

1 Complex interactions between local adaptation, plasticity, and sex affect vulnerability to
2 warming in a widespread marine copepod

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13 **Keywords:**

14 Thermal adaptation, climate change, copepod, sex-specific response, developmental plasticity,
15 *Acartia tonsa*

16
17 **Abstract:**

18 Predicting the response of populations to climate change requires knowledge of thermal
19 performance. Genetic differentiation and phenotypic plasticity affect thermal performance, but
20 the effects of sex and developmental temperatures often go uncharacterized. We used common
21 garden experiments to test for effects of local adaptation, developmental phenotypic plasticity,
22 and individual sex on thermal performance of the ubiquitous copepod, *Acartia tonsa*. Females
23 had higher thermal tolerance than males in both populations, while the Florida population had
24 higher thermal tolerance compared to the Connecticut population. An effect of developmental
25 phenotypic plasticity on thermal tolerance was observed only in the Connecticut population.
26 Ignoring sex-specific differences may result in a severe underestimation of population-level
27 impacts of warming (i.e. - population decline due to sperm limitation). Further, despite having a
28 higher thermal tolerance, southern populations may be more vulnerable to warming as they lack
29 the ability to respond to increases in temperature through phenotypic plasticity.

30
31 **Introduction:**

32 Temperature has a profound effect on organismal performance [1,2]. Rapid climate warming
33 represents a significant challenge for organisms, increasing average environmental temperatures
34 [3] and the frequency of extreme climatic events (i.e. - heat waves) [4]. Predicting organismal
35 responses to these changes depends on our understanding of the factors affecting thermal
36 tolerance. Acute thermal tolerance is known to be affected by phenotypic plasticity [5] and
37 genetic differentiation [6], as well as diet, behavior and individual sex [7,8,9]. Spatial variation
38 in the thermal environment should generate adaptive differences in thermal performance between
39 populations from different environments [2]. The Climate Variability Hypothesis (CVH) [10,11]
40 states that increased thermal tolerance should correspond with increased mean environmental
41 temperature, while plasticity should evolve in response to variability in the thermal environment.
42 This is supported in terrestrial and freshwater aquatic systems [12, 13], but there have been fewer

43 tests in marine systems [14,15,16], yielding only limited support. Additionally, no tests of the
44 CVH have examined how individual sex factors into observed variation in thermal performance.

45
46 Here we examine the effects of genetic differentiation, developmental phenotypic plasticity, and
47 individual sex on thermal tolerance in the widespread copepod *Acartia tonsa*. This species
48 dominates coastal and estuarine systems around the globe. With a geographic range covering a
49 large latitudinal thermal gradient, this is a good model system to explore the contributions of
50 various adaptive mechanisms to thermal adaptation [15]. Our results show that complex
51 interactions between these variables strongly affect our ability to predict organismal responses to
52 climate change.

53 54 **Methods:**

55 Plankton samples were collected from surface tows at field sites in Groton, Connecticut, and
56 Punta Gorda, Florida (Table 1) during July and August of 2017 using a 250 μ m mesh plankton
57 net. Temperature data for both sites was obtained from the AQUA-MODIS satellite database
58 [17]. Initial laboratory populations, comprised of >1500 mature adults to minimize the effects of
59 genetic drift, were established from collected animals. Cultures were maintained under common
60 garden conditions (30 psu, 12:12 light:dark, 18°C) for several generations, and fed a diet of the
61 microalgae *Tetraselmis* sp., *Rhodomonas* sp., and *Thalassiosira weissflogii*. Eggs were collected
62 from the 18°C culture and moved to 22°C to develop. All other factors were held constant. Once
63 mature, individuals from both developmental conditions (18 and 22°C) were exposed to a 24-
64 hour acute heat stress. Individuals were carefully transferred to a microcentrifuge tube with 1.5
65 mL of filtered seawater, then transferred to heat blocks set to a constant temperature (18-38°C at
66 degree intervals). Each individual experienced a single temperature. Individual survivorship was
67 measured after 24 hours as binary data (1 = survival, 0 = mortality).

