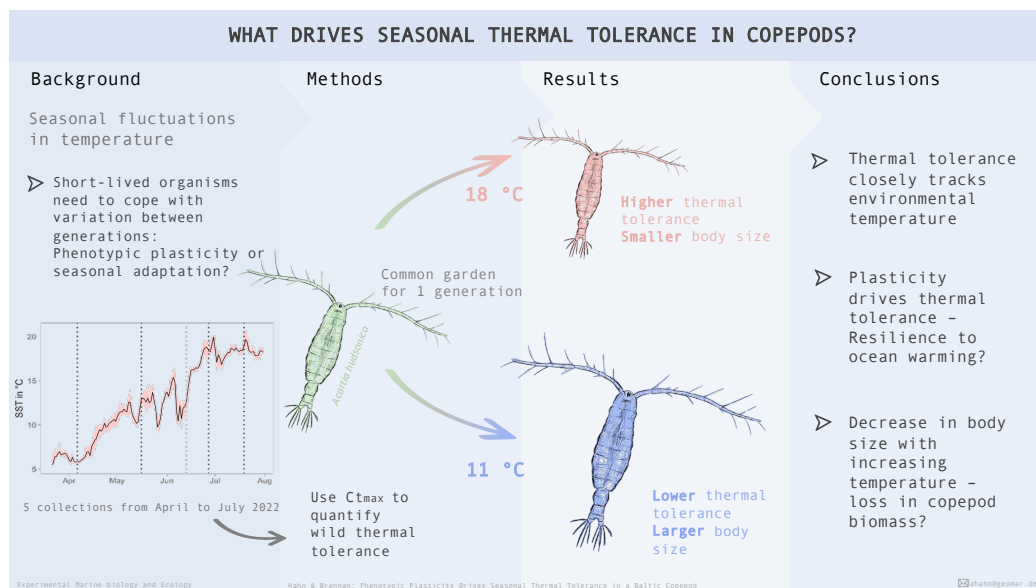


1 Graphical Abstract

2 Phenotypic Plasticity Drives Seasonal Thermal Tolerance in a Baltic Copepod

3 Alexandra Hahn, Reid S. Brennan



5 Highlights

6 **Phenotypic Plasticity Drives Seasonal Thermal Tolerance in a Baltic** 7 **Copepod**

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- 9 • *Acartia hudsonica* shows strong seasonality in thermal tolerance.
- 10 • The observed seasonal differences in CT_{max} are driven by phenotypic
11 plasticity not adaptation.
- 12 • Body size in *A. hudsonica* is negatively correlated to environmental
13 and developmental temperature.
- 14 • This is the first record of *A. hudsonica* in the Baltic Sea known to the
15 authors.

16 Phenotypic Plasticity Drives Seasonal Thermal
17 Tolerance in a Baltic Copepod

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19 **Abstract**

20 Seasonal changes in environmental conditions require substantial physiologi-
21 cal responses for population persistence. Phenotypic plasticity is a common
22 mechanism to tolerate these changes, but for organisms with short gener-
23 ation times rapid adaptation may also be a contributing factor. Here, we
24 aimed to disentangle the impacts of adaptation from phenotypic plasticity
25 on thermal tolerance of the calanoid copepod *Acartia hudsonica* collected
26 throughout spring and summer of a single year. We used a common garden
27 (11 °C and 18 °C) design to determine the relative impacts of plasticity ver-
28 sus adaptation. *Acartia hudsonica* were collected from five time points across
29 the season and thermal tolerance was determined using critical thermal max-
30 imum (CT_{max}) followed by additional measurements after one generation of
31 common garden. As sea surface temperature increased through the season,
32 field collected individuals showed corresponding increases in thermal toler-
33 ance but decreases in body size. Despite different thermal tolerances of wild
34 collections, common garden animals did not differ in CT_{max} within thermal
35 treatments. Instead, there was evidence of phenotypic plasticity where higher
36 temperatures were tolerated by the 18 °C versus the 11 °C treatment animals

37 across all collections. Acclimation also had significant effects on body size,
38 with higher temperatures resulting in smaller individuals, consistent with
39 the temperature size rule. Therefore, the differences in thermal tolerance
40 and body size observed in field collected *A. hudsonica* were likely driven by
41 plasticity rather than adaptation. However, the observed decrease in body
42 size suggests that nutrient availability and ecosystem functioning could be
43 impacted if temperatures consistently increase with no change in copepod
44 abundance. This is the first record of *A. hudsonica* in the Baltic Sea known
45 to the authors.

46 *Keywords:* CT_{max} , Seasonality, *Acartia*

47 1. Introduction

48 Environmental variation is ubiquitous across habitats and organisms are
49 able to respond to and tolerate this variation in multiple ways. When vari-
50 ation is both predictable and experienced within the lifespan of an individ-
51 ual, it is expected that plasticity will evolve (Pereira et al., 2017; Bitter
52 et al., 2021). Conversely, if environmental variation is unpredictable or at
53 timescales longer than generation time, plasticity is unlikely to evolve but
54 selection should act with each environmental fluctuation. In this latter case,
55 the resulting changes in selective pressure through time can lead to fluctuat-
56 ing selection, which can not only drive rapid adaptation but also contribute
57 to the maintenance of genetic diversity in populations (Bergland et al., 2014).

58 One of the major drivers of fluctuating selection in the wild is seasonal
59 change. In this scenario, the environmental changes that occur within a
60 year result in divergent selective pressures at different temporal periods. For

61 example, summer months may favor warm tolerant genotypes while cooler
62 spring or fall temperatures may favor genotypes that have higher perfor-
63 mance at low temperature. While these changes are relatively consistent
64 on a yearly basis, the temporal scale is beyond many organisms' lifespan,
65 which can lead to adaptation to different periods within the fluctuating sea-
66 sonal environmental change. This phenomenon has been observed in diverse
67 species and ecosystems, including marine copepods (Hairston and Dillon,
68 1990), *Lonchopterid* flies (Niklasson et al., 2004), dandelion (Vavrek et al.,
69 1996), two-spotted ladybugs (Brakefield, 1985), and swallows (Brown et al.,
70 2013), among others (Siepielski et al., 2009; Bell, 2010). There is also evi-
71 dence for fluctuating selection at the genomic level: in *Drosophila* fruit flies
72 genome-wide allele frequencies consistently and repeatedly shift between sea-
73 sons due to selection, helping to maintain genetic variation within popula-
74 tions (Johnson et al., 2023). Thus, adaptive responses to seasonal change
75 may be a common phenomenon across taxa with short generation times.

76 In addition to contributing to the maintenance of genetic variation within
77 populations, the mechanisms underlying rapid seasonal adaptation can help
78 shed light on how populations may respond to ongoing anthropogenic driven
79 global change. For example, many species experience yearly temperature
80 changes with an amplitude greater than those predicted under global warm-
81 ing (Bujan et al., 2020) and across terrestrial and aquatic ectotherms, popula-
82 tions routinely achieve increased thermal tolerance in warmer seasons (Hop-
83 kin et al., 2006; Bujan et al., 2020). This suggests that mechanisms enabling
84 seasonal responses, such as plasticity and adaptation, might similarly drive
85 resilience to global warming.

