *P. cresphontes*
577 than the northern range model. Similar results have been found for other species, with model
578 accuracy and performance increasing with larger study extents (VanDerWal et al., 2009; Connor
579 et al., 2019). This is likely a result of smaller environmental gradients in the northern range,
580 which causes the model to have a greater difficulty detecting differences between presences
581 and absences, and in turn leads to difficulties predicting presences and absences in novel areas
582 (Smith & Santos, 2019). Prevalence can also decrease with larger study extents (Barbet-Massin
583 et al., 2012). However, the accuracy of the two models was still different using TSS, a metric
584 that is not sensitive to prevalence (Table S4; Figure 3). Nevertheless, even if model
585 performance was lower in the northern range relative to the full range, the model was still valid
586 and performed adequately across all metrics.

587 While growing degree-days and precipitation explain a lot of the variation in habitat
588 suitability for *P. cresphontes*, we may have underestimated the role of cold tolerance at the
589 northern range edge for three reasons. First, low temperature thresholds, like the CT_{min},
590 although not lethal, could still impact *P. cresphontes'* survival in natural conditions due to the
591 enhanced risk of predation or starvation linked with the loss in mobility at temperatures below
592 2°C. While a possibility, this hypothesis depends on a high likelihood of predation, which is
593 unknown across *P. cresphontes'* range (Hazel et al., 1998; McAuslane, 2009). Low temperature
594 may also lead to sublethal effects on developmental success, energy status and behaviour.
595 Second, it is also possible that more prolonged or repeated exposure to low temperatures could

596 impact *P. cresphontes* larval survival or have sublethal effects at the northern range edge
597 because we did not test for the effects of repeated cold injury or chilling. In *Drosophila*, multiple
598 cold events could cause an accumulation of cold injuries resulting in larval death or in sub-lethal
599 effects such as decreased feeding, fecundity and dispersal ability (Marshall & Sinclair, 2012).
600 Third, the SCP estimated here is effectively a theoretical one. Since we could not collect and
601 test the last emerging larvae of the season due to the difficulty in finding them, they might not
602 have reached their peak cold tolerance and therefore *in situ,* SCP could be lower (i.e., their cold
603 hardiness may have been underestimated). On the other hand, the *in situ* SCP may be higher
604 since environmental factors in natural conditions, such as air moisture or exposure to ice
605 nucleators, may further affect survival at temperatures above SCP. Nevertheless, because the
606 low temperature thresholds derived experimentally in this study could be an underestimate, the
607 mechanistic models may not have been optimized perfectly, thus underestimating the role of
608 cold tolerance metrics at a broad scale. However, since temperatures below the SCP (i.e.
609 <-7°C) are very unlikely to occur multiple times before pupation, if at all, low temperatures during
610 autumn remain unlikely to limit the northern range of *P. cresphontes*.
611 Given our lack of knowledge about the overwintering ecology and physiology of this
612 species in its northern range, pupal overwinter survival may be a more important limiting factor
613 for the species' northern range limit. Unfortunately, it is unknown whether they overwinter in the
614 leaf litter on the ground or tree branches above the ground. If on the ground, they would likely be
615 covered in snow (average annual snowfall in Ottawa is 224 cm (Environment and Climate
616 Change Canada)) and therefore buffered against low temperatures. The temperature 30 cm
617 below snow cover is usually -4°C (Flin 2008). This behaviour would increase their chances of
618 surviving the winter. Indeed, West (1996) showed that in Virginia, *P. cresphontes* pupate 3-25
619 cm off the ground on dead branches. In the northern range, it is likely that even at these heights,
620 chrysalids would be covered by snow. While ontogenetic variation in thermal tolerance is