68
69 All analyses were performed using R version 3.5.1 [18]. 1717 individuals were used throughout
70 the experiments. Initially, an ANOVA was run for all data (Survivorship ~ Stress Temperature +
71 Sex + Developmental Temperature + Population, and all two-way interactions). Three-way and
72 four-way interaction were excluded from the ANOVA. ANOVAs were also run for each
73 population separately (Survivorship ~ Stress Temperature * Sex * Developmental Temperature).
74 Thermal performance curves were estimated using logistic regressions. Because of the common
75 garden design, differences in the performance curves between developmental conditions within a
76 population can be attributed to developmental phenotypic plasticity, whereas differences
77 between populations should reflect the effects of genetic differentiation. LD₅₀ (the temperature
78 with 50% mortality) was calculated for each performance curve. The change in LD₅₀ between the
79 two developmental conditions (Δ LD₅₀) was used as a measure of the magnitude of the plastic
80 response.

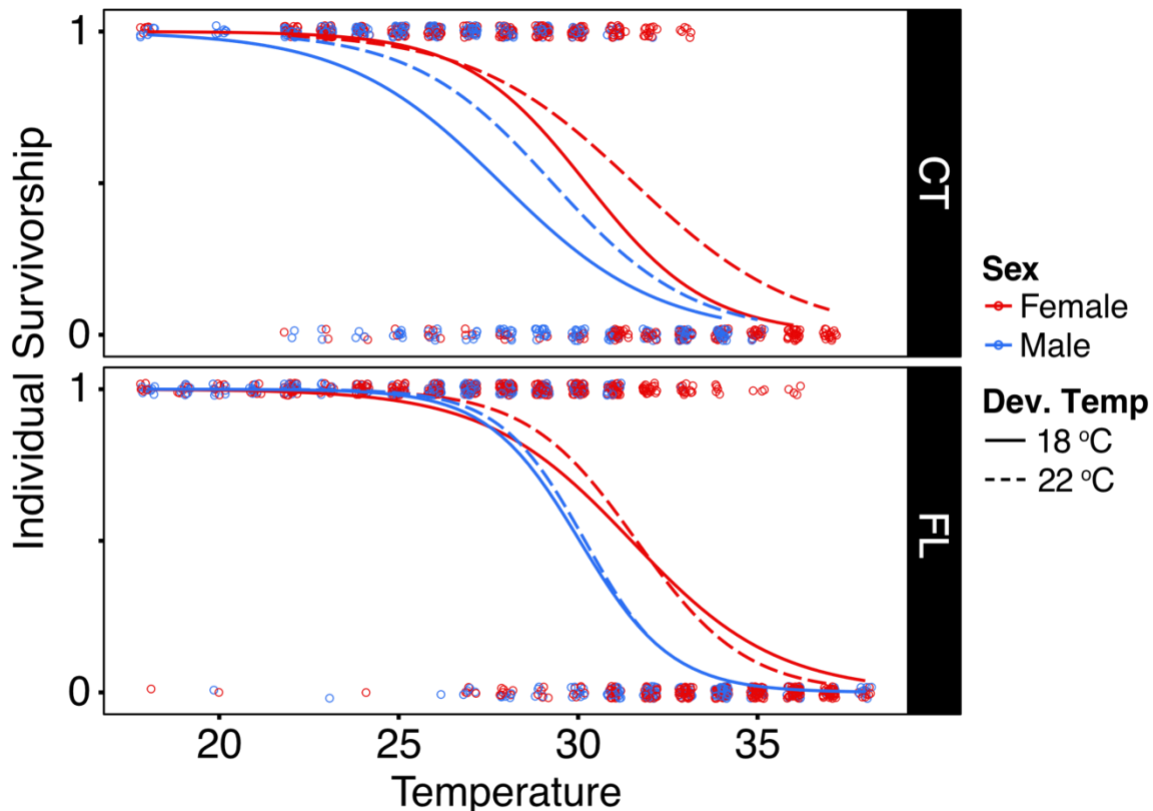
81 82 **Results:**

83 Statistical analyses – In the full ANOVA, significant effects of stress temperature, sex,
84 developmental temperature and population, as well as significant stress temperature x population

85 and developmental temperature x population interaction terms were evident (Table 2). Sex, stress
86 temperature and developmental temperatures were significant in the Connecticut population,
87 with no significant interaction terms. By contrast, only stress temperature and sex and their
88 interaction were significant in the Florida population.

89

90 Thermal performance curves - Males showed lower survival than females in both populations
91 (Fig. 1, red versus blue lines). Developmental temperature had a strong effect on survival in the
92 Connecticut population, but not in the Florida population.

