CLIMATE CHANGE EFFECTS ON COPEPOD PHYSIOLOGY AND TROPHIC TRANSFER

by

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ABSTRACT

Increased anthropogenic carbon dioxide (CO_2) emissions have led to an increasingly acidified ocean and higher average global sea surface temperatures. This alteration of abiotic conditions is directly affecting aquatic organisms through physiological stress and indirectly through reductions in trophic transfer efficiency. Less efficient trophic transfer at the base of the food web would reduce the overall energy available to support higher trophic levels and could be detrimental to the dependent ecosystem. Estuarine ecosystems are subject to harmful algal blooms (HABs). They are also characterized by low species diversity, which lowers ecosystem resilience to environmental perturbations. This results in a system where changes in phytoplankton and their consumers can dramatically impact the health of the local community. Increased temperature and pCO_2 are predicted to change nutritional adequacy and/or toxicity of some HAB species and their copepod consumers. Interactions between *Karlodinium veneficum*, a HAB species present in the Delaware Inland Bays, and its consumer *Acartia tonsa*, a locally-dominant copepod, were used to assess direct changes to physiology and/or indirect changes to trophic transfer. *Acartia tonsa*, toxic prey *K. veneficum*, and non-toxic prey *Storeatula major* were grown in multi-generational laboratory cultures at both ambient conditions (25 °C/400 ppm pCO_2) and those predicted for year 2100 (29 °C/ 1000 ppm pCO_2). Physiological changes were assessed using grazing, respirometry, egg production, and egg hatching success. Grazing experiments indicated there was not a direct toxic effect of the prey on *A.*

tonsa. Respiration rates did not change significantly at higher temperature and pCO_2 values, indicating physiological compensation. Egg production did not significantly differ between treatments, but a significant reduction in egg hatching success was found when *A. tonsa* were fed exclusively *K. veneficum*. Significant reduction of egg production and hatching also occurred as a result of higher temperature and pCO_2. Significant reductions in efficiency of carbon transfer from prey to consumer offspring were found when *A. tonsa* ingested *K. veneficum*, and when *A. tonsa* ingested *S. major* at elevated temperature and pCO_2. In summary, *A. tonsa* acclimated to the elevated pCO_2 and temperature conditions, but changes in resource partitioning led to a lowered transfer of carbon to their offspring. Ingestion of *K. veneficum* also led to a lowered trophic transfer efficiency, irrespective of temperature and pCO_2 level. This indicates that both HABs and increased temperature and pCO_2 from climate change have the potential to alter ecosystem dynamics by reducing trophic transfer efficiency at the base of the food chain.

Chapter 1

INTRODUCTION

Increased anthropogenic carbon emissions have led to a global increase in atmospheric CO_2 and temperature (IPCC 2013). In response to this atmospheric increase, the concentration of CO_2 dissolved in oceanic waters is projected to rise. Increased dissolved CO_2 (pCO_2) lowers the pH of the water, leading to ocean acidification (Broecker and Clark, 2001; Orr et al., 2005) with a projected global average decrease in pH by 0.5 units by year 2100 (Caldeira and Wickett, 2005). Sea surface temperature could rise by as much as 5°C by the end of the century (IPCC 2013). These changes in environmental conditions have the potential to translate into negative effects on the physiology of marine organisms. Environmental stress can result in selective pressure that can in turn alter species composition and abundance, having implications for the entire ecosystem (Walther et al., 2002).

Coastal waters are a valuable natural resource as they are highly productive are play a substantial role in the ocean's carbon cycle (Walsh, 1991). They are also closely tied to the fishing and tourism industries. They are more susceptible than the open ocean to changes in pH due to their lower salinity and alkalinity (Wong, 1979). Coastal areas, such as estuaries, are shallow and closer to sources of nutrient runoff, making them particularly prone to increased primary production. Increased primary production also results in increase microbial decomposition, with further decreases pH (Wallace et al., 2014). Temperature and pCO_2 increases interact with the increased phytoplankton growth and physiology, which could create bottom-up effects on

ecosystems (Riebesell et al., 2007). Some of this increased algal productivity could take the form of harmful algal blooms (HABs) if conditions favor those species. Change in temperature or CO_2 could affect the timing of these blooms, the nutrient quality, and the toxicity of the composing algae (Hallegraeff, 2010; Rossoll et al., 2012; Fu et al,. 2010). It is important to understand how these changes will propagate through the food chain and affect higher trophic levels. Thus, examining the link between HABs and their consumers, the zooplankton community, is crucial to understanding how ecosystems will react to altered climate conditions.

HABs in a Changing Climate

Harmful algal blooms are dominated by species that are already, by definition, thriving in their chosen ecosystem. The fact that these algae form blooms shows they are capable, under the right conditions, of outcompeting native species of phytoplankton. An increase in the frequency of this form of primary productivity has, in some cases, been directly attributed to increased temperature and pCO_2 (Pang et al., 2017; Zue et al., 2017). More often, this increase in frequency is attributed to multiple factors, primarily nutrient loading, that are exacerbated by increases in temperature and pCO_2 as these conditions widen the available niche during which blooms form (Gobler et al., 2017). However, there is evidence from multiple species that their toxicity increases with higher temperature and/or pCO_2. *Alexandrium* in the East China Sea was found to produce a 60% higher concentration of cellular toxins under raised pCO_2 levels (Pang et al., 2017). Zhu et al. (2017) found a California strain of *Pseudo-nitzschia* whose competitive success and toxicity increased with temperature. *K. veneficum* increased production of the more toxic of its two congeners of karlotoxin when raised under elevated pCO_2 and phosphorus limitation (Fu et al., 2010). These

changes in toxicity have the potential to propagate through the food web by affecting the interaction between these HABs and their consumers, the zooplankton.

Zooplankton are the primary link forming the pathway by which energy passes from primary production to higher trophic levels (Mauchline, 1998). Zooplankton also represent an important part of the ocean's biogeochemical cycles (Dam et al., 1995). They make carbon and nutrients available to higher trophic levels, feed the microbial loop with their excretion of dissolved organic matter, and represent a substantial part of the carbon cycle with their respiration (Steinberg and Landry, 2017). Grazing may be reduced by an increase in the toxicity of the algal prey, since avoidance is a documented reaction of zooplankton to increasingly toxic algae (Fiedler, 1982). This would further favor HABs and create a positive feedback loop, as a preference for non-toxic algae would reduce grazing pressure and promote the survival of the toxic species. Sublethal effects of increased toxicity could also impact the efficiency of feeding, and therefore trophic transfer, or reduce reproductive success by decreasing the number of viable offspring produced. Documented sublethal effects of toxic algae on copepods include altered swimming and photobehavior (Cohen et al., 2007), and reduced feeding behavior and rejection of ingested toxic prey (Xu et al., 2017; DeMott and Moxter, 1991; Colin and Dam, 2003; Turriff et al., 1995). Grazers also commonly utilize food sources to obtain important compounds that they cannot synthesize. Since calanoid copepods need to obtain long chain n-3 polyunsaturated fatty acids from ingested algae, changes in the sequestration of these acids in phytoplankton could make them nutritionally inadequate for grazing (Tang et al., 2001; Bermudez et al., 2016).

Thus, increased temperature may not only decrease the efficiency of energy transfer between trophic levels by decreasing the nutritional adequacy of the plankton; it may also increase toxicity concentration in some algal species, creating multiple means of stressing consumers.

Effects of Climate Change on Zooplankton

In addition to indirect effects of ocean acidification and increased temperature on zooplankton by altering their algal food source, there will likely be direct effects to zooplankton of changing environmental conditions. For copepods, a decrease in body size with warmer temperatures has been widely documented across many genera (Horne et al., 2016; Gardener et al., 2011; Sheridan and Bickford, 2011). This is likely due to an increased metabolic rate, which has higher energy demands and could force the organism to prioritize reproduction and physiological maintenance over growth (Sheridan and Bickford, 2011). Though multiple studies have found adult copepod survival to be resilient to levels higher of pCO_2 than those predicted by 2100 (Runge et al., 2016; Pedersen et al., 2013), studies using multiple life stages have found *A. tonsa* nauplii to be more vulnerable than adults (Cripps et al., 2015). In addition, Cripps et al. (2014) found deleterious effects on egg production and nauplii survival when the parents were exposed to high pCO_2. Vulnerability of early life stages to elevated temperature and pCO_2 is seen in other crustaceans as well. High pCO_2 reduced hatching success of the Florida stone crab (Gravinese et al., 2018) and the combination of high pCO_2 and temperature increased swimming speed and feeding rate in larval American lobsters (Waller et al., 2017). Megalopa of the spider crab, *Hyas areneas*, developed slower, had reduced mass, and reduced lipid content when reared at very high pCO_2 (3000 ppm) (Walther et al., 2010). Small et al. (2015) also

found developmental bottlenecks during the larval development of European lobsters, where higher temperature and pCO_2 (1100 µatm at 21℃ versus 420 µatm at 17 ℃) interacted to lower survival and reduce growth at specific stages of development.

