# MULTI-DECADAL, SEASONAL, AND SPATIAL VARIATION OF CALANOID COPEPOD BODY SIZE IN DELAWARE BAY

by

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### ABSTRACT

Size affects feeding, growth, and metabolism in all organisms, and in ectotherms, size is affected by environmental factors. The thermal plasticity of body size in ectothermic species is known as the temperature-size rule (TSR), which states that warmer temperatures lead to a decrease in generation time, resulting in overall smaller size at maturity. I investigate temperature and size variation of dominant copepod species in the Delaware Bay over multiple decades by comparing my results to historical data from the 1930's (Deevey 1960) to assess whether the relationship has changed in response to long term warming. Results for Acartia tonsa and *Centropages hamatus* indicate a decrease in the strength of temperature size relationships over time, resulting in less body size variation in Delaware Bay under current conditions. I also analyze the effects of temperature and food on adult female copepod body size in the current study. While *in situ* temperature is the only significant predictor of seasonal variation in A. tonsa and C. hamatus adult body size, chlorophyll effects spatial body size variation of *A. tonsa* throughout Delaware Bay in August 2016, when bay wide temperatures are more constant. Based on the results of this study and predictions for future temperature and chlorophyll conditions in Delaware Bay, calanoid copepod body size is expected to continue to decrease unless a lower size limit is reached.

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### **Chapter 1**

### **GENERAL INTRODUCTION**

Body size is a fundamental trait that affects feeding, growth, and metabolism in all organisms. From an ecological perspective, larger body sizes are associated with superior performance and fitness. Within natural populations, larger individuals often have greater survival, fecundity and mating success than smaller individuals (Kingsolver & Pfennig 2004). Body temperature is also a key component of organism function due to its influence on the rate of most physiological processes. Ectotherms are organisms that do not retain metabolic heat to regulate body temperature, so more of the energy obtained from food can be used in growth and reproduction, as opposed to endothermic species that use the majority of metabolic energy for thermoregulation (Brusca & Brusca 2003). Ectothermic body temperatures are determined by the thermal conditions outside their bodies, so physiological function is particularly sensitive to the temperature of the surrounding environment (Huey & Kingsolver 1993). Therefore, fitness components like body size are influenced by environmental temperatures.

The thermal plasticity of body size in ectothermic species is a widely-accepted phenomenon known as the temperature-size rule (TSR, Atkinson 1994). This rule states that warmer temperatures lead to a decrease in generation time, resulting in an overall smaller size at maturity. The TSR has been observed across a wide range of aquatic and

terrestrial ectotherms. Temperature induced reduction in body size has been applied to intraspecific geographic variation as well as seasonal fluctuations and has been observed in crustaceans (Moore & Folt 1993), amphibians (Reading 2007), and marine fish (Houde 1989).

In the context of climate change, seasonal fluctuation in TSR is becoming a more prominent concern with atmospheric temperatures rising and warming the oceans. Current trends estimate a global increase of 0.16°C in atmospheric temperature and 0.12°C in sea surface temperature per decade (Rayner et al. 2006, Thorne et al. 2005). The associated decrease in ectothermic body size has also been seen on a global scale, effecting size at the community, population, and individual level by changing species composition, growth and reproduction across diverse biota and ecosystems (Daufresne et al. 2009, Rice et al. 2015). Fecundity and feeding are important fitness components, therefore reduced body size has consequences for individual functioning (Hirst & Kiørbe 2002). Trophic interactions including size-selective predator-prey and size-dependent survival/dispersal (Ohlberger 2013) are put at risk by decreases in mean size of ectothermic populations because smaller individuals may modify the efficiency of these size-dependent interactions. Ectotherms make up around 99% of all species worldwide, therefore it is essential to understand how they respond to a warming climate (Wilson 1992).

Copepods are a dominant group of zooplankton throughout the world's oceans, and thermally induced body size reduction has been observed in many species, in laboratory and seasonal field setting (Hirst et al. 1999, Horne et al. 2015). They are an important food source for fish and other crustaceans, and as a result they are an essential intermediate between primary producers and higher trophic levels. Copepods are particularly important in estuaries where high productivity allows for relatively greater copepod biomass than other aquatic environments (Kleppel et al. 1998). In Delaware Bay, calanoid copepods are the dominant zooplankter across season (Wickline 2016). Calanoid copepods have been shown to be particularly sensitive to increasing temperatures (Horne et al. 2016), making them useful model organisms for studying TSR in this region.

A general mechanism for why temperature-body size variation occurs across ectothermic species is unknown. Calanoid copepods experience determinate growth, with six naupliar stages (NI – NVI) followed by six copepodite stages (CI-CVI). They are considered mature adults in the CVI stage, at which point they no longer molt. Therefore; to understand adult body size variation, which has implications for fitness, it is important to consider the latent effect of early life thermal exposure. Forster et al. (2011) proposed that growth and development rates respond differently to increasing temperature and their decoupling is what leads to smaller size at maturity. At higher temperatures, development rate is more sensitive to temperature and so the rate at which copepods pass through each life stage outpaces the mass they accumulate at each stage. Growth and development rates can be further investigated on a life history basis to identify the naupliar or

copepodite stage that is most thermally sensitive. Previous studies on stage-specific rates in pelagic copepods and brine shrimp (*Artemia franciscana*) found that they have different thermal sensitivities during their early and later larval stages (Forster & Hirst 2012). In early larval stages of *A. franciscana*, body size increased with temperature, contrary to TSR expectations. The effects of TSR become evident in the later larval stages, when development is more temperature dependent than growth. Similar findings for *Acartia tonsa* suggest that the later copepodite stage is more thermally sensitive, so environmental conditions just before maturity, may be the most influential on adult size (Leandro et al. 2006).