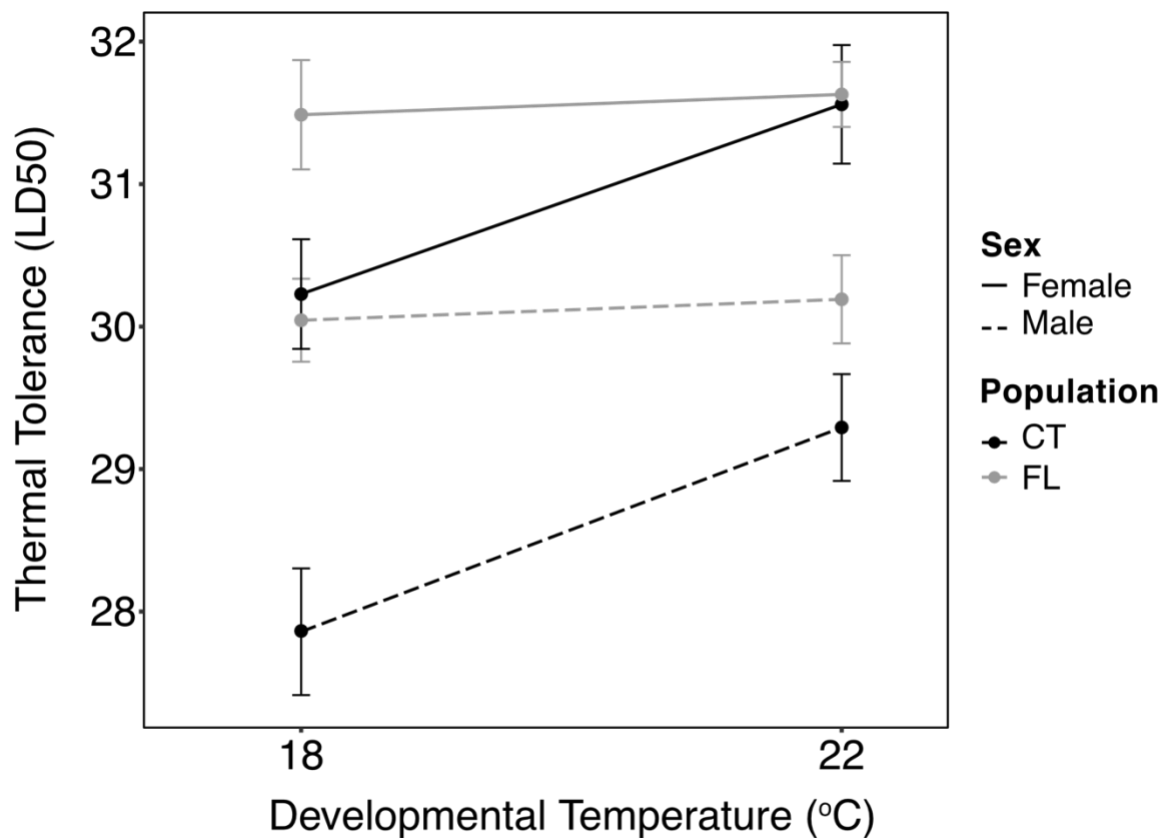


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94 Reaction norms –

95 Thermal tolerance (LD₅₀) reaction norms show clear sex-dependent differences in thermal
96 tolerance (Fig. 2), with females being always more tolerant than males. Higher developmental
97 temperature increased thermal tolerance in the Connecticut population, but not in the Florida
98 population. However, there were no sex-dependent differences in the plastic response between
99 males and females (no difference in slopes), regardless of population.

100



101
102 **Discussion:**
103 The two populations of *Acartia tonsa* used in this study were collected from strongly differing
104 thermal environments - a cool, variable environment (Connecticut), and a warmer, more stable
105 environment (Florida). We observed lower thermal tolerance, but stronger plasticity in the
106 Connecticut population relative to the Florida population, consistent with expectations of the
107 CVH [10,11]. However, we find that individual sex had the largest effect on thermal tolerance.
108 The demographic implications of these results are crucial to consider in predictions of future
109 population dynamics.
110
111 In both populations, females always had a higher thermal tolerance than males. Sex-specific
112 differences in thermal tolerance are observed across diverse systems [8, 19-21]. Within
113 copepods, the few studies that have examined sex-specific thermal tolerance have also found
114 females to be more thermally tolerant than males [9, 22-24], but ours is the first to examine these
115 differences in more than one adaptive mechanism (thermal tolerance and phenotypic plasticity),
116 and in multiple populations. Interestingly, *Acartia tonsa* females have also been found to be
117 more tolerant to toxic dinoflagellates and to starvation [25, 26]. While there are strong
118 differences in male and female thermal tolerance in this study, neither population exhibits
119 significant differences between male and female plastic capacity (ΔLD_{50}). No previous studies

