RISK AND RESOURCES IN THE PLANKTON: BEHAVIORALLY MEDIATED EFFECTS ON COPEPOD POPULATION GROWTH AND ZOOPLANKTON COMMUNITY DYNAMICS

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The Academic Faculty

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RISK AND RESOURCES IN THE PLANKTON: BEHAVIORALLY MEDIATED EFFECTS ON COPEPOD POPULATION GROWTH AND ZOOPLANKTON COMMUNITY DYNAMICS

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To the greatest scientist I know, my husband
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<td>$E$</td>
<td>Encounter rate</td>
</tr>
<tr>
<td>$R$</td>
<td>Reach of an organisms sensor span</td>
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<tr>
<td>$C_T$</td>
<td>Concentration of individuals</td>
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<tr>
<td>$V$</td>
<td>Swimming velocity of copepod</td>
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<td>$\sigma$</td>
<td>Proportion of fertilized females in a population</td>
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<td>$p$</td>
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<td>$K$</td>
<td>Predator’s perceptive distance</td>
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<td>$v_{pred}, v_{prey}$</td>
<td>Swimming velocity of predator and prey</td>
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<td>$s$</td>
<td>Size of prey</td>
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<td>$\omega$</td>
<td>Threshold speed that a predator can detect</td>
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<td>RMS</td>
<td>Root-mean square distance</td>
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<td>$\tau$</td>
<td>Time scale over which an organism’s path is directionally persistent</td>
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<tr>
<td>HAB</td>
<td>Harmful algal bloom</td>
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<td>$I$</td>
<td>Ingestion rate</td>
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$C_1, C_2$  Phytoplankton cell concentration before and after feeding trial

$N$  Number of live copepods at the end of 24 h feeding trial

$B$  Cell biovolume
SUMMARY

In this study, I explored the in situ fertilization success of two marine copepod species, *Temora longicornis* and *Eurytemora herdmani* in coastal Maine. Copepod population growth is of critical importance to marine food webs. Fertilization limitation has been suggested as a constraint on copepod population growth but field surveys describing the prevalence of fertilization limitation are lacking. I collected monthly zooplankton samples and analyzed egg clutches from field-caught females using an egg-staining technique. My results indicate that both species exhibit fertilization limitation in nature but the factors correlated with their fertilization differ and are species-specific. For example, temperature and food concentration were significantly associated with *Temora longicornis* fertilization. In contrast, *Eurytemora herdmani* fertilization was significantly associated with demographic factors such as population density and sex ratio, as well as mysid predator abundance.

To determine a causal relationship between predator density and copepod mating success, I conducted laboratory experiments to determine the effects of a common mysid shrimp predator, *Neomysis americana* on *Eurytemora herdmani* mating success. I subjected males and females to predators or predator cues. I found that the presence of a mysid predator, or only a predator cue, reduces copulation frequency and spermatophore transfer of *E. herdmani*, leading to a 38-61% decrease in *E. herdmani* nauplii production. These results suggest that mysid predators can constrain copepod population growth through non-consumptive processes such as reducing the frequency and success of mating events, highlighting the importance of top-down influences on copepod behavior.
To determine the effect of bottom-up forces on copepod behavior, I measured the behavioral and fitness consequences of *Temora longicornis* ingesting *Alexandrium fundyense*, a phytoplankton species that forms harmful algal blooms in coastal Maine. My results suggest that ingesting *A. fundyense* causes copepods to swim faster and with more directional persistence compared to control algae. *Temora longicornis* increased their average swimming velocity by 24%, which leads to a 24-54% increase in their theoretical encounter rates with predators. Therefore, these findings suggest behaviorally mediated copepod-algae interactions may have significant impacts on harmful algal bloom dynamics and the fate of toxins in marine food webs.
CHAPTER 1

INTRODUCTION

The focus of my thesis research is on the interplay between individual behavior, population dynamics and community-level processes within zooplankton communities in coastal Maine. Planktonic communities have long been recognized for their considerable diversity (Hutchinson 1961) and vital importance for the functioning of oceanic ecosystems (Eppley and Peterson 1979). The target organisms of my thesis work are marine copepods. Copepods are small (1-10 mm) crustaceans that perform the essential ecosystem function of consuming and assimilating primary production (phytoplankton) making it available to higher trophic levels such as commercially important fishes (Mauchline 1998). Copepods belong to the broader classification ‘plankton’, a term derived from the Greek word *planktos*, which means “to wander”. This term implies that plankton have little control over their movements and are subject to the whims of oceanographic processes. However, research conducted in recent decades indicates that plankton such as marine copepods exhibit pronounced and unique behaviors when observed at ecologically-relevant scales. For example, male copepods can find mates with speedy precision by following scent trails left by females (Doall et al. 1998). Copepods can also exhibit extreme escape behaviors that make them the fastest jumpers in the world (Kiørboe et al. 2010).

Due to their small size, copepods experience a physical world that is somewhat non-intuitive to humans. Interactions between copepods take place in a viscous and relatively vast three-dimensional environment (Yen 2000). In this environment,
encounter rates largely govern the population dynamics of copepods (Gerritsen 1980; Gerritsen and Strickler 1977). Therefore, for researchers studying copepod ecology, encounter rate theory is a valuable tool that must be understood within the context of the physical environment as well as the ambit of the organism. In its simplest form, encounter rates in the plankton ($E$) are determined by the rate of the volume swept by an organism, where ($R$) is the reach of an organism’s sensor span, ($C_T$) is the density of encountered organisms and ($V$) is their swimming speed (Gerritsen and Strickler 1977; Visser 2007).

$$E = \pi R^2 C_T V$$

Each term in this equation is influenced by the physical environment as well as the individual behaviors of organisms. For example, at large ($1\text{km} - 100\text{s km}$) scales, seasonal warming and wind-driven deepening of the upper mixed layer supplies nutrients to fuel phytoplankton growth which in turn leads to greater zooplankton densities (Miller 2004). Additionally, large-scale circulation patterns facilitate long dispersal distances for zooplankton. Furthermore, seasonal and climate related alterations in temperature could lead to increases in zooplankton swimming speed, as has been demonstrated in laboratory experiments (Kramer et al. 2011). Finally, wind-driven turbulence can enhance contact rates between individuals (Rothchild and Osborn 1988; MacKenzie and Leggett 1991) or concentrate zooplankton that avoid turbulent layers (Mackas et al. 1993), both of which would increase encounter rates.

At small (cm) to intermediate ($1\text{m} - 100\text{m}$) scales, oceanic features such as thin layers may act as zooplankton aggregation cues (Tiselius 1992; Woodson et al. 2005), facilitating localized increases in zooplankton population densities. At these scales
zooplankton interactions with predators, competitors and resources may affect swimming behavior (van Duren and Videler 1996) as well as mating and escape behaviors – all of which influence zooplankton population density. Therefore, zooplankton encounter rates are governed by both large and small scale processes; the relative importance that researchers assign to these factors greatly depends on the scale at which the measurements are made.

Much of the research addressing the ecology of copepods can be broadly classified into two categories: 1) biological oceanography and 2) behavioral ecology. Biological oceanographers often measure bulk properties of the marine environment such as temperature, salinity and chlorophyll concentration, and relate these environmental parameters to zooplankton distribution and abundance. These observations provide useful insight into how environmental attributes and plankton co-vary at large spatial and temporal scales. However, field measurements taken at these large scales may not be representative of the scale(s) most important to the behavior of individual planktonic organisms. Conversely, behavioral ecologists often bring planktonic organisms into a controlled laboratory or mesocosm setting to study properties of individuals such as physiological rates and behavior. These observations have yielded significant insights into small scale interactions between individuals. However, it is not always clear how these interactions affect large scale processes in the plankton (Kjørboe 2008a).

The overarching aim of my research was to determine how interactions between copepods and adjacent trophic levels (i.e. resources and predators) shape copepod behavior and how these behavioral alterations scale up to impact population dynamics. Despite the importance of predicting copepod population growth for effective fisheries
management, field surveys describing the prevalence of fertilization limitation in coastal copepod species are lacking. Therefore, the aim of Chapter 2 was to determine the prevalence of *in situ* fertilization limitation among two dominant marine copepod species (*Temora longicornis* and *Eurytemora herdmani*) that co-occur in the Damariscotta River estuary (Walpole, Maine) and differ in their reproductive strategy. Additionally, I aimed to determine what population, community and ecosystem level factors were significantly associated with copepod fertilization success in the field.

Predation has long been documented as a key structuring process in aquatic ecosystems through both consumptive (Paine 1966; Carpenter et al. 1985) and non-consumptive mechanisms (Peacor and Werner 2001; Werner and Peacor 2003; Preisser et al. 2005). However, the potential for predators to affect copepod mating behavior and subsequent population growth, has received little attention. Therefore, the aim of Chapter 3 was to determine the effects of a common mysid shrimp predator, *Neomysis americana*, on *Eurytemora herdmani* mating behavior and subsequent offspring production.

In Chapter 4, I explored the behavioral and fitness consequences of copepods ingesting phytoplankton that form harmful algal blooms (HAB). In recent decades, climate change and eutrophication have led to an increase in the frequency and geographic range of HAB blooms (Anderson 1994). Lack of grazing pressure is a key factor responsible for bloom proliferation (reviewed by Roelke 2000). Copepods are important grazers of harmful algal blooms, and much work has been done to determine the effects of harmful algae on copepod feeding, physiology and fitness. However, the effects of ingesting harmful algal bloom species on copepod behavior remain relatively unexplored.
CHAPTER 2

THE PREVALENCE OF FERTILIZATION LIMITATION IN THE COASTAL MARINE COPEPODS TEMORA LONGICORNIS AND EURYTEMORA HERDMANI

Abstract

We measured the in situ fertilization success of two marine copepod species, Temora longicornis and Eurytemora herdmani from May to October 2008 in coastal Maine. Temora longicornis is a free spawning species that releases eggs into the ambient seawater after mating. In contrast, Eurytemora herdmani carries eggs in an egg sac until they hatch. The proportion of fertilized eggs within E. herdmani egg sacs was significantly higher than the freely spawned clutches of T. longicornis, which may be a result of the asymmetrical costs associated with carrying versus spawning an unfertilized egg. There was variation in the proportion of E. herdmani females carrying egg sacs throughout the season indicating that this species does suffer from fertilization limitation and that the presence or absence of an egg sac is a reliable proxy for a whether or not a female is fertilized. Results from our survey suggest that T. longicornis fertilization is significantly associated with temperature and chlorophyll concentration. Eurytemora herdmani fertilization is significantly associated with an interaction between predators and sex ratio and an interaction between population density and sex ratio. Together, these results suggest that E. herdmani and T. longicornis experience fertilization limitation in nature. Furthermore, these results highlight the importance of community and ecosystem level processes in addition to population level processes in dictating the fertilization success and subsequent productivity of copepods.
Introduction

Copepods are the most numerous multicellular organisms inhabiting the world’s oceans (Mauchline 1998). In marine food webs, calanoid copepods perform vital ecosystem functions, including trophically connecting phytoplankton productivity to higher trophic levels including many commercially important species (Runge 1988) and impacting biogeochemical processing by contributing large portions of carbon (in the form of sinking fecal pellets) to the deep ocean (Mauchline 1998; Dagg et al. 2003). The important position copepods hold in marine food webs has prompted investigators worldwide to examine factors limiting their population growth. Traditionally, most population growth experiments have focused on the most basic aspect of copepod production (i.e. female fecundity) by measuring egg production rate (Uye 1982; Huntley and Lopez 1992; Hopcroft et al. 1998, Uye 1982) in relation to environmental factors such as temperature or food concentration (reviewed by Bunker and Hirst 2004). Additionally, researchers often monitor egg hatching success for a more accurate depiction of copepod fitness and potential recruitment. However, results from these studies frequently yield very low hatching success (Dam and Lopez 2003, Ianora et al. 2007, Maps et al. 2005), with the cause for low hatching often not determined (but see Ianora et al. 1989).