In addition to potential differences in thermal sensitivities, there is a distinction in the feeding capabilities between naupliar and copepodite stages that could affect ontogenetic growth rates. Kiørboe & Sabatini (1995) found that certain marine planktonic copepod species grow slower in their naupliar stage due to smaller optimal prey size. As the later larval stage, copepodites are able to handle and ingest larger food particles (Fernández 1979), so it is important to consider the effect of food availability on adult size. Chlorophyll abundance is a commonly accepted proxy for phytoplankton (Roesler 2014), and has been analyzed as an indirect effect of temperature on copepod body size. Previous research in Eastern Atlantic estuaries found that chlorophyll *a* concentrations explain less body size variation than does temperature (Deevey 1960, Heinle 1996, Angilletta 2004, Rice et al. 2015). Deevey (1960) concluded that in regions with wide temperature ranges, like the Delaware Bay and Long Island Sound, temperature is the primary driver of body size variation, while in narrow temperature ranges, chlorophyll may be more influential.

In order to further understand the impacts of climate on ectothermic body size in coastal systems like the Delaware Bay, this study aims to answer two main questions (1) has long-term warming in Delaware Bay changed the temperature-size relationship in ecologically important calanoid copepod species? (2) In addition to temperature, does chlorophyll affect adult copepod body size (a) across seasons in the lower bay, and (b) in summer throughout the bay?

### Chapter 2

# EFFECT OF TEMPERATURE AND CHLOROPHYL VARIATION ON CALANOID COPEPOD ADULT BODY SIZE IN DELAWARE BAY

### 2.1 Introduction

The Delaware Bay is home to over 50 species of copepods, primarily of the order Calanoida, that make up the majority of zooplankton biomass (Deevey 1960, Wickline 2016). They are essential to energy transfer, and provide a food source to many economically valuable species. The most abundant copepod species is *Acartia tonsa*. Individuals are present throughout the bay during all seasons, and are most common during winter and summer months, making them an ideal species for studying seasonal size response (Deevey 1960, Cronin et al. 1962, Wickline 2016). *Centropages hamatus* is another common calanoid species in the Delaware Bay, and is found in estuaries more frequently than other *Centropages* sp. (Durbin & Kane 2007, Wickline 2016). As a cooler water species, *C. hamatus* seasonal abundance is often limited by temperature, making it an interesting species for studying the effects of temperature on size (Gaudy 2007).

Historical zooplankton sampling in Delaware Bay (1929-1933) provides seasonal temperature and body size information for *Acartia tonsa* and *Centropages hamatus* (Deevey 1960). Both species experience seasonal variation in size consistent with TSR expectations. Deevey's (1960) data creates a unique opportunity to revisit body size analysis of these calanoid copepods under current temperature conditions, and better understand the influence of TSR across multiple decades.

Over the past century, the lower Delaware Bay watershed has experienced a ~1°C increase in average air temperature, which in turn heats the sea surface (Najjar et al. 2012). Therefore, studying body size response to long-term warming is relevant in the Delaware Bay. Flood events in this region have also become more frequent over the past century, increasing run-off and phytoplankton biomass (Voynova & Sharp 2012). Climate change models have linked this change in precipitation to temperature (Allan & Soden 2008). Due to the concurrent nature of temperature-induced changes in copepod size and phytoplankton biomass it is important to consider the impact of chlorophyll under current Delaware Bay conditions.

In order to understand body size variation of calanoid copepods in the Delaware Bay, the present study investigates the effects of long-term warming, as well as seasonal fluctuations and spatial distribution of temperature and chlorophyll on adult size. I examined the effect of long-term warming by comparing temperature-size relationships of common copepod species in current study with individuals collected in the early 1930's. I then address seasonal copepod body size variation under current conditions in response to temperature and chlorophyll. In this analysis, I used *in situ* temperature and chlorophyll associated with adult size, as well as satellite derived measurements of these environmental variables from the weeks prior to collection, to account for conditions before maturity. Finally, I investigate copepod body size throughout Delaware Bay in response to *in situ* temperature, chlorophyll and salinity in August 2016 to evaluate the

effect of these environmental variables on size during the summer, when bay wide temperatures are more constant.

### 2.2 Methods

### 2.2.1 Field Measurements and Processing

Sample collection began in November 2014 as a part of a seasonal zooplankton time series (Wickline 2016). Individuals were sampled at 16 stations throughout Delaware Bay (Fig 2.1) in the Spring, early Summer, late Summer and Fall (Table 2.1). Ten of the stations are located along the main channel, ranging from tidal freshwater near Wilmington, DE, to the Atlantic Ocean just outside the mouth of the bay. The remaining stations are located along the Delaware and New Jersey shorelines, with three sites off the coast of each state (Fig 2.1). The seasonal sampling of the entire bay continued through June 2017. From this large subset, I focused on three lower bay focal stations, with locations analogous to the 1929-1933 Delaware Bay sampling reported by Deevey (1960). More frequent sample collection of these focal stations began in July 2016 (Fig 2.1, Table 2.1) to provide higher resolution seasonal data, creating a dataset that reflects the full annual temperature range of the bay. The focal stations are the southernmost Delaware shoreline location, and two main channel stations located inside and outside of the mouth of the bay (Fig 2.1).

