

Phenotypic plasticity drives seasonal thermal tolerance in a Baltic copepod

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ABSTRACT

Seasonal changes in environmental conditions require substantial physiological responses for population persistence. Phenotypic plasticity is a common mechanism to tolerate these changes, but for organisms with short generation times rapid adaptation may also be a contributing factor. Here, we used a common garden design (11 °C and 18 °C) to disentangle the impacts of adaptation from phenotypic plasticity on thermal tolerance of the calanoid copepod *Acartia hudsonica* collected throughout spring and summer of a single year. *Acartia hudsonica* were collected from five time points across the season and thermal tolerance was determined using critical thermal maximum (CT_{max}) followed by additional measurements after one generation of common garden. As sea surface temperature increased through the season, field collected individuals showed corresponding increases in thermal tolerance but decreases in body size. Despite different thermal tolerances of wild collections, after one generation of common garden animals did not differ in CT_{max} within thermal treatments. Instead, there was evidence of phenotypic plasticity where higher temperatures were tolerated by the 18 °C versus the 11 °C treatment animals across all collections. Despite persisting differences between collections due to either adaptation or parental effects, acclimation also had significant effects on body size, with the warm treatment resulting in smaller individuals, consistent with the temperature size rule. Therefore, the differences in thermal tolerance and body size observed in field collected *A. hudsonica* were predominantly driven by plasticity rather than adaptation. However, the observed decrease in body size suggests that nutrient availability for higher trophic levels and ecosystem functioning could be impacted if temperatures consistently increase with no change in copepod abundance. This is the first record of *A. hudsonica* in the Baltic Sea known to the authors.

1. Introduction

Environmental variation is ubiquitous across habitats and organisms respond to and tolerate this variation in multiple ways. When variation is both predictable and experienced within the lifespan of an individual, it is expected that plasticity—the ability for one genotype to express multiple phenotypes in response to the environment, i.e., acclimation (Stearns, 1989)—will evolve (Pereira et al., 2017; Bitter et al., 2021). Conversely, if environmental variation is unpredictable or at timescales longer than generation time, plasticity is unlikely to evolve but instead selection should act with each environmental fluctuation. In this latter case, the resulting changes in selective pressure through time can lead to fluctuating selection, which can not only drive adaptation but also contribute to the maintenance of genetic diversity in populations (Bergland et al., 2014). Here, adaptation is defined as genetic change that improves population fitness and is heritable (Kawecki and Ebert, 2004). In practice, both plasticity and adaptation contribute to

responses to environmental fluctuations and can interact in complex ways that are still being understood (Fox et al., 2019).

One of the major drivers of fluctuating selection in short-lived species in the wild is seasonal change. In this scenario, the environmental changes that occur within a year result in divergent selective pressures at different temporal periods. For example, summer months may favor warm tolerant genotypes while cooler spring or fall temperatures may favor genotypes that have higher performance at low temperatures. While these changes are relatively consistent on a yearly basis, the temporal scale is beyond many organisms' lifespan, which can lead to adaptation to different periods within the fluctuating seasonal environmental change. This phenomenon has been observed in diverse species and ecosystems, including marine copepods (Hairston and Dillon, 1990), *Lonchopterid* flies (Niklasson et al., 2004), dandelion (Vavrek et al., 1996), two-spotted ladybugs (Brakefield, 1985), and swallows (Brown et al., 2013), among others (Siepielski et al., 2009; Bell, 2010). There is also evidence for fluctuating selection at the genomic level: in

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Drosophila fruit flies genome-wide allele frequencies consistently and repeatedly shift between seasons due to selection, helping to maintain genetic variation within populations (Johnson et al., 2023). Thus, adaptive responses to seasonal change may be a common phenomenon across taxa with short generation times.

Population responses to seasonality can also help reveal the fundamental mechanisms that underlie resilience to rapid environmental change, which may be important for understanding resilience to anthropogenic global change. For example, while some species experience yearly temperature changes with an amplitude similar to or greater than those predicted under global warming (Bujan et al., 2020) and terrestrial and aquatic ectotherms routinely achieve increased thermal tolerance in warmer seasons via plasticity (Hopkin et al., 2006; Bujan et al., 2020), in many cases this plasticity will be insufficient to buffer populations completely from global change (Gunderson and Stillman, 2015). Instead, adaptation will be required for population persistence. In the context of short-lived species, population responses to seasonal changes can be used as a natural experiment to understand how plasticity and adaptation contribute to resilience to rapid environmental change. Disentangling the interactions between these processes is critical to understand how populations will respond to rapid changes such as those expected under global change (Williams et al., 2017).

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

In this study, we focus on the calanoid copepod *Acartia hudsonica* Pinhey, 1926. *Acartia* copepods are among the most-studied copepod genera, in part due to their world-wide distribution and high abundance, making them foundational to marine and coastal ecosystems (Walter and Boxshall, 2023). In the Baltic Sea, *Acartia* spp. is one of the dominant zooplankton taxa and critical to local ecosystems (Diekmann et al., 2012; Dzierzbicka-Głowacka et al., 2019). *Acartia hudsonica*, specifically, is a cold adapted species that is generally abundant in winter and spring months, out-competing more warm adapted congeners at low temperatures. The species can tolerate a broad temperature range from at least 4 to 18 °C (Sullivan and McManus, 1986). For populations native to the Eastern United States, *A. hudsonica* produces dormant eggs also referred to as resting eggs at temperatures >16 °C and abundances strongly decline in summer (Sullivan and McManus, 1986).