There are many potential causes of hatching failure in copepod production experiments such as production of resting eggs, maternal assimilation of toxins and the production of unfertilized eggs. The ecological impact of resting eggs (i.e. fertilized eggs that remain dormant in the sediment until favorable conditions induce hatching) on copepod population dynamics has received considerable attention in recent decades.

Marine copepods have internal fertilization, where a male deposits a spermatophore onto a female’s urosome during copulation. The spermatophore empties into the females genital atrium where eggs are fertilized before being extruded (Mauchline 1998). Among marine copepods, species can be classified as having one of two egg production strategies. There are free spawning species that release their eggs into the surrounding seawater and brooding species that carry their eggs in an egg sac until they hatch (Mauchline 1998). Brooding species often have lower egg production rates than spawning species. However, the survival rate is much higher for brooded versus spawned eggs (Kiørboe and Sabatini 1994) with the mortality rate of spawned eggs being as high as 99% at certain times of the year due to predation, sedimentation and cannibalism (Peterson and Kimmerer 1994; Kiørboe and Sabatini 1994). Females that brood their eggs can avert such high egg loss but they, themselves, are at a greater risk. Females carrying egg sacs are often more vulnerable to predation through increased conspicuousness and reduced escape behavior (Magnhagen 1991; Maier et al. 2000). If copepods commonly produce unfertilized eggs in nature when mating opportunities are rare, the cost would likely be much higher for egg brooding species.
Fertilization limitation occurs when population growth becomes limited by successful mating events (Gerritsen 1980). Kiørboe (2006) suggests that one indication that fertilization limitation is occurring in a population is when not all mature females are fertilized. Field observations documenting fertilization limitation within marine copepods have only been conducted on a few species. For example, Hopkins (1982) assessed the fertilization status of the offshore species, *Euchaeta norvegica in situ* by recording the number of females with attached spermatophores. This is a reliable proxy for fertilization in *E. norvegica*, because (unlike most copepod species) females do not discard spermatophores after mating (Hopkins 1982). Furthermore, Uye (1995) used the ratio of *Oithona davisae* females carrying eggs: females without eggs as a proxy for the proportion of breeding females in the population. However, this species is known to carry unfertilized eggs (Uchima 1985, Kiørboe 2007) which highlights the need to determine the fertilization status of clutches for egg carrying species in order to validate the use of egg sac presence versus absence as a metric for fertilization.

Assessing the field fertilization status of coastal, marine copepods is particularly challenging because they discard spermatophores after mating (Ohtsuka and Huys 2001) and many species broadcast spawn their eggs into the ambient seawater. Until recently, assessing fertilization required staining live eggs within an hour of egg deposition (Ianora et al. 1989, Ianora personal communication 2007). Additionally, staining the eggs required multiple steps, such as rinsing several times with fresh seawater. These steps increased egg loss during processing and made it difficult to analyze several samples simultaneously. More recently, Zirbel (2007) modified this staining technique making it
possible to stain preserved eggs in a single step, thus greatly increasing the number of samples that can be processed simultaneously.

Here we investigate the in situ fertilization success of two marine copepod species, *Temora longicornis* and *Eurytemora herdmani* within coastal Maine. Utilizing field observations and laboratory experiments, we asked: 1) What is the prevalence of fertilization limitation within *T. longicornis* and *E. herdmani* populations? 2) What population and community level factors correlate with times of low fertilization? 3) How does the actual measured fertilization success compare with theoretical fertilization success based on published demographic models?

**Methods**

**Survey methods.** Our survey was conducted from the dock at the Darling Marine Center located in the Damariscotta River estuary, Walpole, Maine (43°56’ N, 69°35’ W). The Damariscotta River estuary is a glacially carved drowned river valley that receives very little freshwater input from the Damariscotta Lake. Therefore, conditions in the estuary are very similar to the surrounding coastal waters. Furthermore, the estuary is vertically and horizontally well mixed (McAlice 1977).

Five-day zooplankton surveys were conducted each month May through October 2008 from the dock during ebb tide. Sampling began 2.5 h before sunrise to account for differences in copepod’s diel behavior and to collect animals at the end of the night which is their most active period (Mauchline 1998). The water depth at our sampling location is 10-15 m (at low and high tide respectively). For sample collection, a plankton net (1 m in diameter) with a mesh size of 250 µm and a flowmeter affixed to the center of the net opening was slowly lowered and suspended in the middle of the water column for
app. 15 min. At the end of the sampling period, the cod end was emptied into a bucket containing surface seawater to dilute the zooplankton sample. The net was then rinsed and placed back in the water for a second collection. The purpose of dual collections was to maximize the number of females caught each day while minimizing the opportunity for animals to mate under crowded conditions within the sampling net. Live zooplankton samples were immediately transported to the lab where *Temora longicornis* and *Eurytemora herdmani* females were removed from samples using a wide bore pipette. Females were separated from samples within 20 minutes of sample collection and placed in individual 3 mL well plates in a temperature-controlled room set to ambient temperature (11-18°C). After females were separated, the remaining portion of the zooplankton samples were preserved in 4% buffered formalin.

**Environmental sampling.** Measurements of temperature and chlorophyll concentration were obtained from the Perry Phytoplankton and Optics Lab at the Darling Marine Center. Samples were collected from the dock 3-5 days a week in the morning and analyzed for chlorophyll concentration using a Turner designs fluorometer. Average daily water temperature was recorded on temperature data loggers. Because temperature and food concentrations were not measured at the exact timing of our zooplankton collections, our measurements were inappropriate for addressing small scale temporal differences in these parameters. Therefore, temperature and chlorophyll values were averaged across each sampling day and the 2 days prior. This average more accurately reflects the resolution of our temperature and chlorophyll measurements. Furthermore, these average values are likely more relevant because they encompass the time it takes for food and temperature conditions to affect egg production (Checkley 1980). Salinity
measurements were unavailable during our sampling period. However, previous surveys of our site show that salinity typically varies less than 5 ppt during May through October (Thompson et al. 2006). This fluctuation is much less than values shown to affect behavior (Seuront 2006) or reproduction in other copepods species (Devreker et al. 2009; Holste et al. 2009).

**Egg collection.** Every 40-60 min, females were visually inspected for the presence of eggs. For *Temora longicornis*, which spawns their eggs freely, eggs were collected from the bottom of the wells but females were allowed to remain in wells for the remainder of the incubation period (12 h). Using a drawn Pasteur pipette, eggs were transferred to a 96 well plate and preserved in 4% buffered formalin. All eggs from one female were combined into one well, plates were wrapped in parafilm and vacuum sealed in plastic bags to prevent evaporation during storage. The egg production rate of *T. longicornis* was calculated throughout our survey by counting the total number of eggs produced female⁻¹ 12 h⁻¹. Our egg collection method varied for *Eurytemora herdmani*, which produces an attached egg sac. Females were monitored every 40-60 min until they produced an egg sac. Once a female developed an egg sac, the female + egg sac were placed in 96 well plates and preserved with 4% buffered formalin. As above, well plates were wrapped in parafilm and vacuum sealed in plastic bags.

**Fertilization analysis.** The fertilization status of the preserved clutches was analyzed within 6 months after collection and preservation. To begin analysis, eggs were transferred to a depression slide containing 100 µL of 50 µg of the vital fluorescent stain for visualizing cellular nuclei, Hoechst 33342 (mL 15 ppt filtered seawater)⁻¹. To minimize dilution of the stain, eggs were carefully transferred with minimal media using
a drawn Pasteur pipette and no more than 30 eggs slide$^{-1}$ were analyzed. Slides were covered with a coverslip, sealed in a plastic container and incubated for 10 minutes in the dark at 12˚C. Slides were observed under an Olympus BX-FLA fluorescence microscope with an ultraviolet laser (364 nm $\lambda$). For each egg, the presence of a male and female pronuclei or the presence of a single female pronuclei were observed as a proxy for a fertilized or un-fertilized egg, respectively (Ianora et al. 1989; Ianora and Poulet 1993).

Misidentification of eggs can occur in two ways. First, a polar body could be mistaken as a male’s pronuclei and the egg would therefore, be scored as fertilized when it was unfertilized. Secondly, a fertilized egg that was preserved during fusion could be mistaken as an unfertilized egg. Therefore, preliminary observations were conducted on freshly-laid eggs (both fertilized and unfertilized) and monitored for 4 h at 18˚C. For fertilized eggs, the male and female pronuclei were still visible over an hour after egg deposition, verifying the presence of two nuclei as a good proxy for fertilization. For unfertilized eggs, only one pronuclei was observed (no visible polar body) and remained unchanged for 4 h. To further verify that eggs with just one pronuclei were un-fertilized, oocytes were dissected from 5 gravid females (of both species) for comparison. Unfertilized eggs appeared identical to the dissected oocytes (Ianora 1989).

Zooplankton analysis. Our aim was to obtain an ecological snapshot of the zooplankton community to determine what factors correlate with in situ fertilization success in *Temora longicornis* and *Eurytemora herdmani*. For each sample, the gender and species of adult copepods were identified allowing for estimates of population densities, sex ratios and relative species abundance. These values were corrected for the number of females removed for fertilization analysis. No attempt was made to identify
immature or nauplii stages because our net did not allow for quantitative sampling of these animals and because our main focus was on factors that could affect fertilization success. Non-copepod zooplankton such as polychaetes, predatory mysid shrimp and gelatinous zooplankton were identified to genus or species when possible.

The number of gravid *Eurytemora herdmani* females carrying extruded egg sacs versus gravid females without egg sacs was also determined. In this case, we define gravid as females that possess dark ripened ovaries which represents a female’s potential to be mated. The proportion of gravid females carrying egg sacs out of the total number of gravid females is commonly used as a proxy for mated females (Hopkins 1982, Uye 1995, Kramer et al. 2008). From these females, a subset ($n = 75$) was haphazardly selected and the number of eggs contained within their egg sac was counted to estimate clutch size. To correct for any females that lost their egg sac during preservation or handling, we searched for detached egg sacs within the samples to obtain an egg sac volume$^{-1}$ estimate. Very few detached egg sacs (5 egg sacs out of 60 zooplankton samples) were found. Supplemental observations indicated that egg sacs remain attached to *E. herdmani* females during the preservation process and persist despite intense physical disturbances (such as vortexing the sample media). Additionally, when trying to detach egg sacs from females (for egg staining purposes) the egg sacs often had to be scraped away from the females using a metal probe.

**Fertilization model.** The binary nature of *Eurytemora herdmani* fertilization (see Results) facilitated comparison of the observed proportion of fertilized females with theoretical values predicted by Kiørboe’s (2006) model for the proportion of fertilized
females in a population. Specifically, the proportion of fertilized females in a population ($\sigma$) is described as:

$$\sigma = [1 - \exp\left(-\frac{\beta C_T \phi t}{\phi + 1 + \alpha \beta C_T}\right)]$$

where $\beta$ is the search volume rate of mate-seeking males, $C_T$ is the population density, $\phi$ is the male: female sex ratio, $t$ is the time interval between fertile clutches and $\alpha$ is the spermatophore production time. Population density and sex ratio were parameters measured during our survey. To estimate the remaining parameters, published literature values were used when possible (see Table 1.1). When literature values did not exist or would be inappropriate supplementary experiments were conducted to estimate parameters.