The seasonal time series and the focal station seasonal sampling were done aboard the R/V Daiber. We collected mesozooplankton using a bongo frame with 0.5m nets of 200µm mesh with filtering cod ends and flow meters. The nets were deployed using an oblique tow to ~1-3m above the bottom, held at depth for 30 seconds and recovered. The dual net method is necessary for the continued time series analysis and was only utilized in the focal station sampling to maintain consistency. Once the bongo frame is retrieved from the water, both nets are gently rinsed to concentrate the sample into the cod ends. The content of one cod end is immediately fixed in 4% borax-buffered formaldehyde, excluding any large gelatinous zooplankton such as ctenophores. The content of the other net is frozen for the time series analysis. In addition to the net tows, at each station, we obtained water column profiles of temperature, salinity, turbidity, chlorophyll *a*, dissolved oxygen, and pH using a SBE 19 CTD along with Wet Lab Eco-FlnTurt fluorometer, SBE 43, and SBE 18 sensors. All sensors were attached to a frame, and deployed simultaneously.

The preserved samples were processed using a ZooScan optical scanner and associated Zooprocess software based on the methods reported in Gorsky et al. (2010), as modified by Wickline (2016). Each sample is transferred into freshwater and sieved into three size fractions (>1000  $\mu$ m, >500  $\mu$ m, and >190  $\mu$ m). A subsample of ~1000 individuals are obtained from each size fraction and scanned, generating a greyscale normalized image of the sample. The individual objects from the image are digitally extracted and measured according to 13 object parameters. Plankton ID (PkID) software is used to compare each object to a pre-established Delaware Bay learning set (Wickline 2016), allowing for a semi-automatic identification of the sample. The predictions are manually validated to ensure proper object identification. For the purposes of this study, the digital objects identified as *Acartia tonsa* and *C. hamatus* were isolated and manually

categorized based on sex and life stage. For each species, the full length of 30 adult (CVI) females from the combined size fractions are measured. The full length (mm) is measured from the anterior margin of the prosome to the base of the caudal rami (Fig. 2.2) using ImageJ software applied to the ZooScan generated images.

The CTD data were extracted using SBE Data Processing software. The downcast values for temperature (°C), salinity (psu), and chlorophyll ( $\mu$ g/L) were averaged across depth (m) due to the orientation of the fluorescence sensor, to ensure that the water parameter data accurately represents the conditions from which the organisms were collected. The additional water parameter data were not processed beyond extraction, because they were not pertinent to our analyses of copepod body size.

### 2.2.2 Seasonal Temperature Variation Across Multiple Decades

To investigate calanoid copepod size response to seasonal temperature variation in Delaware Bay, we modeled the adult female full length measurements of *Acartia tonsa* and *Centropages hamatus* with the associated depth-averaged temperature data from the lower bay focal stations, collected over all seasons from 2014-2017 (Table 2.1, Fig 2.1). Due to the significant size differences between sexes, we only measured adult female length, an approach consistent with many investigations of copepod body size (Deevey 1960, Rice et al. 2015, Daufresne et al. 2009, Horne et al. 2016). To compare copepod temperature size response across multiple decades, we selected focal stations that were analogous to sample locations from the 1929-1933 Delaware Bay zooplankton survey described by Deevey (1960). To obtain comparable body size and temperature data from the 1930's, Figure 4 of Deevey (1960) containing adult female *A. tonsa* and *C. hamatus* full length and associated temperature was digitized to extract the data points using Get Data Graph Digitizer (http://getdata-graph-digitizer.com/). It is important to note that Deevey (1960) provides surface values for size and temperature, while we sampled the whole water column. All analyses of body size response to current environmental conditions, utilizes depth-averaged data because it is a more accurate representation of where the copepods were sampled. In order to address this discrepancy, we took all the *in situ* temperature values that were collected from the top meter of the water column and ran a test of collinearity between the complete depth-averaged CTD data and the surface CTD subset (<1m). The results of the collinearity test (VIF > 4) indicate that the depth-averaged and surface values for the current study are not different enough from one another to be considered unique datasets. Therefore, I compared the temperature size response between the two studies, using depth-averaged values for current conditions, and surface values for the Deevey (1960) data.

To identify any differences in seasonal surface water temperature variation between the 1930 - 1932 data and the 2015 - 2017 data, I analyzed temperature averages from the three lower bay stations. The range of years selected from both studies are a subset of the data that have three full years of consecutive data. The Deevey (1960) data for this analysis includes samples collected every month of the year from 1930 - 1932, excluding December. We do not have consecutive monthly *in situ* measurements for the current study, so I used satellite derived data from the middle of each month. Data extracted from January 2015 through December 2017. I compared the yearly temperature

averages between the two studies using a two-factor ANOVA. I also grouped the months by Fall (September to February), and Spring (March to August) and ran a post-hoc Tukey to determine if temperature variation differed between two seasons. Fall and Spring seasonal division determined by months containing the autumnal and vernal equinoxes, respectively. MODIS Aqua satellite data for Delaware Bay was obtained from the NOAA ERDDAP data server (<u>http://basin.ceoe.udel.edu/erddap/index.com</u>).

The temperature size response for *A. tonsa* and *C. hamatus* in both studies were analyzed using linear regressions. I used a Test for Structural Stability to determine if the data contained any natural break points, and fit a single segment or a 2-segment regression accordingly. Analyses were performed in R 3.5.0 (R Core Team 2018) using the strucchange package (Zeileis et al. 2002). The confidence intervals of the slopes, yintercepts, and natural break points identified any significant differences between the regressions.

