Starvation reduces thermal limits of the widespread copepod *Acartia tonsa*

Abstract

Organismal thermal limits affect a wide range of biogeographical and ecological processes. Copepods are some of the most abundant animals on the planet, and play key roles in aquatic habitats. Despite their abundance and ecological importance, there is limited data on the factors that affect copepod thermal limits, impeding our ability to predict how aquatic ecosystems will be affected by anthropogenic climate change. In a warming ocean, one factor that may have particularly important effects on thermal limits is the availability of food. A recently proposed feedback loop known as "metabolic meltdown" suggests that starvation and exposure to high temperatures interact to drastically reduce organismal thermal limits, increasing vulnerability to warming. To investigate one component of this feedback loop, we examined how starvation affects thermal limits (critical thermal maxima: CTmax) of Acartia tonsa, a widespread estuarine copepod. We found that there was no effect of short duration exposure to starvation (up to two days). However, after three days, there was a significant decrease in the CTmax of starved copepods relative to the fed controls. Our results provide empirical evidence that extended periods of starvation reduce thermal limits, potentially initiating "metabolic meltdown" in this key species of coastal copepod. This suggests that changes in food availability may increase vulnerability of copepods to increasing temperatures, amplifying the effects of climate change on coastal systems.

Introduction

The acquisition of nutrition is a fundamental challenge for organisms. While environmental conditions are often considered to impact the ability to find, capture, and ingest food, nutrition also affects how environmental conditions impact organisms. In a changing climate, these feedbacks between feeding and sensitivity to environmental conditions are crucial to consider. [Summary of climate change, temperature, and food availability in the ocean; Are copepods currently or predicted to be exposed to food limitation?].

[Summary of starvation effects on thermal limits literature]. It is vital to understand how copepod thermal limits respond to changes in food availability in order to better model population dynamics in a changing climate.

Copepods are the some of the most abundant animals on the planet, and dominate planktonic communities in the coastal ocean. By nature of their abundance this group plays key ecological roles in aquatic food webs. In many systems, copepods are important consumers of primary productivity, acting as a crucial linkage between phytoplankton and higher trophic levels. Further, many commercial fish larvae depend on copepods as a critical food source during development. Climate change therefore has the potential to impact aquatic ecosystem functions as well as human fishery systems directly through changes in copepod populations. However, despite their abundance and ecological importance, there is limited data on environmental control of copepod thermal limits, including the effects of starvation. This impedes our ability to predict how copepod populations may be affected by co-occurring changes in temperature and food availability over both short (e.g. - seasonal changes) and long timescales (e.g. - anthropogenic climate change). In this study we tracked changes in critical thermal maxima (CTmax) in copepods exposed to extended starvation to test the hypothesis that food deprivation would reduce thermal limits. We observed a clear decline in thermal limits after three days of starvation. Our results suggest that natural variability in food concentrations in the coastal ocean may reduce copepod thermal limits thus increasing vulnerability to increasing temperatures, especially during marine heatwaves.

Methods

The copepods used in this study were collected in July 2020 from Esker Point, Connecticut (lat, long) by surface tow with a 63-um mesh net and solid cod end. Mature *Acartia tonsa* females and males were isolated from the tow contents, and used to initiate a laboratory culture which was maintained in an environmental chamber at 18°C and a 12:12 light:dark cycle, with constant aeration from a small aquarium pump. Copepods were fed *ad libitum* a mixture of three phytoplankton cultured under the same environmental conditions: a green flagellate, *Tetraselmis sp.*; the cryptomonad *Rhodomonas sp.*; and the small diatom *Thalassiosira weissflogii*. This diet is regularly used to maintain large, active laboratory cultures of *A. tonsa* (REF).