Here, we seek to disentangle the impacts of seasonal adaptation from plasticity on thermal tolerance of *A. hudsonica* collected throughout spring and summer of 2022. We predicted that the upper thermal tolerance of wild collected individuals would closely follow the environmental temperature, increasing as environmental temperature increased. We measured thermal tolerance using critical thermal maximum (CT_{max}), which is the temperature at which locomotion ceases

during an acute temperature ramp (Cowles and Bogert, 1944; Morgan et al., 2018). Further, because developmental temperature strongly impacts copepod body size (Horne et al., 2016), we predicted that body size would decrease as temperature increased. To disentangle the relative impacts of plasticity versus adaptation on these traits in wild individuals collected at different points of the season, we used a common garden design at two different temperatures. With phenotypic plasticity as the driver of a population differences, initial differences between wild-caught animals collected throughout the seasons would disappear after common-gardening and traits within each treatment would converge. That is, observed phenotypic differences would be due to environmental rather than genetic factors. In contrast, with adaptation as the driver of phenotypic differences, the differences between sampling times would persist after common-gardening (Fig. 1). Together, these results help to reveal the mechanisms underlying seasonal phenotypic responses of *A. hudsonica*.

2. Material and methods

2.1. Sampling and cultures

All samples were collected by 100 µm WP2 net on board the research vessel *Polarfuchs* in Kiel Fjord, Germany (54°19'50"N 10°09'20"E). Live samples were stored at collection temperature until processing. The experiment included five sampling dates (referred to as collections) from April 2022 to July 2022 that spanned sea surface temperatures (SST) from 5.81 °C to 19.16 °C (Table 1, Hiebenthal et al. (2023)).

For all sampling dates, approximately 440 adult animals were sorted and split into two 6 L culture buckets with air supply and held at their collection temperature. Over the following two days, critical thermal maximum (CT_{max}) assays were run on the wild-caught copepods (details below). After the initial assays, the cultures were moved to a cold (11 °C) or warm (18 °C) culture room, with the cold treatment representing SST occurring early in the growing season of *Acartia* in Kiel Fjord and the warm treatment representing a SST later in the season. No major fluctuations in treatment temperatures occurred throughout the experiment. All cultures were kept on a 12:12 light:dark regime at a common salinity of 15 PSU and were allowed to reproduce, with water changes approximately every 7 days. Feeding was ad libitum with *Rhodomonas* sp. and *Isochrysis galbana* (Holste and Peck, 2006; Ismar et al., 2008; Mahjoub et al., 2014).

After the initial culture establishment at treatment temperatures (2–4 days), the cultures were filtered through a nested 200 µm and 50 µm mesh sieve. Adults were retained on the 200 µm mesh and kept for further culturing. Offspring, eggs and nauplii, of the parental generation were retained on the 50 µm mesh and placed in a new culture bucket to start the F1 generation. Development was monitored to catch the onset of maturation. Once the F1 generation reached adulthood, CT_{max} assays were repeated. Collection 3 collapsed before reaching F1. Therefore, this collection is excluded from further analysis. Similarly, the condition of the cold treatment of collection 2 was in decline during the same time period. Though the experiments were conducted for this treatment and the data included for transparency, the results should be treated cautiously.

2.2. Acute thermal tolerance assays

Critical thermal tolerance, CT_{max}, was used as a proxy for thermal tolerance. CT_{max} is defined as the temperature at which locomotion is affected in a way that prevents the individual to move away from harmful conditions, eventually resulting in death (Cowles and Bogert, 1944). In this study, CT_{max} was the temperature at which the individuals showed no visible response to a stimulus. For each collection and treatment the CT_{max} of twenty males and twenty females was quantified. Adult individuals were sorted under temperature-controlled conditions and placed in 12 mL glass tubes filled with 5 mL of 15 PSU filtered

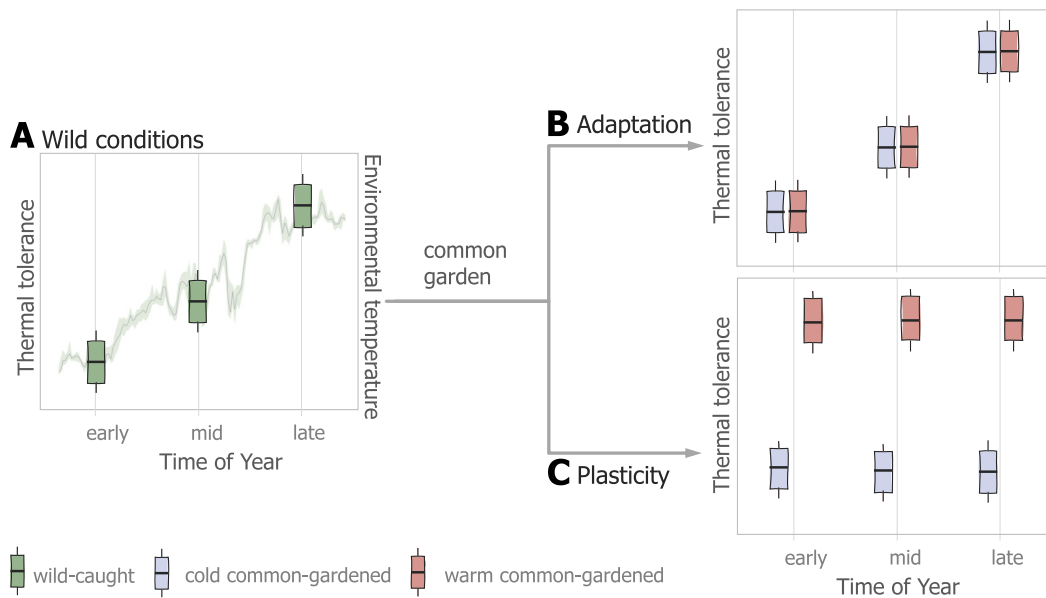


Fig. 1. Conceptual figure showing the expected outcomes of the common garden experiment. Coloured boxes correspond to the treatments. (A) Under wild conditions, thermal tolerance is expected to correlate with environmental temperature throughout the year. Thermal tolerance is shown as box-plots with environmental temperature displayed as the background line. (B) If seasonal differences are due to adaptation, differences in thermal tolerance between time points would persist after common-gardening, indicating the traits are due to genetic causes. (C) Conversely, if differences are due to plasticity, thermal tolerances would converge within treatments, indicating that the original differences were due to environmental effects.