120 have examined differences in male and female developmental phenotypic plasticity, but higher
121 acclimatory capacity was observed in females in a different copepod species [9]. This difference
122 in sex-dependence of the different adaptive mechanisms suggests that their physiological basis is
123 different.

124
125 Multiple factors affect acute thermal stress tolerance. Understanding these factors, and how they
126 vary among populations, has critical implications for predictions of future population dynamics.
127 Lower male thermal tolerance creates an asymmetrical vulnerability to climate change, which
128 could lead to population declines under less intense warming due to sperm-limitation [27]. Our
129 results also suggest that, despite having a higher thermal tolerance, southern populations may be
130 more vulnerable to predicted warming. The southern population exists near their thermal limit
131 under present conditions. As they are also unable to respond to increased temperatures through
132 developmental phenotypic plasticity, any further increase in temperature is likely to have
133 significant negative effects on population survival. Furthermore, males have a significantly lower
134 thermal tolerance, lowering the temperature threshold that would bring the onset of temperature-
135 driven population dynamic changes. Both sexes in the Connecticut population, however, have
136 thermal tolerance values well above the current temperature maximum, and have a robust plastic
137 capacity to increase thermal tolerance, potentially decreasing deleterious effects of warming on
138 this population. Without examining multiple adaptive mechanisms and factors affecting thermal
139 tolerance in several different populations, the complex variation in vulnerability to warming
140 cannot be predicted.

141
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145
146 **Author Contributions:**

147 M.S. collected and cultured all copepods. H.G.D. and M.S. designed the study. K.R. and S.H.
148 performed the experiments. M.S. analyzed the data. M.S. and H.G. Dam drafted the manuscript.

149
150 **Data Accessibility:**

151 All data is accessible on Dryad (doi:10.5061/dryad.v5g6r80)

152
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155 research fellowships from the University of Connecticut.

156
157 **Competing Interests:**

158 We have no competing interests.

159
160 **Ethics Statement:**

161 Permits to collect individuals were not required. Standard protocols were used for copepod
162 culturing and thermal tolerance assays. Ethics committee approval was not required.

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Table Legends:

Table 1 – Site name, geographic coordinates, mean annual temperature, mean annual maximum, and mean annual temperature range for all collection locations.

Table 2 - ANOVA results for the logistic regression relating survivorship to stress temperature, population, developmental temperature, and individual sex. Significant terms ($p < 0.05$) are bolded.

Figure Legends:

Figure 1 - Survivorship data for *Acartia tonsa* individuals is indicated by the points (1 = survival, 0 = mortality). Thermal performance curves are estimated by logistic regression. Color and line type indicate individual sex and developmental temperature respectively.

Figure 2 - Reaction norms showing thermal tolerance (LD_{50}) as a function of developmental temperature for both sexes (line type) from the two populations (color). Points are thermal tolerance \pm SE from the logistic regression models. Reaction norm slope is the magnitude of plasticity.

References:

1. Hochachka, P. W. & Somero, G. N. 2002 *Biochemical adaptation: mechanism and process in physiological evolution*. New York.
2. Angilletta, M. J. 2009 *Thermal adaptation: a theoretical and empirical analysis*. New York: Oxford University Press Inc.
3. Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R, Church JA, Clarke L, Dahe Q, Dasgupta P, Dubash NK. Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change. IPCC; 2014.
4. Meehl, G. A. 2004 More Intense, More frequent, and longer lasting heat waves in the 21st century. *Science* **305**, 994–997. (doi:10.1126/science.1098704)
5. Stillman, J. H. 2003 Acclimation capacity underlies susceptibility to climate change. *Science* **301**, 65–65. (doi:10.1126/science.1083073)
6. Hochachka, P. W. & Somero, G. N. 1968 The adaptation of enzymes to temperature. *Comparative biochemistry and physiology* **27**, 659–668. (doi:10.1016/0010-406X(68)90605-1)
7. Andersen, L. H., Kristensen, T. N., Loeschke, V., Toft, S. R. & Mayntz, D. 2010 Protein and carbohydrate composition of larval food affects tolerance to thermal stress and desiccation in adult *Drosophila melanogaster*. *Journal of Insect Physiology* **56**, 336–340. (doi:10.1016/j.jinsphys.2009.11.006)