We used five replicate experiments to test our hypothesis that starvation would reduce copepod thermal limits over time. Each experiment involved measuring a baseline thermal limits for the culture (as critical thermal maxima: CTmax), and then tracking CTmax over time in two groups of copepods, a fed control group and a starved treatment group. To initiate each experiment, 10 mature females were isolated from the laboratory culture and maintained for 24 hours in 200 mL of an *ad libitum* food solution (800 ug C / L of *Tetraselmis*). Preliminary work showed that short exposure to three different prey options (*Tetraselmis sp.*, *Rhodomonas sp.*, and *Oxyrrhis marina*, a heterotrophic dinoflagellate) did not affect copepod thermal limits (Supp. Fig. 1). After 24 hours, thermal limits were measured as Critical Thermal Maxima (CTmax; described in the following section), which served as a baseline initial value for each experiment. On the same day as the baseline CTmax measurements, an additional ~90 mature females were isolated and divided into six groups. The six groups were randomly assigned to either the starvation treatment or the fed control treatment. The starvation groups were maintained in 0.2-um filtered sea water. The control group was provided a food solution comprising 800 ug C / L of *Tetraselmis*, identical to the conditions used to assess baseline CTmax. Each group was held in a 100 mL cup with a plastic cylinder nested within. The bottom end of the cylinder was covered by a 150-um mesh screen. Similar set-ups are often used to prevent egg cannibalization during egg production assays, as eggs of *A. tonsa* sink through the mesh to the base of the cup; in our case, this prevented females in the starvation group from acquiring nutrition via egg cannibalism. All groups were transferred to fresh media (either filtered sea water or food solution) on a daily basis throughout the experiment by gently removing the meshed column and placing it into a new 100 mL cup.

Thermal limits were measured each day for five days, starting 24 hours after the females were isolated using a custom setup. The apparatus used has three components: a reservoir, a water bath, and a temperature sensor (Supp. Fig. 2). We used a 5 gallon bucket covered with a neoprene sleeve as our reservoir. The reservoir was filled with ~20 L of water, which is then slowly warmed using a 300 watt aquarium water heater (BRAND). In this arrangement, the temperature ramping rate is determined by the interaction between the power output of the aquarium heater and the volume of water in the reservoir. The reservoir also contains two aquarium pumps (BRAND), one of which circulates water within the reservoir while the other pumps water from the reservoir into the water bath, which sits atop the reservoir. The water bath is a transparent plexiglass box that fits over the opening of the reservoir. Water is pumped up from the reservoir at a rapid rate, flooding the water bath. A recession cut into one of the edges of the box allows water to spill back into the reservoir. The water bath contains several test-tube holders that are used to secure the experimental vessels (50 mL flat-bottom glass vials) during the CTmax trials. Because the box is transparent, individuals are easily monitored through the side of the water bath throughout the trial, eliminating the necessity to remove experimental vessels from the water bath, potentially inducing temperature fluctuations. The final component of this apparatus is a small Arduino computer system that logs temperature with three independent sensors at 5 second intervals. These sensors are small enough to be placed inside the same experimental vessels used during the CTmax trials, providing a continuous record of the temperatures within the vessels. No horizontal gradients in temperature were detected within the water bath, and temperatures are averaged across the three sensors.

Using this setup, we measured individual CTmax values for ten copepods per day, selected at random from the six cups. One replicate experiment (replicate 2) was ended after only ___ days when all individuals from the starvation treatment died. At the beginning of each CTmax trial, the reservoir was filled with 20 L of water and adjusted to 18°C. After the reservoir reached the proper temperature, experimental vessels containing 10 mL of 0.2 um filtered sea water were placed in the water bath, which was then flooded with water, bringing the experimental vessels to the correct temperature as well. Individual copepods were then