Table 1.1 Description of symbols used in the paper along with parameter estimates and sources.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Units</th>
<th>Source</th>
<th>Value (mean ± s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma_o, \sigma_p$</td>
<td>Proportion of fertilized females</td>
<td>-</td>
<td>Observed this study, predicted from Kiørboe 2006</td>
<td>0.73 ± 0.11</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Search volume rate</td>
<td>L/day$^{-1}$</td>
<td>Calculated from Kiørboe and Bagøen 2005</td>
<td>72 ± 3</td>
</tr>
<tr>
<td>$C_T$</td>
<td>Density of adults</td>
<td>m$^{-3}$</td>
<td>Observed this study</td>
<td>104 ± 20</td>
</tr>
<tr>
<td>$\phi$</td>
<td>Male: female sex ratio</td>
<td>-</td>
<td>Observed this study</td>
<td>1.8 ± 0.9</td>
</tr>
<tr>
<td>$t$</td>
<td>Time interval between clutches</td>
<td>days</td>
<td>Estimated from Lloyd 2005</td>
<td>7.2 ± 0.7</td>
</tr>
<tr>
<td>$r$</td>
<td>Radius of detection (maximum)</td>
<td>cm</td>
<td>Measured this study</td>
<td>0.46 ± 0.07</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Spermatophore production time</td>
<td>days</td>
<td>Estimated from Choi and Kimmerer 2009</td>
<td>1.06 ± 0.14</td>
</tr>
<tr>
<td>$u$</td>
<td>Male swimming speed</td>
<td>cm s$^{-1}$</td>
<td>Measured this study</td>
<td>0.66 ± 0.03</td>
</tr>
</tbody>
</table>
To estimate the search volume rate of *Eurytemora herdmani*, an equation from Kiørboe and Bagøien (2005) was used for a cruising male searching for a female’s pheromone plume, consistent with observations of *E. herdmani* mating interactions described by Katona (1973). For males detecting a female’s pheromone plume, search volume rate ($\beta$) can be described as:

$$\beta_{\text{Sphere, cruising}} = \pi r^2 u$$

where $r$ is the radius of the pheromone sphere surrounding a female and $u$ is the male’s swimming velocity.

**Supplemental experiments.** Supplementary experiments were conducted to determine the swimming speed of male and female *Eurytemora herdmani* and *Temora longicornis* at the lowest and highest temperatures measured in our survey (i.e. 11˚ and 18˚ C, respectively) because temperature can affect swimming speed within some copepod species and have impacts on their mate encounter rates (Kramer et al. 2011).

Copepod swimming speed was measured at high and low temperatures in a Schlieren optical system described by Strickler (Strickler 1998) and further described by (Doall et al. 1998). Copepods were first allowed to acclimate to appropriate temperatures by adjusting incubation temperatures by no more than 2˚ C day$^{-1}$. Observations were recorded onto DVDs and digitized using Prism Video Converter 1.82 software. Films were split into clips using SolveigMM Video Splitter. When necessary, clips were further processed in Adobe Premier Pro. CS5.5 to enhance contrast. Swimming paths were analyzed using LabTrack software. A total of 40 trajectories from 4 replicate trials were analyzed. An analysis of variance (ANOVA) determined that there were no differences between replicate trials; therefore we pooled data across trials.
(Kramer et al. 2011). Swimming speed values used in the model were drawn from a truncated normal distribution with a mean and standard error of our measured swimming speeds and used to calculate the search volume rate (β).

To determine the signal size (r) for Eurytemora herdmani, mating experiments were conducted using the same optical setup and analysis described above. The maximum distance at which a mate seeking male detected a female E. herdmani (0.59 cm) was measured based on a total of 5 mating events from 7 replicate trials.

The time interval between fertile clutches (t) was approximated using an equation describing how the reproductive cycle of a congeneric species, Eurytemora affinis, depends on temperature (Lloyd 2005).

\[
t = 169.07 \times T^{-1.3114}
\]

The time interval, t is the sum of the interclutch duration (the time between a female’s clutch hatching and the production of a new clutch) and the egg hatching time which depends on the temperature (T). This reference lacked an estimate for the variation surrounding the total cycle duration. Therefore, we used the standard error of t estimated by Devreker et al. (2009), who reports the reproductive cycle length of Eurytemora affinis. To estimate the spermatophore production time (\( \alpha \)), we used the mean and standard error values measured by Choi and Kimmerer (2009) for Eurytemora affinis (1.06 ± 0.14 days).

The fertilization model was then used to estimate fertilization for each sample date based on the observed population density, sex ratio and temperature. A parametric bootstrap method was used to assess uncertainty in predicted fertilization estimates.

Swimming speed (u) and spermatophore production rate (\( \alpha \)) were randomly drawn from
truncated normal distributions with observed mean and standard error. The distribution was truncated at the upper and lower observed values to avoid nonsensical values. Maximum detection radius (r) was assumed to be fixed. The median, upper and lower confidence intervals for predicted fraction fertilized were taken from the distribution of 10,000 bootstrapped estimates.

**Statistical model.** All statistics were performed in R (R Core Development Team, 2011). To determine differences between the proportions of fertilized eggs within *Temora longicornis* versus *Eurytemora herdmani* clutches, we fit a generalized linear model (GLM) with logit-link function for binomial distribution with species as our explanatory variable.

For *Eurytemora herdmani*, we further aimed to determine if the presence or absence of an egg sac is an appropriate indicator that a female is fertilized. To determine this, we asked whether *E. herdmani* fertilization varies within a female’s clutches (indicating that females are laying a high proportion of unfertilized eggs) or if fertilization within clutch is predictably high but the proportion of females carrying egg sacs within a population varies. We compared the coefficient of variation of the fertilization status of female’s clutches versus the proportion of fertilized females within the population to determine the most appropriate proxy to use. We found the proportion of fertilized eggs to be predictably high within *E. herdmani* clutches (see Results) and thus used the presence or absence of an egg sac as a proxy for fertilization for all subsequent analyses.

For both *Eurytemora herdmani* and *Temora longicornis*, we determined what factors were associated with fertilization success by fitting a generalized linear mixed
model (GLMM) with a logit-link function for binomial distribution. We considered population density, sex ratio, relative species abundance, predator presence, temperature, chlorophyll concentration and tidal height as fixed explanatory variables (along with *a priori* selected interactions, See Table 1.2). We used month as a random variable to account for variation within the data associated with the sampling period. All data were pooled by sampling date. The benefit of comparing measurements pooled by day is that this lessens the error associated with sampling a highly patchy environment.

Table 1.2 Description of *a priori* selected interaction terms to include the generalized linear mixed model assessing the effect of ecological and environmental parameters on *Temora longicornis* and *Eurytemora herdmani* fertilization success.

<table>
<thead>
<tr>
<th>Interactions</th>
<th>Primary justification for inclusion in model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density x Sex ratio</td>
<td>Low densities may have more of an effect when the sex ratio is skewed (and vice versa).</td>
</tr>
<tr>
<td>Density x Chlorophyll concentration</td>
<td>Higher food concentration likely supports higher copepod densities.</td>
</tr>
<tr>
<td>Density x Relative abundance</td>
<td>A combination of a low population density and low relative abundance may increase the occurrence of heterospecific mating events (gamete wastage).</td>
</tr>
<tr>
<td>Density x Mysid abundance</td>
<td>Mysids may reduce copepod densities through predation.</td>
</tr>
<tr>
<td>Sex ratio x Mysid abundance</td>
<td>Mysids may skew copepod sex ratio by preying differentially on a gender.</td>
</tr>
<tr>
<td>Sex ratio x Relative abundance</td>
<td>A combination of a male-skewed sex ratio and low relative abundance may increase the occurrence of heterospecific mating events (gamete wastage).</td>
</tr>
<tr>
<td>Temperature x Chlorophyll concentration</td>
<td>A combination of high temperatures and high food concentration may increase egg production rate, giving females less time be mated.</td>
</tr>
</tbody>
</table>
Due to differences in egg production, the response variables used differed between species. *Temora longicornis* lays unfertilized eggs within their clutch. Therefore a female’s fertilization status is the proportion of fertilized eggs within her clutch. The number of female clutches that could be stained was ultimately dictated by the number of gravid females caught in the sample and whether or not those females laid eggs during the incubation period. The number of females analyzed varied from 1-8 females for each sample. Therefore, we added an additional random factor of date to our *T. longicornis* analysis to account for the fact that different numbers of females were analyzed on our sampling dates.

*Eurytemora herdmani* egg sacs contained a very high proportion of fertilized eggs (0.95 ± 0.01, mean ± s.e., See Results). As such, a female’s fertilization status can be thought of as a binary response (1 = fertilized female, 0 = unfertilized female). Therefore, the proportions of females carrying egg sacs out of the total number of females were used. The total number of females scored per sampling day was 91 ± 11, (mean ± s.e.).

Results from previous studies suggest that temperature and food concentration can have substantial effects on egg production rate (Bunker and Hirst 2004). Furthermore, temperature and chlorophyll concentration were found here to be significantly associated with *Temora longicornis* fertilization success (See Results, Table 1.3). Therefore, to analyze differences in the number of eggs produced, we used a zero-inflated Poisson regression with chlorophyll concentration and temperature as our explanatory variables. The number of eggs produced by *T. longicornis* in 12 h$^{-1}$ was used as our response variable. There were a large number of zeros (females that did not produce eggs) within
this dataset. Therefore, the zero-inflated Poisson regression was necessary because it takes into account the binary of the component of the data (i.e. females that produced or did not produce eggs) as well as numerical component of the data (i.e. the number of eggs produced).

**Results**

On average, 95% of *E. herdmani* eggs were fertilized. However, the proportion of females carrying egg sacs within the population was lower (0.73 ± 0.11, mean ± s.e.) and had a higher coefficient of variation (15.6% versus 6.7%). Therefore, we used the presence or absence of an egg sac as a proxy for a fertilized or un-fertilized female, respectively.

![Figure 1.1](image)

Figure 1.1 Average number of fertilized (white) and unfertilized (grey) eggs within the clutches of an egg-brooding copepod, *Eurytemora herdmani* and a free spawning copepod, *Temora longicornis*. Females were captured from the field and their clutches were stained with a nuclei specific probe (*n* = 70 and *n* = 134 for *E. herdmani* and *T. longicornis*, respectively). Proportion of fertilized eggs were compared using a generalized linear model with a binomial distribution and logit-link function (*Z* = -13.45, *p* < 0.001).
During our survey, the average temperature ranged from 11˚ to 18˚ C with maximum temperatures observed in July and minimum temperatures in May and October. Average chlorophyll concentrations ranged from 1.4 to 6.1 µg carbon L⁻¹ with minimum and maximum values observed in September and August respectively. The most abundant predator present in our samples was the mysid shrimp, Neomysis americana. Mysid abundance ranged from 0 to 226 individual m⁻³ with minimum and maximum densities observed in May and August, respectively.

*Temora longicornis* population densities ranged from 3 to 116 individuals m⁻³ with minimum densities observed in May and October and maximum densities observed in June and July. *Temora longicornis* sex ratio ranged from 0.67 to 2.38 (male: female) with minimum and maximum values in August and October respectively (Fig. 1.2).

Figure 1.2 Seasonal dynamics of the fertilization status of *Temora longicornis* (A), chlorophyll concentration (B) and temperature (C) from the Damariscotta river estuary in coastal Maine, USA. Two replicate samples were collected for 5 consecutive days from May to October, 2008. Factors that affect fertilization success were determined by fitting a generalized linear mixed model (GLMM) with a logit-link function for binomial distribution. Month and sampling day were considered as random variables. Chlorophyll concentration negatively affected *Temora longicornis* fertilization (GLMM, p-value = 0.02, binomial) and temperature positively affected fertilization (GLMM, p-value = 0.002, binomial).
*Eurytemora herdmani* had higher maximum population densities ranging from 4 to 822 individuals m$^{-3}$ with maximum densities in July and August and minimum densities in May and September. *Eurytemora herdmani* sex ratio ranged from 0.47 to 3.94 (male: female) with highest values in May and lowest values in August.