- 202 8. Johnstone, M., Schiffer, M. & Hoffmann, A. A. 2017 Separating multiple sources of
203 variation on heat resistance in *Drosophila hydei*. *Journal of Insect Physiology* **96**, 122–127.
204 (doi:10.1016/j.jinsphys.2016.11.001)
- 205 9. Bradley, B. P. 1978 Increase in range of temperature tolerance by acclimation in the
206 copepod *Eurytemora affinis*. *The Biological Bulletin* **154**, 177–187.
- 207 10. Janzen, D. H., 1967 Why mountain passes are higher in the tropics. *Am. Nat.* **101**, 233 – 249.
- 208 11. Stevens GC. 1989 The latitudinal gradient in geographical range: how so many species
209 coexist in the tropics. *Am. Nat.* **133**, 240 – 256. (doi:10.1086/ 284913)
- 210 12. Shah, A. A. et al. 2017 Climate variability predicts thermal limits of aquatic insects across
211 elevation and latitude. *Functional Ecology* **31**, 2118–2127. (doi:10.1111/1365-2435.12906)
- 212 13. Sunday, J. M., Bates, A. E. & Dulvy, N. K. 2011 Global analysis of thermal tolerance and
213 latitude in ectotherms. *Proceedings of the Royal Society B: Biological Sciences* **278**, 1823–
214 1830. (doi:10.1098/rspb.2010.1295)
- 215 14. Stillman, J. H. & Somero, G. N. 2000 A comparative analysis of the upper thermal
216 tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: influences of latitude,
217 vertical zonation, acclimation, and phylogeny. *Physiological and Biochemical Zoology* **73**,
218 200–208. (doi:10.1086/316738)
- 219 15. Compton, T. J., Rijkenberg, M. J. A., Drent, J. & Piersma, T. 2007 Thermal tolerance
220 ranges and climate variability: A comparison between bivalves from differing climates.
221 *Journal of Experimental Marine Biology and Ecology* **352**, 200–211.
222 (doi:10.1016/j.jembe.2007.07.010)
- 223 16. Pereira, R. J., Sasaki, M. C. & Burton, R. S. 2017 Adaptation to a latitudinal thermal
224 gradient within a widespread copepod species: the contributions of genetic divergence and
225 phenotypic plasticity. *Proceedings of the Royal Society B: Biological Sciences* **284**,
226 20170236–8. (doi:10.1098/rspb.2017.0236)
- 227 17. NASA Goddard Space Flight Center, Ocean Ecology Laboratory, Ocean Biology Processing
228 Group. 2014 Sea-viewing Wide Field-of-view Sensor (SeaWiFS) ocean color data. NASA
229 OB.DAAC, Greenbelt, MD, USA. (doi:10.5067/ORBVIEW-2/SEAWIFS_OC.2014.0)
- 230 18. R Core Team. 2016 *R: A language and environment for statistical computing*. R Foundation
231 for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org/>
- 232 19. Jeffries, K. M., Hinch, S. G., Martins, E. G., Clark, T. D., Lotto, A. G., Patterson, D. A.,
233 Cooke, S. J., Farrell, A. P. & Miller, K. M. 2012 Sex and proximity to reproductive maturity
234 influence the survival, final maturation, and blood physiology of Pacific salmon when
235 exposed to high temperature during a simulated migration. *Physiological and Biochemical*
236 *Zoology* **85**, 62–73. (doi:10.1086/663770)