### 2.2.3 Seasonal Variation in Temperature and Food

To understand the role of seasonal variation in temperature and food on copepod body size under current conditions, I modeled adult female full length measurements of *Acartia tonsa* and *Centropages hamatus* with associated depth-averaged temperature and chlorophyll *a* data from the focal stations, collected over all seasons from 2014-2017 (Table 2.1, Fig 2.1). These data were fit by a multiple linear regression with full length (mm) as the response variable and *in situ* depth-averaged temperature (°C) and chlorophyll a (µg/L) as the explanatory variables. I ran separate multiple linear regressions for *A. tonsa* and *C. hamatus*.

In order to consider the effect of environmental conditions before maturity on adult size, I modeled A. tonsa and C. hamatus adult female full length again, adding satellite derived (MODIS Aqua, ERDDAP data server) sea surface temperature and chlorophyll from the weeks prior to each *in situ* sampling as explanatory variables. The duration of ontogeny for ectothermic organisms varies with temperature (Forster et al. 2011). Previous studies on development found that A. tonsa take 20 days at 15°C (Landry 1983) and 10 days at 20°C (Paffenhöfer 1991), while C. hamatus takes 26 days at 15°C (Halsband-Lenk et al. 2002) and 16 days at 20°C (Fryd et al. 1991). To account for differences in development duration, I include averages of surface water conditions from a total of 16 days prior to copepod collection, extracted as two consecutive 8-day averages. For every sampling event (Table 2.1), there is the *in situ* CTD data at  $(t_0)$ , the average from the previous 8 days  $(t_{.8/0})$ , and an additional average from days 16 to 8 before copepod collection  $(t_{-16/-8})$ , for both temperature and chlorophyll The *in situ* and satellite data were fit by a multiple linear regression of A. tonsa and C. hamatus full length (mm) vs temperature (°C) and chlorophyll a ( $\mu$ g/L) at time of collection (Temp  $t_0$ , Chl  $t_0$ ), 8-day average prior to collection (Temp  $t_{-8/0}$ , Chl  $t_{-8/0}$ ), and average of days 16 to 8 prior to collection (Temp *t*<sub>-16/-8</sub>, Chl *t*<sub>-16/-8</sub>). *A. tonsa* and *C. hamatus* regression analysis run separately.

### 2.2.4 Bay Wide Copepod Size Variation in August 2016

To further investigate copepod adult female body size variation, I modeled full length measurements of *Acartia tonsa* and associated water parameter data from 13 stations throughout Delaware Bay from August 2016, when bay wide temperatures range from 20°C to 30°C. Of the 16 sampling stations (Fig 2.1), the three northern most tidal freshwater stations (3-M, 4-M, 5-M) were excluded from the model analysis due to the lack of individuals (3-M), and the potential presence of an *A. tonsa* cryptic sub lineage (Plough et al. 2018) found in salinities below 10 psu (4-M, 5-M).

The bay wide investigation focuses on *Acartia tonsa*, the most dominant copepod in Delaware Bay (Wickline 2016), in August 2016 (Table 2.1), extending the scope of body size analysis from just the lower bay stations, to the whole estuary. Salinity is added as an associated water parameter variable to account for any differences in copepod body size observed between regions of the bay. Data from the 13 stations were fit by a multiple linear regression with *A. tonsa* full length (mm) as the response variable, and *in situ* depth-averaged temperature, chlorophyll *a*, and salinity as the explanatory variables. I ran a one-factor ANOVA with full length as the response variable and station as the explanatory variable to determine any significant differences in body size throughout the bay.

### 2.3 Results

#### 2.3.1 Seasonal Temperature Variation Across Multiple Decades

In the current study (2015 - 2017), average water temperature in the lower Delaware Bay is  $1.47^{\circ}$ C warmer (two-factor ANOVA, P = 0.001) than in the Deevey (1960) study (1930 - 1932, Fig 2.3). In the Fall season (September to February), average warmer temperature is  $2.35^{\circ}$ C (post-hoc, P = 0.002), while in the Spring season (March to August), there is no significant difference between the studies (P = 0.191). Field sampled adult female *Acartia tonsa* and *Centropages hamatus* follow a general seasonal decrease in full length with an increase in temperature, consistent with the TSR and the temperature size response seen in the Deevey (1960) data (Fig 2.3). The average full length of *A. tonsa* is significantly smaller (P = 0.001, Rank Sum test) in the current study ( $1.08\pm0.01$ mm) than in the Deevey study ( $1.14\pm0.01$ mm). Average full length for *C. hamatus* in the current ( $1.29\pm0.03$ mm) and Deevey ( $1.37\pm0.03$ mm) studies are not significantly different (P = 0.055).

I used a simple linear regression to describe *Acartia tonsa* adult female full length as a function of temperature at the time of collection in the current and Deevey (1960) studies (Table 2.2, Fig 2.4). I analyzed the regressions by comparing the slope (95% CI) and y-intercept (95%CI) generated the current and Deevey data (Table 2.3). The CI for these values do not overlap suggesting that the regressions are significantly different. I used the regression equations to calculate the percent change in full length between the two studies as a function of temperature (Fig 2.5). Based on average percent change across all temperatures, *A. tonsa* is 6.79% smaller in the current study than in the Deevey study.