![Graph A](image1.png)  
![Graph B](image2.png)  
![Graph C](image3.png)  
![Graph D](image4.png)

Figure 1.3 Seasonal dynamics of the fertilization status of *Eurytemora herdmani* (A), *E. herdmani* population density (B) mysid predator abundance (C) and male: female sex ratio (D) from the Damariscotta river estuary in coastal Maine, USA. Two replicate samples were collected for 5 consecutive days from May to October, 2008. Factors that affect fertilization success were determined by fitting a generalized linear mixed model (GLMM) with a logit-link function for binomial distribution. Month was considered a random variable. Variation in the proportion of fertilized *E. herdmani* females is best explained by mysid abundance (GLMM, $p = 0.003$, binomial) and an interactions between mysid x sex ratio (GLMM, $p = 0.001$, binomial) and density x sex ratio (GLMM, $p = 0.03$, binomial).
*Temora longicornis* egg production rate was highest in May (39 ± 4 eggs female$^{-1}$, mean ± s.e.) and lowest in October (10 ± 2 eggs female$^{-1}$, mean ± s.e.). *Eurytemora herdmani* clutch size was greatest in May (49 ± 8 eggs female$^{-1}$, mean ± s.e.) and smallest in July (11 ± 1 eggs female$^{-1}$, mean ± s.e.). The fertilization status of *Eurytemora herdmani* egg sacs was significantly higher than the freely spawned clutches of *Temora longicornis* (Fig. 1.1. $Z = -13.45, p < 0.001$, GLM binomial distribution).

Table 1.3 Results from a generalized linear mixed describing factors associated with fertilization in two dominant marine copepod species, *Temora longicornis* and *Eurytemora herdmani*. Below is the full model output with only non-significant interactions removed.

<table>
<thead>
<tr>
<th>Species</th>
<th>Term</th>
<th>Coefficient</th>
<th>St. Error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Temora longicornis</em></td>
<td>Intercept</td>
<td>-1.97</td>
<td>2.22</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Population density</td>
<td>0.01</td>
<td>0.009</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Sex ratio</td>
<td>0.42</td>
<td>0.56</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Chlorophyll concentration</td>
<td>-0.48</td>
<td>0.19</td>
<td>0.009 **</td>
</tr>
<tr>
<td></td>
<td>Relative abundance</td>
<td>-3.9</td>
<td>6.29</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>Mysid abundance</td>
<td>-0.0007</td>
<td>0.002</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>0.26</td>
<td>0.12</td>
<td>0.03 *</td>
</tr>
<tr>
<td></td>
<td>Tidal height</td>
<td>0.09</td>
<td>0.23</td>
<td>0.68</td>
</tr>
<tr>
<td><em>Eurytemora herdmani</em></td>
<td>Intercept</td>
<td>0.45</td>
<td>0.95</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Population density</td>
<td>0.47</td>
<td>0.32</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Sex ratio</td>
<td>0.27</td>
<td>0.24</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Chlorophyll concentration</td>
<td>0.01</td>
<td>0.18</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Relative abundance</td>
<td>-0.43</td>
<td>1.79</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Mysid abundance</td>
<td>-1.3</td>
<td>0.4</td>
<td>0.003 **</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>0.04</td>
<td>0.06</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Tidal height</td>
<td>-0.07</td>
<td>0.06</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Density x Sex ratio</td>
<td>-0.41</td>
<td>0.19</td>
<td>0.03 *</td>
</tr>
<tr>
<td></td>
<td>Sex ratio x Mysid</td>
<td>0.98</td>
<td>0.3</td>
<td>0.001 **</td>
</tr>
</tbody>
</table>
Results from our field survey indicate that *Temora longicornis* fertilization is positively associated with temperature (Table 1.3, GLMM, *p*-value = 0.002, binomial) and negatively associated with chlorophyll concentration (Table 1.3, GLMM, *p*-value = 0.02, binomial). Variation in the proportion of fertilized *E. herdmani* females is negatively correlated with mysid abundance (Table 1.3, GLMM, *p*-value = 0.003, binomial). An interaction between low (female-skewed) sex ratios and mysid abundance is negatively associated with fertilization (Table 1.3, GLMM, *p*-value = 0.001, binomial). Male-skewed sex ratios combined with high population densities were negatively associated with fertilization (Table 1.3, GLMM, *p*-value = 0.03, binomial). The zero-inflated Poisson regression model predicting the number of eggs produced by *Temora longicornis* in 12 h from chlorophyll concentration and temperature showed a significant positive association between egg production and an interaction between chlorophyll concentration and temperature ($\chi^2 = 198.97$, df = 2, *p* < 0.001).
Figure 1.4. Predicted versus observed proportion of fertilized *Eurytemora herdmani* females. Values were predicted based on demographic model by Kiørboe (2006) describing how population density, sex ratio and swimming behavior affect the probability that a female is mated. Confidence intervals were generated from uncertainty in some of our parameter estimates. Observed values were obtained from 5 day field surveys of *E. herdmani* populations conducted at 3 week intervals from May to October 2008 (n = 30) in coastal Maine, USA. Dotted horizontal line represents the maximum fraction fertilized (i.e. 1). Dashed diagonal line represents 1:1 relationship between observed and predicted values.

Kiørboe’s (2006) model predicted higher fertilization values than those observed during our survey (Fig 1.4). When we plot the observed and predicted values together versus our three model input values we see that the model overestimates the fraction fertilized based on the observed sex ratio and temperature values (Fig. 1.5).

Average *Temora longicornis* swimming speed at 18° C was 0.61 ± 0.01 cm s<sup>-1</sup> (mean ± s.e.) This was significantly higher than the average speed at 11° C, 0.49 ± 0.04, mean ± s.e., (t-test, t = -4.5, p < 0.001). There was no difference between *Eurytemora herdmani* swimming at high and low temperatures (t-test, t = 1.1, p = 0.3).
Figure 1.5. Observed (blue circles) and predicted (black circles) proportion of fertilized *Eurytemora herdmani* females versus the observed model inputs population density (A), male:female sex ratio (B) and temperature (C) obtained from 5 day field surveys of *E. herdmani* populations conducted every 3 weeks from May through October 2008 in coastal Maine, USA.

**Discussion**

The attached egg sacs of *Eurytemora herdmani* contained a significantly higher percentage of fertilized eggs compared to the freely spawned clutches of *Temora longicornis* (Fig. 1.1). This could reflect the different costs associated with producing unfertilized eggs for these species. For an egg brooding female, carrying around an egg sac can increase her vulnerability to predators and increase metabolic demands (Magnhagen 1991; Maier et al. 2000). It is likely that females would be selected to avoid this when eggs are unfertilized and the gain of enhanced offspring survival is not possible. Therefore, *E. herdmani* may possess a physiological mechanism to prevent carrying an unfertilized clutch. It is unknown whether *E. herdmani* releases unfertilized eggs into the water or if they are able to hold on to eggs within their oviducts until fertilization occurs. Nonetheless, the fertilization status of *E. herdmani* eggs sacs being
high means that the presence versus absence of an egg sac is an appropriate proxy for a fertilized female.

The cost of eggs going unfertilized is likely lower for *Temora longicornis*. Producing eggs is unlikely to increase their vulnerability towards predators or increase the cost of swimming because *T. longicornis* spawn their eggs freely. However, females are still investing valuable nutrients in these eggs. The question is why don’t they hold on to their eggs or resorb them? Oosorption, the process of re-absorbing mature eggs to conserve nutrients during times of starvation or to maintain a constant supply of newly matured eggs, has been well documented in insects (Moore et al. 2007). Egg resorption has also been demonstrated in the marine copepod, *Calanus finmarchicus* in response to days of starvation (Niehoff 2004). However, this process has never been demonstrated in response to lack of mating in copepods which could be due to the short time window that some copepod species are able to produce eggs. Even under optimal laboratory conditions, *T. longicornis* females can only produce eggs for approximately 18 d (Sichlau and Kiørboe 2011). Therefore, it may be a better strategy to continue to produce high quality eggs during this time window even when mating rates are rare.

Our results indicate that not being recently mated is a factor that prevents *Eurytemora herdmani* and *Temora longicornis* from reproducing at the full capacity. In this system, *T. longicornis* fertilization is associated with food concentration and temperature (Table 1.3, Figs 1.2). Increased temperatures were positively correlated with *T. longicornis* fertilization (Table 1.3). This could in part be due to increased encounter rates resulting from *T. longicornis* increasing their swimming speed in warmer temperatures. Furthermore, if males produce more spermatophores in response to higher
temperatures, then this could lead to higher fertilization rates. However, it is unknown how temperature affects spermatophore production rate.

Food concentration (in the form of chlorophyll concentration) was negatively associated with *T. longicornis* fertilization (Table 1.3). Low quality foods such as some dinoflagellate species have been shown to decrease spermatophore quality and subsequent fertilization success in the congener, *Temora stylifera* (Ianora et al. 1999; Caldwell et al. 2004). However, we do not have data to address this hypothesis here. An increase in food concentration coupled with warmer temperatures increased the number of eggs *T. longicornis* produced. *Temora longicornis* can produce 25 ± 4 (mean ± s.e.) fertilized eggs after one mating event (unpublished observations). Therefore, speeding up egg production may decrease the amount of time that females have to mate in between spawning events. However, egg production alone cannot explain the effect of food and temperature on fertilization. At times during our survey when temperature was high but food concentration was not, egg production rate was lower. Having a lowered egg production rate could give *T. longicornis* more time to mate between spawning events.

*Eurytemora herdmani* fertilization was negatively correlated with mysid abundance and an interaction between sex ratio and density and an interaction between sex ratio and mysids (Table 1.3). The interaction between density and sex ratio actually had a significant negative effect on *E. herdmani* fertilization indicating that when population densities were high and sex ratio was male-skewed fertilization success was lower (Fig. 1.3). This seems counterintuitive. However, during our survey, we commonly observed substantially male-skewed samples (i.e. 4x more males than females). Therefore, it is possible that when populations are dense and male-skewed,
males may actually negatively impact fertilization success of females through male-male competition (i.e. rough handling of females inducing stress). In behavioral observations, males often grab mating males with their antennae (personal observation). Furthermore, in many species, females can become damaged by mating events that are too frequent (Arnqvist and Nilsson 2000; Parker 2006). However, this has not been fully explored for marine copepods.

The negative effect of mysid predators on *E. herdmani* fertilization could be due to predators consuming copepods, reducing density and thus, reducing mating events. However, we did not detect a significant interaction between mysid presence and population density from our survey results (Table 1.3). Furthermore, mysids were most abundant during the month of August when *E. herdmani* density was at a seasonal high (Fig 1.3). There was a significant interaction between mysids and sex ratio (Table 1.3). This interaction indicates that at times of high mysid abundance and low (female-skewed) sex ratio, *E. herdmani* fertilization was negatively affected. In other copepod species such as *Oithona daviseae*, female fertilization is limited when populations are strongly female-skewed (Kiørboe 2007). Mysids could also negatively impact fertilization status by differentially preying on male *E. herdmani* and skewing the sex ratio. It is unclear whether or not mysids are responsible for skewing the sex ratio through male ingestion. Lasley-Rasher and Yen (2012) found no difference between the number of male and female *E. herdmani* ingested by *Neomysis americana* in small (15-1000 mL) containers. However, in the field male *E. herdmani* may encounter mysid predators more because they swim significantly faster than females (unpublished observation). Finally, *E. herdmani* may have reduced their mating frequency in response to elevated mysid
predator abundance. In manipulative experiments, *Eurytemora herdmani* reduced mating frequency and offspring production in the presence of a mysid predator cue even in the absence of an actual predator (Lasley-Rasher and Yen 2012).