Ecosystem Insights

Climate change studies on systems with multiple trophic levels are useful for providing insight about energy fluxes through an ecosystem. These fluxes between trophic levels determine the total energy of the system, which can have implications for ecosystem diversity and composition. While both primary producers and secondary consumers are expected to undergo changes in physiology as a result of changing water temperature and pH, studies that couple these factors can expose effects that are inherently different but may dictate how the ecosystem functions under a different climate regime (Vizzini et al., 2017; Ullah et al., 2018).

Organization of the Thesis

In this thesis, I take a multigenerational approach to understanding climate change effects on trophic transfer in the pelagic food web. In Chapter 2, I assess whether an increased temperature and pCO_2 regime have negative physiological consequences for the copepod *A. tonsa* when they are exposed to these conditions for multiple generations, and whether this regime changes the trophic transfer efficiency between *A. tonsa* and the toxic prey alga *K. veneficum*.

Chapter 2

PHYSIOLOGY AND TROPHIC TRANSFER

2.1 Introduction

Predicted rises in temperature and pCO_2 in the ocean are expected to have several distinct impacts on marine organisms. Increasingly acidified water is expected to decrease calcification rates as calcium carbonate becomes undersaturated in the water. This would affect many invertebrates, such as mollusks and crustaceans, as well as calcifying phytoplankton, such as foraminifera. Evidence has already been seen for slower calcification rates and thinner shells (Langdon et al., 2000, Riebesell et al., 2000). Change in the pH of ocean waters is also predicted to have an effect on acid-base regulation. Higher pCO_2 values in the water will cause more CO_2 to diffuse into the body, possibly affecting the pH of the animal's internal fluids. The ability to compensate with acidosis of the body fluid is highly variable, and is potentially very energetically costly (Fabry et al., 2008; Pörtner and Reipschläger, 1996; Hand, 1991). A result of expending extra energy on regulating internal pH is the reduction of growth and reproduction (Hand, 1991), which could affect the long-term survival of the species. There is also a vertical gradient of pCO_2 in the ocean, which means vertical migrators (like many copepods) may be exposed to more variable pCO_2 levels than would be predicted by using only surface values (Fabry et al., 2008). Changes in temperature directly affect metabolism in many ectotherms, as well as change rates of crucial biological reactions and molecular stability (Hochachka and Somaro, 2002).

Interactions between HABs and their consumers under an altered climate scenario are important to understanding how physiological changes at the cellular and organismal level could translate into changes at the ecosystem level. Previous

research concerning climate change effects on HABs has found evidence for changes in physiology and toxicity for a species that grows in the Delaware Inland Bays (Handy et al., 2008). *Karlodinium veneficum* is a mixotrophic dinoflagellate that can form high-density blooms in the estuary and is known to produce two congeners of karlotoxins (Handy et al., 2008; Fu et al., 2010). Karlotoxins induce pore formation in cell membranes with high concentrations of 4-desmethyl sterols and are known to be harmful to fish (Deeds et al., 2002; Deeds et al., 2006). Studies have shown that *K. veneficum* exhibits increases in cellular C:N and carbohydrate:protein ratios when raised under elevated pCO_2 and temperature conditions (Coyne et al., in prep). This suggests a change in resource partitioning, which could change the nutritional content of this species for organisms that graze on it (Coyne et al., in prep; Urabe et al., 2003).

Copepods are dominant within the zooplankton community (Wickstead, 1976), and *Acartia tonsa* is, numerically, the most abundant species both seasonally and geographically within the Delaware Bay estuary (Herman et al., 1984; Wickline et al., submitted). *Acartia tonsa* is a calanoid copepod with 13 isochronal stages of development (Fig. 2.1). The egg stage is followed by six naupliar stages (NI-VI), followed by six copepodite (CI-CVI) stages, where the final copepodite stage (CVI) is a sexually-mature adult. Their generation time is relatively short; only 7-10 days are required to mature from egg to reproducing adult at 25.5 ℃ (Heinle, 1966), and at 30.7 ℃ that generation time shortens to 4 days (Miller et al., 1977). This makes them a practical study species as they can be cultured for multiple generations in the lab. The ability to maintain a culture for multiple generations under the treatment conditions is essential for understanding any adaptation that may result, as this

reaction could take longer than one generation to develop (Dam, 2013) and could be an important factor in mitigating negative effects (Thor and Dupont, 2015).

Trophic transfer is the movement of organic matter from a lower trophic level to a higher one. This occurs through the ingestion of the lower level by the higher one. Trophic transfer efficiency (TTE) is how much organic matter gets transferred from the lower to higher level. Most ecosystems have five or fewer trophic levels (Sugihara, 1989), and this is partially constrained by TTE. TTE limits the amount of higher level predators that can be supported by an ecosystem, which means understanding TTE and how it may change with climate is crucial to assessing ecosystem resilience (Cropp and Gabric, 2002). For this work, TTE refers to the movement of carbon from the lowest trophic level (primary producers) to the first heterotrophic level (primary consumer). This is the autotroph-heterotroph interface. To quantitatively describe this interface, carbon values are needed for both the prey and consumer. To measure how efficiently the consumer uses the carbon to grow or reproduce, carbon requirements for their offspring and metabolism are required. These measures describe not only how much carbon was transferred from prey to consumer, but also how much carbon the consumer provides for the next trophic level. Studying the autotroph-heterotroph interface is valuable for predicting climate change effects on an ecosystem because it reveals energetic changes in the base of the food chain (i.e. where energy changes happen first), that could affect the available energy to support higher trophic levels.

To examine possible differences in trophic transfer efficiency between zooplankton and different primary producers under a climate change scenario of increased temperature and pCO_2, I cultured two algal prey species (palatable and non-

toxic cryptophyte *Storeatula major*, and HAB-forming *K. veneficum*) and a copepod grazer (*A. tonsa*) over multiple generations at either ambient levels (25 ℃/400 ppm) or elevated levels (29 ℃ /1000 ppm). Using these organisms, I tested the hypotheses that multigenerational rearing under elevated temperature and pCO_2 results in (1) increased copepod respiration rates, (2) reduced copepod grazing and egg production rates when provided *K. veneficum*, and (3) reduced efficiency of carbon transfer from producer to consumer. Collectively, this work explores trophic implications of a predator/prey interaction simulating one which could occur with a potentially harmful prey under future climate conditions.

2.2 Methods

2.2.1 pH Stat Culturing

pH Stat System

For both Ambient (25 ℃/400 ppm) and Elevated (29 ℃ /1000 ppm) treatments, copepods and algae were maintained in a custom pH stat system modified from Hoadley et al. (2016) (Fig. 2.2). For copepods, sumps (n=3) connected to each copepod culture (n=3) had pCO_2 levels controlled by the input of air and CO_2 gas. The pH of each sump was measured every 30 seconds (Thermo-Scientific Orion Ross Ultra pH glass electrode) and integrated into software (KSGrowStat) that controlled delivery of air, CO_2-free air, or CO_2 gas, thereby stating pH at the setpoint. Carbonate chemistry was confirmed using spectrophotometric pH measurements (Lamandé et al., 2015) and dissolved inorganic carbon (DIC) measurements to calculate pCO_2 using CO2Sys (Lewis et al., 1998). Probes were recalibrated once weekly using seawater buffers (Dickson et al., 2007). Each sump exchanged water with its respective

copepod culture by peristaltic pump, with residence times of ~4.5 hours in both treatments. This system had one algal culture (n=1) of *S. major* (isolated from the Chesapeake Bay, obtained from A.R. Place), which was statted directly.

Karlodinium veneficum was reared under the same temperature and pCO2 values as the copepods using a separate pH stat system (Qubit Systems) by another member of this project. These cultures were all continuous and statted directly. Carbonate chemistry was confirmed using the same methods described above. Probes were recalibrated weekly using NBS buffers (Sigma-Aldrich).