621 present in other species (Vernon & Vannier, 1996; Rinehart et al., 2006; Terblanche et al. 2006;
622 Marais et al. 2009; MacLean et al., 2016; but see Ouimette, 2018) and only larvae were tested
623 in this study, *P. cresphontes* larvae purge their food and liquids before pupation, thus they are
624 likely to be even more cold tolerant at the pupal stage. Other members of *Papilio* are more cold
625 tolerant than what we determined for the larval stage of *P. cresphontes. P.canadensis* and
626 *P.glaucus,* are considered to be freeze tolerant at the pupal stage and have an SCP of -23°C to
627 -27°C (Kukal et al., 1991). *P. xuthus* is freeze avoidant with an SCP of -25°C (Shimada 1988).
628 Therefore, the pupae of *P. cresphontes* are likely to have even greater cold hardiness than the
629 larvae and thus be able to survive the winter at the northern range if they pupate at similar
630 heights as the populations in Virginia.
631
632 *Conclusions*
633 Our results demonstrate that the cold tolerance of *P. cresphontes* larvae is well matched
634 to its environment in the northern part of its range and that it is unlikely that low temperatures in
635 autumn are limiting its range. This suggests that the warming of autumn temperatures over the
636 past decade is unlikely to have been the factor that led to the recent range expansion of
637 *P.cresphones*, as originally hypothesized by Finkbeiner (2011). Instead, the warming of winter
638 temperatures could have ameliorated overwintering survival, leading to an improvement in
639 habitat suitability. Further study on determining the key factor(s) limiting the northern range of
640 *P. cresphontes* should focus on defining the cold hardiness of the pupal stage, the overwintering
641 behaviour and the ecological relevance of the physiological thresholds found in this study.
642 Determining the ultimate factors that limit species' distributions will be critical in accurately
643 predicting species' range shifts in response to future climate change.
644
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949 **Tables**

950 Table 1. Survival rates and developmental success for the low-temperature assays. Test
951 temperature and generation are shown. The percentage of initial larvae that survived the first 24
952 hours after the experiment, successfully pupated and eclosed as adults is also shown. All
953 percentages are based on the number of larva that began the experiment.

Test	Generation	Number of larvae	Larval survival (%)	Pupation (%)	Eclosion (%)
-2°C	July	23	93	78.5	52
-6°C	July	10	70	60	30
	August	17	100	100	0
-8°C	July	8	12.5	12.5	12.5
	August	10	10	10	0

954

Table 2. Original list of variables initially considered for the modelling and their acronyms. The data source is shown as well as the calculation used to produced the final rasters. All variables are averaged over the period from 1980-2010 and the original map projection of all variables was Lambert Conical Conic except for NDVI, which was sinusoidal.