The observed proportion of fertilized *Eurytemora herdmani* females was lower than that predicted by the demographic model (Fig 1.4). The effect of population density agrees reasonably well (Fig 1.5 A). However, we often detected sex ratios that were quite male-skewed and at these times fertilization success was much lower than expected (Fig 1.5 B). This could indicate that sex ratio is only important to a threshold level of males and beyond that threshold, other factors control fertilization success. Alternately, this may indicate that there is a deleterious effect of having too many males in the population, warranting further behavioral experiments.

*Eurytemora herdmani* fertilization did not follow an expected trend with our observed temperature values. However, we made some key assumptions when estimating parameters, so it is important to interpret these results prudently. In our model, we used an equation developed for *E. affinis* to estimate the reproductive cycle duration in response to temperature. If *E. herdmani* cycles are shorter than our estimates, this could lead to an overestimation of fertilized females due to there being less time to mate between clutches. We also used an estimate of spermatophore production time based on *E. affinis* from Choi and Kimmerer (2009). However, it is possible that *E. herdmani* males produce spermatophores at a slower rate which would lead to an overestimation of fertilized females. Finally, if there are factors that cause *E. herdmani* to drop fertilized egg sacs, this may have skewed our results. For example, Devreker et al. (2012) found that *E. affinis* would release fertilized egg sacs when incubated in warm
(18˚ C) temperatures. However, over 160 individual females have been incubated and monitored until egg hatching success at temperatures ranging from 11˚-18˚ C (in this study and Lasley-Rasher and Yen 2012) and we have never observed a dropped egg sac except in cases where the female died. Compared to the multitude of studies focused on *Eurytemora affinis*, there are relatively few studies on *E. herdmani*. Future work targeting the reproductive and behavioral dynamics of this species is needed.

In this study, we determined that both *Temora longicornis* and *Eurytemora herdmani* populations experience fertilization limitation in nature. The factors that are associated with copepod fertilization vary depending on the species being considered. The factors of importance (i.e. temperature, food abundance, predator presence, population density and sex ratio) span individual, demographic and ecological scales. Furthermore, our results indicate *E. herdmani* fertilization is considerably lower than predicted values based on demographic models. This research highlights the need to look beyond demography (i.e. population density and sex ratio) when addressing constraints on copepod population growth.
CHAPTER 3

PREDATION RISK SUPPRESSES MATING SUCCESS AND OFFSPRING PRODUCTION IN THE COASTAL MARINE COPEPOD EURYTEMORA HERDMANI

Abstract

We investigated the effects of a common mysid predator, Neomysis americana on the mating success of an estuarine copepod, Eurytemora herdmani. The presence of a mysid predator, or only a predator cue, reduced copulation frequency and spermatophore transfer success of E. herdmani, and led to a substantial decrease in E. herdmani nauplius production. Thus, mysid predators can influence copepod population growth through non-consumptive processes by reducing the frequency and success of mating events. This highlights the need to look beyond population-level demographic factors (i.e., sex ratio and population density) and consider community-level ecological factors (such as predation risk) when modeling population growth rates of prey species critical to marine food webs.

Introduction

Predation has long been documented as a key structuring process in aquatic ecosystems, demonstrated as a loss due to direct consumption by the predator of its prey (Paine 1966; Carpenter et al. 1985). More recent empirical studies demonstrated that predators also produce visual, physical or chemical cues that confer a ‘predation risk’ to surrounding prey; these non-consumptive effects generate significant changes in prey populations that cascade through entire ecosystems, and often equal or exceed the effects
of predation alone (Peacor and Werner 2001; Werner and Peacor 2003; Preisser et al. 2005). Often, the threat of predation causes prey to reduce activities that make them vulnerable to predators, such as altering the timing and spatial extent of their migration (Ohman et al. 1983; Neill 1990), foraging (Trussell et al. 2002), refuge use (Lima and Dill 1990), or mating behavior (Sih et al. 1990; Koga et al. 1998). Mating and mating-related behaviors are particularly influenced by perceived predation risk because these behaviors are often highly conspicuous and leave prey vulnerable to predators (Magnhagen 1991). As a result, animals may reduce the frequency or duration of their mate-searching (Maier et al. 2000), mate-signaling (Ryan et al. 1982), and copulation behavior (Sih et al. 1990) to avoid detection by predators. Thus, when predation is intense, individual shifts in prey behavior may scale to affect the reproductive success and growth of an entire prey population (Lima 1998). It is especially important to understand these behavioral effects on the population dynamics of species that occupy basal trophic positions in food webs.

Marine copepods play a vital role in coastal food webs as food for a diverse array of predators (e.g., whales, juvenile fish, bivalves, shrimp, etc.), many of which are commercially important species (Runge 1988; Mauchline 1998). Consequently, researchers have identified important factors that influence the productivity of copepod populations (Mauchline 1998). Traditionally, population growth experiments have centered on the effects that environmental factors such as temperature and food concentration have on copepod egg production rate (Checkley 1980; Huntley and Lopez 1992; Mauchline 1998). However, more recent theoretical and experimental studies indicate that demographic factors, such as population density and sex ratio, also strongly
affect population growth rates for copepods (Choi and Kimmerer 2008; Kiørboe 2008b; Kramer et al. 2008).

Field surveys of zooplankton communities indicate that predators mediate the density and species diversity of copepods (Ohman 1986; Hirst and Kiørboe 2002). However, these field surveys primarily assessed the consumptive effects of predators on copepod prey, in terms of reducing population size (Ohman 1986; Hirst and Kiørboe 2002) or skewing copepod sex ratio (Hirst et al. 2010). Recent studies indicate that changes in copepod sex ratio and density can decrease mate encounter rates and thus successful mating events (Choi and Kimmerer 2008, 2009; Kiørboe 2008b). However, the potential for predators to affect copepod-mating behavior via non-consumptive effects, and thus alter subsequent population growth, has received little attention.

Here, we investigate the role of predation risk on the mating success of *Eurytemora herdmani*. Using a series of laboratory and field experiments, we asked: 1) Does the presence of a mysid predator or predator cue interfere with the mating success of *E. herdmani*? 2) What is the nature of the cue responsible for eliciting this effect? 3) Does the presence of a predator cue influence copepod population growth via reduced mating success?

**Methods**

**Study site and organisms.** *Eurytemora herdmani* were collected in the Damariscotta River estuary, Walpole, Maine (43°56’ N, 69°35’ W) using a plankton net with a mesh size of 250 µm, towed obliquely by boat at approximately 30 m depth. Upon collection, animals were immediately transferred into 20 L containers of surface seawater and transported to the lab. *Eurytemora herdmani* was chosen as our target species.
because of its high abundance in our study site, reaching peak densities over 1000 individuals per m$^3$ (See Results, Chapter 2). *Eurytemora herdmani* were sorted from mixed samples under a dissecting microscope and placed in containers filled with filtered seawater at densities less than 25 individuals L$^{-1}$. Containers were gently aerated and placed in a temperature-controlled room set to match ambient water temperature (15˚C). Copepods were fed mixtures of *Rhodomonas lens* and *Tetraselmis* sp. and provided a 14:10 h light-dark cycle. To ensure virgin status for mating experiments, copepodite stage V (CV) females were reared in the absence of males until they reached sexual maturity (1-4 d). Prior to experiments, females were visually inspected under a microscope to be sure that only mature females (i.e., possessing dark oocytes) were used in experiments. Males were held in aerated containers of filtered seawater, separated from females for 1-2 d to allow enough time for spermatophore generation and increase their eagerness to mate during experiments.

To assess consumptive and non-consumptive predator effects on copepod mating success, mating experiments were conducted in the presence of a mysid shrimp, *Neomysis americana*. *Neomysis americana* were used in these assays because they are common in our study area, can achieve high swarm densities up to 10$^5$ m$^{-3}$ in some areas (Jumars 2007), and readily feed on adult *Eurytemora herdmani* (personal observation). Furthermore, mysids are important copepod predators in many systems (Takahashi 2004). *Neomysis americana* were collected at night via vertical plankton tows from the dock at the Darling Marine Center in Walpole, Maine, (43°56’ N, 69°35’ W). A plankton net with 250 µm mesh size was lowered into the water column and kept in the middle of the water column for several minutes and then pulled to the surface. Mysids were kept in
aerated 20 L buckets and fed mixed zooplankton and mixtures of *Rhodomonas lens* and *Tetraselmis* sp. phytoplankton. Mysids were measured under a microscope and only individuals between 8-10 mm were used for experiments.

**Laboratory: Mating behavior in the presence of a predator and predator cue.**

To determine the effects of predation and predation risk on the mating success of *Eurytemora herdmani* 1 male and 1 virgin female were incubated in either 1) the presence of a *Neomysis americana*, 2) a physical predator mimic, 3) a chemical predator cue, or 4) a combined physical and chemical predator cue. To mimic the physical cue of a mysid predator, *Artemia salina* (8-10 mm) were used because they swim around the experimental vessel frenetically and create a feeding current (Barlow and Sleigh 1980), thus producing fluid shear that copepods will escape from. Supplemental experiments confirmed that there was no difference between the frequency of copepod escapes from physical cues generated by *A. salina* and the actual predator, *N. americana* (*t* = 0.53, df = 30, *p* = 0.9, *n* = 16, *t*-test). To apply a chemical cue generated by *N. americana* to appropriate treatments, *N. americana* were incubated with male and female *Eurytemora herdmani* (at a density of 10 mysids + 20 copepods l⁻¹) in filtered, autoclaved seawater for 24 h, allowing *N. americana* to hunt and feed on *E. herdmani*. Seawater was then filtered through a 100 µm sieve to remove animals, and further vacuum-filtered with a 0.4 µm filter paper to remove particulates. This conditioned seawater was stored at 4ºC and used in experiments in an un-diluted form within 12 hours.

Trials were conducted in small volumes (20 mL) to facilitate high encounter rates and because this volume is comparable to volumes that yielded maximum mating success in a previous study on the congeneric *Eurytemora affinis* (Choi and Kimmerer 2009). A
consumptive predator treatment (predator present), non-consumptive predator treatments (physical predator mimic, chemical predator cue, combined physical + chemical predator cue) and a control (predator absent) were randomly assigned to containers ($n = 30$ per treatment) and copepods were randomly assigned to each treatment. Experiments were run in the dark at 15˚C for two hours. Animals were inspected under a dissecting scope every 20 min and males and females were scored as alive and healthy (i.e., swimming normally), dead or missing. Females were further inspected for the presence of a spermatophore attached to their urosome, indicating a successful mating event.

Females without attached spermatophores were individually incubated for 24 hours to see if they developed an egg sac. If these females did develop an egg sac, we assumed that they received a spermatophore during the mating trial and that the spermatophore fell off. To test this assumption, we conducted supplementary experiments to determine if virgin females would develop an egg sac without first receiving a spermatophore. The behavior of 50 couples (1 male + 1 virgin female) was observed under infrared light; virgin females that did not receive a spermatophore during the trial ($n = 15$) were subsequently incubated for 24 hours to determine whether or not they would develop an egg sac. No females developed an egg sac without receiving a spermatophore first.