The current data for *C. hamatus* were fit with a linear regression while the Deevey data were fit with a 2-segment linear regression. I used a test if structural stability and found a natural breakpoint in the regression for the Deevey data at 8.30°C (Table 2.2). The slope for *C. hamatus* current data is significantly different from region 1 and region 2 slopes of the Deevey data (95% CIs, Table 2.3). Under current conditions, *C. hamatus* full length steadily decreases with warming until 15°C where size begins to plateau, suggesting a lower size limit. In the Deevey data, full length increases with warming below 8.30°C and proceeds to decrease in the warmer temperatures without a lower size limit

### 2.3.2 Seasonal Variation in Temperature and Food

A multiple linear regression model was generated to predict the full length (mm) of adult female *Acartia tonsa* and *Centropages hamatus* based on seasonal changes of *in situ* temperature (Temp  $t_0$ ) and chlorophyll *a* (Chl  $t_0$ ) obtained at the time of collection. For both copepod species, Temp  $t_0$  and Chl  $t_0$  are not collinear (VIF < 4), so both can be used in the model. A stepwise linear regression analysis indicated that *A. tonsa* and *C. hamatus* full length is best explained (lowest AIC) by Temp  $t_0$  alone (Table 2.4), indicating that seasonal variation in temperature is a significant predictor of adult size while chlorophyll is not. Based on the model's coefficient estimates, *A. tonsa* adult

female full length decreases 0.003 mm for each 1°C and *C. hamatus* adult female full length decreases 0.013 mm for each 1°C.

The multiple linear regression predicting *A. tonsa* and *C. hamatus* full length was ran again including satellite derived sea surface temperature (°C) and sea surface chlorophyll (µg/L) from the weeks before the adult copepods were collected, to account for environmental conditions before maturity. The *in situ* and satellite temperature data shown in Figure 2.6 (B) are similar at each collection date and follows a consistent annual pattern with seasonal maximums in September of each year. Chlorophyll (Fig 2.6 C) has more variation between the *in situ* and satellite data at each collection date and annual trends vary from year to year. Seasonal maximums can be seen in May of 2015 and 2017.

The test for collinearity indicates that Temp  $t_0$ , Chl  $t_0$ , Chl  $t_{.8/0}$ , and Chl  $t_{.16/-8}$  are not collinear (VIF < 4) and can be included in the model for *A. tonsa* and *C. hamatus*. The satellite derived sea surface temperatures are not different enough from the *in situ* temperature to be included in the model. The stepwise regression analysis for *A. tonsa* identified a multiple linear regression as the best-fit model, with full length (mm) as the response variable and Temp  $t_0$ , Chl  $t_{.8/0}$ , and Chl  $t_{.16/-8}$  the explanatory variables (Table 2.5). Based on this model's estimates, *A. tonsa* adult female full length decreases 0.004mm with each 1°C increase in *in situ* temperature (P = 0.005) as the only significant explanatory variable. Stepwise regression analysis of *C. hamatus* determined a multiple linear regression best-fit model with full length (mm) as the response variable, with Temp  $t_0$  and Chl  $t_{.8/0}$  as explanatory variables (Table 2.5). Based on the coefficient estimates, *C.* 

*hamatus* adult female full length decreases 0.012mm with each 1°C increase in *in situ* temperature (P < 0.001). Both multiple linear regressions indicate that seasonal variation of temperature at the time of collection is the significant predictor of adult female body size for both *A. tonsa* and *C. hamatus*. A notable difference between the two species is that *C. hamatus* shows a greater decrease in size per 1°C increase in *in situ* temperature than *A. tonsa*.

### 2.3.3 Bay Wide Copepod Size Variation in August 2016

Contour maps of the explanatory variable across Delaware Bay for August 2016 are given in Figure 2.7. *In situ* temperature and chlorophyll follow a similar gradient through the bay, with high values in the upper estuary that decrease towards the ocean. The opposite trend is seen for salinity, with low values from freshwater input in the upper estuary moving to marine salinities at the mouth of the bay.

The stepwise regression identified a multiple linear regression as the best fit model, with *A. tonsa* full length (mm) as the response variable and *in situ* depth averaged-chlorophyll (Chl  $t_0$ ) and Salinity (Sal  $t_0$ ) as the explanatory variables (Table 2.6). Based on the model's coefficient estimates, *A. tonsa* adult female full length decreases 0.008 mm with each 1µg/L increase in chlorophyll (P = 0.013) throughout the bay in August 2016. *Acartia tonsa* full length also increases 0.004 mm with increasing salinity (P = 0.045), which is evident in Figure 2.8, where significantly larger body sizes are seen at stations in close proximity to the mouth of the bay (one-factor ANOVA, P < 0.001).

### 2.4 Discussion

Deevey (1960) reported seasonal decreases in copepod body size with increasing temperatures in the lower Delaware Bay, using data collected from 1929-1933. This pattern fits the temperature size rule (TSR) which states that increased temperatures lead to a decrease in generation time, resulting in overall smaller size at maturity (Atkinson 1994). Through quantification of the Deevey data, and replication of comparable information under current conditions, I was able to analyze this temperature-size relationship over time.

In the current study, I found evidence of long-term warming in Delaware Bay with significantly higher temperatures during fall-winter months. Temperature induced body size reduction of calanoid copepod species has occurred in Delaware Bay over the last eight decades, causing a decrease in magnitude of the temperature-size response. As a result, there is less seasonal body size variation of *Acartia tonsa* and *Centropages hamatus* under current conditions. I observed a lower size limit for *C. hamatus* at high temperatures in the current study. *Acartia tonsa* body size variation throughout the bay is determined by chlorophyll in August 2016, when bay wide water temperatures are more constant.