Copepod Collection and Maintenance

Acartia tonsa was cultured in stage-tracked cultures for at least 2 generations before grazing experiments were conducted. Slightly different culturing methods were used for the different temperature and pCO_2 treatments. For the Ambient treatment, copepods were collected at dusk on a flood tide from a stationary davit on a dock in the Broadkill River just inside of Roosevelt Inlet near the mouth of Delaware Bay, USA (38º 49'N, 75º 12' W). Collections were done using a 75cm diameter, 335µm mesh conical plankton net set horizontally at the surface for 20 minutes. The salinity and temperature at time of collection were 25 psu and 20 ºC. Copepods were diluted in 20 psu, 0.2µm-filtered seawater (FSW) and incubated overnight while temperature was increased at a rate of 1 ºC every 5 hours. Adult copepods were sorted the next day into the culture carboys (8 L) (n=3), where they were kept in a submerged column within the carboy with a false bottom of 150 µm nitex mesh. This gave water flow to the adults but allowed eggs to fall to the bottom of the carboy, preventing egg cannibalism. These adult copepods were fed with 500 µgC of *S.major* and incubated at 25 ºC for 24 hours. After this 24 hr period, adults were removed and the carboys

were attached to the pH stat system. Every other day, cultures were fed 500 µgC of *S. major* kept in a semi-continuous culture in exponential growth at the same temperature and pCO_2 as the copepods. *Storeatula major* was grown in low phosphorus and nitrogen f/2 medium to match the treatment of the *K. veneficum* and keep its nutrient levels representative of environmental conditions (Fu et al., 2010). The culture water was changed every four days by removing three-fourths of the water using a siphon and replacing it with FSW.

For the Elevated treatment, copepods were collected in the late fall and were sparse in Roosevelt Inlet. For this reason, these copepods were collected from Delaware Bay (39° 0.4'N, 75° 8.1'W). This collection was done by vertically towing the plankton net up through the water column. These animals were also diluted to 20 psu by adjusting water salinity by 5 psu day^{-1} and 25 ℃ by ramping 1º every 5 hours. *Acartia tonsa* were then sorted and kept in a 15 L FSW culture for approximately five months (~15 generations) at 25 ℃ with weekly water changes and feeding every 2-3 days with 500 µgC of batch-cultured *S. major*. When the Elevated experiment was started, adults were sorted into an egg-laying setup similar to that described above, except in 2 L, and incubated at 25 ℃ for 24 hours. Adults were then removed, eggs were poured into the 4 L bottles to be used for the culturing, and bottles were incubated while ramping the temperature at a rate of 1º every 5 h until 29 ºC was reached. Once cultures reached this temperature, they were attached to the pH stat system described below and kept at 1000 ppm for the duration of the experiment.

2.2.2 Grazing

Grazing experiments at both Ambient and Elevated treatments were carried out after two generations to determine *A. tonsa* ingestion rates for a given quantity and

toxicity of algae. The palatable alga grazing experiment used varying concentrations of S. major to determine ingestion and egg production as a function of food concentration. A toxicity gradient experiment using both *S. major* and *K. veneficum* in a gradient of varying ratios was used to determine ingestion and egg production in the presence of a toxic alga. Palatable-alga grazing experiments had six levels of *S. major* at 100, 200, 300, 400, 500, and 600 µgC/L. Toxicity gradient experiments had five levels of an *S. major*/*K. veneficum* mixture in a non-toxic: toxic ratio of 0%, 25%, 50%, 75%, and 100%, and all levels were carbon normalized to 500 ugC/L. Carbon values for *S. major* and *K. veneficum* at 25 ℃ and 29 ℃ were obtained from elemental combustion analysis of continuous and semi-continuous algal cultures (Costech Analytical Tech.). In both experiments, 8 females and 2 males were placed in a 315 mL culture flask with a vented cap to allow air exchange (n=3 flasks at each level, one from each copepod culture). Control bottles had the same algal composition, but without copepods (n=3). The medium was FSW at their experimental temperature and pCO_2. Algae were kept in suspension by rotating the bottles end-over-end on a plankton wheel rotating at 0.5 rev min^{-1}. Elevated experiments were run for approximately 16 hours, while Ambient experiments were run for 24 hours. The difference in experiment length was to allow the maximum amount of time for ingestion while preventing eggs released during the experiment from hatching and developing past the first naupliar stage. Eggs and first nauplii do not feed (Landry 1983), so they do not affect algal cell counts. The difference in development time was related to the temperature, as *A. tonsa* develops faster at 29 ℃ than 25 ℃ (see Results). The algal concentrations in the control and experimental flasks were measured by manually counting samples preserved in 2% glutaraldehyde on a

Sedgewick-Rafter counting cell. At least 400 cells were counted from each sample. Ingestion rates were calculated using the equations of Frost (1972). The medium in each bottle was poured through a 20 µm sieve to collect the eggs and nauplii. Nauplii were either sedated with magnesium chloride and counted immediately or fixed in 4% formaldehyde to be counted later. Eggs were kept in petri dishes of FSW and monitored for up to 4 days to determine hatching success.

For analysis of the direct toxicity of *K. veneficum*, ingestion of *S. major* versus egg production (from the palatable-alga grazing experiment) was plotted alongside *S. major* egg production to ingestion relationship (from the toxicity gradient grazing). This layout describes ingestion of a palatable alga and resulting egg production in the presence of a toxic alga (Jónasdóttir et al., 1998). The design is intended to illuminate any direct toxic effects resulting from the presence of *K. veneficum* on grazing of *S. major*. This includes paralysis of the consumer (preventing further ingestion/ egg production), death of the consumer, and ingestion without assimilation. Experimental values above or along the regression line indicate nutritional adequacy at that ingestion level. Values that fall below the line indicate lower egg production than would be expected for that ingestion level if the ingested prey was nutritionally sufficient (Colin and Dam, 2002).

2.2.3 Respiration Experiments

Adults from the F0, F1, and F2 generations were sieved out of each culture and sorted using a wide-bore Pasteur pipette. For each experiment, 10 females and 10 males from each culture were used. All sorting and experiments were done using sump water from the pH stat culture setup to keep the pCO_2 level of experimental water consistent with culture water. This water was first syringe-filtered at 0.2 µm to

remove microbes that could affect dissolved oxygen levels. The 20 adults were first sorted onto well-slides and each was photographed for determination of prosome length using ImageJ software (NIH). Then they were loaded into 200 µl wells of a glass microplate with integrated oxygen-sensor spots (Loligo Systems). Four additional wells were filled with the same seawater and used as copepod-free controls. Experiments were run for several hours, during which dissolved oxygen in each well was measured at intervals of 30 s to 1 min. Respiration rates (pmol/g dry weight/hr) were determined using the RespR package in R statistical software (https://januarharianto.github.io/respR/articles/respR.html), with dry weight calculated from prosome length according to Berggreen et al. (1988). Rates were always determined using the period during which oxygen concentration fell between approximately 60% and 80% saturation to assure all animals were experiencing independent respiration. Statistical analysis of rates excluded F0 individuals as these animals were maintained or collected at a lower temperature than that of the experimental conditions.

2.2.4 Trophic Transfer Efficiency

Trophic transfer between *A.tonsa* and *K. veneficum* was calculated according to the methods of Cripps et al. (2016). Respiration rates (R) were calculated from the respiration experiments for F2 adults for both Ambient and Elevated treatments. Respiration rates were converted into respiratory carbon equivalents (ngC/ngC/day) using a respiratory quotient of 0.97 (Frangoulis, 2010). Egg production rates were used to calculate a carbon-based (ngC/ngC/day) growth rate (G). These were the egg production/female/day values from the 100:0 (*S. major* only) and 0:100 (*K.veneficum* only) levels in the Ambient and Elevated grazing experiments. Ingestion rates (I)

were taken from the ingestion rates during the same grazing levels as the egg production values. These were converted into carbon equivalents (ngC/ngC/day) using the mass measurements of F2 adults from the respiration experiments used to calculate R. These vital rates were used to calculate **gross growth efficiency** (GGE), as the proportion of ingested invested into reproduction (and somatic growth, here assumed to be zero for adult copepods), where:

$$GGE=G/I \quad \text{Eq. 2.1}$$

net growth efficiency (NGE), as the proportion of assimilated carbon allocated to reproduction (and growth, here assumed to be zero for adult copepods), where:

$$NGE=G/(G+R) \quad \text{Eq. 2.2}$$

and **assimilation efficiency** (AE), as the proportion of ingested carbon that is assimilated for reproduction (and growth, here assumed to be zero for adult copepods) and physiological maintenance, where:

$$AE=(G+R)/I \quad \text{Eq. 2.3}$$

These efficiencies were calculated for each copepod culture, under each treatment condition, for *S. major* and *K. veneficum* as the prey of *A. tonsa*. Differences were assessed by comparing 95% confidence intervals for each of these cases.

2.3 Results

All statistical tests were run in SigmaPlot 14.0 (Systat Software).

2.3.1 pH Stat Culture

DIC, spectrophotometric pH measurements, and calculated pCO_2 values are detailed in Table 2.1. Average pCO_2 values for copepod cultures (n=3) were 536±58 ppm and 1150±44 ppm for the Ambient and Elevated treatments respectively.