To determine at what stage mating success was interrupted by predation or perceived predation risk, a subset of the mating trials for each treatment ($n = 15$ per treatment) were observed in a darkened room using an infrared light to illuminate the animals and score their mating behavior. No more than 3 pairs were observed at once so that the observer could carefully score the following mating behaviors: mate captures
(i.e., male grasps female with his antennule), mating pair formations (male and female assume copulatory position) and spermatophore transfer (male attaches a spermatophore to female’s urosome). During the trial, males were allowed to capture their female multiple times. In contrast, we only allowed copepods to form copulatory pairs once during the trial. After observing pair formation, copulation, and separation, we immediately scored females for the presence or absence of an attached spermatophore. Therefore, trials in which pair formation occurred were inherently shorter in duration. We calculated capture frequency by dividing the number of captures by the number of minutes observed to facilitate comparison across trials of different durations.

**Field: Effects of predator cue on copepod fitness.** To examine the effects of a mysid-predator cue on *Eurytemora herdmani* mating success under conditions more closely resembling those in the field, 3 male and 3 female *E. herdmani* were placed in 1 L polyethylene bottles containing either 1) 10 mysid predators, 2) a combined chemical + physical cue consisting of 10 predator mimics swimming in mysid exudates, or 3) a filtered-seawater control (*n* = 15 per treatment). We chose this copepod density, (6 individuals L⁻¹) to be high enough to facilitate adequate mate encounter rates during trials and to increase the odds that some copepods would survive in treatments containing predators. We chose this predator density, 10 mysids L⁻¹ to represent a medium density swarm of *Neomysis americana* (Jumars 2007). As with all other experiments, *Neomysis americana* between 8-10 mm were used as the predator and *Artemia salina* 8-10 mm were used to mimic the physical cue given off by the predator. However, to create a chemical cue matching the amount of exudates created during this experiment, the cue was created by allowing 10 mysids to forage on 6 *E. herdmani* for 2 h in 1 L of seawater;
this conditioned water was filtered as described above and added in full to appropriate treatments. Containers were sealed and placed in individual mesh bags that were suspended from the dock at the Darling Marine Center during ebb or flood tide to subject the bottles to natural water motion. These larger (1 L) floating containers were used to more closely replicate natural conditions for encounter rates that may be constrained in the small (20 mL) vessels containing still water. All experiments were conducted at night. After two hours, animals were carefully removed from experimental bottles. For each container, we noted the number of animals missing (presumed eaten) from predator treatments. All copepods were recovered from our control and predator-cue treatments.

All females were individually incubated in 20 mL containers until their eggs hatched (2-3 d). Females that never developed an egg sac were incubated for 3 days. On day three, all females were removed from their container and 2 mL of acetic acid was added to the container to stain and fix the nauplii (Maps et al. 2005). All nauplii were counted.

**Statistical Analyses.** For binomial data, i.e. mating success, pair formation, and spermatophore transfer, we fitted generalized linear models (GLM) with binomial distribution (i.e., success = 1, failure = 0) and logit-link function followed by post hoc contrasts using SAS software v.9.1 (SAS Institute Inc, 2007). To estimate the magnitude of our treatment effects we calculated odds ratios (OR) with corresponding 95% confidence intervals (CI). Odds ratios that fall within 3.2-10 indicate substantial evidence and 10-100 indicate strong evidence (Jeffreys 1961).

For datasets comprised of continuous response variables with normal error distributions (i.e., capture frequency and clutch size), we compared treatments using a
one-way analysis of variance (ANOVA). The total number of nauplii produced per replicate failed to meet normality assumptions even after transformation attempts. This is a typical problem for count data containing lots of zeros (Crawley 2005), so these data were analyzed with a generalized linear model using R (R Development Core Team, 2009) with a logarithmic link function and a quasi-Poisson distribution to compensate for overdispersion (Crawley 2005) followed by post hoc contrasts. To estimate the effect sizes of our treatments with continuous data, we calculated Cohen’s $d$ ($d$). Cohen’s $d$ is commonly used in meta-analyses to depict the relative magnitude of treatment effects. Cohen’s $d$ values greater than 0.8 (or -0.8) are indicative of strong effects (Cohen 1988).

**Results**

The presence of a mysid predator significantly reduced *Eurytemora herdmani* mating success (Fig. 2.1, control versus predator OR = 16.0, 95% CI = 4.5-56.7, $X^2$ = 18.45, df = 1, $p < 0.001$, GLM binomial distribution). *Neomysis americana* consumed at least one of the two copepods in 30% of the predator treatments and did not differentially prey on males or females ($d = 0$, 95% CI = -0.69-0.71, $U = 97.0$, df = 28, $p = 0.9$, Mann-Whitney $U$-test).
Figure 2.1. Effects of predator cues on mating success. Percentage of individual *Eurytemora herdmani* females that successfully mated during a 2 h incubation with one conspecific male in the presence of a mysid predator *Neomysis americana*, a chemical predator cue, a physical predator cue, or combined physical + chemical predator cues, relative to controls (n = 30 for all treatments). Percentages were analyzed using a generalized linear model with a binomial distribution and logit-link function with post hoc contrasts ($X^2 = 25.98$, df = 4, $p < 0.001$). Different lower case letters indicate differences at the 0.05 alpha level.

In the absence of an actual predator, *E. herdmani* mating success was significantly reduced by combined (chemical + physical) treatments (Fig. 2.1, control versus combined cue OR = 8.0, 95% CI = 2.5-25.9, $X^2 = 12.07$, df = 1, $p = 0.0005$, GLM binomial distribution), indicating that this effect is not simply due to consumption or injury caused by predatory attacks.
Figure 2.2. Effects of predator cues on mating behavior. Percentage of *Eurytemora herdmani* couples that formed mating pairs (A) and successfully transferred spermatophores (B) in the presence of a mysid predator *Neomysis americana*, a chemical predator cue, a physical predator cue, or combined physical + chemical predator cues, relative to controls (*n* = 15 for all treatments). Percentages were analyzed using a generalized linear model with a binomial distribution and logit-link function with post hoc contrasts (*X^2^* = 12.48, *df* = 4, *p* < 0.05) and (*X^2^* = 23.15, *df* = 4, *p* < 0.001) for (A) and (B), respectively. Different lower case letters indicate a significant difference at an alpha level of *p* < 0.05.

Visual observations in a subset of experiments revealed that there was no significant difference in the number of mate captures min⁻¹ (i.e., male grasps female with his antennae) (*d* = 0.69, 95% CI = -0.13-1.25, *F*₄,₇₀ = 1.83, *p* = 0.1, ANOVA). However, male and female *Eurytemora herdmani* formed copulatory pairs significantly less often in the presence of a combined (chemical + physical cue), relative to predator-free controls (Fig. 2.2A, control versus combined cue OR = 17.9, 95% CI = 2.7-116.9, *X^2^* = 9.06, *df* = 1, *p* = 0.003, GLM binomial distribution). Furthermore, the chemical cue alone also reduced the frequency of *E. herdmani* pair formations (Fig. 2.2A, control versus chemical cue OR = 7.4, 95% CI = 1.2-45.1, *X^2^* = 4.76, *df* = 1, *p* = 0.03, GLM binomial distribution). In contrast, *E. herdmani* did not significantly reduce pair formation
frequency in response to a physical cue alone (Fig. 2.2A, control versus physical cue OR = 4.3, 95% CI = 0.7-26.5, $X^2 = 2.52$, df = 1, $p = 0.1$, GLM binomial distribution). The presence of an actual predator did not significantly reduce copulatory pair formation (Fig. 2.2B, control versus predator OR = 5.7, 95% CI = 0.9-34.5, $X^2 = 3.58$, df = 1, $p = 0.06$, GLM binomial distribution), but did decrease the number of successful spermatophore transfers among copulating pairs (Fig. 2.2B, control versus predator OR = 56.0, 95% CI = 5.1-611.7, $X^2 = 10.89$, df = 1, $p = 0.001$, GLM binomial distribution) indicating that the predator interferes with the process of spermatophore transfer. *Neomysis americana* consumed at least one copepod in 7% of the predator treatments. We did not detect a statistical difference in the duration of mating events across any treatment (Fig. 2.3, $d = 0.47$, 95% CI = -0.35-1.25, $F_{4,34} = 1.82$, $p = 0.9$, ANOVA).

![Figure 2.3](image.png)

**Figure 2.3.** Effects of predator cues on mating duration. The time spent mating (mean ± 95% CI) between male and female *Eurytemora herdmani* in the presence of a mysid predator *Neomysis americana*, a chemical predator cue, a physical predator cue, or combined physical + chemical predator cues, relative to controls ($n = 15$ per treatment). Analyzed by one-way analysis of variance ($F_{4,34} = 1.82$, $p = 0.9$).
In field incubation experiments, the total number of offspring produced per replicate was suppressed by the presence of both predator and predator cue treatments (Fig. 2.4A, control versus predator $d = 1.45$, 95% CI = 0.78-2.12, $t_{43} = 3.73$, df = 1, $p < 0.001$; control versus cue $d = 0.76$, 95% CI = 0.05-1.59, $t_{43} = 2.04$, df = 1, $p < 0.05$, GLM quasi-Poisson distribution). *Neomysis americana* consumed one of six copepods in 21% of the replicates, consumed two of six copepods in 14% of the replicates and consumed more than two of six copepods in 7% of the replicates. In summary, actual predation on *E. herdmani* adults during the experiment contributed to the subsequent reduction in offspring in 43% of the predator replicates. We did not detect a difference in the number of nauplii per clutch (Fig. 2.4B, $d = 0.22$, 95% CI = -0.57-0.96, $F_{2,34} = 0.51$, $p =0.61$, ANOVA).
Figure 2.4. Effects of predator cues on offspring production. The (A) total number of nauplii and (B) number of nauplii per clutch (mean ± 95% CI) produced by female *Eurytemora herdmani* after a 2 h incubation with equivalent densities of males in containers incubated in the field housing either a mysid predator *Neomysis americana*, a chemical predator cue, or a predator-free control. Copepods were removed post-incubation and kept in isolation until eggs hatched (2-3 days) in laboratory containers. Total number of nauplii (A) analyzed by generalized linear model with quasi-Poisson distribution and logarithmic link function and post hoc contrasts, ($F_{2,42} = 6.43, p = 0.003$). Number of nauplii per clutch (B) analyzed by a one-way analysis of variance ($F_{2,34} = 0.51, p = 0.3$). Different lower case letters indicate a significant difference at alpha level $p < 0.05$. 
Discussion

The presence of a combined predator cue (chemical exudates + physical cue mimic), considerably reduced the mating success and subsequent offspring production of *Eurytemora herdmani* (Figs. 2.1 and 2.4). These effects were observed in the absence of an actual predator, indicating that a reduction in copepod mating success can occur by non-consumptive mechanisms, and need not involve attacks from predators or predator-induced changes in mate density or sex ratio. These results suggest that non-consumptive effects of *N. americana* on its prey generate a significant proportion of the overall patterns we observed in our study. Our findings corroborate a growing number of studies demonstrating that non-consumptive effects of predators have large effects on prey population growth (Peacor and Werner 2001; Werner and Peacor 2003; Preisser et al. 2005) and highlight the importance of perceived risk in reducing offspring production and subsequent population growth.

Recent copepod-population growth models suggest that small population densities (Choi and Kimmerer 2008, 2009) and biased sex ratios (Kiørboe 2007, 2008b) decrease copepod mating success and subsequent population growth by reducing mate-encounter rates (Kiørboe and Bagøien 2005; Visser and Kiørboe 2006; Kramer et al. 2011). Here we show in small (20 mL) vessels, where encounter rates are not limiting, that *E. herdmani* mating success is significantly reduced by the presence of a predator cue. While we acknowledge that changes in encounter rates have obvious and important consequences for copepod mating success, our results suggest that predator cues play an important, but underestimated, role in reducing copepod mating success by reducing the ability or willingness of copepods to mate even when encounter rates are high.
Our results highlight the importance of non-consumptive effects of predators on copepod mating success (Figs. 2.1, 2.2, 2.4). Broadly, there are two possible explanations for this effect. First, predator cues alter the behavior of copepods, leading them to adopt more inconspicuous behaviors and delay reproduction to ensure their own survival. Second, predator cues interfere with the ability of copepods to successfully mate by disrupting mate finding, pair formation or spermatophore transfer. How we distinguish between these two mechanisms varies depending on the stage of mating being examined (Buskey 1998).