86 Copepods provide an ideal model to understand population level re-
87 sponses to fluctuating selection across seasons. These organisms are short-
88 lived with generation times of a few weeks leading to multiple generations
89 per year, each subjected to a unique thermal regime. Previous work on ther-
90 mal tolerance of copepods has found contributions of both plasticity and
91 adaptation. For instance, rearing temperature drives plastic responses and
92 significantly influences thermal tolerance (González, 1974), egg production
93 (Holste and Peck, 2006) and growth (Sasaki and Dam, 2020). Temperature
94 also affects adult body size with warmer temperatures leading to smaller
95 individuals (Viitasalo et al., 1995; Sasaki et al., 2019), consistent with the
96 temperature-size rule (Atkinson, 1994). Conversely, there is ample evidence
97 for local adaptation to temperature (Lonsdale and Levinton, 1985; Pereira
98 et al., 2017; Karlsson and Winder, 2020) as well experimental evolution stud-
99 ies showing adaptive responses to high temperature after only a few gener-
100 ations (Dam et al., 2021; Brennan et al., 2022b). Finally, planktonic cope-
101 pods are an integral part of marine food webs and function as an essential
102 link between primary production and higher trophic levels (Turner, 2004;
103 Dzierzbicka-Głowacka et al., 2019). Therefore, our understanding of drivers
104 of copepod responses to temperature change has important implications for
105 the resilience of marine ecosystems as a whole.

106 In this study, we focus on the calanoid copepod *Acartia hudsonica* (Pin-
107 hey, 1926). *Acartia* copepods are among the most-studied copepod genera,
108 in part due to their world-wide distribution and high abundance, making
109 them foundational to marine and coastal ecosystems (Walter and Boxshall,
110 2023). In the Baltic Sea, this group is one of the dominant zooplankton and

111 critical to local ecosystems (Diekmann et al., 2012; Dzierzbicka-Głowacka
112 et al., 2019). *Acartia hudsonica*, specifically, is a cold adapted species that is
113 generally abundant in winter and spring months out-competing more warm
114 adapted congeners at low temperatures. The species can tolerate a broad
115 temperature range from at least 4 to 18 °C (Sullivan and McManus, 1986).
116 For populations native to the Eastern United States, *A. hudsonica* produces
117 resting eggs at temperatures > 16 °C and abundances strongly decline in
118 summer (Sullivan and McManus, 1986).

119 Here, we seek to disentangle the impacts of adaptation from plasticity in
120 thermal tolerance of *A. hudsonica* collected throughout spring and summer of
121 2022. We hypothesized that the thermal tolerance of wild collected individ-
122 uals would closely follow the environmental temperature. Further, because
123 developmental temperature strongly impacts copepod body size (Horne et al.,
124 2016), we predicted that body size would decrease as temperature increased.
125 We used common garden conditions at two different temperatures to deter-
126 mine if the observed thermal tolerance and body size shifts between wild
127 individuals were driven by adaptation or plasticity. Together, these results
128 help to reveal the underlying mechanisms driving seasonal thermal tolerance
129 of *A. hudsonica* and provide insight into how this species may respond to
130 warming conditions in the future.

131 **2. Material & Methods**

132 *2.1. Sampling and cultures*

133 All samples were collected by 100 μm WP2 net on board the research
134 vessel *Polarfuchs* in Kiel Fjord (54°19'50"N 10°09'20"E). Live samples were

135 stored at collection temperature until processing. The experiment included
136 five sampling dates (referred to as collections) from April 2022 to July 2022
137 that spanned SST from 5.81 °C to 19.16 °C (Table 1, Hiebenthal et al. un-
138 published data).

Table 1: Overview of sampling dates and corresponding SST averages.

Collection name	Collection date	Daily mean	2-week mean
Collection 1	06 Apr 2022	5.81 ±0.05 °C	6.36 ±0.47 °C
Collection 2	16 May 2022	12.66 ±0.53 °C	11.44 ±0.84 °C
Collection 3	13 Jun 2022	12.37 ±0.33 °C	12.86 ±1.52 °C
Collection 4	27 Jun 2022	18.35 ±0.13 °C	16.55 ±1.83 °C
Collection 5	19 Jul 2022	19.16 ±0.79 °C	18.11 ±0.68 °C

139 For all sampling dates, approximately 440 adult animals were sorted and
140 split into two 6 L culture buckets with air supply and held at their collection
141 temperature. Over the following two days, CT_{max} assays were run on the
142 wild-caught copepods. After the initial assays, the cultures were moved to
143 a cold (11 °C) or warm (18 °C) culture room. No major fluctuations in
144 temperature occurred throughout the experiment. All cultures were kept on
145 a 12:12 light regime at a common salinity of 15 and were allowed to reproduce,
146 with water changes approximately every 7 days. Feeding was *ad libitum* with
147 *Rhodomonas sp.* and *Isochrysis galbana* (Holste and Peck, 2006; Ismar et al.,
148 2008; Mahjoub et al., 2014).

149 After the initial culture establishment at treatment temperatures (2-4

150 days), the cultures were filtered through a nested 200 μm and 50 μm mesh
151 sieve. Adults were retained on the 200 μm mesh and kept for further cultur-
152 ing. Offspring, eggs and nauplii, of the parental generation were retained on
153 the 50 μm mesh and placed in a new culture bucket to start the F1 gener-
154 ation. Development was monitored to catch the onset of maturation. Once
155 the F1 generation reached adulthood, CT_{max} assays were repeated. Collec-
156 tion 3 collapsed before reaching F1. Therefore, this collection is excluded
157 from further analysis.

158 2.2. Temperature assays

159 CT_{max} was used as a proxy for thermal tolerance. CT_{max} is defined as
160 the temperature at which locomotion is affected in a way that prevents the
161 individual to move away from harmful conditions, eventually resulting in
162 death (Cowles and Bogert, 1944). In this study, CT_{max} was the temperature
163 at which the individuals showed no visible response to a stimulus (details
164 below). For each collection and treatment the CT_{max} of twenty males and
165 twenty females was quantified. Individuals were sorted under temperature-
166 controlled conditions and placed in 12 ml glass tubes filled with 5 ml of filtered
167 seawater. Ten individuals, five males and five females were simultaneously
168 run per trial. The starting temperature of the experimental tank matched
169 the wild collection or culturing temperature. To minimize bias, an assistant
170 placed the individuals in random order in the experimental tank, leaving the
171 experimenter blinded to the animals' sex until after the experiment. Heating
172 was monitored with a thermometer (PCE-HPT1, PCE instruments, Men-
173 schede, Germany) placed into an additional glass tube filled with filtered
174 seawater. The animals were given a 30-minute acclimation period before

175 heating was started. With a 300 W and 500 W heater, heating was con-
176 sistent at ~ 0.2 °C/min (Fig. S.1). Once 22.5 °C was reached, the 300 W
177 heater was removed, slowing the ramping temperature to ~ 0.1 °C/min to
178 facilitate monitoring the individuals. Throughout the experiment, the exper-
179 imenter was blinded to the exact temperature. Animals were continuously
180 monitored and when movement ceased, gentle pipetting was used to trigger
181 a reaction. If still no movements occurred, the corresponding temperature
182 was considered CT_{max} .

183 After the experiment, the sex for all animals was confirmed and the indi-
184 viduals were preserved in 95% ethanol and later photographed using a Nikon
185 imaging microscope and Nikon imaging software (NIS-Elements v. 5.20.00).
186 All images were obtained using the same magnification to ensure consistency.
187 From the pictures, the prosome length was measured for each copepod using
188 ImageJ (Schneider et al., 2012). Three measurements were obtained per in-
189 dividual and averaged in the later analysis. While formalin is usually used to
190 preserve plankton samples for length analysis (Connolly et al., 2017; Aguilera
191 et al., 2020), previous work on zooplankton has shown no effect of ethanol
192 preservation on body size (Black and Dodson, 2003) and any size effect would
193 be consistent across the experiment. Further, ethanol preservation allows for
194 downstream genetic analyses, which is essential when dealing with cryptic
195 copepod species.

196 *2.3. Genotyping*

197 To confirm species identity, 4-15 copepods per collection were genotyped
198 using the mtCOI region (Table S.1). For the DNA extraction, ethanol-
199 preserved copepods were rinsed with ultra-clean water and rehydrated for

200 one hour. Individual copepods were transferred into 100 μ l of 5% Chelex
201 solution, incubated for 20 minutes at 95 °C in a water bath, then centrifuged
202 for 5 minutes at 8000 rpm. PCR reactions were conducted in 20 μ l volume
203 with 9.8 μ l ultra clean water, 2 μ l dNTPs, 2 μ l Buffer, 2 μ l LCO1490 for-
204 ward primer or HCO2198 reverse primer (5mM, Folmer et al. (1994)), 0.2 μ l
205 DreamTaq DNA polymerase (Thermo Fisher Scientific Inc., Massachusetts,
206 US), and 2 μ l of DNA.