2.3.2 Grazing

Palatable-alga ingestion using *S. major* at multiple carbon levels was significantly higher at elevated conditions than at ambient (two-Way ANOVA, $p<0.001$, Holm-Sidak post-hoc $p<0.05$). In the Ambient treatment, ingestion rates increased with carbon level (Fig. 2.3A) (Kruskal-Wallis ANOVA on ranks, $p<0.05$, Tukey post hoc test, $p<0.05$). The same pattern was found in the Elevated treatment (Fig. 2.3B) (one-way ANOVA, $p<0.05$). Likewise, egg production in the Ambient treatment increased with carbon level (Fig. 2.3C) (one-way ANOVA, $p<0.001$, Holm-Sidak $p<0.05$), but in the Elevated treatment egg production was static, showing no significant differences (one-way ANOVA, $p=0.709$) (Fig. 2.3D). When comparing egg production between Ambient and Elevated treatments, there was a significant reduction in egg production with elevated conditions at the four highest carbon levels (two-way ANOVA, $p=0.002$, Holm-Sidak, $p<0.05$). The egg hatching success in these experiments (Fig. 2.3 E, F) was significantly reduced with elevated conditions (two-way ANOVA, $p<0.05$, Holm-Sidak, $p<0.001$). Therefore, *A. tonsa* ingested more carbon, but produced fewer viable eggs when reared at elevated temperature and pCO_2 conditions.

In the toxicity gradient grazing experiments (Fig. 2.4 A, B), ingestion rates of *K. veneficum* increased with proportion of this alga in the Ambient treatment (one-way ANOVA, $p<0.05$, Holm-Sidak, $p<0.05$). However, ingestion of *K. veneficum* did not increase with proportion under elevated conditions (one-way ANOVA, $p=0.248$). Differences in *S. major* ingestion were found between proportion levels in both Elevated and Ambient treatments (one-way ANOVAs, $p<0.05$, Holm-Sidak, $p<0.05$). Though there was not a clear pattern in the Elevated treatment, ingestion of *S. major* in the Ambient treatment generally decreased with *S. major* proportion (Fig. 2.4 A, B).

Between treatments, there was a reduction for *S. major* ingestion at the 25:75 and 75:25 levels under elevated conditions (two-way ANOVA, $p<0.05$, Holm-Sidak, $p<0.005$). However, no significant interactions were found between treatment and proportion for *K. veneficum* ingestion (two-way ANOVA, $p>0.05$). Egg production was not significantly different between the two treatments (Mann-Whitney, $p>0.05$), and no differences were found between levels within treatments (one-way ANOVAs, $p>0.05$) (Fig.2.4 C, D). Hatching success was not significantly different between levels in the Elevated treatment (ANOVA, $p>0.05$) (Fig. 2.4 E, F). In the Ambient treatment, a decrease was found only between levels 100:0 and 0:100 (Kruskal-Wallis ANOVA on ranks, $p<0.05$). There was a significant reduction in the mean hatching success at elevated conditions (two-way ANOVA, $p<0.05$, Holm-Sidak, $p=0.002$). Overall, the ingestion rates in the Ambient treatment generally followed a trend of increasing ingestion of a prey with increased concentration of said prey. This trend was not reflected in the Elevated treatment ingestion rates. Since hatching success was reduced, this indicates that, as was true in the palatable alga experiments, higher temperature and pCO_2 resulted in fewer viable offspring produced.

For analysis of the direct toxicity of *K. veneficum*, ingestion of *S. major* versus egg production (from the palatable-alga grazing experiment; Fig. 2.3 A-D) was plotted alongside *S. major* egg production to ingestion relationship (from the toxicity gradient grazing; Fig. 2.4 A-D). This layout describes ingestion of a palatable alga and resulting egg production in the presence of a toxic alga (Jónasdóttir et al., 1998). In both Ambient and Elevated treatments, experimental values are above the palatable-prey regression line, indicating there is no direct toxic effect of *K. veneficum* on *A. tonsa* grazing activity at the concentrations used in this experiment (Fig. 2.7).

2.3.3 Respiration Experiments

Prosome lengths of adults in the F1 and F2 generations only were analyzed; F0 adults were excluded from analysis because they developed at a different temperature than the treatment dictated. Males were significantly smaller than females (two-way ANOVA, $p<0.05$, Holm-Sidak, $p<0.001$). Females were significantly smaller at the Elevated treatment (two-way ANOVA, $p<0.05$, Holm-Sidak, $p<0.001$), however there was no difference for males (two-way ANOVA, $p<0.05$, Holm-Sidak, $p=0.591$).

Weight-specific respiration rates were analyzed in the same manner as prosome lengths. Females had significantly higher rates than males across both treatments (two-way ANOVA, $p<0.05$, Holm-Sidak, $p<0.001$). However, there was no difference in rates due to treatment (two-way ANOVA, $p<0.05$, Holm-Sidak, $p=0.541$) (Fig. 2.6).

2.3.4 Trophic Transfer Calculations

For trophic transfer between *A. tonsa* and *S. major*, GGE significantly decreased from ambient conditions to elevated (1.02 to 0.3 in Ambient, 0.54 to -1.4 in Elevated). For *A. tonsa* to *K. veneficum*, no changes were significant (95% CI). AE was significantly different between *S. major* and *K. veneficum* at ambient conditions (0.82 to 0.58). No significant differences were found between prey species or treatment in NGE (Table 2.2).

2.4 Discussion

Temperature and pCO_2 Effects on *A. tonsa* Physiology

Grazing with a palatable alga was significantly different across treatments, indicating *A. tonsa* consumed fewer cells per volume when consumer and prey were reared at a higher temperature and pCO_2. Since egg production was also significantly

higher at four of the six carbon levels in the Ambient treatment, this indicates that at ambient conditions, *A. tonsa* needed less food to produce more eggs. In addition, ingestion and egg production are positively associated at ambient conditions, but this relationship disappears at elevated conditions. Egg production was not only depressed overall in the Elevated treatment, but also static across different carbon levels. This is indicative of more energy being put towards metabolic processes, leaving less available for reproduction. However, it also indicates that egg production is prioritized over physiological maintenance, as egg production does not decrease across concentrations within the Elevated treatment even at the lowest food level.

The indication of less energy available for reproduction is consistent with the results from the respiration experiments. If ingestion increases, but metabolism and reproduction do not, then more energy must be being diverted into keeping those rates stable. Therefore, metabolic rates for Elevated treatment copepods indicated they are expending more energy for physiological maintenance than those at Ambient treatment conditions.

Since no significant differences in respiratory rates were found between treatment or generation using F1 and F2 adults, *A. tonsa* does not appear to have a higher metabolic rate at the Elevated treatment conditions. This is reflected in the Q_{10} value of 1.12, calculated from the F2 adults. A Q_{10} value relates metabolic rate and temperature according to the equation:

$Q_{10} = (k_1/ k_2) \text{ ^ } 10/(t_1-t_2)$ From Harris et al., 2000; Eq. 2.4

where k_1 and k_2 are the respiration rates of *A. tonsa* at temperatures t_1 and t_2, which in this case are 25 and 29 ℃ respectively. A Q_{10} of 1 would indicate no dependence of the metabolic rate on temperature. Most biological reactions fall in the range of 2-3

(Prosser, 1961), while low Q_{10} values are typical for metabolisms of eurythermic species (Rao and Bullock, 1954; Precht et al., 1973). A low Q_{10} is advantageous when animals are faced with frequent temperature fluctuations, as can happen in shallow, coastal environments, because it maintains metabolic processes at lower temperatures and saves energy at higher temperatures (Hiromi et al., 1988). This Q_{10} is slightly lower than acute Q_{10} values found in Gaudy et al. (2000) for both *A. tonsa* and *A. clausi* in the Mediterranean Sea. They found Q_{10} values of around 2 for both species across the salinities of 15, 25, and 35 psu, calculated from respiration rates at 10 and 20 °C (Gaudy et al., 2000).

The lack of an increase in metabolism with temperature signifies that respiratory compensation must be occurring at the elevated conditions. Maintenance of the routine metabolism at a higher energetic cost could be a result of hypercapnia and/or thermal stress. Since I only tested these factors synchronously, it is impossible to delineate the relative strength of the two stressors. However, as these factors are causally linked in the environment, their interactive effect is the one of relevance.