Table 1

Overview over sampling dates and corresponding sea surface temperature averages.

Collection name	Collection date	Daily mean	2-week mean
Collection 1	06 April 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 June 2022	12.37 ±0.33 °C	12.86 ±1.52 °C
Collection 4	27 June 2022	18.35 ±0.13 °C	16.55 ±1.83 °C
Collection 5	19 July 2022	19.16 ±0.79 °C	18.11 ±0.68 °C

seawater. Ten individuals, five males and five females, were simultaneously run per trial. The starting temperature of the experimental tank matched the wild collection or culturing temperature. To minimize bias, an assistant placed the individuals in random order in the experimental tank, leaving the experimenter blinded to the animals' sex until after the experiment. Heating was monitored with a thermometer (PCE-HPT1, PCE instruments, Menschede, Germany) placed into an additional glass tube filled with filtered seawater. The animals were given a 30-minute acclimation period before heating was started. With a 300 W and 500 W heater, heating was consistent at ~ 0.2 °C/min (Fig. S.1). Once 22.5 °C was reached, the 300 W heater was removed, slowing the ramping temperature to ~ 0.1 °C/min to facilitate monitoring the individuals. Throughout the experiment, the experimenter was blinded to the exact temperature. Animals were continuously monitored and when movement ceased, gentle pipetting was used to trigger a reaction. If still no movements occurred, the corresponding temperature was considered CT_{max} .

After the experiment, the sex for all animals was confirmed and the individuals were preserved in 95% ethanol and later photographed using a Nikon imaging microscope and Nikon imaging software (NIS-Elements v. 5.20.00). All images were obtained using the same magnification to ensure consistency. From the pictures, the prosome length was measured for each copepod using ImageJ v.1.53 k (Schneider et al., 2012). Three measurements were obtained per individual and averaged in the later analysis. While formalin is usually used to preserve plankton samples for length analysis (Connolly et al., 2017; Aguilera et al., 2020), previous work on zooplankton has shown no effect of ethanol

preservation on body size (Black and Dodson, 2003) and any size effect would be consistent across the experiment. Further, ethanol preservation allows for downstream genetic analyses, which is essential when dealing with cryptic copepod species.

2.3. Genotyping

To confirm species identity, 4–15 copepods per collection were genotyped using the mtCOI region (Table S.1). For the DNA extraction, ethanol-preserved copepods were rinsed with ultra-clean water and rehydrated for one hour. Individual copepods were transferred into 100 μ L of 5% Chelex solution, incubated for 20 minutes at 95 °C in a water bath, then centrifuged for 5 minutes at 8000 rpm. PCR reactions were conducted in 20 μ L volume with 9.8 μ L ultra clean water, 2 μ L dNTPs, 2 μ L Buffer, 2 μ L LCO1490 forward primer or HCO2198 reverse primer (5 mM, Folmer et al. (1994)), 0.2 μ L DreamTaq DNA polymerase (Thermo Fisher Scientific Inc., Massachusetts, US), and 2 μ L of DNA.

Amplification conditions were: 3 minutes of denaturation at 94 °C followed by 33 cycles of 45 s denaturation at 94 °C, 45 s annealing at 48 °C, 60 s extension at 72 °C and a final extension at 72 °C for 7 minutes. Samples were sequenced on a Sanger sequencing platform by Eurofins Deutschland (Germany). Sequences were checked and aligned to generate consensus sequences using CodonCodeAligner (CODONCODE, 2010). The *Acartia* genus consists of multiple cryptic species, making species identification difficult. Therefore, we followed Figueroa et al. (2020) and used a bayesian approach to determine species and clade for all samples. Using known samples from Figueroa et al. (2020), we aligned all raw reads with MUSCLE (Edgar, 2004), converted to NEXUS format in R v.4.2.2 using APE (Paradis and Schliep, 2019), and used MrBayes (Ronquist et al., 2012) to build a phylogenetic tree with *Acartia dana* as the outgroup. Tree plots were made using ggtree (Yu et al., 2017). Since thermal tolerance measurements showed outliers in collections 4 and 5, 38 experimental outliers and additional trial animals from those collections were genotyped. All outliers that did not belong to the species *A. hudsonica* were excluded from the analysis, this includes the entirety of the warm acclimated collection 5.

2.4. Data analysis and statistics

Data manipulation, visualization, and statistics were conducted in R version 4.2.2 (R Core Team, 2022).

To assess the impact of various factors on CT_{max} in *Acartia hudsonica*, a linear mixed-effects model was employed. The model included fixed effects for treatment (wild, cold, warm), collection (1–5), sex, length, and their interactions. To account for potential sources of variability, random effects were incorporated for vial number, time of day, and tank used for the trials. Model significance and variance decomposition were evaluated using the *Anova* function. Visualizations of random effects were produced using the *plot_model* function. The initial model's random effects were found to contribute minimally to the overall fit as indicated by both Akaike's information criterion (AIC) and Bayesian information criterion (BIC) and visual inspection. Subsequently, the random effects were dropped, resulting in a more parsimonious full model that considered only fixed effects. Considering biological relevance, we further narrowed down the final model, comparing AIC and BIC values for the full and biological model. Model diagnostics, including normality tests of residuals, assessment of auto-correlation, and visual inspection of residuals, were performed to ensure the adequacy of the model. Analysis of variance (ANOVA) was conducted to assess the significance of the fixed effects. Post-hoc analyses were conducted using the *emmeans* package (Lenth, 2023), including pairwise comparisons of treatment effects within collections. A similar analysis was performed for the impact of fixed effects on length. To explore the influence of mean collection temperature as a continuous variable, rather than discrete collections, on thermal performance, similar linear models than described above were fitted for both CT_{max} and length. Since these different approaches resulted in similar model outputs, we focused on the discrete model to facilitate post-hoc comparisons between different treatments and collections. Finally, to further disentangle, adaptation and potential parental effects on the F1 generation, we employed linear models including effects of paternal temperature and treatment on length and CT_{max} for only F1 individuals.