Lacking a continuous record, I estimate an increase in surface water temperatures of 0.02 °C year <sup>-1</sup> based on lower bay mean annual values from the 1929-1933 Deevey (1960) and 2015- 2017 current studies. This warming rate is similar to the increase in mean air temperature of 0.011 °C year <sup>-1</sup> observed for Delaware over the last century according to DNREC climate change analysis report (2011). A study from the Long

Island Sound found a similar increase in surface water temperatures of 0.03 °C year <sup>-1</sup> over the course of five decades (Rice et al. 2015). In the Delaware Bay, significant warming occurred during the fall-winter months (September to February), and we see consistently higher temperatures from August through January in the current study (Fig 2.3). These warming trends are similar to the nearby Long Island Sound where increased temperatures were seen from June to December (Rice et al. 2015).

Higher temperatures affect individual copepod development times. The rate at which sub-adult copepods pass through each life stage, outpaces the mass they accumulate at each stage, so ultimately, they mature smaller (Forster et al. 2011). As global warming progresses, average adult size within a given population is expected to decrease. Reductions in copepod body size has been shown to effect fecundity and feeding, which are important fitness components (Kingsolver & Huey 2008). In the calanoid species *Pseudocalanus* sp. and *Eurytemora affinis*, bigger females produce larger clutches of eggs, which in turn contributes to population growth (McLaren 1965, Hirche 1992). Within a species, larger copepods are able to consume larger, as well as a wider size range of food particles, so smaller individuals experience less efficient feeding and growth (Berggreen et al. 1988). Therefore, smaller body sizes have potential consequences for individual fitness.

It is important to note that the Deevey (1960) data was collected in the 1930's and preserved in formalin for thirty years, putting the samples at risk of shrinkage. In order to address this issue, I analyzed size against temperature as opposed to the percent change in size over time. In the current study, I see body size reductions consistent with long-term

warming in the lower estuary (Fig 2.4). Rice et al. (2015) also reported significantly smaller *Acartia* sp. in Long Island Sound over the course of multiple decades. Both calanoid species show body size response consistent with the predictions of the temperature size rule in the context of global warming, despite the potential issue of Deevey (1960) sample shrinkage.

In the context of climate change, the temperature-size rule predicts long-term warming will cause a general decrease in the mean size of copepods at the population level (Daufresne et al. 2009). With observed warming and associated size reduction, the overall seasonal variation in copepod body size decreases. Consistently smaller organisms could result in less carbon available to higher trophic levels threatening the efficiency of energy transfer and overall food web dynamics within estuarine ecosystems (Rice et al. 2015). Zooplankton predators in Delaware Bay such as larval Atlantic croaker (*Micropogonias undulatus*) and menhaden (*Brevoortia* sp.) have been known to select prey based on particle type and size (Govoni et al. 1986, Stoecker & Govoni 1984). Therefore, a shift in the mean size of copepods, the dominant zooplankter, could alter predator-prey interactions.

Over the course of eight decades, we have seen significant decrease in the magnitude of the temperature-size relationship for *A. tonsa* and *C. hamatus* in Delaware Bay. As a result, we see decreased variability in copepod size across all seasons. The strength of the temperature-size relationship for *A. tonsa* decreases 37.5% between the Deevey (1960) and current study (Fig 2.4). These findings are consistent with the interannual decrease in *Acartia* sp. reported in the Long Island Sound in response to

increases in temperature. For *C. hamatus*, there is not a distinct positive temperature-size relationship at colder temperatures in the current study due to the absence of data below 5°C, and the linear decrease in body size with increasing temperature is 30% less than seen in the Deevey (1960) study. A decrease in the strength of the temperature size relationship for both species, means less change in body size across all seasons (Fig 2.4). These reductions in seasonal size variability for *A. tonsa* and *C. hamatus* support Daufresne (2009) predictions that long-term warming may lead to a decrease in mean body size of copepod populations, and threaten the efficiency of trophic interactions in Delaware Bay.

Under current conditions, *C. hamatus* appears to reach a lower size limit at 15°C, above which body size does not drop below 1.1mm (Fig 2.4). This body size plateau at high temperatures is inconsistent with the Deevey (1960) study and with TSR predictions. An explanation can be offered by looking at changes in phytoplankton size as opposed to chlorophyll concentration. *Centropages* sp. have the ability to selectively feed based on prey size (Summer 1973, Saage et al. 2009), and have been shown to select for larger microplankton ( $20 - 200 \mu m$ ) particles (Kleppel 1993). In the Delaware Bay, phytoplankton communities at higher temperatures ( $25^{\circ}$ C -  $28^{\circ}$ C) are dominated by nanoplankton ( $2 - 20 \mu m$ , Pennock & Sharp 1986). Therefore, under these conditions food availability for *C. hamatus* is limited.

Previous studies suggest that food limitation is the main factor responsible for decreases in copepod growth rate, and declines in growth have been shown to correspond with increases in adult size (Richardson & Verheye 1999). In warmer conditions, the

negative effect of low food availability on growth resulting in larger size at maturity, could explain why we do not see a decrease in *C. hamatus* body size at temperatures above 15°C. In the Long Island Sound, change in body size of *Temora longicornis* during summer months was linked to decreases in the availability of larger phytoplankton which supports the prediction that food particle size effects growth at high temperatures (Dam & Peterson 1991). I saw a similar body size response to low food availability with *A. tonsa* in August 2016, when bay wide temperatures were above 25°C (Fig 2.7). Near the mouth of the bay, low chlorophyll concentrations were associated with significantly larger individuals, indicating that food limitation is a significant predictor of adult size at high temperatures despite TSR expectations.