207 Amplification conditions were: 3 min of denaturation at 94 °C followed
208 by 33 cycles of 45 s denaturation at 94 °C, 45 s annealing at 48 °C, 60 s
209 extension at 72 °C and a final extension at 72 °C for 7 min. Samples were
210 sequenced on a Sanger sequencing platform by Eurofins Deutschland (Ger-
211 many). Sequences were checked and aligned to generate consensus sequences
212 using CodonCodeAligner (CODONCODE, 2010). The *Acartia* genus consists
213 of multiple cryptic species making species identification difficult. Therefore,
214 we followed Figueroa et al. (2020) and used a bayesian approach to determine
215 species and clade for all samples. Using known samples from Figueroa et al.
216 (2020), we aligned all raw reads with MUSCLE (Edgar, 2004), converted
217 to NEXUS format in R using APE (Paradis and Schliep, 2019), and used
218 MrBayes to build a phylogenetic tree with *Acartia dana* as the outgroup.
219 Tree plots were made using *ggtree* (Yu et al., 2017). Since thermal tolerance
220 measurements showed outliers in collections 4 and 5, 38 experimental outliers
221 and additional trial animals from those collections were genotyped.

222 2.4. Data analysis and statistics

223 Data manipulation, visualization, and statistics were conducted in R ver-
224 sion 4.2.2 (R Core Team, 2022).

225 To understand the factors influencing CT_{max} and length we used gener-
226 alized linear models. Model selection was done by comparing model fit and
227 Akaike's information criterion (AIC) as well as considering biological rele-
228 vance. The models included main effects of collection number (1-5), treat-
229 ment (wild, warm, cold), sex, length, and the interaction of treatment and
230 collection. Random effects of vial number, tank used for the trial, and time
231 of day did not improve model fit and were therefore excluded from the final
232 models. A similar model was used to determine the factors influencing cope-
233 pod length. Here, we included main effects of developmental temperature
234 and sex. Pairwise post-hoc testing was done comparing the model means
235 using the *emmeans* package (Lenth, 2023).

236 3. Results

237 The sampling days spanned SST values from 5.81 °C to 19.16 °C (Fig. 1A;
238 see Table 1 for all values and standard deviations). The thermal tolerance of
239 wild-caught individuals mirrored the environmental temperature (Fig. 1B).
240 CT_{max} values were lowest in Collection 1 (25.9 °C \pm 1.4 °C), where the tem-
241 perature over the two weeks prior to sampling averaged 6.36 °C \pm 0.47 °C.
242 CT_{max} then increased throughout the collections (Col-2: 27.9 °C \pm 0.7 °C;
243 Col-3: 27.9 °C \pm 1.0 °C; Col-4: 29.1 °C \pm 0.6 °C), reaching its maximum at
244 Collection 5 (29.8 °C \pm 2.0 °C), where the two-week SST average was 18.11
245 °C \pm 0.68 °C (Spearman's $\rho = 0.758$, $p < 0.001$). The prosome length of wild
246 individuals showed an opposite trend, with size decreasing as SST increased
247 (Fig 1C, Spearman's $\rho = -0.572$, $p < 0.001$). Two week averages were chosen
248 to characterize SST as this is the approximate the amount of time it takes

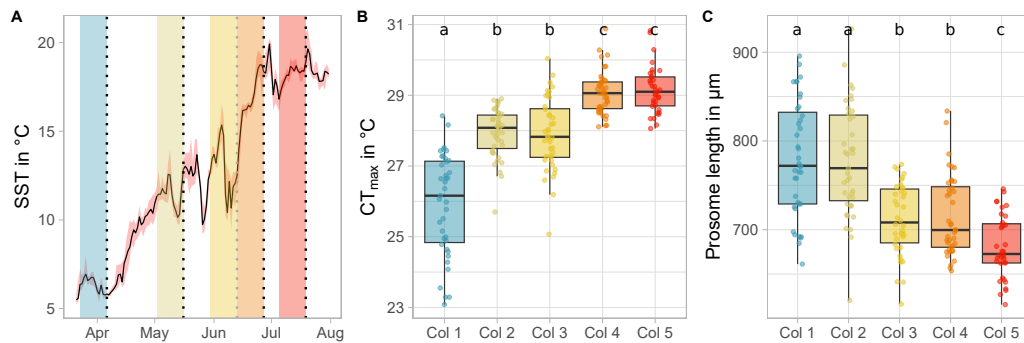


Figure 1: Thermal tolerance follows the seasonal changes in temperature. (A) Sea surface temperature at the collection site where sampling points are indicated by dotted lines and shaded boxes indicate the two-week period prior to sampling. (B) Critical thermal maxima and (C) mean prosome length for wild collected individuals. Boxplot colors correspond to sampling date and compact letters show the results from post-hoc tests where shared letters indicate no significant difference between measurements.

249 for copepods to mature from egg to adult and can therefore be considered
250 developmental temperature; the two-week average showed a similar trend to
251 daily mean temperatures (Fig. S.2).

252 Following the common garden, there was a significant effect of treatment,
253 collection and the interaction between treatment and collection on CT_{max}
254 (Fig. 2A, $p < 0.001$ for all, see Table S.3) for detailed report). While CT_{max}
255 of the wild individuals varied depending on the SST around sampling, the
256 thermal tolerance measurements within the cold and warm treatment were
257 similar across collections. The weighted model means for all warm treatments
258 did not significantly differ from each other (col-1: $28.7\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$, col-2: 28.6
259 $^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$, col-4: $29.0\text{ }^{\circ}\text{C} \pm 0.6\text{ }^{\circ}\text{C}$, $p \geq 0.578$) and were comparable to the
260 wild collections 4 and 5 ($29.1\text{ }^{\circ}\text{C} \pm 0.6\text{ }^{\circ}\text{C}$ and $29.2\text{ }^{\circ}\text{C} \pm 0.7\text{ }^{\circ}\text{C}$ respectively, p