3. Results

The sampling days spanned SST values from 5.81 °C to 19.16 °C (Fig. 2A; see Table 1, Table S.2). The thermal tolerance of wild-caught individuals mirrored the environmental temperature (Fig. 2B). CT_{max} values were lowest in Collection 1 (25.9 °C \pm 1.4 °C), where the

temperature over the two weeks prior to sampling averaged 6.36 °C \pm 0.47 °C. CT_{max} then increased throughout the collections (Col-2: 27.9 °C \pm 0.7 °C; Col-3: 27.9 °C \pm 1.0 °C; Col-4: 29.1 °C \pm 0.6 °C), reaching its maximum at Collection 5 (29.2 °C \pm 0.7 °C), where the two-week SST average was 18.11 °C \pm 0.68 °C (correlation between CT_{max} and collection temperature: Pearson's $r = 0.766$, $p < 0.001$). The prosome length of wild individuals showed an opposite trend, with size decreasing throughout the collections (Fig. 2C, correlation between prosome length and collection temperature: Pearson's $r = -0.528$, $p < 0.001$). Two week averages were chosen to characterize SST as this is the approximate amount of time it takes for copepods to mature from egg to adult and can therefore be considered developmental temperature; the two-week average showed a similar trend to daily mean temperatures (Fig. S.2). However, we note that developmental times are slower at colder temperatures and our estimates here likely do not capture the entire developmental period for the colder collections.

Considering both wild animals and common-garden treatments, there was a significant effect of treatment, collection, sex, and the interaction between treatment and collection on CT_{max} (Fig. 3A, $p < 0.001$ for all), regardless if collection was included as discrete variable or continuous collection temperature (Table 2, Table S.5). Effects of treatment on CT_{max} and prosome length were present in both male and female animals. Across all treatments and collection, females consistently had significantly higher thermal tolerance and larger body size compared to males (CT_{max} females: 28.4 °C \pm 1.2 °C, males: 27.7 °C \pm 1.2 °C, length females: 744 μ m \pm 69 μ m, males: 694 μ m \pm 44 μ m, $p < 0.001$ for both).

If seasonal adaptation was the driver of thermal tolerance, post-hoc tests would reveal significant differences between collections under the same common garden treatments. Conversely, under plasticity, no significant differences between collections within the same treatment would be expected (Fig. 1). While CT_{max} of the wild individuals varied depending on the SST before sampling, the thermal tolerance measurements within the cold and warm treatment were mostly similar across collections with the exception of cold collection 2 (Table S.6). For prosome length, the effect of treatment, collection, and their interaction were significant (Fig. 3B, $p < 0.001$ for all, Table 2). In contrast to the trends observed for CT_{max} , post-hoc tests showed no clear similarities of prosome length within treatments, suggesting a more complicated connection between prosome length, seasonality and common garden temperature including potential parental effects (Table S.7).

To further disentangle the impacts of plasticity, adaptation and po-

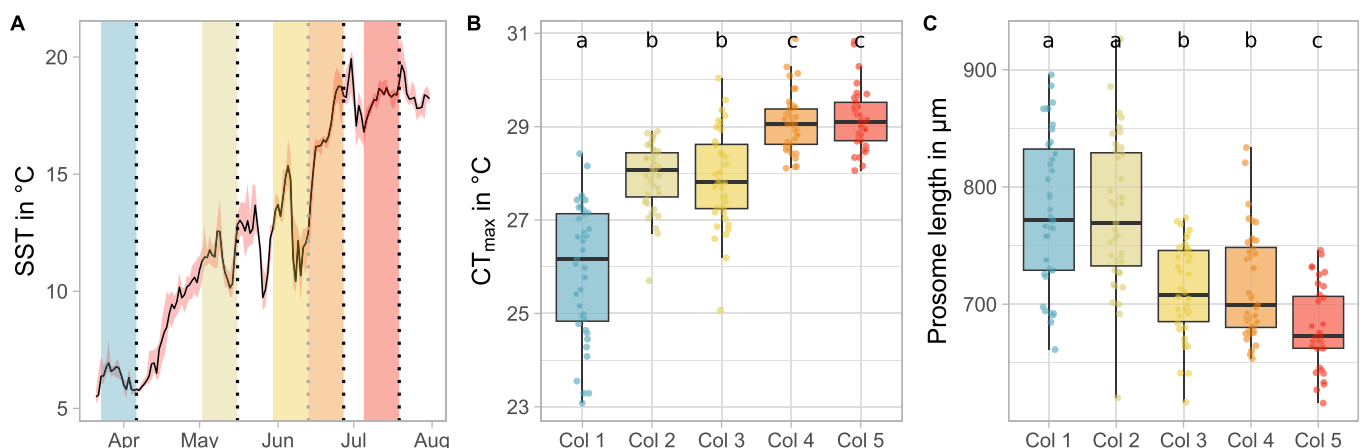


Fig. 2. Thermal tolerance follows the seasonal changes in temperature. (A) Sea surface temperature at the collection site where sampling points are indicated by dotted lines and shaded boxes indicate the two-week period prior to sampling. (B) Critical thermal maxima and (C) mean prosome length for wild 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.