At the earliest stage of mating, we found no significant difference in the frequency of mate captures among any of the treatments indicating that males are able to locate females equally well in the presence of a predator and are willing to pursue females in the presence of predator cues. Therefore, it does not appear that predators diluted pheromones by mixing the water or masked pheromones with their kairomones in our study. In contrast, once mate capture occurred, there was a reduction in mating pair formation in the presence of a combined (chemical + physical) predator cue as well as a chemical cue alone (Fig. 2.2A); this could occur because females are less willing to mate, males voluntarily release females, or predators (and mimics) disturb the surrounding water and make it difficult for males to ‘hold on’. There was no difference in mating pair formation when copepods were exposed to a physical mimic alone (Fig. 2.2A), suggesting that they have the ability to mate in the presence of a physical disturbance. Therefore, the reduction in pair formation was likely due to a reduction in the willingness to mate by the male, the female or both.
In the final stage of mating, copepods suffered reduced spermatophore transfer success when exposed to predators and all predator cue types (Fig. 2.2B). Again, this could be due to females rejecting spermatophores, males voluntarily releasing females before transferring, or predators (and mimics) disturbing the surrounding water and interfering with the male’s ability to properly place and fasten his spermatophore. Our data suggest that when copepods are exposed to predator cues they behaviorally reduce their spermatophore transfer rate and when they are exposed to an actual predator these effects are due to both behavior and actual consumption or injury. Therefore, we conclude that at nearly every stage in the mating process, the threat of predation alters mating behavior and hinders mating success.

The risk associated with forming copulatory pairs (i.e., heightened conspicuousness and diminished escape ability) is shared equally by males and females. Therefore, it is plausible that males and females would separate when they perceive elevated predation risk. However, a female incurs an additional cost after mating due to the development of a large egg sac that makes her more visually conspicuous to predators and reduces her escape ability (Magnhagen 1991; Maier et al. 2000). Therefore, females may escape from copulatory pairings more often than males, or reject spermatophores to reduce these extended reproductive costs (Maier et al. 2000).

In our study, copepods did not alter their mating duration in the presence of predators or predator cues (Fig. 2.3). These results are similar to those found by Maier (2000) for the freshwater cyclopoid *Cyclops vicinus*, which did not alter mating durations in the presence of a *Chaoborus* predator. Together, these results suggest that individual copepods do not alter their mating durations in response to proximate predator cues.
However, over evolutionary time scales freshwater copepod populations have adapted to lakes with high predator densities by having shorter mating durations (Jersabek et al. 2007), suggesting that natural selection favors individuals that copulate quickly when predation is intense. In these freshwater systems, there is a tradeoff between assured paternity through post-copulatory mate guarding and the elevated risk of predation associated with prolonged copulation (Jersabek et al. 2007). In our study, *E. herdmani* males always released females shortly after spermatophore transfer, suggesting that mate guarding does not occur in *E. herdmani* and that copulation duration is set by the amount of time it takes for a male to transfer a spermatophore.

Combined (chemical + physical) predator cues interfered with *Eurytemora herdmani* mating success (Figs. 2.1, 2.2, 2.4). In laboratory assays, the presence of *N. americana* chemical exudates alone reduced copulatory pair formation (Fig. 2.2A) suggesting that predator kairomones or alarm cues generated by injured conspecifics can alter mating behavior. Predator kairomones and alarm cues are important in eliciting prey behavioral responses in aquatic systems (Brönmark and Hansson 2000). Often, prey show elevated behavioral responses to predator cues if predators are consuming prey conspecifics or closely related prey species, relative to predator exudates generated from starved predators (Schoepchner and Relyea 2005; Smee and Weissburg 2006). It is important to note that the presence of a chemical cue reduced copulatory pair formation, whereas an actual predator did not (Fig. 2.2A). We suggest that this may be due the fact that predator trials were conducted in clean seawater. Therefore, there was no ‘scent’ of a prior predation event in the water at the beginning of the trial. This may indicate chemical cues from an actual predation event elicit more of a behavioral response than
chemical cues from a predator alone. However, in this study, we did not include a 
starved predator treatment or a killed-conspecifics treatment to disentangle these effects. 
Therefore, we cannot distinguish whether the chemical cue released from the predator, 
injured conspecifics, or a combination of both is necessary to elicit the changes in 
copepod mating success

There is evidence from both marine and freshwater systems that copepods alter 
their behavior in response to predator exudates by migrating to a depth refuge (Neill 
1990), reducing grazing activity (Cieri and Stearns 1999) or reducing swimming speed 
(van Duren and Videler 1996). A reduction in swimming activity has important 
consequences for mating success because it directly affects the probability of encounter 
between males and females (Gerritsen and Strickler 1977; Kiørboe and Bagøien 2005; 
Visser and Kiørboe 2006). We did not directly monitor swimming activity in this study. 
However, a reduction in E. herdmani swimming activity in response to Neomysis 
americana exudates would be more important in our larger field containers (1 L) than in 
the small vessels used in laboratory studies (20 mL), where encounter rates were 
inherently high.

To mimic both the hydromechanical and tactile cue of a Neomysis americana 
predator, we exposed Eurytemora herdmani couples to an herbivorous brine shrimp. The 
brine shrimp provided tactile stimulation by swimming up to the copepods and directly 
touching them (Lasley-Rasher pers. obs.); their swimming also produced fluid shear, 
which is known to be the primary hydrodynamic cue responsible for eliciting escape 
responses in copepods (Yen et al. 1992; Fields and Yen 1997; Fields 2010). The physical
cue alone significantly reduced mating success (Fig. 2.1, 2.2B) indicating that a physical predator cue can interfere with copepod mating success.

Combined chemical and physical predator cues consistently reduced mating success (Figs. 2.1, 2.2, 2.4) in all field and laboratory experiments, whereas, chemical and physical cue treatments produced intermediate responses (in terms of effect sizes). Therefore, *E. herdmani* may perceive the combined chemical and physical cue treatment as a more risky environment than either cue treatment alone. It is possible that *E. herdmani* detects predator kairomones which then heightens its sensitivity to the physical cue of a nearby predator (and vice versa). However, little is known about the ability of copepods to simultaneously process chemical and mechanical information in terms of predator-prey interactions. Nonetheless, prey individuals should be favored by natural selection to alter the magnitude of their behavioral response (i.e., reduce mating frequency) in response to the level of perceived risk so as not to waste valuable mating opportunities (Magnhagen 1991).
CHAPTER 4
INTOXICATED COPEPODS: INGESTING TOXIC PHYTOPLANKTON LEADS TO RISKY BEHAVIOR

Abstract

Harmful algal blooms (HABs) have devastating consequences on coastal economies and pose a significant human health risk. Therefore, there has been a great deal of research conducted on the species that graze HABs such as marine copepods. Typically, the response variables that are measured in such studies are the physiological and direct fitness consequences of copepods ingesting HABs. Less attention has focused on how ingesting HABs can alter copepod behavior, which could produce indirect fitness effects. According to encounter rate models, small alterations in zooplankton swimming behavior could have large impacts on copepod dispersal and encounter rate with predators. We measured changes in the behavior of the marine copepod, *Temora longicornis* after ingesting *Alexandrium fundyense*. Following *A. fundyense* exposure, *T. longicornis* increased their swimming speed and the straightness of their swimming path. Models suggest that these changes could lead to a 24-54% increase in encounter frequency between copepods and their predators. This work highlights the need to determine how ingesting HAB species alters grazer behavior as this can have significant impacts on fate of HAB toxins in marine systems.
Introduction

Harmful algal blooms (HABs) negatively impact coastal economies and human health (Anderson 1989). In recent decades, climate change and eutrophication have led to an increase in the frequency and geographic range of HAB blooms (Anderson 1994). As such, a great deal of research has been targeted at elucidating mechanisms that control bloom formation, propagation and termination. A key factor responsible for bloom proliferation is the lack of adequate grazing pressure (reviewed by Roelke 2000). Therefore, understanding interactions between grazers and harmful algal bloom species is of critical importance.

Copepods are important grazers of harmful algal blooms, and much work has been done to determine the effects of harmful algae on copepod feeding, physiology and fitness. Behavioral studies have mostly assessed immediate reactions of copepods to HAB species, such as cell rejection and avoidance (Huntley et al. 1986; Schultz and Kjørboe 2009). Fewer studies have documented the effects of ingesting harmful algae on copepod swimming behavior (but see Cohen et al. 2007). Due to the high search volume rates of marine copepods (app. 100 L day$^{-1}$, Kjørboe and Bagøien 2005) and the patchy nature of harmful algal blooms (Cembella et al. 1994; Luerssen et al. 2005; Thomas et al. 2010), copepods may experience intermittent exposure to patches containing HAB species followed by HAB-free areas. However, little is known about the lasting effects of HAB exposure on copepod swimming behavior. Effects of HABs on copepod behavior could be important, as swimming behavior has a direct impact on copepod dispersal and encounter rates with predators (Gerritsen and Strickler 1977; Visser and Kjørboe 2006;
Visser 2007), both of which determine the propensity of copepods to graze HABs and transfer HAB toxins through marine food webs.

*Alexandrium fundyense* is a neurotoxic dinoflagellate that causes harmful algal blooms along Northeastern United States and Southeastern Canada. These bloom events have devastating impacts on the economy of the Northeastern United States due to shellfish fisheries closures, and outbreaks of Paralytic Shellfish Poisoning which can cause severe human health effects (Anderson 1994; Anderson et al. 2005). Furthermore, the frequency and intensity of *A. fundyense* blooms have increased in recent years (Anderson et al. 2005). The outcome of grazing interactions between *Alexandrium* sp. and copepods are varied and context-dependent. Some copepod species behaviorally reject *Alexandrium* sp. (Huntley et al. 1986) or become incapacitated after feeding (Ives 1987) while others are unaffected or even increase their ingestion rates (Teegarden et al. 2008). Furthermore, *Alexandrium* sp. can have detrimental (Dutz 1998), positive (Avery and Dam 2007) or no effect on egg production (Avery pers. comm.). These effects vary not only between species (Teegarden and Cembella 1996) but within a species according to gender (Avery et al. 2007) and historical exposure (Colin and Dam 2003).

In this study we ask the following questions: 1) Does the common calanoid copepod *Temora longicornis* exhibit altered swimming behavior in response to *Alexandrium fundyense* exposure? 2) Is this behavioral change explained by nutritional inadequacies or toxicity of the phytoplankton? 3) How does this behavioral change affect copepod encounter rates with predators?
Methods

Collection and culture of organisms. Our target grazer species, *Temora longicornis* is a calanoid copepod that co-occurs with *Alexandrium fundyense* in Gulf of Maine waters. We collected *T. longicornis* from the Damariscotta River estuary, Walpole, Maine (43°56’ N, 69°35’ W) using a plankton net with a mesh size of 250 µm, towed obliquely by boat at approximately 30 m depth. Upon collection, animals were immediately transferred into 20 L containers of surface seawater and transported to a temperature-controlled room. Within 24 h of collection, animals were transferred to 1L polyethylene bottles. Sealed bottles were packed in an insulated box containing ice packs and cushioning material to maintain a cool temperature (app. 5-10°C) and minimize stress. Animals were shipped to the Georgia Institute of Technology, Atlanta, GA within 24 h. Once received, animals were diluted with artificial seawater and allowed to acclimate to their natural temperature (usually 4-6°C warmer than their shipping temperature) over 24 h. Copepods were fed mixed cultures of *Tetraselmis* sp. and *Isochrysis galbana*. After a 24 h acclimation period, adult *Temora longicornis* were sorted from mixed samples under a dissecting microscope and placed in containers filled with filtered seawater at densities less than 25 individuals L⁻¹.