Hypercapnia is the increase in pCO_2 of the hemolymph as a result of a concomitant pCO_2 increase in the external medium (i.e. the seawater). The increase of pCO_2 in the seawater reduces pH, which compromises CO_2 excretion and leads to the build-up of CO_2 in the hemolymph. This CO_2 forms carbonic acid, which dissociates into hydrogen ions [H^+] and bicarbonate [HCO_3^-]. The acid-base balance of the hemolymph is maintained by ion exchange (Na^+ for H^+, Cl^- for HCO_3^-) across either the hemolymph-cell interface or the hemolymph-external medium interface. This is energetically costly, however, as continual uptake of bicarbonate from seawater depends on ion gradients which must be maintained via active ion-transport (Whiteley,

2011). There is also documentation of a buffering effect provided by dissolution of the calcium-carbonate shell, however since bicarbonate is readily available from seawater, this buffering technique is thought to be important only in air-breathing crustaceans (Cameron, 1985). The same ion-regulatory mechanisms that maintain acid-base balance are used to maintain osmoregulation within the animal. Therefore, it is predicted that animals with a higher resilience to hypercapnia stress will be those animals that already have a well-developed osmoregulation system (Whiteley, 2011). Since copepods can inhabit a wide range of salinities, they potentially have the capacity to regulate acid-base balance in the hemolymph with their existing physiology.

Copepods are poikilotherms, meaning they allow their body temperature to fluctuate with thermal changes in the surrounding environment. Elevated temperatures commonly result in smaller body size in ectotherms (Atkinson, 1994). This is reflected in the change from F0 adults to F1 under Elevated and Ambient conditions, as F0 adults were maintained at a temperature 5 ºC higher than F1 in both cases (Fig. 2.5). However, the expected decrease in prosome length with increased temperature, represented by the Elevated treatment conditions, was absent in this experiment. This lack of a difference within sex between the two treatments could be a result of too small a temperature change to invoke a size difference, physiological limitation, or an ameliorating effect of abundant food concentration. The difference in temperature between the Elevated and Ambient treatments was 4 ºC. It is possible that *A. tonsa* does not exhibit a significant change in body size for this interval. It is also possible that the temperature of 29 ºC is near the thermal tolerance limit of *A. tonsa*, and that their ability to decrease body size as an adaptive measure to offset thermal

stress is reduced or inhibited at this temperature. This is unlikely, however, given thermal tolerance data for *A. tonsa* in other locations. When acclimated to 20 ºC, *A. tonsa* from Mt. Hope Bay (Massachusetts-Rhode Island) had a critical thermal maximum of ~ 35 ºC (González, 1974). In addition, critical thermal maxima increased with increasing acclimation temperature (González, 1974). According to this pattern, 29 ºC acclimated *A. tonsa* should be well-within their thermal tolerance limit. Thirdly, change in body size or mass is known to be affected by food concentration in addition to temperature (Hirst and Bunker, 2003). Since copepods in this study were provided with a saturating amount of carbon (500 µgC/L) in the form of a palatable alga for the duration of their lifecycle, this abundance of food may have offset the need to reduce body size at 29 ºC.

Effects of Elevated Treatment on Trophic Transfer Efficiency

GGE shows how much carbon the animal produced in relation to how much carbon they took in. If all ingested carbon were allocated to growth (i.e. reproduction for an adult female *A. tonsa*), then GGE would equal one. In reality, some energy must be used for physiological maintenance, so GGE values should realistically be <1. Indeed, Cripps et al. (2016) reported a GGE of 0.12 for *A. tonsa* fed palatable prey at 400 ppm and 0.026 at 1000ppm. Kiørboe et al. (1985) found a GGE of 0.41 when they fed *A. tonsa* with palatable prey at a carbon level of 367 µgC/L, slightly lower than the 500 µgC/L used in the present study. The significant drop in GGE between ambient and elevated conditions for *A. tonsa* grazing on *S. major* shows that the elevated conditions lower GGE on a palatable prey. The drop in *S. major* GGE is not explained by the change in *S. major* cellular carbon content, as it is negligible between and Ambient and Elevated cultures. This suggests the change is related to

physiological changes at the copepod level instead of the prey level, and is in accordance with the results above.

AE indicates how efficiently the consumer assimilated carbon from the prey source. *Acartia tonsa*'s AEs did not change with increased temperature and pCO_2 when *A. tonsa* was fed a palatable prey item.

NGE shows how much of the total carbon intake (growth + metabolic costs) is allocated to growth (egg production in the case of adults). Net growth efficiency did not change significantly across treatment. Regardless of the environmental stressors, *A. tonsa* always allocated approximately 70-90% of ingested carbon to egg production. This means they are prioritizing reproduction over physiological maintenance. Conversely, NGE decreased significantly in Cripps et al. (2016). Cripps et al. (2016) found a decrease from 0.518 ± 0.27 to 0.301 ± 0.21 (same prey species) with elevated pCO_2 (1000 ppm). However, Cripps et al. (2016) did not manipulate temperature, and conducted all experiments at 24.5 ℃. This suggests temperature may interact with pCO_2 in directing NGE.

Effect of Elevated Treatment on Trophic Transfer Efficiency with a Toxic Alga

In the toxicity gradient *S. major*:*K. veneficum* ratio experiments, the trend for *K. veneficum* ingestion was the same between treatments, though the quantity ingested was lower overall at higher temperature and pCO_2. This is consistent with the palatable-prey data, and suggests that *A. tonsa* will continue to consume *K. veneficum* in relation to the quantity offered, but will consume less overall at climate change conditions. Grazing on *K. veneficum* was similar in magnitude to that found in Waggett et al. (2008). A notable aspect of the *K. veneficum* grazing is that the highest ingestion level of *K. veneficum* by carbon (0 :100 ambient conditions) was the only

ingestion level to have a significant decrease in egg hatching success. This could indicate the level at which *K. veneficum* concentration negatively affects *A. tonsa* physiology at the population level.

Direct toxicity of *K. veneficum* on *A. tonsa* ingestion of *S. major* was shown to be negligible (Fig. 2.7). This indicates *K. veneficum* cells were not causing paralysis of *A. tonsa* or hindering ingestion by another mechanism. This could be a result of the variable toxicity of the lab-cultured *K. veneficum* cells. Since positive ingestion rates of *K. veneficum* were found in the toxicity gradient experiment under ambient conditions (Fig. 2.6), it is unlikely that ingestion of *K. veneficum* is being suppressed in the same manner found in Waggett et al. (2008). Since ingestion of a confirmed-toxic strain of *K. veneficum* reduced total grazing significantly when present in concentrations of ≥50%, the authors concluded that these *K. veneficum* cells must be acting as a chemical deterrent (Waggett et al., 2008). Since toxicity was not confirmed for the *K. veneficum* strain used in this experiment, and since it is known to vary with abiotic conditions (Bachvaroff et al., 2009), it is possible this strain either was less toxic overall than Waggett et al.'s (2008) strain or that the ratio of intracellular to extracellular karlotoxin was different.

Gross growth efficiency decreased with the ingestion of *K. veneficum*. When *A. tonsa* ingests this HAB species, they transfer less carbon to their egg production than when *S. major* was ingested under comparable temperature/pCO_2 conditions. This is much lower than *S. major* at ambient conditions (1.02) but higher than *K. veneficum* (0.3). The difference in GGE resulting from *S. major* versus *K. veneficum* ingestion shows that *A. tonsa* is more efficient at producing eggs when eating *S. major* at ambient conditions. GGE for *K. veneficum* does not change between treatments.

Since these values are quite low (.3 and -1.4) it is possible GGE is already at a theoretical minimum for *K.veneficum* ingestion.

Net growth efficiency did not change significantly between *S. major* and *K.veneficum*. The similarity of values within each treatment (Table 2.2) indicates that the amount of carbon allocated to eggs does not change with prey source. This is interesting given the relative amounts of carbon in *S. major* and *K. veneficum* (~1:3). NGE values here are comparable to that from Kiørboe et al. (1985) (e.g., 0.74; see Table 2.2).

The only significant difference in AEs was between *K. veneficum* at Ambient and Elevated treatments. Assimilation efficiency was again similar to Kiørboe et al. (1985) (0.55). The lack of decrease in AE when *A. tonsa* was fed *K. veneficum* at elevated conditions indicates that the combination of climate change conditions and a toxic prey did not interact to decrease TTE.

Multi-Generational Effects and Sensitivity of Juvenile Life Stages

The observed changes in TTE with treatment suggest a change in resource allocation at higher temperature/pCO2 conditions. This effect could be amplified or dampened over multiple generations, showing the need for multi-generational studies. Additionally, most literature on climate-change mediated effects on crustaceans has focused on the adult stage. Nauplii and copepodite stages are likely to be more vulnerable to environmental stressors than adults, as they still must allocate energy towards somatic growth. In addition, they might have less developed ion-transport mechanisms available to maintain acid-base balance.