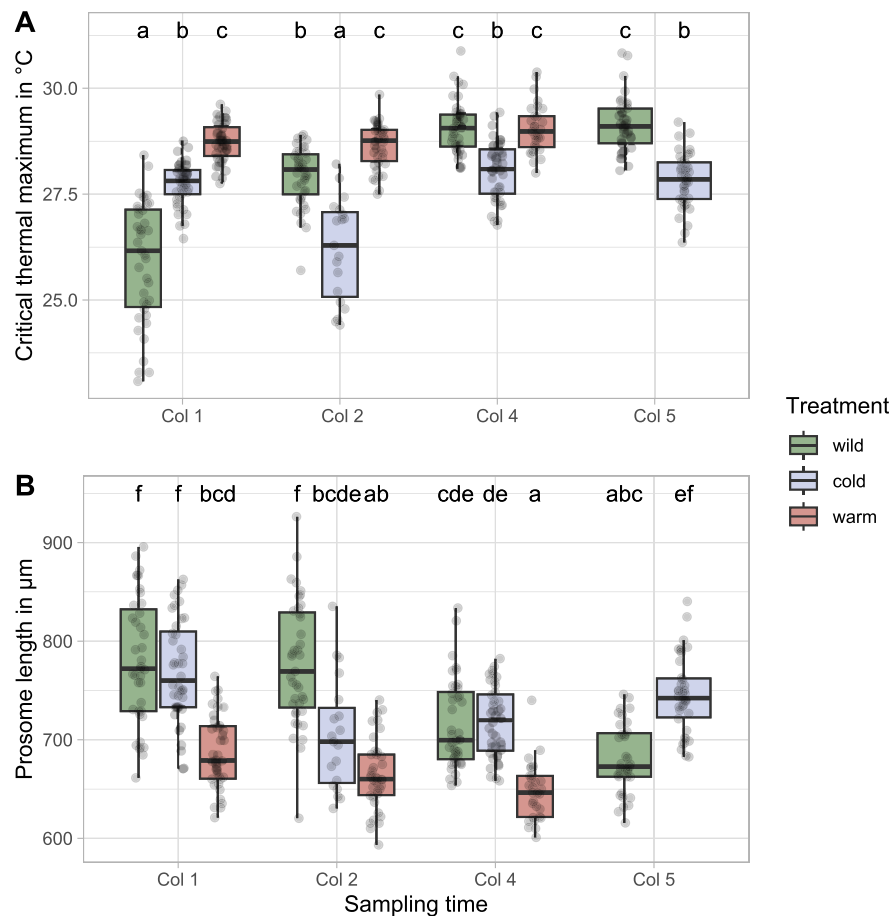


Fig. 3. Phenotyping results following common garden for (A) critical thermal maxima and (B) prosome length. Colors of boxes correspond to treatment where “wild” are field collected animals, “cold” are F1 animals at 11 °C, “warm” are F1 animals at 18 °C. As only *A. hudsonica* data points are included in the analysis, individual *Acartia tonsa* outliers and warm col-5 are excluded. Compact letters show the results from post-hoc tests where shared letters indicate no significant difference between measurements.

Table 2
ANOVA type III results for the main model outputs investigating effects of collection, treatment, sex, and length on CT_{max} and effects of collection, treatment, and sex on prosome length, models only include *A. hudsonica*. Significant *p*-values are bolded.

	d.f.	Sum. Sq.	F-value	Pr (>F)
CT _{max}				
collection	3	194.66	141.10	<2e⁻¹⁶
treatment	2	121.74	132.36	<2e⁻¹⁶
collection x treatment	5	178.76	77.75	<2e⁻¹⁶
sex	1	36.50	79.38	<2e⁻¹⁶
length	1	0.58	1.26	0.26
Length				
collection	3	273,014	67.02	<2e⁻¹⁶
treatment	2	197,310	72.65	<2e⁻¹⁶
collection x treatment	5	195,872	28.85	<2e⁻¹⁶
sex	1	305,356	224.88	<2e⁻¹⁶

tential paternal effects, we considered the reaction norms of F1 common garden populations (Fig. 4). Here, common garden treatment temperature had a significant positive effect on thermal tolerance ($p < 0.001$, Fig. 4A, Table 3). Despite an initial effect of parental developmental temperature on F1 CT_{max} ($p < 0.001$), this pattern was driven by the cold collection 2 where low performance was likely due to poor culture

condition prior to the experiments. After excluding these animals, no effect of parental developmental temperature on F1 thermal tolerance remained ($p = 0.23$), suggesting that observed effects are due to plasticity and not carryover effects. Similarly, prosome length was negatively affected by treatment temperature ($p < 0.001$, Fig. 4B), but the effects of parental developmental temperature remained significant even after the removal of cold collection 2. While there was a size reduction related to treatment, individuals from collection 1 were consistently larger than individuals from collection 4 (cold-1: $770 \mu\text{m} \pm 52 \mu\text{m}$, cold-4: $718 \mu\text{m} \pm 36 \mu\text{m}$, warm-1: $687 \mu\text{m} \pm 38 \mu\text{m}$, warm-4: $648 \mu\text{m} \pm 29 \mu\text{m}$). This indicates that F1 prosome length could be influenced by either adaptation or parental effects in addition to plasticity.