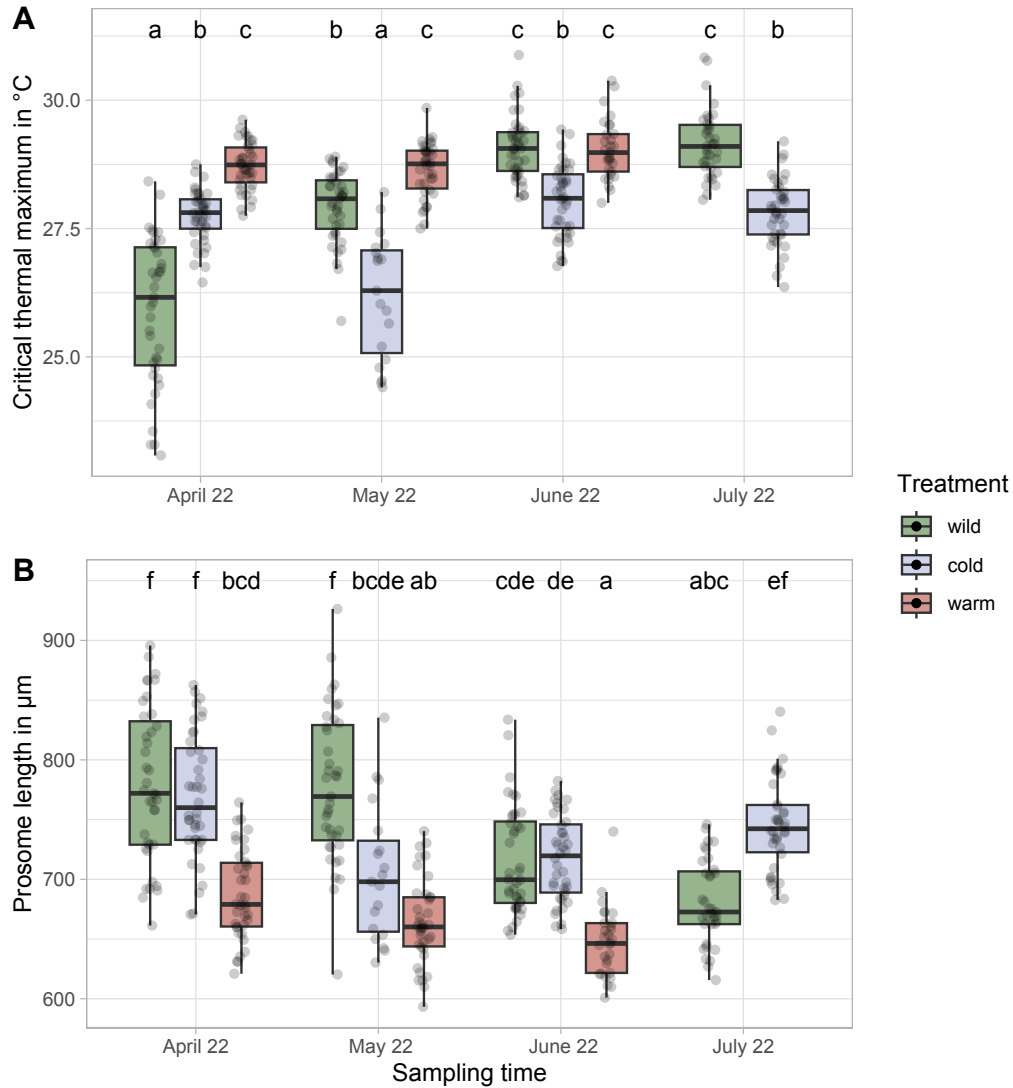


Figure 2: Phenotyping results following common garden for (A) critical thermal maxima and (B) prosome length. Colors of boxes correspond to treatment where "wild" are field collected animals, "cold" are F1 animals at 11°C, "warm" are F1 animals at 18°C. Compact letters show the results from post-hoc tests where shared letters indicate no significant difference between measurements.

261 ≥ 0.153) where the mean temperature resembled the warm treatment (warm
262 treatment 18 °C, wild SST 16.55 °C \pm 1.83 °C, and 18.11 °C \pm 0.68 °C respec-
263 tively). For the cold treatment, weighted means did not differ significantly
264 (col-1: 27.7 °C \pm 0.5 °C, col-4: 28.0 °C \pm 0.7 °C, col-5: 27.8 °C \pm 0.6 °C, $p \geq$
265 0.789) and were comparable to the thermal tolerance of the wild collection
266 2 (27.9 °C \pm 0.7 °C, $p \geq 0.992$) where the developmental temperature was
267 similar (cold treatment 11 °C, wild SST 11.44 °C \pm 0.84 °C). The exception
268 was the cold treatment of collection 2, which was significantly lower than
269 the other cold collections ($p < 0.001$). Here, CT_{max} was comparable to the
270 mean of the wild individuals within collection 1 (col-1: 25.9 °C \pm 1.4 °C, col2:
271 26.2 \pm 1.2 °C, $p = 0.965$) despite the treatment temperatures differing by \sim
272 4 °C. The effect of treatment, collection on prosome length and the inter-
273 action of both terms were significant (Fig. 2B, $p < 0.001$ for all, see Table
274 S.3). However, post-hoc testing revealed that there were no clear similari-
275 ties within treatments, suggesting a more complicated connection between
276 prosome length, seasonality and common garden temperature.

277 There was a strong plastic effect of developmental temperature on thermal
278 performance and prosome length ($p < 0.001$, Fig. S.4). The reaction norms
279 for treatment effect on CT_{max} showed a positive effect of treatment temper-
280 ature (Fig. 3A). There was a significant interaction between collections that
281 was driven by the outlier col-2 and when col-2 was removed there was no
282 interaction (with col-2: $p < 0.001$, without col-2: $p = 0.897$). Prosome length
283 was negatively affected by treatment temperature (Fig. 3B). Again, a signif-
284 icant interaction was driven by col-2, that was not present when removing
285 the outlier collection (with col-2: $p = 0.019$, without col-2: $p = 0.372$). The

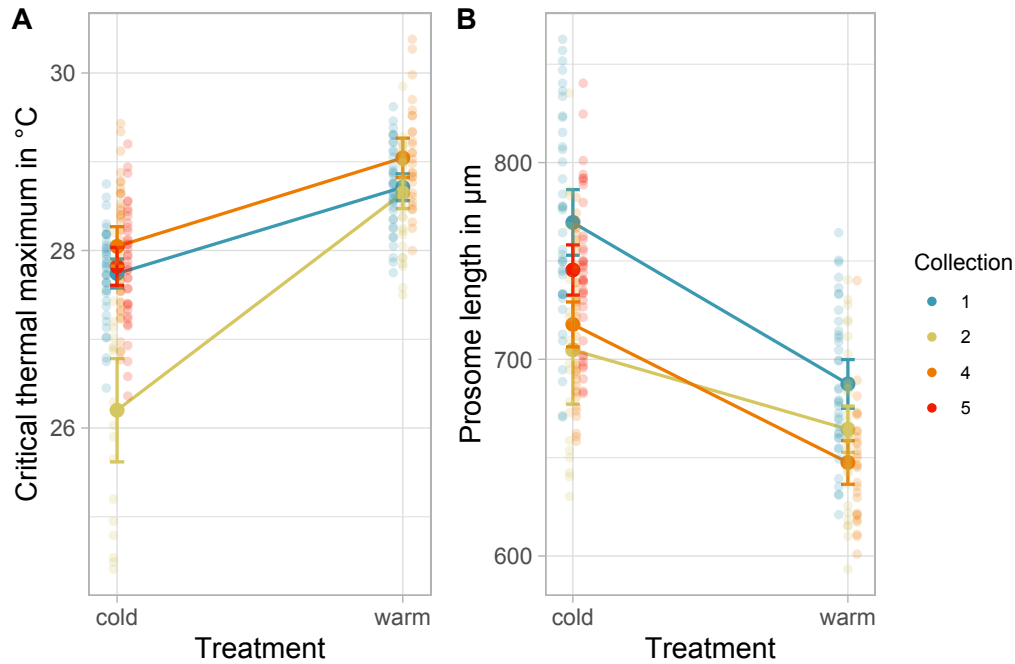


Figure 3: Reaction norms for treatment effect on (A) Critical thermal maximum; (B) Prosome length; bold points indicate mean values, error bars indicate 0.95 % confidence interval.

286 similar slopes of reaction norms and the absence of an interaction between
287 treatment and collection support the presence of plasticity.

288 Furthermore, there was an increase of CT_{max} with increasing developmen-
289 tal temperature (Spearman's $\rho = 0.718$, $p < 0.001$). Conversely, prosome
290 length was negatively correlated with developmental temperature, where
291 higher temperatures during development led to smaller individuals (Spear-
292 man's $\rho = -0.566$, $p < 0.001$). These effects were present in both male and
293 female animals, however, females had significantly higher thermal tolerance
294 and larger body size compared to males (CT_{max} females: $28.4 \text{ }^\circ\text{C} \pm 1.21 \text{ }^\circ\text{C}$,
295 males: $27.7 \text{ }^\circ\text{C} \pm 1.21 \text{ }^\circ\text{C}$, length females: $744 \text{ } \mu\text{m} \pm 69 \text{ } \mu\text{m}$, males: $694 \text{ } \mu\text{m}$

296 $\pm 44 \mu\text{m}$, $p < 0.001$ for both).