Multiple studies have found evidence for decreased survival at early life stages, which could translate into population effects when a local copepod population

is forced through this bottleneck. Egg hatching success and nauplii survival has been documented to decrease with increased pCO_2. Kurihara et al. (2004) found a reduction in egg production with dramatically increased pCO_2 (2,000 and 10,000 ppm) in *Acartia steurei* that could be reversed by returning the female to ambient seawater. The authors also found a reduction in egg hatching success and N1 survival of *Acartia erythraea* with increasing CO_2 concentration (Kurihara et al., 2004). Egg viability and naupliar development of *Acartia bifilosa* were negatively impacted by a higher temperature (20 ℃, compared to a control of 17 ℃), but not lower pH (7.6 treatment, 8.0 control) in Vehmaa et al. (2013). A pCO_2 of 8000 ppm was found to have no effect on the egg production of *Calanus finmarchicus*, but dramatically decreased hatching rate (Mayor et al., 2007). Weydmann et al. (2012) found similar results with *Calanus glacialis*. Egg production remained stable with reduced pH, but hatching success decreased (Weydmann et al., 2012). Though both these studies looked at ocean acidification without the confounding effects of temperature increase, these results align with the reduction in hatching success found in the palatable-prey feeding experiments under elevated conditions. I believe this provides evidence that egg hatching is uniquely sensitive to environmental stressors, and may become the bottleneck for copepod populations in the future.

The most common experimental setup for examining pH and temperature stressors on copepods is to use a short-term exposure (a few days) on adults and measure their physiological and reproductive response. While this gives some insight into population effects, much of this work is representative of an acute stress response. An acute response may not be representative of copepod reactions to these stressors on the timescale for which they will naturally take place. Measuring animal responses on

evolutionary timescales is not usually practical, however using a multiple-generation approach to study copepod reactions to environmental stressors can begin to illuminate population-level responses. This approach is becoming more common in climate-change literature. Several studies have used two generations of copepods reared under altered pCO_2 regimes (Pedersen et al., 2014; Fitzer et al., 2013; Thor and Dupont, 2015; Kurihara and Ishimatsu, 2008) and one study to date has used four generations (Li et al., 2017). Kurihara and Ishimatsu (2008) did not find any significant effects of a raised pCO_2 (2380 µatm) on *Acartia tsuensis*, but the other studies all found changes in the second generation when pH was decreased. Naupliar production and female fecundity decreased with pH in the second generations of *Tisbe battagliai* and *Pseudocalanus acuspes* respectively (Fitzer et al., 2013; Thor and Dupont, 2015). Pedersen et al. (2014) found both immediate negative consequences on the physiology of *C. finmarchicus* in response to pCO_2 (growth, feeding, metabolism), and also the potential for long-term adaptation to ocean acidification stress. Though the first generation showed developmental delays, this effect was not present in the F2 generation, indicating acclimation was possible over a relatively short time span (Pedersen et al., 2014). This study in particular highlights how single-generation and multi-generation effects may be vastly different.

One approach to getting around the practical difficulties of multi-generational rearing of animals in the lab is to use laboratory-derived vital rates to inform population models. These models can be used to predict the chronic consequences of an acute stress reaction. Though these models depend heavily on assumptions, they still provide insight into population-level effects resulting from an individual-level environmental response. Fitzer et al. (2012) used measured naupliar production from

two generations of lab-cultured *T. battagliai* under different pCO_2 scenarios to generate a mixed-effects model which simulated approximately 2430 generations (100 years). This model predicted a gradual decline in naupliar production, which was contradictory to the immediate rise in naupliar production with decreased pCO_2 (Fitzer et al., 2012). This increase was attributed to a hormesis-type response (Fitzer et al., 2012). These differences highlight the need to place short-term exposure into long-term context before making conclusions about a population's resilience to climate change.

Conclusion

In conclusion, grazing experiments using *A. tonsa* and palatable alga *S. major* reared under a raised temperature and pCO_2 regime demonstrated, through reduced viable egg production, that more energy is needed for physiological maintenance at these conditions. However, the metabolism of *A. tonsa* did not appear to be dependent on temperature, indicating respiratory compensation in response to either thermal/hypercapnic stress or some combination. Trophic transfer efficiency was altered when *A. tonsa* was reared under a raised temperature and pCO_2 regime and when *A. tonsa* ingested the toxic alga *K. veneficum*. In these cases, the same percentage of carbon ingested was allocated to egg production, signifying the priority of reproduction, but less total carbon was transferred to egg production. This decrease in the amount of carbon to enter the next generation of *A. tonsa* could be exacerbated or ameliorated over time. Long-term, multi-generational studies will be needed to parse out the population-level importance of these trophodynamic changes under an altered climate regime.

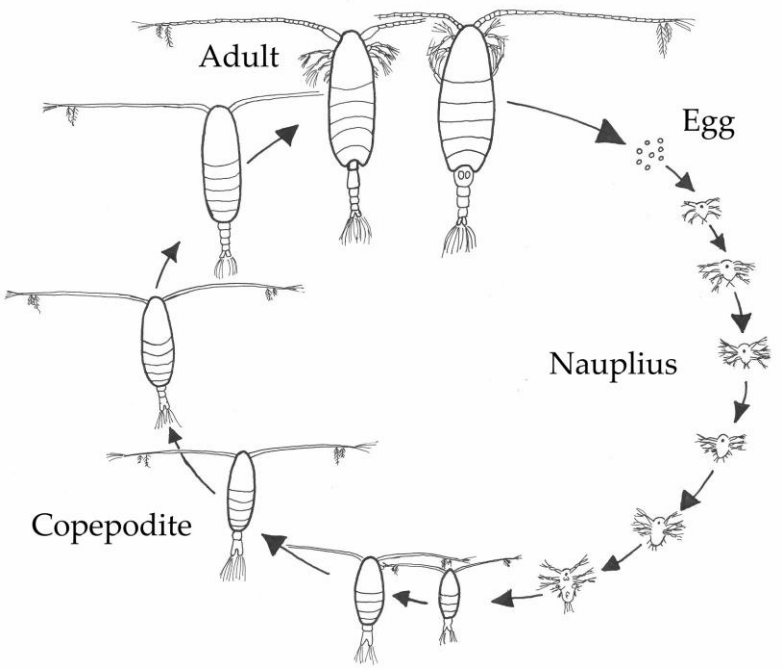

Figure 2.1. **Calanoid Copepod Life Cycle**. Image from NOAA.gov.

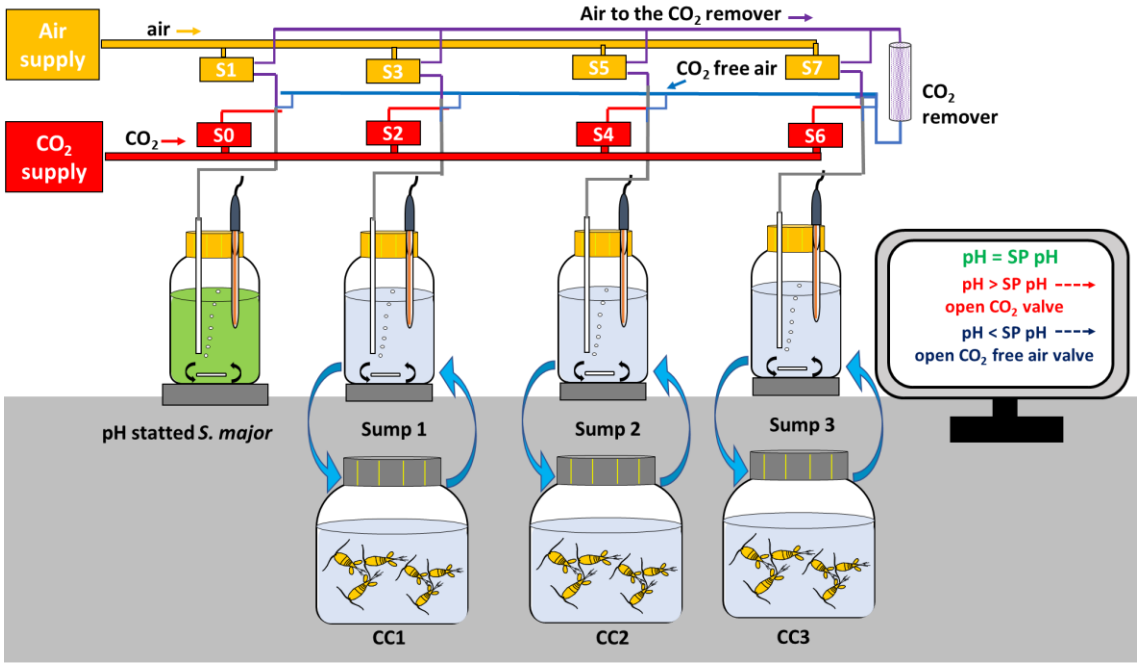

Figure 2.2. **Schematic Diagram of pH Stat Culturing System for Copepods and *S. major*.** S0-6 markers represent solenoids. CC1, CC2, and CC3 represent copepod cultures 1, 2, and 3 respectively.