4. Discussion

We have shown that seasonal fluctuations in temperature have strong effects on thermal tolerance and body size in *A. hudsonica*. We predicted that the thermal tolerance of copepods would closely correlate with their developmental temperature and that these observed physiological changes would be due to plastic or genetic mechanisms. As predicted, the CT_{max} of *A. hudsonica* increased in parallel with environmental temperature. However, under common garden conditions collections showed similar levels of plasticity and converged on common thermal tolerances, indicating that differences in tolerance between collection times were driven by plasticity with no concrete evidence for rapid seasonal adaptation. Conversely, while collections showed similar levels of plasticity in body size, population differences persisted under

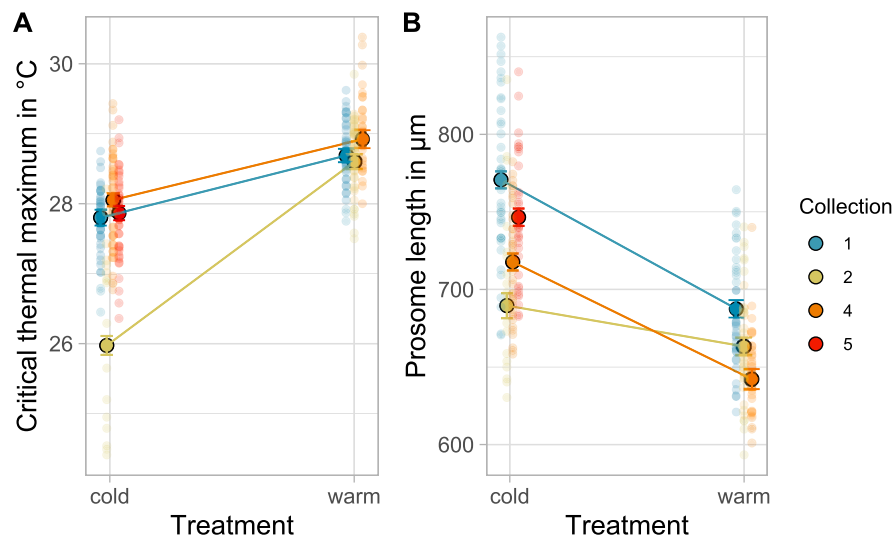


Fig. 4. Reaction norms for treatment effect on (A) Critical thermal maximum; (B) Prosome length; bold points indicate mean values, error bars indicate 0.95% confidence interval. As only *A. hudsonica* data points are included in the analysis, individual *A. tonsa* outliers and warm col-5 are excluded. Model means and standard error are derived using the *emmeans* package.

Table 3
ANOVA type III results for the effects of parental developmental temperature on thermal tolerance and prosome length of the F1 generation, for the second part of the analysis cold col-2 was excluded due to its poor condition prior to the experiments. Significant p-values are bolded.

	d.f.	Sum. Sq.	F-value	Pr (>F)	d.f.	Sum. Sq.	F-value	Pr (>F)
F1 CT_{max}	all				cold col-2 removed			
treatment	1	15.55	29.04	1.72e⁻⁰⁷	1	3.14	12.31	0.0006
parental temp	1	6.19	11.55	0.0008	1	0.38	1.48	0.23
sex	1	4.29	8.01	0.005	1	15.5	60.8	2.6e⁻¹³
length	1	5.73	10.69	0.001	1	0.28	1.08	0.30
treatment x par temp	1	0.00	0.002	0.96	1	0.07	0.29	0.59
F1 Length	all				cold col-2 removed			
treatment	1	22,926	14.98	0.0001	1	36,913	33.36	2.62e⁻⁸
parental temp	1	21,886	14.30	0.0002	1	33,544	30.3	1.03e⁻⁷
sex	1	87,006	56.86	9.93e⁻¹³	1	115,293	104.20	<2e⁻¹⁶
treatment x par temp	1	3690	2.41	0.12	1	1740	1.57	0.21

common garden, either due to adaptation or trans-generational effects. Together, these results indicate that *A. hudsonica* has substantial phenotypic plasticity to rapidly acclimate to large changes in external temperature.

4.1. Seasonal variation in thermal tolerance

For both marine and terrestrial ectotherms, the ability to rapidly acclimate to changes in environmental temperature is common and adaptive (Gunderson and Stillman, 2015). In coastal marine organisms, particularly those from temperate environments, the presence of thermal plasticity is essential as shallow waters tend to have high variance in their temperatures, requiring rapid phenotypic responses (Reusch, 2014). This is particularly true for intertidal copepods such as *Tigriopus* which can experience daily temperature changes of >10 °C (Leong et al., 2017) and therefore have high thermal tolerance and plasticity (Healy et al., 2019). Further, across copepods there is near universal presence of thermal plasticity that is dependent on environmental temperature (Sasaki and Dam, 2021b). For *Acartia* copepods specifically, *A. tonsa* and *A. hudsonica* from the east coast of the United States are typically plastic in their thermal tolerance (Sasaki and Dam, 2020) and laboratory studies on *A. tonsa* show large effects of acclimation on thermal tolerance (Sunar and Kir, 2021). Thus, plasticity plays an important role in enabling most marine copepods to tolerate environmental temperature fluctuations.

While the Baltic Sea experiences only small wind-driven and irregular tides, the variation in water temperature at our collection site is nevertheless high, ranging from 5.65 °C to 20.70 °C during the study period. This high and relatively predictable variation likely favors the evolution of plasticity observed in the population and similar patterns of plasticity have been found in other studies (Bitter et al., 2021). For example, Sasaki and Dam (2020) found large seasonal variation in thermal LD50 of *A. hudsonica* from the east coast of North America ranging from 23 °C to 27 °C; the authors did not disentangle if these differences were due to plasticity or adaptation. However, we cannot directly compare measures from LD50 to CT_{max}. Similarly, the degree of plasticity observed in our study largely matches expectations of other studies on *A. hudsonica*. González (1974) showed that acclimation leads to a 0.14 °C increase in CT_{max} per 1 °C as compared to the 0.10 °C acclimation effect observed here. Previous work on related *Acartia* species has shown that populations from less variable low latitude thermal environments harbor lower levels of phenotypic plasticity than those from more variable high latitude sites (Sasaki and Dam, 2020). Therefore, the plasticity that *A. hudsonica* harbors to respond to changing temperature across the season is likely adaptive in this environment and is present across multiple populations.