297 In addition to the correlation of developmental temperature, there was
298 a negative correlation between prosome length and CT_{max} in wild animals
299 where smaller individuals had significantly higher thermal tolerance ($p <$
300 0.001 , Pearson' $r = -0.229$, Fig. 4A). While this result was likely driven by
301 the aforementioned relation of developmental temperature and length, the
302 negative trend was also present, though weaker, when looking only at the
303 warm treatment common gardened animals ($p = 0.038$, Fig. 4B); no effect
304 was observed within cold common gardened treatments ($p = 0.108$, Fig. 4B).

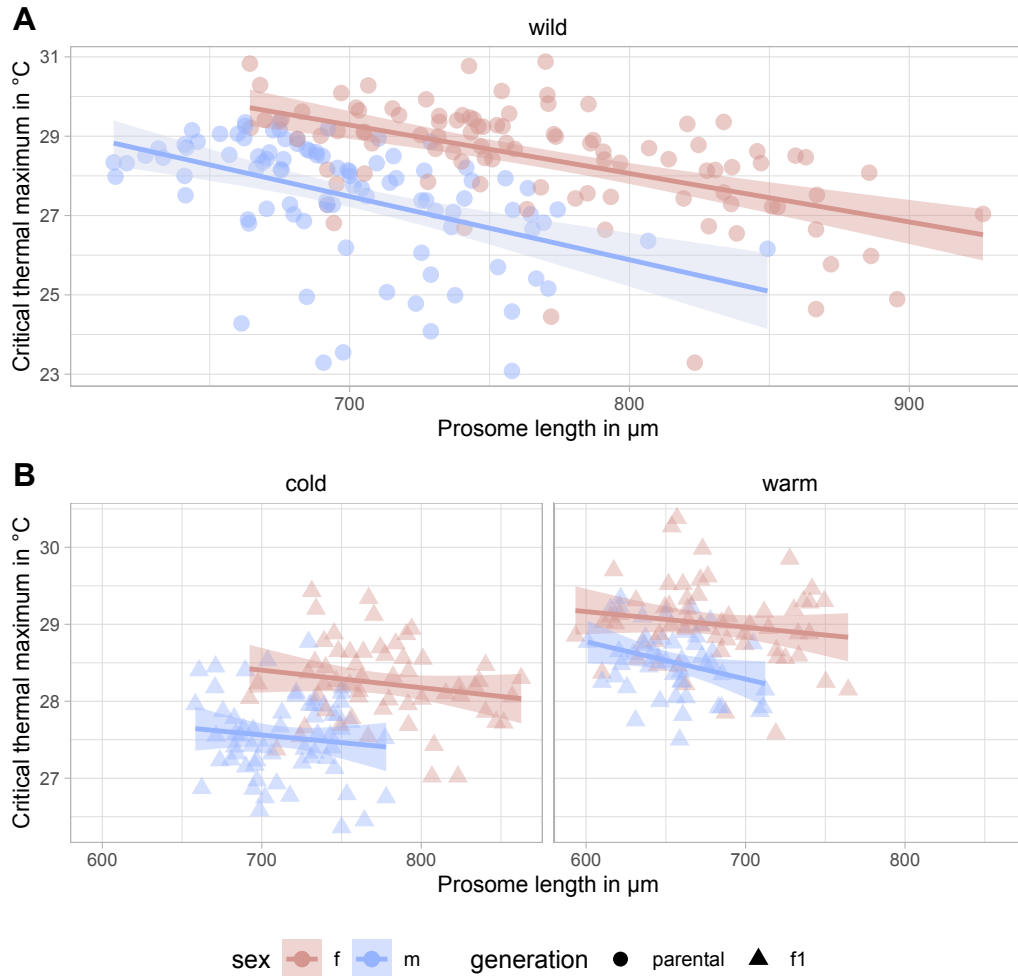


Figure 4: Correlation between prosome length and CT_{max} in *A. hudsonica*, (A) Parental generation, length has a significant effect on CT_{max} ($p < 0.001$, $R^2 = 0.35$) (B) F1 generation; cold treatment: no significant effect of length on CT_{max} ($p = 0.108$, $R^2 = 0.323$); warm treatment: a significant effect of length on CT_{max} ($p = 0.038$, $R^2 = 0.242$). Linear regression per sex with 95% confidence interval.

305 4. Discussion

306 Seasonal fluctuations have strong physiological effects on organisms occu-
307 pying these variable conditions. We hypothesized that the thermal tolerance
308 of copepods would closely mirror their developmental temperature and that
309 both plastic and genetic mechanisms would contribute to the physiological
310 change observed. As predicted, the CT_{max} of *A. hudsonica* increased in
311 parallel with environmental temperature. However, under common garden
312 conditions collections showed similar levels of plasticity and converged on
313 common thermal tolerances and body sizes, indicating that phenotypic dif-
314 ferences between collection times were driven by plasticity with no evidence
315 for rapid adaptation. Together, these results indicate that *A. hudsonica* has
316 substantial phenotypic plasticity to rapidly acclimate to large changes in
317 external temperature.

318 4.1. Seasonal variation in thermal tolerance

319 For both marine and terrestrial ectotherms, the ability to rapidly ac-
320 climate to changes in environmental temperature is common and adaptive
321 (Gunderson and Stillman, 2015). In coastal marine organisms, particularly
322 those from temperate environments, the presence of thermal plasticity is
323 essential as shallow waters tend have high variance in their temperatures, re-
324 quiring rapid phenotypic responses (Reusch, 2014). This is particularly true
325 for intertidal copepods which can experience daily temperature changes of
326 nearly 10°C (Leong et al., 2017) and therefore have high thermal tolerance
327 and plasticity (Healy et al., 2019). Further, across copepods there is near
328 universal presence of thermal plasticity that is dependent on environmental

329 temperature (Sasaki and Dam, 2021). For *Acartia* copepods specifically, *A.*
330 *tonsa* and *A. hudsonica* from the east coast of the United States are typically
331 plastic in their thermal tolerance (Sasaki and Dam, 2020) and laboratory
332 studies on *A. tonsa* show large effects of acclimation on thermal tolerance
333 (Sunar and Kir, 2021). Thus, plasticity plays an important role in enabling
334 most marine copepods to tolerate environmental temperature fluctuations.

335 While the Baltic Sea experiences only small wind-driven and irregular
336 tides, the variation in water temperature at our collection site is neverthe-
337 less high, ranging from 5.65 °C to 20.70 °C during the study period. This
338 high and relatively predictable variation likely favors the evolution of plas-
339 ticity observed in the population (Bitter et al., 2021). Indeed, previous work
340 has shown that *Acartia tonsa* from less variable low latitude thermal envi-
341 ronments harbor lower levels of phenotypic plasticity than those from more
342 variable high latitude sites (Sasaki and Dam, 2020), consistent with the lati-
343 tudinal hypothesis of plasticity (Janzen, 1967; Ghalambor et al., 2006). Sim-
344 ilarly, Sasaki and Dam (2020) found that seasonal variation in thermal LD50
345 of *A. hudsonica* from the east coast of North America was driven by plasticity
346 rather than adaptation. Therefore, the plasticity that *A. hudsonica* harbors
347 to respond to changing temperature across the season is likely adaptive in
348 this environment and is present across multiple populations.

349 The lack of seasonal adaptation is in contrast to the evidence of this phe-
350 nomenon in other systems including both terrestrial *D. melanogaster* (John-
351 son et al., 2023) and the sister species to *A. hudsonica*, *A. tonsa* (Sasaki and
352 Dam, 2020). For *A. tonsa*, Sasaki and Dam (2020) found that collections
353 from different time points differed in their plasticity. It is unclear exactly

354 what drove the seasonal patterns in *A. tonsa*, but it may be due to the pres-
355 ence of cryptic lineages emerging or surviving at different temperatures. The
356 comparison to *D. melanogaster* is also interesting as terrestrial organisms
357 typically have a better ability than marine organisms to buffer their thermal
358 environment via behavioral mechanisms, known as the Bogert effect (Bogert,
359 1949). *Acartia* copepods have enormous populations sizes that are regularly
360 in the hundreds of individuals per cubic meter of water (Möllmann, 2002).
361 Therefore, the efficiency of selection may be similar between *Acartia* and
362 *Drosophila*. Given this, one might expect similar signals of seasonal adapta-
363 tion in our system relative to *Drosophila*; we observed no evidence to support
364 this expectation.