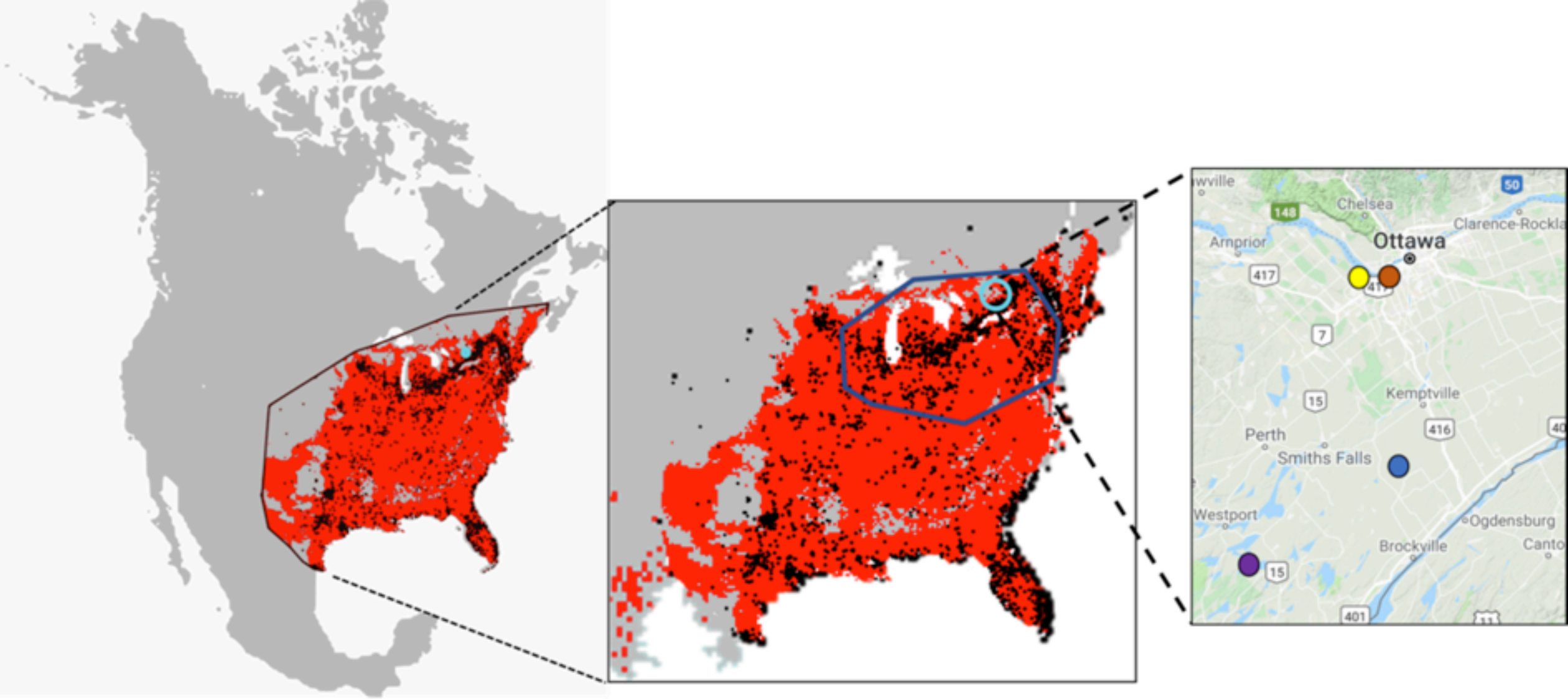
Acronym	Full name (units)	Data source	Calculation	Importance
NDVI	Normalized Difference Vegetation Index	Modis/Terra project	Averaged monthly NDVI raster's over the time frame.	Seto 2004, Pettorelli 2005
bFFP	beginning of frost free period (FFP; day of year)	Databasin	The day of the year on which FFP begins	Hayes 1982, Westwood & Blair 2010
eFFP	end of frost free period (day of year)	Databasin	The day of the year on which FFP ends	Hayes 1982, Westwood & Blair 2010
FFP	frost free period (days)	Databasin	The number of days between the last spring frost and the first autumn frost	Hayes 1982, Westwood & Blair 2010
NFFD	number of frost free days	Databasin	Number of days above 0°C.	Hayes 1982, Westwood & Blair 2010
PAS	average precipitation as snow (mm)	Databasin	Accumulated snowfall averaged across the time frame	Roland & Matter 2016
Precip	average precipitation as rainfall (mm)	Databasin	Accumulated rainfall averaged across the time frame	Storch et al. 2003
EMT	extreme minimum temperature (°C)	Databasin	Lowest temperature recorded for every given year, averaged over the timeframe.	Crozier 2003
EXT	extreme maximum temperature (°C)	Databasin	Highest temperature recorded for every given year, averaged over the timeframe.	Malcolm et al. 1987
MCMT	mean temperature of the coldest month (°C)	Databasin	Average daily temperature for the coldest month	Crozier 2004; Luoto et al., 2006
MWMT	mean temperature of the warmest month (°C)	Databasin	Average daily temperature for the warmest month	Crozier 2004
octtre	average temperature in October (°C)	Daymet	Average daily temperature for October.	Larsen & Lee 1994
GDD	average growing degree-days of base 10°C	Daymet	((Tmax + Tmin) / 2) -10	Luoto et al., 2006
SCP	average number of days per year below -6.6°C (days)	Daymet	Based on the experimentally derived SCP; a day was counted if the average daily temperature reached -6.6°C. Days were counted and averaged across time frame	Ungerer et al., 1999
CT_{min}	average number of days per year below 2.14°C (days)	Daymet	Based on the experimentally derived CT_{min}; a day was counted if the average daily temperature reached 2.14°C. Days were counted and averaged across time frame	Andersen et al. 2015
PLLT	Potential lower lethal temperature; Average number of days per year below -8°C (days)	Daymet	Based on the experimentally derived potential lower lethal temperature; a day was counted if the average daily temperature reached -8°C. Days were counted and averaged across time frame	Andersen et al. 2015

Table 3. Contribution of the environmental variables in explaining habitat suitability across different model extents and approaches. Shown is the rank order of variable importance and mean (± S.E) proportion of variance explained. In bold is the variable that explains the most amount of variation for each model type.