- 237 20. Blanckenhorn, W. U., Gautier, R., Nick, M., Puniamoorthy, N. & Schäfer, M. A. 2014
238 Stage- and sex-specific heat tolerance in the yellow dung fly *Scathophaga stercoraria*.
239 *Journal of Thermal Biology* **46**, 1–9. (doi:10.1016/j.jtherbio.2014.09.007)
- 240 21. Winne, C. T. & Keck, M. B. 2005 Intraspecific differences in thermal tolerance of the
241 diamondback watersnake (*Nerodia rhombifer*): effects of ontogeny, latitude, and sex.
242 *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*
243 **140**, 141–149. (doi:10.1016/j.cbpb.2004.11.009)
- 244 22. Willett, C. S. 2010 Potential fitness trade-offs for thermal tolerance in the intertidal copepod
245 *Tigriopus californicus*. *Evolution* **64**, 2521–2534. (doi:10.1111/j.1558-5646.2010.01008.x)
- 246 23. Damgaard, R. M. & Davenport, J. 1994 Salinity tolerance, salinity preference and
247 temperature tolerance in the high-shore harpacticoid copepod *Tigriopus brevicornis*. *Mar*
248 *Biol* **118**, 443–449. (doi:10.1007/BF00350301)
- 249 24. Hall, C. J. 2001 Effects of salinity and temperature on survival and reproduction of
250 *Boeckella hamata* (Copepoda: Calanoida) from a periodically brackish lake. *Journal of*
251 *Plankton Research* **23**, 97–104. (doi:10.1093/plankt/23.1.97)
- 252 25. Avery, D. E., Altland, K. K. & Dam, H. G. 2008 Sex-related differential mortality of a
253 marine copepod exposed to a toxic dinoflagellate. *Limnology and Oceanography* **53**, 2627–
254 2635. (doi:10.4319/lo.2008.53.6.2627)
- 255 26. Finiguerra, M. B., Dam, H. G., Avery, D. E. & Burris, Z. 2013 Sex-specific tolerance to
256 starvation in the copepod *Acartia tonsa*. *Journal of Experimental Marine Biology and*
257 *Ecology* **446**, 17–21. (doi:10.1016/j.jembe.2013.04.018)
- 258 27. Kiørboe, T. 2007 Mate finding, mating, and population dynamics in a planktonic copepod
259 *Oithona davisae*: There are too few males. *Limnology and Oceanography* **52**, 1511–1522.
260 (doi:10.4319/lo.2007.52.4.1511)

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264 Table 1 –

Population	Coordinates	Mean Annual Temperature (°C)	Mean Maximum Temperature (°C)	Mean Annual Temperature Range (°C)
Connecticut (CT)	41.320591, -72.001564	13.3	22.7	22.5
Florida (FL)	26.940398, -82.051036	24.9	31.4	15.3

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266 Table 2 –
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All Data

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
Stress Temperature	1	928.89	1715	1409.7	0.00
Individual Sex	1	54.7	1714	1355	1.40E-13
Developmental Temperature	1	7.74	1713	1347.2	0.005388
Population	1	17.8	1712	1329.4	2.45E-05
Dev. Temp. x Population	1	5.46	1711	1324	0.019462
Sex x Dev. Temp	1	0.27	1710	1323.7	0.60482
Sex x Population	1	3.82	1709	1319.9	0.050578
Stress Temp. x Population	1	8.38	1708	1311.5	0.003801
Stress Temp. x Dev. Temp.	1	0.06	1707	1311.5	0.809781
Stress Temp. x Sex	1	2.29	1706	1309.2	0.130522

CT Model

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
Stress Temperature	1	318.6	725	666.22	0.00
Individual Sex	1	29.42	724	636.8	5.82E-08
Developmental Temperature	1	10.49	723	626.31	0.001202
Stress Temp. x Sex	1	0	722	626.31	0.960815
Stress Temp. x Dev. Temp.	1	0.14	721	626.17	0.71148
Sex x Dev. Temp.	1	0.23	720	625.94	0.631601
Stress Temp. x Sex x Dev. Temp.	1	1.75	719	624.2	0.186143

FL Model

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
Stress Temperature	1	646.63	988	706.5	0.00
Individual Sex	1	20.51	987	685.99	5.94E-06
Developmental Temperature	1	0.63	986	685.36	0.42597
Stress Temp. x Sex	1	4.86	985	680.5	0.02744
Stress Temp. x Dev. Temp.	1	2.97	984	677.52	0.08465
Sex x Dev. Temp.	1	0.22	983	677.31	0.64267
Stress Temp. x Sex x Dev. Temp.	1	0.23	982	677.08	0.63395

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