Table 2.1 Carbonate Chemistry for pH Stat System. DIC and pH^{Total} were measured every 2-4 days and used to calculate corresponding pCO_2 value. ± values indicate standard deviations.

Treatment	ID	DIC	pH	pCO_2 (ppm)
Ambient	*S.major*	2258.94±65.3	8.14±0.02	392.96±81.34
	Sump1	2285.24±25.93	8.058±0.02	494.01±88.1
	Sump2	2272.68±9.24	8.13±0.02	402.65±15.71
	Sump3	2204.08±79.36	8.10±0.01	425.57±23.81
	CC1	2253.51±30.95	7.99±0.03	572.39±45.29
	CC2	2238.60±39.13	8.05±0.05	511.54±73.45
	CC3	2249.26±42.62	8.03±0.03	524.21±53.84
Elevated	*S.major*	2569.07±35.95	7.79±0.008	1077.46±33.18
	Sump1	2385.77±10.88	7.76±0.007	1069.03±21.24
	Sump2	2435.14±26.9	7.76±0.01	1098.26±34.91
	Sump3	2372.02±19.82	7.81±0.01	948.48±26.17
	CC1	2345.43±20.3	7.74±0.01	1109.25±35.99
	CC2	2368.14±18.53	7.70±0.02	1251.26±52.8
	CC3	2353.10±18.12	7.75±0.01	1089.21±41.67

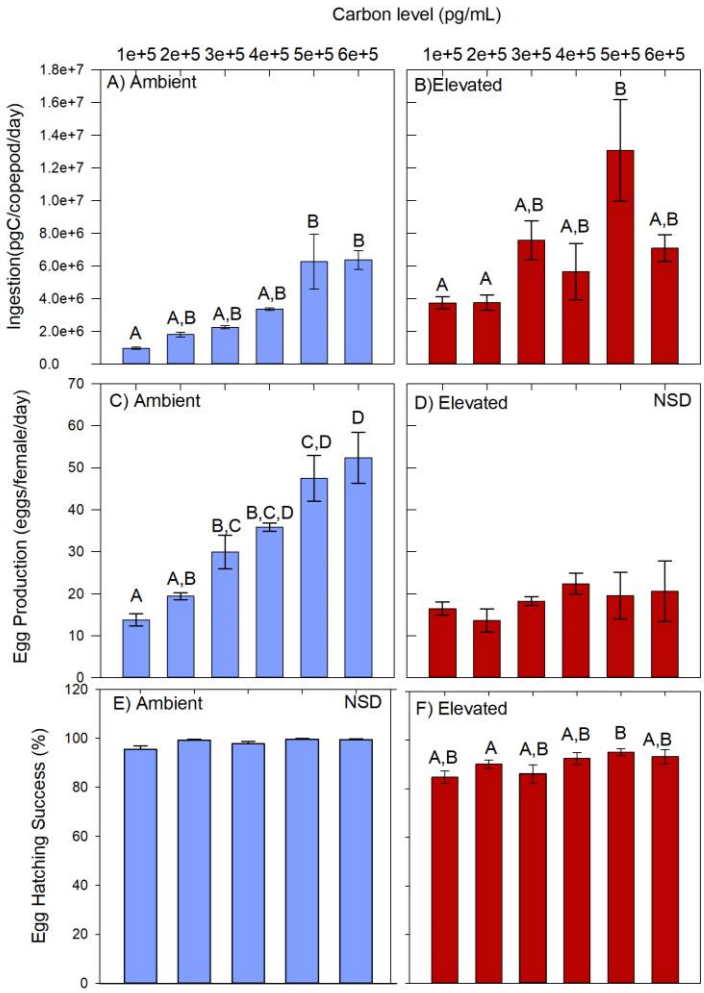

Figure 2.3. **Palatable-Alga Grazing, Egg Production, and Hatching Success for *A. tonsa* fed *S. major*.** Ingestion as a function of carbon concentration in A) Ambient conditions and B) Elevated conditions. Egg production and hatching success from the same experiment under C, E) Ambient and D, F) Elevated conditions. All error bars represent standard deviation (n=3). Significant differences between bars shown by different letters (A-Kruskal-Wallis, $p=0.006$; Tukey, $p<0.05$), (B, C-one-way ANOVA, $p<0.05$; Holm-Sidak, $p<0.05$). D, E) NSD indicated no significant differences were found (one-way ANOVA, $p<0.05$; Holm-Sidak, $p<0.05$). Blue bars indicate Ambient conditions (25 ºC /400ppm) and red bars indicate Elevated conditions (29 ºC /1000ppm).

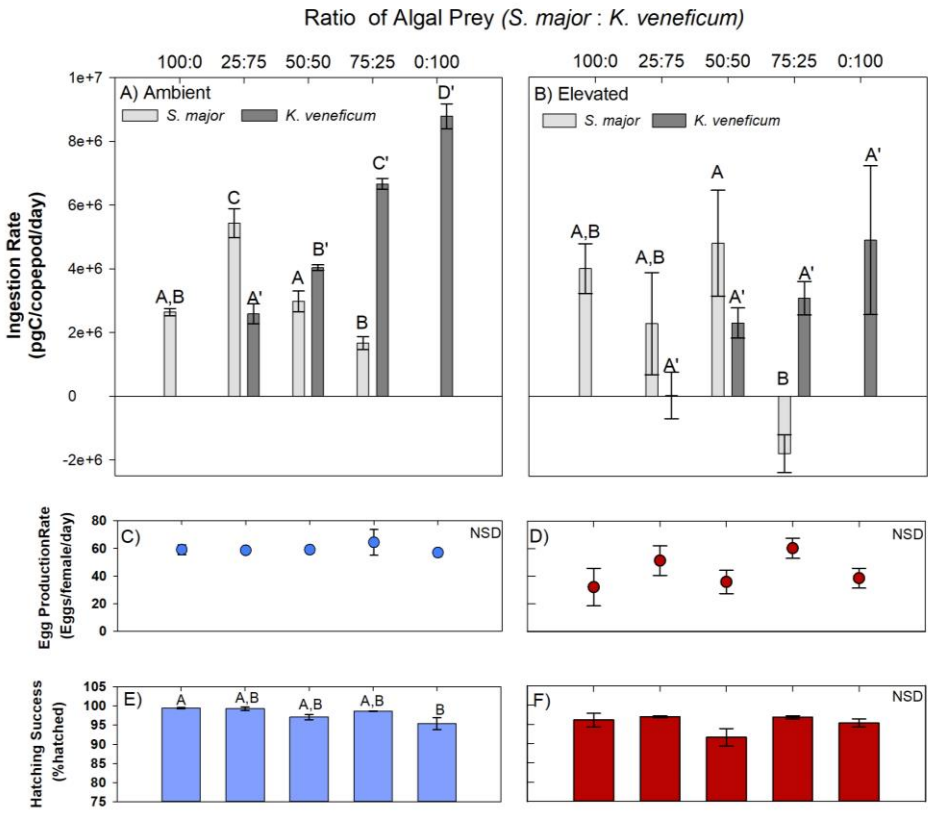

Figure 2.4. **Toxicity Gradient Grazing, Egg Production, and Hatching Success for *A. tonsa* fed a Carbon-Normalized Ratio of *S. major* to *K. veneficum*.** A) Grazing experiment using prey and consumer reared under Ambient conditions (25 ºC /400ppm). B) Grazing experiment using prey and consumer reared under Elevated conditions (29 ºC /1000ppm). C, E) Egg production and hatching success of eggs produced during grazing experiment in A. Blue color indicates Ambient conditions. D, F) Egg production and hatching success of eggs from grazing experiment in B. Red color indicates Elevated conditions. Error bars are standard error (n=3). Different letters above bars indicate significant differences in data (A, B-One-way ANOVAs, $p<0.05$; Holms-Sidak, $p<0.05$; E-Kruskal-Wallis, $p<0.05$; Tukey, $p<0.05$). Where no significant differences were present, NSD is written (C, D, F).