placed into the vials, and let acclimate for 10 minutes at constant temperature. All copepods were checked during this time period for normal behavior. Individuals exhibiting abnormal behaviors were excluded from further analysis. After this resting phase, the water heater was turned on, initiating the temperature ramp. Simultaneously, the temperature logger began to record temperature and a stop watch began recording the time passed. Individuals were continuously monitored as water temperature increased. CTmax is generally defined as the temperature at which an individual ceases to respond to physical stimuli (REF), indicating the onset of "ecological death" (the inability to escape lethal temperatures, predators, etc.). In *A. tonsa* this is indicated by cessation of movement, a lack of response to gentle physical stimuli (e.g. - slow flushing of the water in the tube with a transfer pipette), and abnormal body configuration (specifically - antennules pressed against the sides of prosome and a distinct dorsal tilt of the urosome). The time at which an individual began to exhibit these characteristics was recorded and that individual's experimental vessel removed from the water bath. After all individuals reached their CTmax, copepods were photographed using a camera attached to an inverted scope (or the equivalent), and body size estimated using a scale micrometer and the software ImageJ (REF). Individuals from replicate experiment 5 were not photographed due to a malfunction with the imaging software.

The times recorded during the trials were converted to the CTmax values in degrees C using temperatures recorded on the temperature logging system. There is an "uncertainty window" for each measurement, however, that also has to be accounted for - as there are between 1-10 vials being monitored at any point in the trial, the time at which an individual was recorded as having stopped responding to stimulus corresponds with the latest time (and therefore highest temperature) it could have reached its CTmax. The period of time during which an individual could have reached its CTmax extends from this definite end point to the last time the individual was checked. As it generally takes around 5 seconds to check whether an individual has stopped responding, the duration of this uncertainty window was estimated for each individual as the number of vials remaining in the water bath multiplied by 5 seconds. This uncertainty window decreased in length as the trial went on, until, for the final individual, the window includes just the amount of time it took to check whether the individual had stopped responding. CTmax is estimated as the average temperatures recorded by all three temperature sensors throughout the uncertainty window. We used this time-based method instead of directly monitoring the temperatures to reduce any sub-conscious bias stemming from past knowledge or expectations about copepod thermal limits.

Statistical Analysis

Results

The custom setup produced consistent ramping rates across assays (Supp. Fig. 3). Ramping rates did however vary within each assay, decreasing over time due to the imperfect insulation of the bucket reservoir. Ramping rates always between the target ramping rates of 0.1 - 0.3°C per minute, however, which have been used previously to measure CTmax for copepods (REF).

A total of 254 CTmax measurements were made across the five replicate experiments (153 from the fed controls and 101 from the starved treatment). Average CTmax gradually decreased over the course of the five day starvation period (Figure 1). There was no difference between thermal limits of fed and starved individuals on days 1 or 2. Thermal limits were ~1°C lower by day 3 and continued to decrease by ~2°C per day after that. By day 5, starved individuals had thermal limits that were ~5°C lower than those of control individuals. There was, however, also a large increase in the variance of individual thermal limits in the starved treatment; several individuals maintaining thermal limits similar to those observed in the control individuals while others had thermal limits as low as 22°C. No changes were observed between the fed controls and the baseline CTmax values during any of the experiments (Supp. Fig. 4). There was no significant effect of size on CTmax during any of the experimental days (Supp. Fig. 5).