The limited evidence for seasonal adaptation in thermal tolerance found in this study is contrasted by examples of this phenomenon in other systems including both terrestrial *D. melanogaster* (Johnson et al., 2023) and the sister species to *A. hudsonica*, *A. tonsa* (Sasaki and Dam,

2020). For *A. tonsa*, Sasaki and Dam (2020) found that collections from different time points differed in their plasticity. It is unclear exactly what drove the seasonal patterns in *A. tonsa*, but it may be due to rapid adaptation or the presence of cryptic lineages emerging or surviving at different temperatures. The comparison to *D. melanogaster* is also interesting as terrestrial organisms typically have a better ability than marine organisms to buffer their thermal environment via behavioral mechanisms, known as the Bogert effect (Bogert, 1949). This behavior buffering might then lead to weaker selective pressures and less adaptation to temperature in terrestrial organisms relative to marine, as has been found previously (Sasaki et al., 2022). Therefore, we might expect stronger selective pressures in marine relative to terrestrial organisms. Further, *Acartia* copepods have enormous population sizes that are regularly in the hundreds of individuals per cubic meter of water (Möllmann, 2002). As larger population sizes increase the efficiency of and response to selection, one might expect that if selection was present across seasons, copepods would show signals of adaptation similar to *Drosophila*; population sizes are likely not substantially different between these species. Despite these expectations, we observed no evidence to support the presence of seasonal adaptation of thermal tolerance in *A. hudsonica*.

There are a number of possible explanations for the lack of seasonal adaptation of CT_{max} in our study. First, *A. hudsonica* may have sufficient plasticity in CT_{max} to respond to the seasonal changes. The thermal plasticity of this population indicates a large capacity to buffer the against thermal stress that may be encountered in the wild. The highest two-week mean SST in our study was 18.11 °C with the highest measured temperature of 20.70 °C, which is substantially lower than the lowest CT_{max} of individuals collected during this time period and the lowest thermal tolerance recorded for any wild individual throughout the whole experiment (28.1 °C and 23.1 °C respectively). Therefore, it is possible that CT_{max} was not directly under selection and that an alternative phenotype would show a seasonal adaptive response, as has been observed in other systems (Hairston and Dillon, 1990; de Villemereuil et al., 2020). It is important to note that the SST measures in this study might not represent the full spectrum of temperature variation experienced by *A. hudsonica*. While this species undergoes diel vertical migration and utilizes the entire water column (Bollens et al., 1992) and thus temperature gradient, there may be fine scale, unpredictable temperature variation, for example in shallow waters created by wind-driven tidal fluctuations, that our SST measurements do not capture. As a result, there may be scenarios in which this population approaches its thermal limits locally, which could explain the high CT_{max} observed here; it is unknown at which frequency these high temperature exposures might occur.

An alternative explanation for the lack of evidence of seasonal variation may be due to a lack of heritable genetic variation for thermal tolerance in this population. However, populations of *A. hudsonica* from North America can rapidly evolve to elevated temperatures under laboratory conditions (deMayo et al., 2023) and there is evidence for local adaptation to temperature along latitudinal gradients in the sister species, *A. tonsa* (Sasaki and Dam, 2019). Therefore, it is likely that heritable variation in thermal tolerance is also present in this species and population. Finally, much of the evidence for seasonal adaptation in *D. melanogaster* has been found at the genomic level (Johnson et al., 2023). Given this, our populations may similarly be experiencing fluctuating selection that would be detectable using genomic approaches. Future studies should focus on additional phenotypes that may be evolving seasonally as well as the genomic evidence for seasonal adaptation in this species.

While there was plasticity for CT_{max} in *A. hudsonica*, it remains unclear if these copepods have sufficient plasticity to respond to future ocean warming without adaptive responses (Gunderson and Stillman, 2015). Indeed, deMayo et al. (2023) showed that under global warming conditions, plasticity alone is insufficient to maintain high population fitness in *A. hudsonica*. However, the species can rapidly adapt after just

four generations to recover fitness levels. Therefore, it is likely that both plasticity and adaptation will be required to tolerate future environmental conditions and more work is needed to understand the relative contribution of each to overall resilience.

4.2. Body size and potential impacts of warming

Body size showed both plastic and persistent effects under common garden conditions. As temperatures increased, body size decreased in *A. hudsonica* (Fig. 2), a common phenomenon across ectotherms known as the temperature-size rule (Angilletta and Dunham, 2003; Rubalcaba and Olalla-Tárraga, 2020). This concept applies to copepods (Escribano and McLaren, 1992; Viitasalo et al., 1995), including those from tropical (Ortega-Mayagoitia et al., 2018) and temperate environments (Riccardi and Mariotto, 2000). While there were similar levels of plasticity between collections, differences between collections persisted under common garden condition (Fig. 4). These differences could be due to either rapid adaptation across the season or trans-generational effects. For example, in zooplankton it is known that smaller adults produce smaller eggs, which in turn produce smaller offspring (Cooney and Gehrs, 1980; Gabsi et al., 2014). However, because we used only a single generation of common garden, we cannot distinguish between these mechanisms. Other factors such as phytoplankton density may also affect the body size of individuals in the wild (Deevey, 1964, 1966), though this would not have affected the common garden animals in our study.