Over the course of multiple decades, seasonal warming in the Delaware Bay has led to a reduction in body size of the common calanoid species *Acartia tonsa* and *Centropages hamatus* as well as a decrease in the magnitude of the temperature-size response. These observed changes result in overall smaller individuals across temperature, consistent with the predictions of long-term warming on ectotherms according to TSR. *Acartia tonsa* also shows an inverse relationship between body size and chlorophyll concentration throughout the bay in August 2016. The Delaware Bay is expected to experience continued surface water warming in response to increased atmospheric  $CO_2$  (Najjar et al. 2000) and increase in phytoplankton biomass due to higher frequency of major flood events and associated nutrient discharge (Voynova & Sharp 2012). Based on the TSR trends observed in the current study, and future

projections for Delaware Bay conditions, average size of these ecologically important copepod species is expected to continually decrease, unless a lower size limit is reached.





CRUISE DATE	CRUISE	TEMPERATURE	CHLOROPHYLL
	IYPE	RANGE (°C)	RANGE (µG/L)
November 2014	Whole Bay	6.71 - 8.78	2.08 - 4.80
April 2015	Whole Bay	4.28 - 6.30	4.52 - 6.85
June 2015	Whole Bay	16.15 - 22.39	2.42 - 8.81
August 2015	Whole Bay	21.08 - 23.91	5.46 - 11.96
November 2015	Whole Bay	14.07 - 15.11	2.47 - 5.37
April 2016	Whole Bay	9.23 - 10.9	3.78 - 7.05
July 2016	Lower Bay	20.71 - 23.92	2.05 - 6.61
September 2016	Lower Bay	23.08 - 23.72	2.11 - 3.48
October 2016	Lower Bay	18.87 - 19.38	1.55 - 2.13
February 2017	Lower Bay	4.72 - 5.96	3.79 - 6.14
April 2017	Whole Bay	11.88 - 15.02	24.35 - 27.20
June 2017	Whole Bay	16.85 - 22.44	2.43 - 4.84
July 2017	Lower Bay	17.30 - 24.93	0.80 - 4.07
January 2018	Lower Bay	0.80 - 2.91	16.92 - 24.49

Table 2.1: Available samples of cruise-collected copepods with corresponding depth averaged temperature and chlorophyll range.



Figure 2.2: Zooscan Images of adult female *Acartia tonsa* and *Centropages hamatus* collected from the Delaware Bay. Body size as full length is measured from the anterior margin of the prosome to the base of the caudal rami.



Figure 2.3: Average surface water temperatures collected each month from 1930 to 1932 (Deevey) and 2015 to 2017 (Current) from the lower Delaware Bay stations. Data for each month are mean  $\pm$  SE. Two-way ANOVA (Study, P = 0.001). Post-hoc Tukey (Fall Season, P = 0.002).



Figure 2.4: Acartia tonsa (left), Centropages hamatus (right) adult female body size as a function of water temperature for the current and historical (Deevey 1929-1933) data. Current data are means  $\pm$  S.E. of body size and water temperature for *A. tonsa* (n=4-30 copepods/mean) and *C. hamatus* (n=2-30 copepods/mean). *A. tonsa* (y = 1.123 – 0.003x, R<sup>2</sup> = 0.135) and historical (y = 1.244 – 0.008x, R<sup>2</sup> = 0.481.*C. hamatus* (y = 1.445 – 0.013x, R<sup>2</sup> = 0.537) while the Deevey data were fit with a 2-segment linear regression (y1 = 1.464 + 0.0118x, R<sup>2</sup> = 0.103 for x < 8.30; y2 = 1.918 – 0.043x. R<sup>2</sup> = 0.813 for x > 8.30).

Table 2.2: Full length vs temperature regression selection for *Acartia tonsa* and *Centropages hamatus* in the current and historical (Deevey) studies. Asterisk denote best fit model. The test for structural stability looks for break points in the regression and P-value <0.05 suggests a break point exists.

Species	Parameters	Current Data (2014-2018)	Deevey Data (1929-1933)
Acartia tonsa	Linear regression model	<i>P</i> = 0.029*	<i>P</i> < 0.0001*
	Test for structural stability	<i>P</i> = 0.280	<i>P</i> = 0.661
Centropages hamatus	Linear regression model	<i>P</i> = 0.0001*	<i>P</i> < 0.0001*
	Test for structural stability	<i>P</i> = 0.121	P < 0.0001*
	2 Segment linear regression model (break point)		$P = 0.0002*(8.30^{\circ}\text{C})$

Table 2.3: Regression analysis of *Acartia tonsa* and *Centropages hamatus* results in the current and historical Deevey data. Asterisks denote significant differences between the two studies.