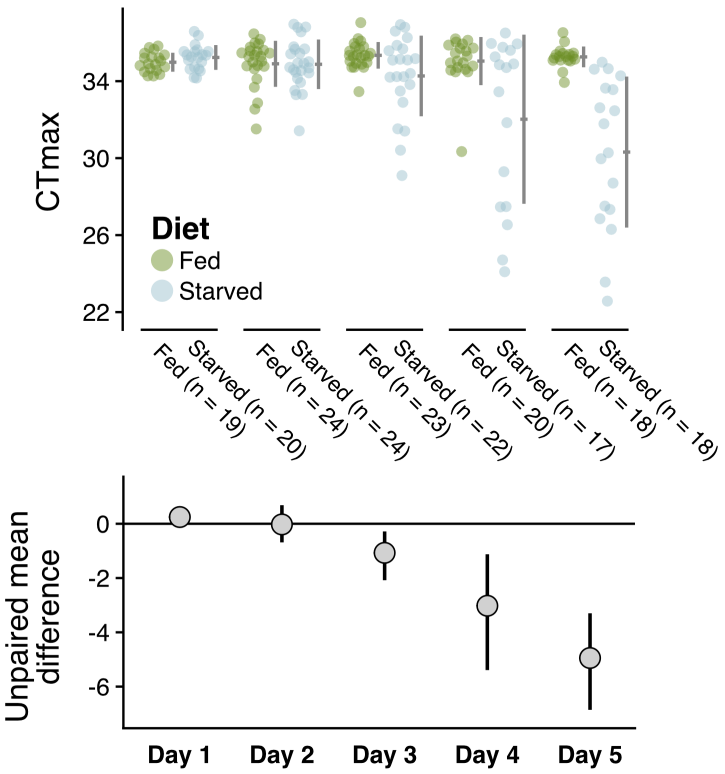
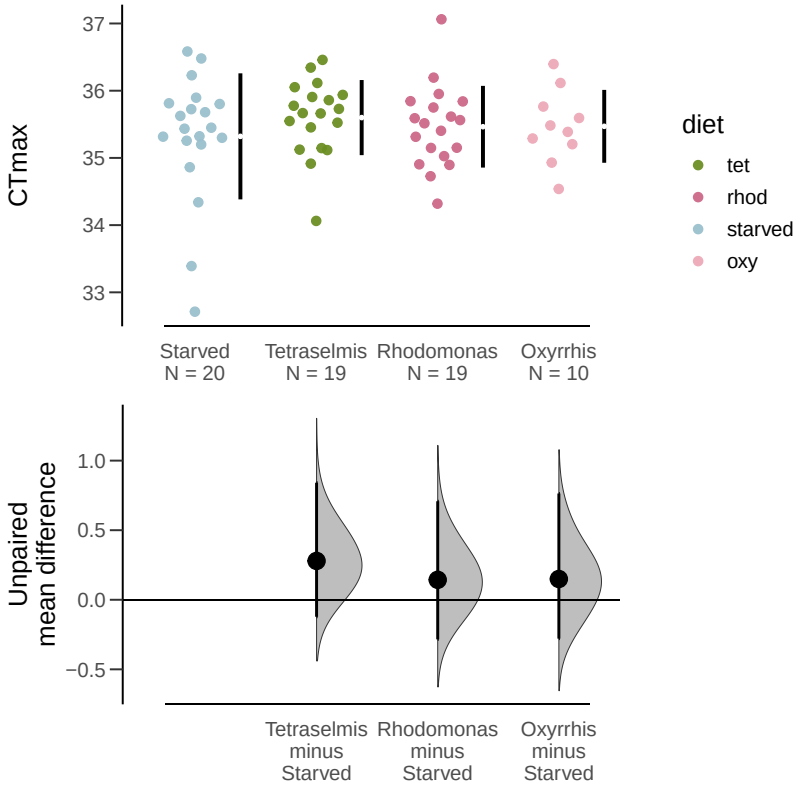


Figure 1: Estimation plots depicting the gradual reduction in thermal limits relative in the starvation group relative to the control group.