Variables	North America				Northern range			
	Correlative		Mechanistic		Correlative		Mechanistic	
	Rank order	Variation explained (SE)	Rank order	Variation explained (SE)	Rank order	Variation explained (SE)	Rank order	Variation explained (SE)
Growing degree-days	**1**	**31.33 (0.19)**	**1**	**27.34 (0.23)**	**1**	**37.17 (0.08)**	**1**	**35.03 (0.06)**
Precipitation	2	24.87 (0.08)	2	21.70 (0.085)	2	23.94 (0.08)	2	22.47 (0.07)
Extreme maximum temperature	3	17.54 (0.06)	3	16.22 (0.06)	6	2.38 (0.02)	7	2.41 (0.02)
Normalized Difference Vegetation Index	4	15.45 (0.10)	4	15.87 (0.11)	4	16.77 (0.11)	4	14.89 (0.11)
Mean temperature of the coldest month	NA	NA	NA	NA	3	16.9 (0.06)	3	15.57 (0.05)
Precipitation as snow	5	10.80 (0.07)	5	10.05 (0.07)	5	2.84 (0.02)	6	3.46 (0.02)
CT_{min}*	NA	NA	NA	NA	NA	NA	5	5.97 (0.03)
Potential lower lethal temperature*	NA	NA	6	8.80 (0.04)	NA	NA	8	0.16 (0.004)