Species	Parameters	Current Data (2014-2018)	Deevey Data (1929-1933)	Significance Comparison
Acartia tonsa	Regression	P = 0.029 (linear)	<i>P</i> < 0.0001 (linear)	
	Slope	-0.003	-0.008	95% CI*
	(95% CI)	(-0.0055, -0.0004)	(-0.0087, -0.0065)	
	Y-intercept	1.123	1.244	95% CI*
	(95% CI)	(1.081, 1.165)	(1.210, 1.277)	
Centropages hamatus	Regression	P = 0.0001 (linear)	P = 0.0002 (2 segment)	
	Slope	-0.0130	x < 8.3°C: 0.0118	95% CI*
	(95% CI)	(-0.0182, -0.008)	(0.004, 0.020)	
			x > 8.3°C: -0.043	
			(-0.048, -0.038)	95% CI*
	Break Point		8.30	
	(95% CI)		(6.150, 10.45)	



Figure 2.5: Percent change in full length between the Deevey (1929 – 1933) and the Current study (2014 - 2018) for *Acartia tonsa* and *Centropages hamatus* (y = 100 - (Current /Deevey \*100). Positive values above the grey reference line indicate Deevey full length is greater than Current full length at that given temperature.

Table 2.4: Summary of multiple linear regressions of *Acartia tonsa* and *Centropages hamatus* full length (mm) vs temperature (°C, Temp  $t_0$ ) and chlorophyll *a* (µg/L, Chl  $t_0$ ) at time of collection. Bold lines indicate best fit models (lowest P-value and AIC).

Species	Model	F	df	P-value	AIC
	full length ~ Temp t <sub>0</sub>	5.20	35	0.029	-205.89
Acartia tonsa	full length ~ Temp $t_0$ + Chl $t_0$	2.71	34	0.081	-204.23
Centropages hamatus	full length ~ Temp $t_0$	26.70	23	<0.0001	-122.67
Centropuges numutus	full length ~ Temp $t_0$ + Chl $t_0$	13.13	22	0.0002	-121.05



Figure 2.6: Seasonal full length (mm, A) of *Acartia tonsa* and *Centropages hamatus*, Temperature (°C, B) and chlorophyll *a* ( $\mu$ g/L, C) data at time of collection (Temp *t*<sub>0</sub>, Chl *t*<sub>0</sub>), 8-day average prior to collection (Temp *t*<sub>-8/0</sub>, Chl *t*<sub>-8/0</sub>), and average of days 16 to 8 prior to collection (Temp *t*<sub>-16/-8</sub>, Chl *t*<sub>-16/-8</sub>) averaged (±SE) from the lower Delaware Bay focal stations from 2014 to 2018.

Table 2.5: Summary of multiple linear regression of *Acartia tonsa* and *Centropages hamatus* full length (mm) vs temperature (°C) and chlorophyll *a* ( $\mu$ g/L) at time of collection (Temp  $t_0$ , Chl  $t_0$ ), 8-day average prior to collection (Temp  $t_{-8/0}$ , Chl  $t_{-8/0}$ ), and average of days 16 to 8 prior to collection (Temp  $t_{-16/-8}$ , Chl  $t_{-16/-8}$ ). Bold lines indicate best fit models (lowest P-value and AIC).

Species	Model	F	df	P-value	AIC
Acartia	full length ~ Temp $t_0$ + Chl $t_{-8/0}$ + Chl $t_{-16/-8}$	3.63	33	0.023	-207.32
tonsa	full length ~ Temp $t_0$ + Chl $t_{-16/-8}$ + Chl $t_{-8/0}$ + Chl $t_0$	2.88	32	0.038	-206.13
	full length ~ Temp $t_0$ + Chl $t_{-16/-8}$	3.32	34	0.048	-205.35
	full length ~ Temp $t_0$	5.20	35	0.029	-205.33
Centropages	full length ~ Temp $t_0$ + Chl $t_{-8/0}$	17.15	22	<0.0001	-124.9
hamatus	full length ~ Temp $t_0$ + Chl $t_{-8/0}$ + Chl $t_0$	26.7	23	0.0001	-123.75
	full length ~ Temp $t_0$ + Chl $t_{-8/0}$ + Chl $t_0$ + Chl $t_{-16/-8}$	11.03	21	0.0004	-123.06
	full length ~ Temp $t_0$	8.231	20	< 0.0001	-122.67



Figure 2.7: August 2016 contour plots for temperature, chlorophyll, and salinity measured as *in situ* depth averaged values at time of collection. Black dots indicate sampling locations.

Table 2.6: Summary of August 2016 multiple linear regression of *Acartia tonsa* adult female full length (mm) vs temperature (°C), chlorophyll *a* ( $\mu$ g/L) and salinity at time of collection (Temp *t*<sub>0</sub>, Chl *t*<sub>0</sub>, Sal *t*<sub>0</sub>) at each Delaware Bay sampling station. Bold lines indicate significant models (P < 0.01).

Model	F	df	P-value	AIC
full length ~ Chl $t_0$ + Sal $t_0$	8.90	10	0.006	-87.67
full length ~ Chl $t_0$ + Sal $t_0$ + Temp $t_0$	5.45	9	0.021	-85.84
full length ~ Chl $t_0$	9.03	11	0.012	-84.17
full length ~ Temp $t_0$	8.90	11	0.012	-84.08
full length ~ Chl $t_0$ + Temp $t_0$	5.14	10	0.029	-83.57
full length ~ Sal $t_0$	5.00	11	0.047	-81.25



Figure 2.8: Average full length (mm) ( $\pm$ SE) of adult female *Acartia tonsa* at each of the 15 stations labeled on the map of Delaware Bay. Stations ordered by proximity to the mouth of the bay. 4-M and 5-M excluded from analysis (tidal freshwater, n=3-5 copepods/station). 6-M through 12-M (n=4-30 copepods/station) included in comparison of body size at each station (one-factor ANOVA). Asterisks indicate stations with significantly larger body size than stations without asterisks (P < 0.001). Data is from August 15<sup>th</sup> and 16<sup>th</sup>, 2016.

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