Discussion

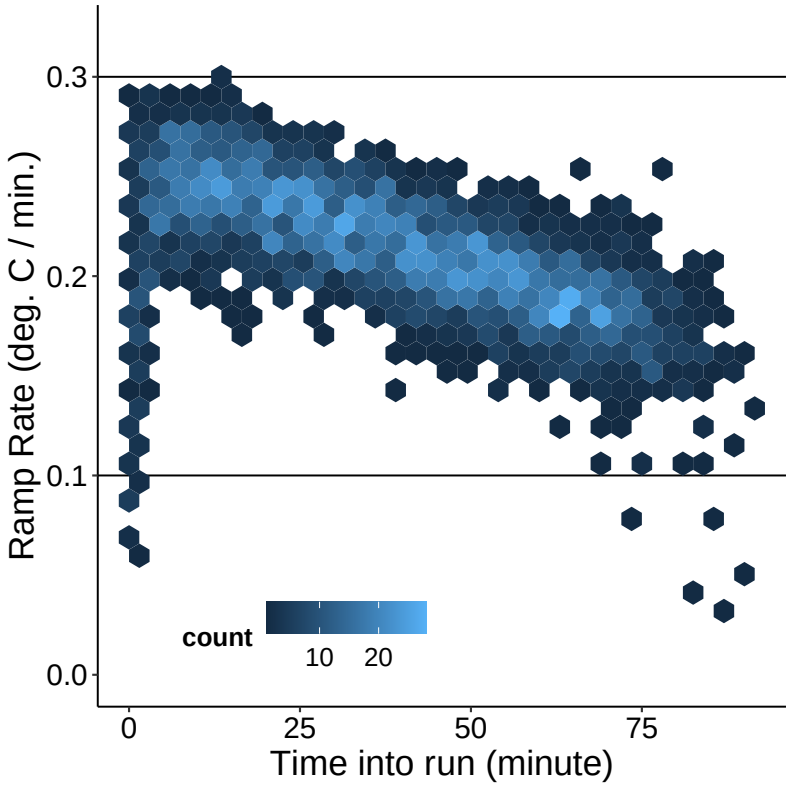
Supplementary Material

Supp. Fig. 1 - Diet figure

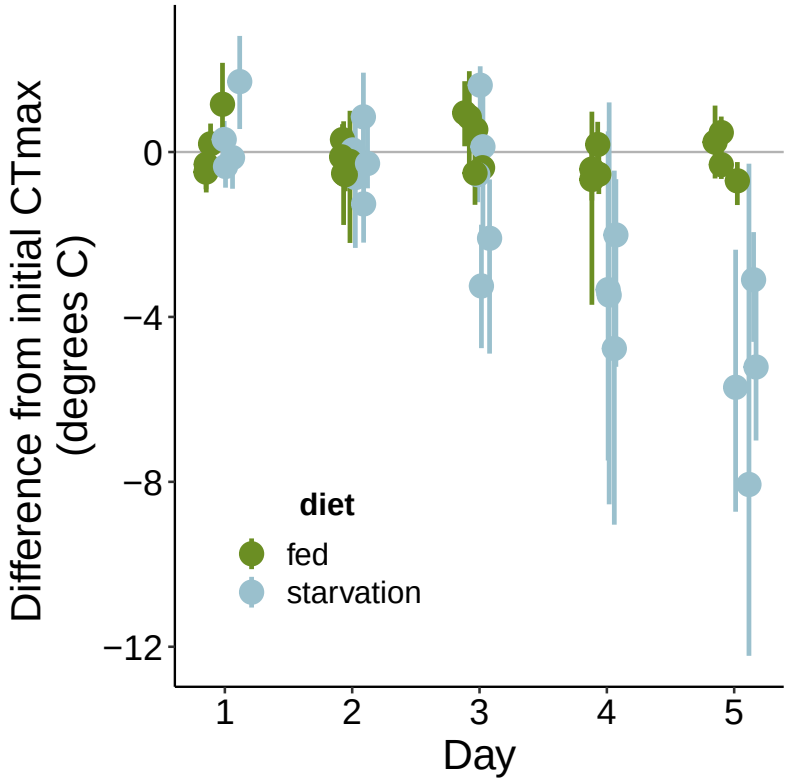


Supp. Fig. 2 - Schematic of the custom device used to measure copepod CTmax values.

Supp. Fig. 3 - Ramping rates during these trials (the increase in temperature per minute) decrease over time due to the imperfect insulation of the bucket reservoir. Ramping rates were always between the target ramping rates of 0.3 and 0.1 degrees C per minute. The plot below is a hexagonal heatmap of ramping rate throughout the CTmax trials. The plane is divided into regular hexagons, which are shaded according the frequency of the encompassed ramping rates.



Supp. Fig. 4 - CTmax vs baseline



Supp. Fig. 5 - Thermal limits (CTmax) plotted against individual body size for each day, separated

by treatment. A linear regression is shown, along with the associated p-value for each day. At no point throughout the experimental duration (day 1 through 5) is thermal limit associated with length.