365 There are a number of possible explanations for the lack of seasonal adap-
366 tation in our study. First, *A. hudsonica* may have sufficient plasticity to
367 respond to the seasonal thermal environment. Given the Bogert effect, envi-
368 ronmental temperature is directly experienced to a greater degree in marine
369 systems and selection for plasticity may be strong and result in a highly
370 flexible phenotype. Alternatively, there may be no heritable genetic varia-
371 tion for thermal tolerance in this population. However, populations of *A.*
372 *hudsonica* from North America can rapidly evolve to elevated temperatures
373 under laboratory conditions (deMayo et al., in press) and there is evidence
374 for local adaptation to temperature along latitudinal gradients in the sister
375 species, *Acartia tonsa* (Sasaki and Dam, 2019). Therefore, it is likely that
376 heritable variation in thermal tolerance is also present in this species and
377 population. An alternative explanation is that the phenotypes of focus were
378 not under selection or sensitive enough to capture any adaptive responses.

379 The temperatures at the collection site did not approach the critical ther-
380 mal limit measured in the lab (maximum SST during sampling: 20.70 °C).
381 Therefore, it is unlikely that CT_{max} was directly under selection. It is possi-
382 ble that an alternative phenotype would show a seasonal adaptive response
383 as has been observed in other systems (Hairston and Dillon, 1990; de Ville-
384 mereuil et al., 2020). Finally, much of the evidence for seasonal adaptation
385 in *D. melanogaster* has been found at the genomic level (Johnson et al.,
386 2023). Given this, our populations may similarly be experiencing fluctuating
387 selection that would be detectable using genomic approaches.

388 While we observed high levels of plasticity for CT_{max} , under future tem-
389 perature conditions it is unlikely that most ectotherms have sufficient plastic-
390 ity to respond to temperature changes without adaptive responses (Gunder-
391 son and Stillman, 2015). Indeed, DeMayo et al showed that under warming
392 conditions, plasticity alone is insufficient to maintain high population fitness
393 in *A. hudsonica* (deMayo et al., in press). However, the species can rapidly
394 adapt after just four generations to recover fitness levels. Therefore, it is
395 likely that both plasticity and adaptation will be required to tolerate future
396 environmental conditions and more work is needed to understand the relative
397 contribution of each to overall resilience.

398 *4.2. Plasticity in body size and potential potential impacts of warming*

399 As temperatures increased, body size decreased in *A. hudsonica* (Fig.
400 1), a common phenomenon across ectotherms known as the temperature-size
401 rule (Angilletta and Dunham, 2003; Rubalcaba and Olalla-Tárraga, 2020).
402 This concept applies to copepods (Escribano and McLaren, 1992; Viitasalo
403 et al., 1995), including those from tropical (Ortega-Mayagoitia et al., 2018)

404 and temperate environments (Riccardi and Mariotto, 2000). However, other
405 factors such as phytoplankton density may affect the body size of individ-
406 uals in the wild (Deevey, 1964, 1966), though this would not have affected
407 the common garden animals in our study. The reduction in body size in
408 response to increasing temperature may be driven by the disproportionate
409 increase in respiration and metabolism relative to ingestion and assimilation
410 of nutrients (Lehman, 1988), leading to lower overall energy available for
411 growth and therefore a smaller body size. Further, there may be a trade-off
412 that favors smaller individuals at high temperatures; reproductive efficiency,
413 the ratio of egg production and respiration, is maximized at smaller body
414 sizes and therefore may be adaptive in warmer temperatures. Similarly, at
415 higher temperatures oxygen availability (aerobic scope) may be decreased in
416 larger individuals relative to smaller individuals, favoring smaller body sizes
417 (Rubalcaba et al., 2020). Alternatively, in cold temperatures growth periods
418 may be prolonged while the growth rate remains relatively stable, leading to
419 larger individuals under cold conditions (Vidal, 1980).

420 Regardless of the mechanism, as temperatures warm due to anthropogenic
421 causes, decreases in body size may affect ecosystem interactions. This is par-
422 ticularly true in the Baltic Sea where the heating rate is around three times
423 higher than the ocean average due to its unique topography (Reusch et al.,
424 2018; Szymczycha et al., 2019; Dutheil et al., 2022). A size reduction in
425 *A. hudsonica*, or other prey organisms, might impact higher trophic level
426 predators, for example by requiring the consumption of more individuals to
427 maintain the same amount of nutrient intake (Garzke et al., 2015). If abun-
428 dance does not increase with decreasing body size, nutrient availability may

429 be reduced for consumers who will also require increased energy needs un-
430 der higher temperature (Brown et al., 2004). Further, Garzke et al. (2015)
431 observed that large copepods from colder temperatures clear algae biomass
432 more efficiently than smaller individuals, exerting top-down control on phy-
433 toplankton. This in turn suggests that smaller copepods are less efficient
434 grazers, with less control over the planktonic community. As copepods are
435 important grazers on large phytoplankton and microzooplankton (Sommer
436 et al., 2003; Armengol et al., 2017), less efficient grazing or a shift to different
437 prey size classes might have unforeseen cascading effects across ecosystems.

438 Finally, the correlation between body size and CT_{max} (Fig. 4) suggests
439 that body size itself may influence the thermal tolerance of an individual
440 with small body size being of advantage in warm environments. While the
441 effect was weak in comparison with the effects of developmental temperature
442 and the mechanistic link between body size and thermal tolerance remains
443 unknown, this relationship could potentially be used to predict an individ-
444 ual's thermal tolerance. This may be of interest when analyzing historical
445 samples where body size measurements from the same location across years
446 could enable predictions about past thermal tolerance and environmental
447 temperatures. More work would be needed to develop these predictions.

448 *4.3. Outliers and mixed species*

449 The outliers in collection 2 under cold conditions, characterized by un-
450 expectedly low thermal tolerance and small body sizes, were most likely the
451 result of an impending culture collapse. This was potentially caused by poor
452 food quality during that period due to ciliate and bacteria growing in the
453 algae cultures used for feeding. The negative effects of ciliates on copepod

454 fitness are well described with effects ranging from decreased egg produc-
455 tion (Burris and Dam, 2014) to increased adult mortality (Visse, 2007). The
456 species identity of the ciliates in this study could not be determined. How-
457 ever, ciliate peak abundances correlated with culture collapse, and after the
458 establishment of more frequent water changes, the cultures improved. Since
459 the survival and fitness of the animals was clearly affected by external factors
460 unrelated to the experiment, length and thermal tolerance measurements for
461 the cold treatment in collection 2 should be treated with caution and were
462 therefore excluded from parts of the analysis.

463 In the wild collection 5, five individuals showed a CT_{max} above 33 °C,
464 which was well beyond the distribution of values for any other collection
465 (Fig. S.5). In the F1 generation, the warm treatment of collection 4 and
466 5 and the cold treatment of collection 5 also had similar high performing
467 individuals (11, 40, and 2 individuals, respectively) (Fig. S.5). These ex-
468 treme outliers suggested a mixed species composition, which was confirmed
469 by genotyping individuals of each collection as well as high thermal outliers
470 (see Supplement *Mixed species*). High thermal outlier individuals were *A.*
471 *tonsa* without exception (Table S.1, Fig. S.6). The increased thermal toler-
472 ance for *A. tonsa* relative to *A. hudsonica* is consistent with other studies.
473 On the east coast of North America, *A. hudsonica* dominates plankton com-
474 munities early in the year when water temperatures are low and is replaced
475 by *A. tonsa* as temperatures increase (Borkman et al., 2018; Sullivan and Mc-
476 Manus, 1986). This pattern appears to be similar for copepod communities
477 in Kiel Bight as well. Since *A. tonsa* was present only in later collections and
478 just as individual outliers, this paper could not determine how thermal tol-

479 erance of *A. tonsa* changes within a season. However, we would hypothesize
480 that *A. tonsa* follows an overall similar trend than shown for *A. hudsonica*
481 with thermal tolerances shifted towards warmer temperatures. Additional
482 experiments would be required to test this hypothesis.