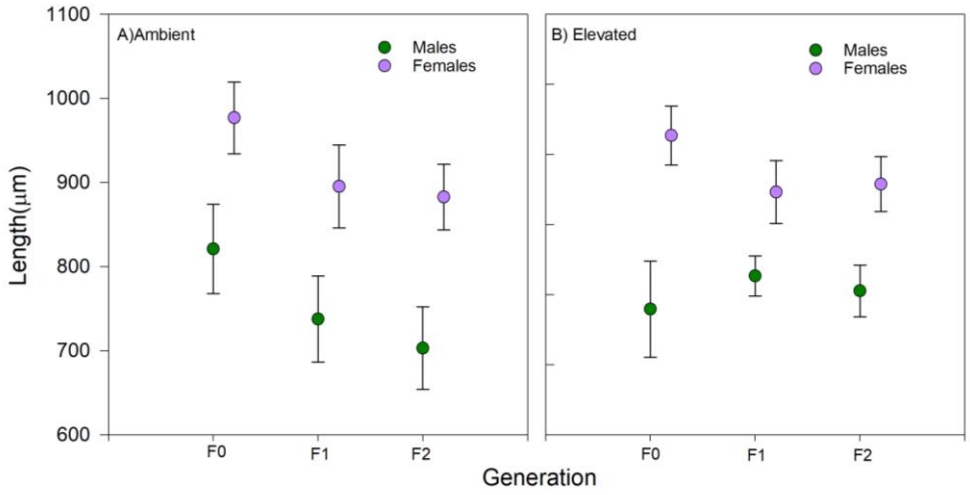

Figure 2.5. **Average Prosome Lengths (n=30) in the F0, F1, and F2 Generation Adults used in Respiration Experiments at both A) Ambient and B) Elevated Treatments.** Error bars (n=30) indicate standard deviation. Purple circles indicate females, green circles indicate males. F1 and F2 females in A) Ambient treatment are significantly larger than F1 and F2 females in the B) Elevated treatment (two-way ANOVA, $p<0.05$, Holm-Sidak, $p<0.001$). F1 and F2 females were significantly larger than F1 and F2 males in both treatments (two-way ANOVA, $p<0.05$, Holm-Sidak, $p<0.001$).

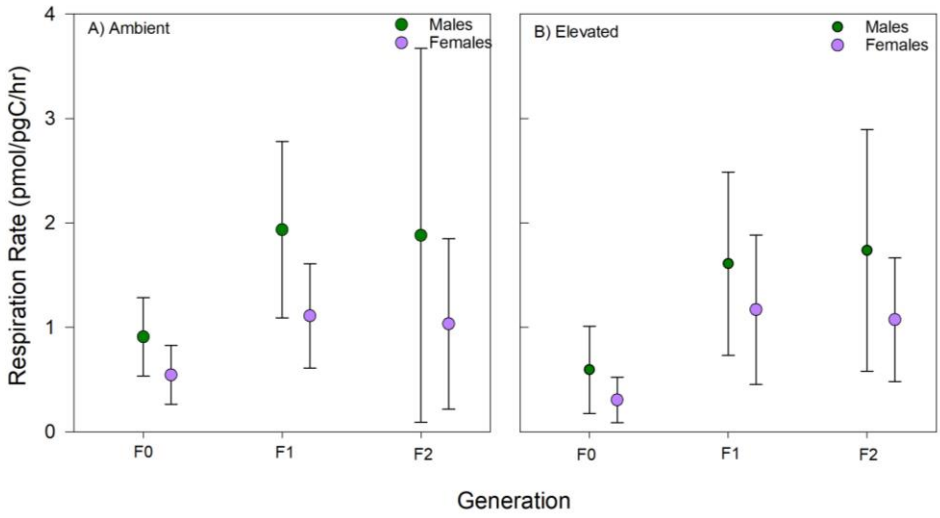

Figure 2.6. Weight-Specific Respiration Rates (n=30) of Adult Males (green circles) and Females (purple circles) at both A) Ambient and B) Elevated Treatments at F0, F1, and F2 Generations. Error bars indicate standard deviations (n=30). Statistical test carried out using only F1 an F2 adults. Females had significantly higher rates than males across both treatments (two-way ANOVA, $p<0.05$, Holm-Sidak, $p<0.001$). No difference in rates due to treatment were found (two-way ANOVA, $p<0.05$, Holm-Sidak, $p=0.541$).

Table 2.2. **Trophic Transfer Calculations**. Values presented are averages (n=3) for each treatment and prey source, with the exception of R. Since respiration rates were calculated only on adults from the statted cultures, there is no comparison for *K. veneficum*. The R value for each treatment is an average (n=30) of adults fed only *S. major*. R, I, and G values are presented with standard errors (n=3). GGE, NGE, and AE values are averages (n=3) with 25% CI and 75% CI values in parentheses.

Treatment	Prey	R(ngC/ngC/day)	I (ngC/ngC/day)	G(ngC/ngC/day)	GGE	NGE	AE
Ambient	*S. major*	0.12 ± 0.04	0.59 ± 0.03	0.60 ± 0.02	1.02 (0.89,1.1)	0.84 (0.72,0.93)	0.82 (0.65,1.0)
	K. veneficum	0.12 ± 0.04	1.97 ± 0.07	0.58 ± 0.02	0.30 (0.25,0.33)	0.84 (0.7,0.93)	0.58 (0.53,0.61)
Elevated	*S. major*	0.13 ± 0.03	0.97 ± 0.15	0.51 ± 0.05	0.54 (0.48,0.67)	0.80 (0.73,0.87)	0.66 (0.52,0.75)
	K. veneficum	0.13 ± 0.03	1.20 ± 0.56	0.55 ± 0.1	-1.4 (-4.7,0.3)	0.81 (0.73,0.90)	0.55 (0.37,0.77)

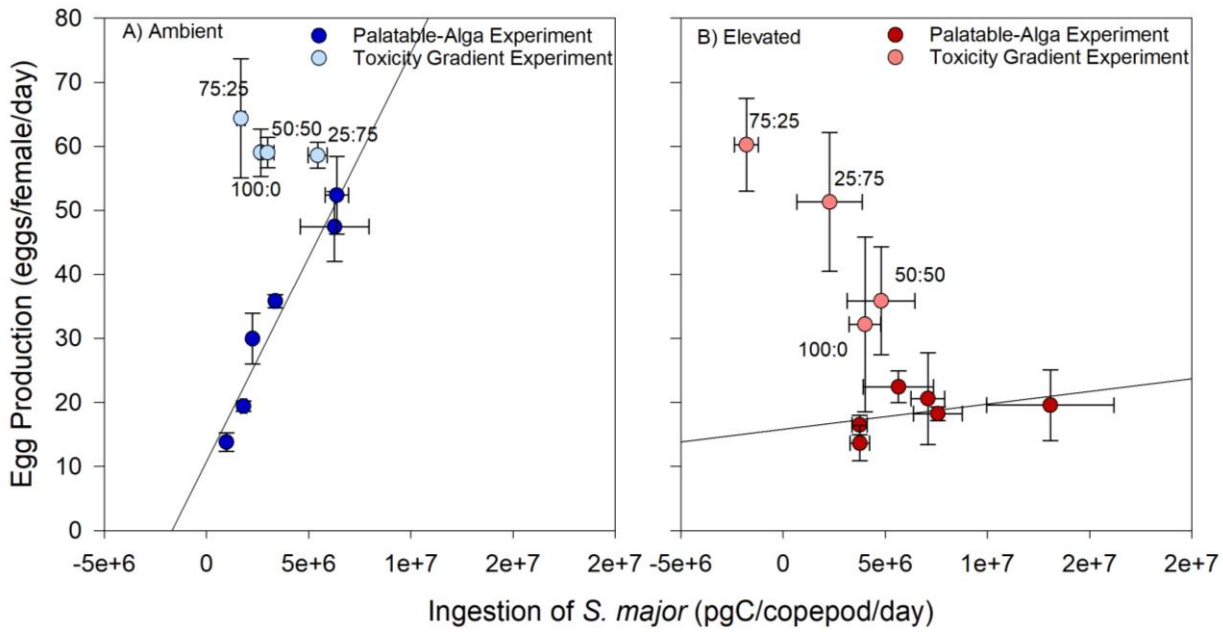

Figure 2.7. **Assessment of Direct *K. veneficum* Toxicity to *A. tonsa* Grazing on *S. major*.** Mean ingestion of *S. major* by *A. tonsa* is plotted as a function of egg production for (A) Ambient and (B) Elevated treatments. The black lines are linear regressions for *A. tonsa* ingestion/egg production when feeding solely on *S. major* over a range of concentrations (Palatable-Alga experiment). Thus, the area above the regression line refers to nutritional adequacy and the area below it refers to nutritional inadequacy. Lighter colored circles are data from the toxicity gradient experiments, interpreted with respect to these areas. When light circles fall above or along the black line, that value of egg production/ingestion is nutritionally adequate. If light circles fell below the black line, this would indicate a directly toxic effect of the prey ingested at that data point. All error bars are standard error (n=3).

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