The reduction in body size in response to increasing temperature may be driven by the disproportionate increase in respiration and metabolism relative to ingestion and assimilation of nutrients (Lehman, 1988), leading to lower overall energy available for growth and therefore a smaller body size. At higher temperatures aerobic scope may be decreased in larger individuals relative to smaller individuals, favoring smaller body sizes (Rubalcaba et al., 2020; Verberk et al., 2021). Alternatively, in cold temperatures growth periods may be prolonged while the growth rate remains relatively stable, leading to larger individuals under cold conditions (Vidal, 1980).

As temperatures increase due to anthropogenic causes, decreases in body size may affect ecosystem interactions. This is particularly true in the Baltic Sea where the heating rate is around three times higher than the ocean average due to its unique topography (Reusch et al., 2018; Szymczycha et al., 2019; Dutheil et al., 2022). A size reduction in *A. hudsonica*, or other prey organisms, might impact higher trophic level predators, for example by requiring the consumption of more individuals to maintain the same amount of nutrient intake (Garzke et al., 2015). If abundance does not increase with decreasing body size, nutrient availability may be reduced for consumers who will also require increased energy needs under higher temperature (Brown et al., 2004). Further, Garzke et al. (2015) observed that large copepods from colder temperatures clear algae biomass more efficiently than smaller individuals, exerting top-down control on phytoplankton. This suggests that smaller copepods are less efficient grazers, with less control over the planktonic community. As copepods are important grazers on large phytoplankton and microzooplankton (Sommer et al., 2003; Armengol et al., 2017), less efficient grazing or a shift to different prey size classes might have unforeseen cascading effects across ecosystems.

4.3. Outliers and mixed species

The outliers in collection 2 under cold conditions, characterized by unexpectedly low thermal tolerance and small body sizes, were most likely the result of an impending culture collapse. This was likely caused by poor food quality during that period due to ciliate and bacteria growth in the algae cultures used for feeding. The negative effects of ciliates on copepod fitness are well described with effects ranging from decreased egg production (Burris and Dam, 2014) to increased adult mortality (Visse, 2007). The species identity of the ciliates in this study

could not be determined. However, ciliate peak abundances correlated with culture collapse, and after the establishment of more frequent water changes, the cultures improved. Since the survival and fitness of the animals was clearly affected by external factors unrelated to the experiment, length and thermal tolerance measurements for the cold treatment in collection 2 should be treated with caution and were therefore excluded from parts of the analysis.

In the wild collection 5, five individuals showed a CT_{max} above 33 °C, which was well beyond the distribution of values for any other collection (Fig. S.4). In the F1 generation, the warm treatment of collection 4 and 5 and the cold treatment of collection 5 also had similar high performing individuals (11, 40, and 2 individuals, respectively) (Fig. S.9). These extreme outliers suggested a mixed species composition, which was confirmed by genotyping individuals of each collection as well as high thermal outliers (see Supplement *Mixed species*). High thermal outlier individuals were *A. tonsa* without exception (Table S.1, Fig. S.10). The increased thermal tolerance for *A. tonsa* relative to *A. hudsonica* is consistent with other studies. On the east coast of North America, *A. hudsonica* dominates plankton communities early in the year when water temperatures are low and is replaced by *A. tonsa* as temperatures increase (Borkman et al., 2018; Sullivan and McManus, 1986). This pattern appears to be similar for copepod communities in Kiel Fjord as well. Since *A. tonsa* was present only in later collections and just as individual outliers, we could not determine how thermal tolerance of *A. tonsa* changes within a season. However, we would hypothesize that *A. tonsa* follows an overall similar trend than shown for *A. hudsonica* with thermal tolerances shifted towards warmer temperatures. Additional experiments would be required to test this hypothesis.

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

This study represents the first known record of *A. hudsonica* in the Baltic Sea. First, *A. hudsonica* may have recently invaded the Baltic Sea either via migration from the North Sea or due to ballast waters from shipping. Secondly, *A. hudsonica* historically may have been misidentified in the Baltic Sea. Copepods from the genus *Acartia* have a record of mis-identifications and are difficult to distinguish morphologically (Figuerola et al., 2020). Before the 1970's, *A. hudsonica* was a subspecies of *A. clausi*, but is now considered its own species (Bradford, 1976; Ueda, 1986). Given this, it is possible that *A. hudsonica* is native to the Baltic Sea but has been, and still is, commonly identified as *A. clausi*.

If *A. hudsonica* is newly established, understanding how plasticity and adaptation contribute to persistence in a new environment could contribute to understanding how populations will respond to ongoing environmental change and range shifts. However, more work is needed to reveal the origin of this local population of *A. hudsonica*.

5. Conclusions

Here we showed that the thermal tolerance of *Acartia hudsonica* is plastic in response to changing temperature within a single year. We found that thermal tolerance is closely correlated with environmental temperature, indicating that *A. hudsonica* has capacity to tolerate increasing temperatures that fall within the current range experienced in nature. Conversely, while body size was plastic in response to temperature, there were also population differences across the season, suggesting the presence of either evolved or trans-generational effects. This observed decrease in body size could impact nutrient availability and ecosystem functioning if temperatures consistently increase with no change in copepod abundance. By focusing on the relative impacts of plasticity and adaptation to population responses to temperature change we can begin to understand the resilience of populations and ecosystems to ongoing global change.

CRedit authorship contribution statement

Alexandra Hahn: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Reid S. Brennan:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data set and scripts can be found on https://github.com/HahnAlexandra/Plasticity_Acartia_hudsonica/tree/v.1.1.0. The individual sequences are uploaded on NCBI GenBank <https://www.ncbi.nlm.nih.gov/genbank/>: find individual accession numbers in Table S.1. The complete data set of sea surface temperature from Kiel Fjord is published by our collaborator Claas Hiebenthal on <https://doi.pangaea.de/10.1594/PANGAEA.963281>.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jembe.2024.152014>.

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