483 4.4. First record of *A. hudsonica* in the Baltic Sea

484 From our literature and database review, the molecular barcoding in this
485 study is the first record of *A. hudsonica* in the Baltic Sea. There are two
486 possible explanations for this novel species presence. First, *A. hudsonica*
487 may have recently invaded the Baltic Sea. In the North Sea, *A. omori* – a
488 species that co-occurs with *A. hudsonica* off the Japanese coast (Ueda, 1987)
489 – was first described by Seuront (2005) in the early 2000. The successful
490 invasion of *A. omori* suggests that *A. hudsonica* could similarly have been
491 introduced to the North Sea. As the North Sea and Baltic Sea are connected,
492 it is possible that *A. hudsonica* then moved to the Baltic Sea. Alternatively,
493 there is ample shipping in the region and an independent local introduction
494 could have occurred.

495 Secondly, it cannot be ruled out that *A. hudsonica* has historically been
496 misidentified in the Baltic Sea. Copepods from the genus *Acartia* have a
497 record of mis-identifications in databases and are difficult to distinguish mor-
498 phologically (Figueroa et al., 2020). Instead, phylogenetic methods are re-
499 quired for accurate species identification. Before the 1970's, *A. hudsonica*
500 was a subspecies of *A. clausi*, but is now considered its own species (Brad-
501 ford, 1976; Ueda, 1986). Given this, it is possible that *A. hudsonica* is native
502 to the Baltic Sea but has been, and still is, commonly identified as *A. clausi*.

503 **5. Conclusions**

504 Here we showed that *Acartia hudsonica* has high phenotypic plasticity
505 in response to changing temperature within a single year. We found that
506 thermal tolerance closely tracks environmental temperature, indicating that
507 *A. hudsonica* has capacity to tolerate increasing temperatures that fall within
508 the current range experienced in nature. However, the observed decrease in
509 body size suggests that nutrient availability and ecosystem functioning could
510 be impacted if temperatures consistently increase with no change in copepod
511 abundance. By focusing on the relative impacts of plasticity and adaptation
512 to population responses to temperature change we can begin to understand
513 the resilience populations and ecosystems to ongoing global change.

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522 **Conflicts of interest**

523 The authors declare that there is no conflict of interest to disclose.

524 **Data accessibility**

525 The data set and scripts can be found on [https://github.com/HahnAlexandra/](https://github.com/HahnAlexandra/Plasticity_Acartia_hudsonica)
526 [Plasticity_Acartia_hudsonica](#). The individual sequences are uploaded on
527 NCBI GenBank <https://www.ncbi.nlm.nih.gov/genbank/>, find individ-
528 ual accession numbers in Table S.1. SST data from Kiel Fjord will be made
529 available on <https://www.pangaea.de> by our collaborator Claas Hiebenthal.

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Table 1: Overview over sampling dates and corresponding SST average.

Collection name	Collection date	Daily mean	2-week mean
Collection 1	06 Apr 2022	5.81 \pm 0.05 °C	6.36 \pm 0.47 °C
Collection 2	16 May 2022	12.66 \pm 0.53 °C	11.44 \pm 0.84 °C
Collection 3	13 Jun 2022	12.37 \pm 0.33 °C	12.86 \pm 1.52 °C
Collection 4	27 Jun 2022	18.35 \pm 0.13 °C	16.55 \pm 1.83 °C
Collection 5	19 Jul 2022	19.16 \pm 0.79 °C	18.11 \pm 0.68 °C

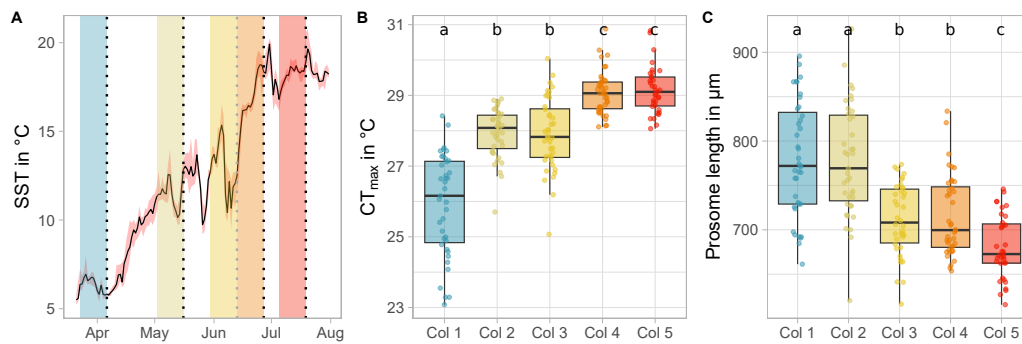


Figure 1: Thermal tolerance follows the seasonal changes in temperature. (A) Sea surface temperature at the collection site where sampling points are indicated by dotted lines and shaded boxes indicate the two-week period prior to sampling. (B) Critical thermal maxima and (C) mean prosome length for wild collected individuals. Boxplot colors correspond to sampling date and compact letters show the results from post-hoc tests where shared letters indicate no significant difference between measurements.