* variable derived experimentally

963 **Figures**
964
965 **Figure 1.** The geographic distribution and northern range of *Papilio cresphontes*. The first panel
966 shows the entire range of *P. cresphontes* encompassed by the minimum convex polygon (thin
967 black line). The second panel shows the position of the northern range, denoted by the solid
968 blue line, relative to the entire range. The blue circle indicates the locations of the field sites. In
969 the first two panels, the occurrence points (black) are shown against the predicted habitat
970 suitability (red). The third panel shows the field sites where larvae were collected at the northern
971 range limit: the blue dot indicates the Brockville site (44.84952, -75.75226), red indicates the
972 Queen's University Biological Station site (44.56747, -76.32454), brown indicates the Mud lake
973 site (5.37192, -75.79451) and yellow indicates the Shirley's Bay site (45.36546, -75.88302).
974
975 **Figure 2.** Overview of cold tolerance experiments conducted in this study. Shown are the
976 supercooling point (SCP), low temperature survival assays, and critical thermal minimum
977 experiments (CT_{min}). Sites included in the experiments are: Brockville, Queen's University
978 Biological Station (QUBS), Mud lake and Shirley's Bay. For number of larvae in each
979 experiment, please refer to Table S1.
980
981 **Figure 3.** A comparison of model accuracy for the two model extents (northern and full range)
982 and approaches (correlative and mechanistic) using all three evaluation metrics (AUC (Area
983 under the receiver operating characteristic curve), Kappa, TSS (True skills test). Mean accuracy
984 scores and standard error bars across 100 iterations are shown with the y-axis cropped at 0.2.
985 Grey and black bars represent the mechanistic and correlative models, respectively. Letters
986 indicate that mean scores are significantly different from each other ($p<0.05$).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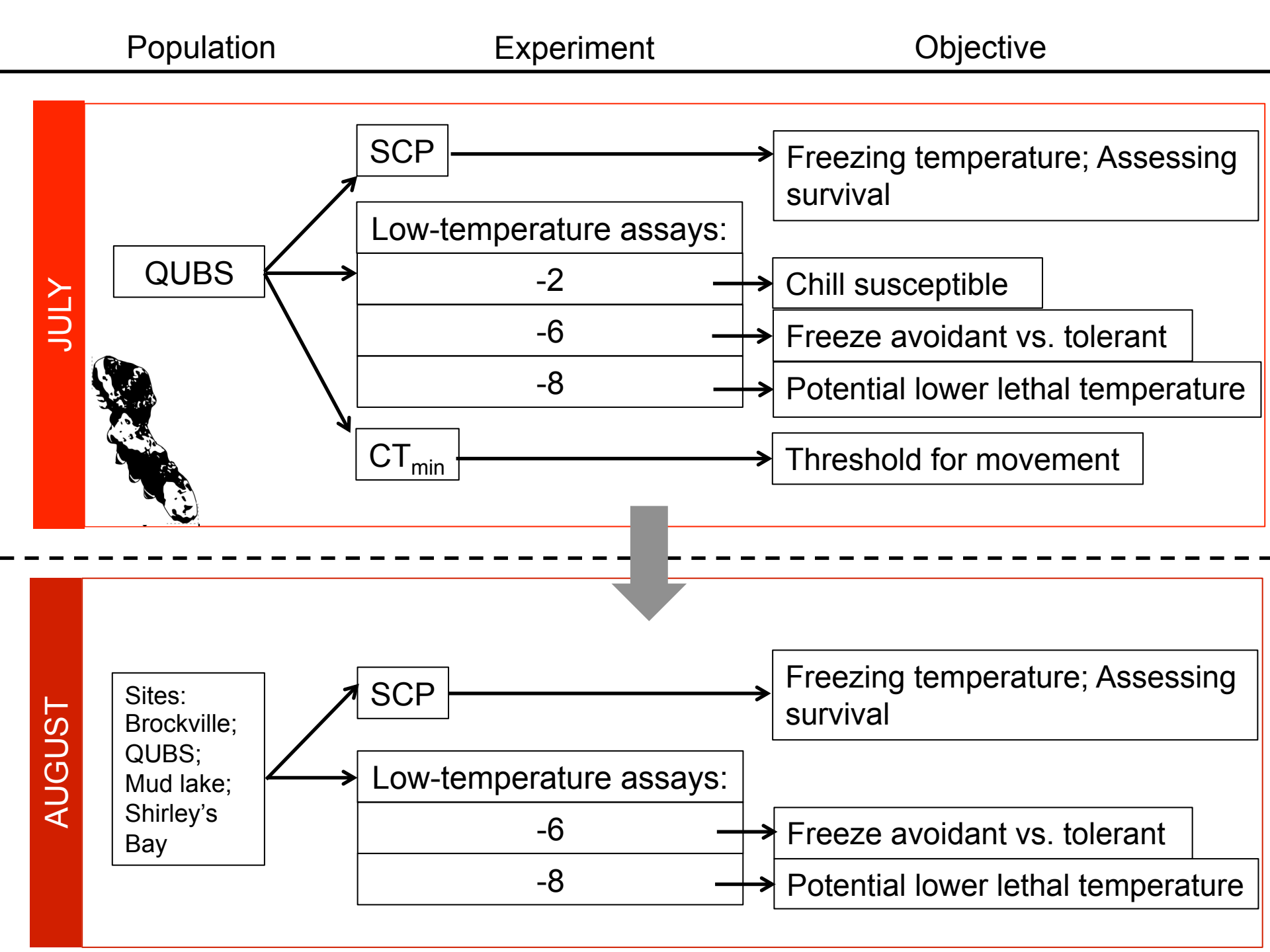
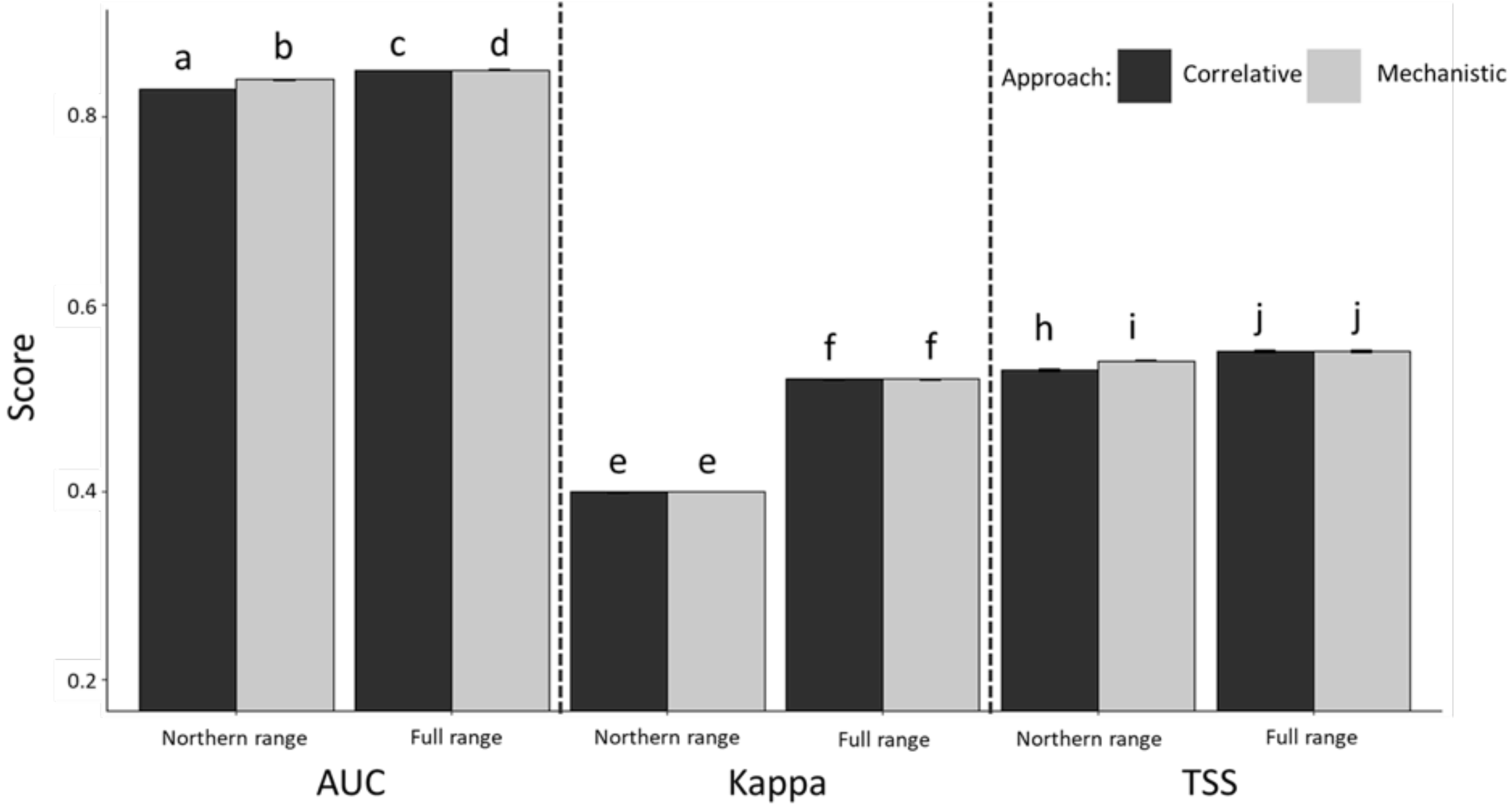

Population
Experiment
Objective
JULY
QUBS
SCP
Freezing temperature; Assessing survival
Low-temperature assays:
-2
Chill susceptible
-6
Freeze avoidant vs. tolerant
-8
Potential lower lethal temperature
CTmin
Threshold for movement
AUGUST
Sites: Brockville; QUBS; Mud lake; Shirley's Bay
SCP
Freezing temperature; Assessing survival
Low-temperature assays:
-6
Freeze avoidant vs. tolerant
-8
Potential lower lethal temperature


Approach:
Correlative
Mechanistic
Score
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Northern range
Full range
Northern range
Full range
Northern range
Full range
AUC
Kappa
TSS