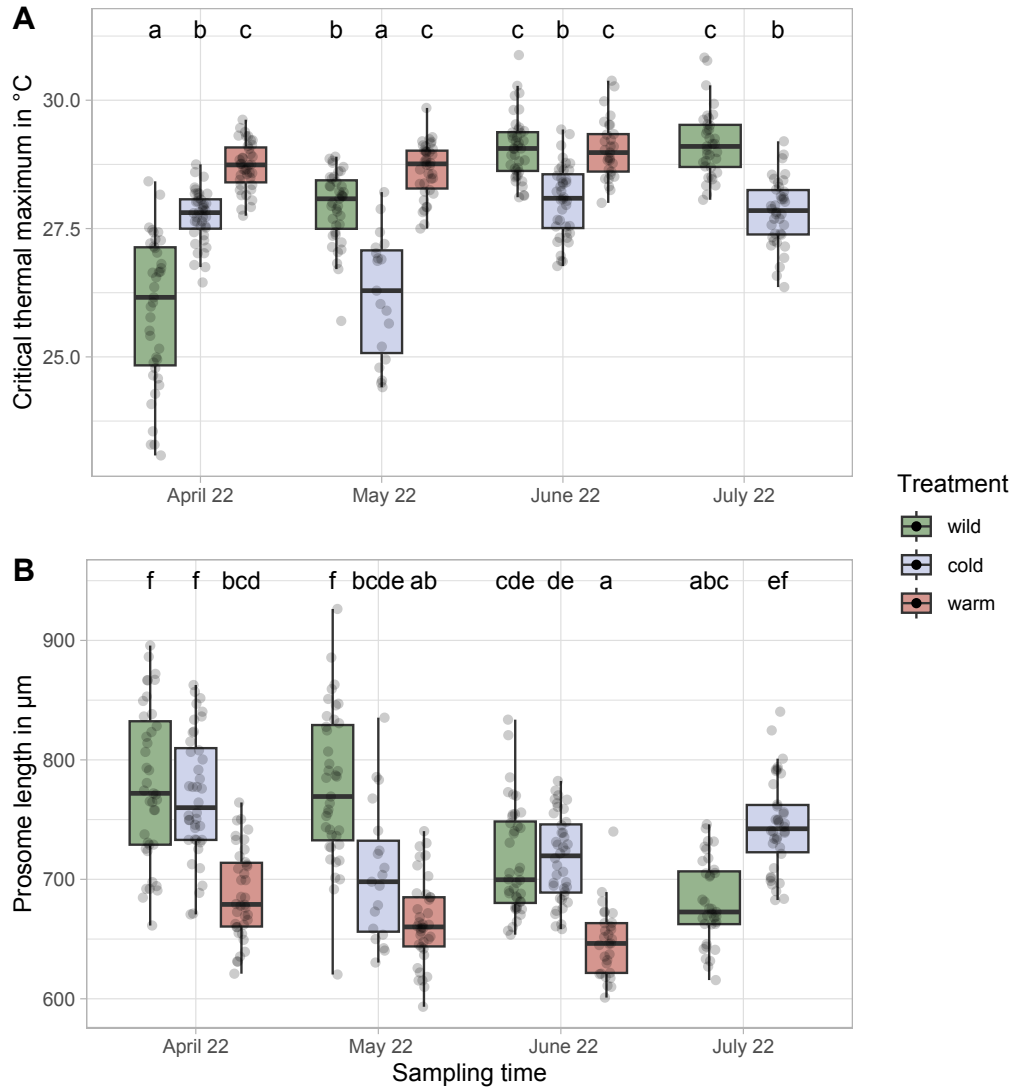


Figure 2: Phenotyping results following common garden for (A) critical thermal maxima and (B) prosome length. Colors of boxes correspond to treatment where "wild" are field collected animals, "cold" are F1 animals at 11°C, "warm" are F1 animals at 18°C. Compact letters show the results from post-hoc tests where shared letters indicate no significant difference between measurements.

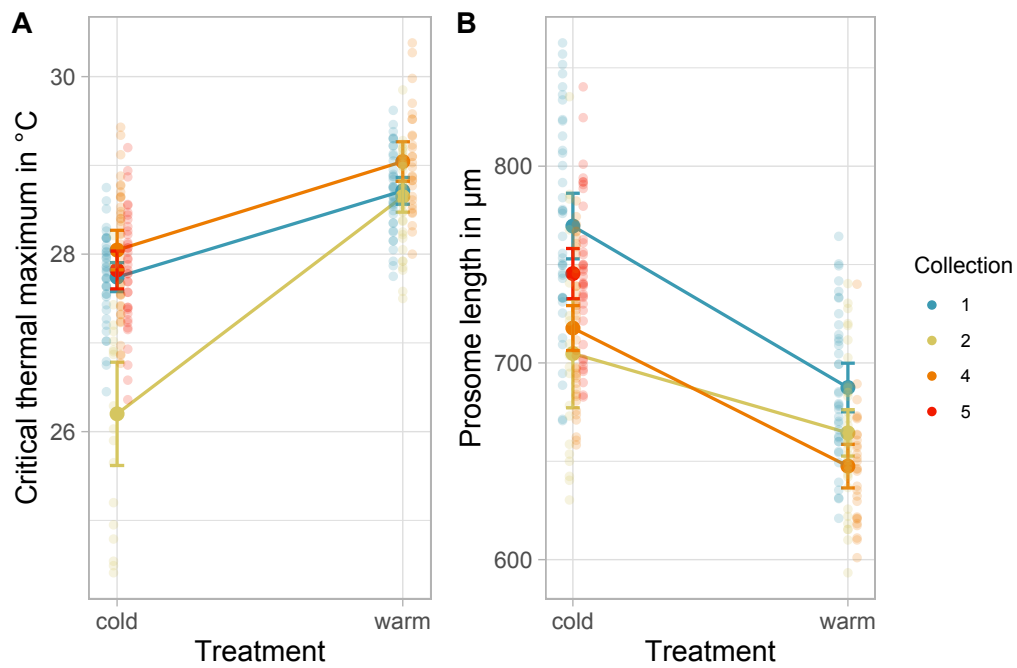


Figure 3: Reaction norms for treatment effect on (A) Critical thermal maximum; (B) Prosome length; bold points indicate mean values, error bars indicate 0.95 % confidence interval.

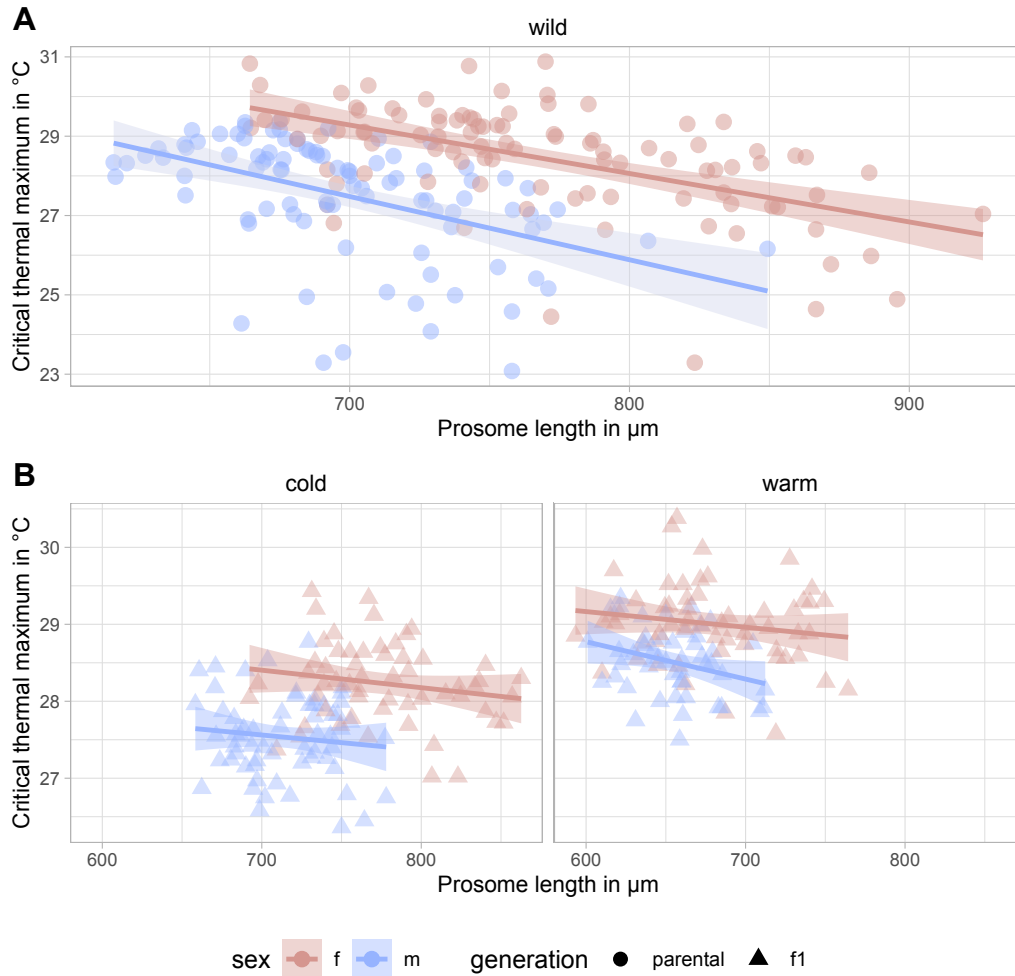


Figure 4: Correlation between prosome length and CT_{max} in *A. hudsonica*, (A) Parental generation, length has a significant effect on CT_{max} ($p < 0.001$, $R^2 = 0.35$) (B) F1 generation; cold treatment: no significant effect of length on CT_{max} ($p = 0.108$, $R^2 = 0.323$); warm treatment: a significant effect of length on CT_{max} ($p = 0.038$, $R^2 = 0.242$). Linear regression per sex with 95% confidence interval.