*Alexandrium fundyense* was chosen as our ‘HAB’ species because it is known to contain Paralytic Shellfish Toxins (PST) (Anderson et al. 2005). The strain we used (NCMA 1719), was obtained from the National Center for Marine Algae and Microbiota, Boothbay Harbor, Maine. Cell biovolume (6.61x10³ µm³) was calculated treating the cell as a rotational ellipsoid. Cell dimensions (26.6 ± 1.9 µm, 21.8 ± 0.5 µm, [mean ± s.e.]) were then measured from images using a FlowCAM (n = 12). *Rhodomonas lens*
(NCMA 739, cell biovolume $3.36 \times 10^2 \mu m^3$ calculated as a rotational ellipsoid from dimensions $13.5 \pm 1.8 \mu m$, $6.9 \pm 0.3 \mu m$, [mean ± s.e., $n = 7$]) was used as our non-toxic ‘control’ algae because it is commonly fed to copepods in culture due to its nutritional quality (Koski et al. 1998). All phytoplankton species were cultured in L1 Media (Guillard and Hargraves 1993), exposed to a 14:10 light:dark cycle and maintained at 14˚ C (for *A. fundyense*) and 21˚ C (for *R. lens*).

Carbon: nitrogen ratios for *A. fundyense* and *R. lens* were analyzed by Micro-Dumas combustion at the University of Georgia Institute of Ecology, Athens, GA. Culturing and harvesting conditions were kept constant during all experiments to minimize any physiological differences within phytoplankton species. All phytoplankton cultures were in late exponential phase when harvested for experiments. Maximum culture densities were $4.8 \times 10^7$ cells L$^{-1}$ and $1.3 \times 10^8$ cell L$^{-1}$ for *A. fundyense*, and *R. lens*, respectively.

**Toxin analysis.** To verify the toxicity of our *Alexandrium fundyense* stock culture, 2 samples were shipped overnight to Greenwater Laboratories in Palatka, Florida on two different occasions. The first sample was analyzed using a competitive ELISA (enzyme-linked immunosorbant assay) to detect the presence of saxitoxin (the most potent of the PST derivatives). However, ELISA is not reactive with all PST derivatives and often underestimates the total toxins present. Due to logistical constraints, a detailed toxin profile was only analyzed for the second sample. The toxins examined were *N*-sulfocarbamoyl-11-hydroxysulfate toxins (C1 and C2), gonyautoxin 1-4 (GXT1-4), saxitoxin (STX), neosaxitoxin (neoSTX) and other decarbamoyl derivatives (dcGTX2, dcGTX3, dcSTX). Toxins were analyzed by high-pressure liquid chromatography.
(HPLC) with fluorescence detection following a pre-column oxidation method (AOAC 2005).

**Swimming behavior.** The aim of our behavioral experiment was to determine if the swimming behavior of *Temora longicornis* is altered by exposure to *Alexandrium fundyense*. Fifteen male and fifteen female *Temora longicornis* were placed in a 1 L glass tank containing filtered seawater and fed one of the following treatments: 80% *Alexandrium fundyense* (320 cell mL$^{-1}$) + 20% *R. lens* (1,120 cell mL$^{-1}$) or 100% *Rhodomonas lens*, (5,600 cell mL$^{-1}$). We calculated the diet composition using biovolume equivalents. Therefore, copepods were given equivalent total biovolumes of food across all treatments. Copepods were starved for 12 h prior to experiments. We chose 320 cell mL$^{-1}$ for our *A. fundyense* treatment to ensure that copepods were not food limited. Although this concentration is quite high for *A. fundyense* blooms in the Gulf of Maine, where maximum cell densities are typically approximately 10 cell mL$^{-1}$ (Anderson 2005), this is well within the range of *A. fundyense* cell densities measured in other areas (Hattenrath et al. 2010). Furthermore, we simultaneously offered *T. longicornis* an alternate food, *R. lens*, to avoid ‘force-feeding’ the copepods *A. fundyense*.

After allowing copepods to feed for 2h, we carefully transferred them to a tank with filtered seawater amended with $2.5 \times 10^4$ cell mL$^{-1}$ of the alga *Tetraselmis* sp. This cell density has been used as a saturating food level in previous studies using *Tetraselmis* sp. and copepod grazers (Buttino et al. 2009). *Tetraselmis* sp. is a non-toxic green flagellate that is commonly used as food for copepod cultures. Furthermore, *Tetraselmis* sp. has been fed to copepods during depuration periods in other *Alexandrium fundyense* grazing experiments (Dam and Haley 2011). Tanks were covered with parafilm and
placed in a temperature-controlled room where copepods were allowed to feed on this nutritious food for 15 h before being used in behavioral experiments. We repeated this exposure process with four replicate tanks for both treatments.

We chose a 2 h exposure time to minimize the physiological incapacitation or severe impairment observed in other studies (Ives 1987; Dutz 1998). The purpose of a 15 h recovery period was to allow copepods to feed on control algae so that changes in their behavior would not be due to differences in hunger levels (caused by any differences in the nutritional value of *Alexandrium fundyense* versus *Rhodomonas lens*). Fifteen hours is much longer than the average gut clearance time for marine copepods (app. 1-2 h, Mauchline 1998) but less than the average residence time of saxitoxins harbored in copepod guts and tissues (app. 33 h, Dam and Haley 2011).

Following the recovery period, we transferred copepods to 1 L experimental tanks containing filtered seawater. The experimental tanks containing copepods were visualized in a Schlieren optical system developed by Strickler (Strickler and Hwang 1998) and further described by (Doall et al. 1998). We conducted three replicate trials for each treatment to account for inter-individual variability (Seuront et al. 2004). Observations were conducted in the dark with a near-infrared laser used to illuminate the copepods. A green laser beam was directed down the center of the vessel to attract copepods to the center of the tank and reduce effects of vessel walls (Doall et al. 1998). Preliminary observations revealed that the light encouraged copepods to swim in the center of the tank without inducing strong aggregative behavior. Observations were recorded onto DVDs and digitized using Prism Video Converter 1.82 software and split into clips using SolveigMM Video Splitter. When necessary, clips were further
processed in Adobe Premier Pro. CS5.5 to enhance contrast. Swimming paths were analyzed using LabTrack software. Our path selection criteria required that copepods were swimming in the center of the tank for at least 10 s. We then constructed 3-dimensional tracks by matching the common z position from superimposed mirror images of individual copepods.

We calculated instantaneous swimming velocity $V$ (cm s$^{-1}$) of the animals using the distances between position in the x, y and z plane over a given time step $t$.

$$V = \sqrt{(x_{t+1} - x_t)^2 + (y_{t+1} - y_t)^2 + (z_{t+1} - z_t)^2} \times p$$

Where $x_p, y_p, z_t$ and $x_{t+1}, y_{t+1}, z_{t+1}$ correspond to the copepod’s position at time $t$ and time $t + 1$, respectively, and $p$ is the frame acquisition rate of our camera (33 frames s$^{-1}$). Instantaneous velocities were averaged over an individual track to obtain a mean swimming velocity. Swimming speed has important consequences for *T. longicornis* distribution and survival because increased swimming speed leads to greater dispersal distances, increased encounter rates with predators and increased conspicuousness (Yen and Strickler 1996; Visser and Kiørboe 2006, Visser 2007). We calculated the effect of changes in swimming velocity on encounter rates between *T. longicornis* and their predators ($E$) using the following equation (Gerritsen and Strickler 1977):

$$E = \pi K^2 (v_{pred}^2 + v_{prey}^2)^{0.5}$$

Where $K$ is the perceptive distance of the predator and $v_{pred}$ and $v_{prey}$ are the swimming velocities of the predator and prey, respectively. The relative importance of the prey’s swimming speed depends on type of predator encountered (Visser 2007). For a visual cruising predator, such as a fish larvae the encounter rate $\beta$ increases proportionally as prey velocity $v_{prey}$ increases (Visser 2007). However, for a rheotactic
 predator that relies on hydrodynamic cues from prey (such as a hovering mysid shrimp),
the predator’s detection distance ($K$) scales as

$$K \approx s \left( \frac{v_{prey}}{\omega} \right)^{0.5}$$

where ($s$) and ($v_{prey}$) are the prey’s size and swimming velocity and ($\omega$) is the sensitivity
(threshold speed) that the predator can detect (Visser 2007). Therefore, a faster
swimming organism is more conspicuous to predators. In this case, the predator’s
encounter rate is proportional to $v_{prey}^2$.

Additionally, we calculated the net:gross displacement ratio (NGDR) for each of
the tracks. This is a commonly used metric that varies from 0-1 and describes the degree
degree of path tortuosity (Buskey 1984). An NGDR of 1 represents a perfectly straight path,
whereas an NGDR of 0 describes Brownian motion. Since net:gross displacement ratios
are inherently scale-dependent, we controlled for this scale-dependency by analyzing the
only the first 10 s of each path. Additionally, we calculated the directional persistence of
copepods’ swimming paths from different food treatments by estimating the correlation
time scale. The correlation time scale was estimated by plotting the root-mean-square
displacement (RMS) over time and fitting Taylor’s formula for continuous random walk
(Taylor 1921, Kiørboe 2008b).

$$
\text{Root mean square displacement (RMS)} = \left\{ 2V^2 \tau [t - \tau \left( 1 - e^{-\left(\frac{t}{\tau}\right)} \right)] \right\}^{0.5}
$$

Where $V$ is the copepod’s effective swimming speed and $t$ is the time interval
between measurements. The root-mean-square displacement (RMS) is the square root of
the average net distance travelled by copepods at each time step. This is analogous to the
standard deviation of the position of the copepods (Kiørboe 2008a). The correlation time
scale ($\tau$) is estimated from the above equation and can be thought of as the time scale over which an animal’s path is directionally persistence (i.e. straight). High directional persistence of swimming paths indicates increased encounter rates between individuals (Visser and Kiørboe 2006; Selander 2011).

**Survivorship experiments.** To determine the effects of ingesting *Alexandrium fundyense* on copepod survival, we incubated *Temora longicornis* in 250 mL beakers containing filtered artificial seawater amended with one of the following treatments: 100% *Rhodomonas lens* containing 5,600 cell mL$^{-1}$, 80% *Alexandrium fundyense* (320 cells mL$^{-1}$) + 20% *R. lens* (1,120 cells mL$^{-1}$), low food control containing 1,120 cells mL$^{-1}$ of *R. lens* and a starved control (containing only filtered seawater). The percentages were based on biovolume equivalents to account for the different sizes of the phytoplankton cells. The 100% *R. lens* treatment serves as a control by having the same amount of total food (by biovolume) as the *A. fundyense* treatment, whereas the low food *R. lens* serves as a control by having the same amount of nutritious food as the *A. fundyense* treatment.

To control for feeding history, *Temora longicornis* were incubated in filtered seawater for 12 h prior to experiments. Three male and three female *T. longicornis* were randomly assigned to 250 mL beakers ($n = 5$). Beakers were covered with parafilm and placed in a 14°C incubator (interspersed with respect to treatment) and exposed to a 14:10 h light:dark cycle. Every 24-25 h, copepods were visually inspected under a dissecting microscope and scored as either dead or alive. Live copepods were transferred to new beakers containing fresh phytoplankton and dead copepods were discarded. Experiments were conducted for 4 days.
Ingestion experiment. We measured copepod ingestion rate in a subset of our beakers ($n = 5$) between 24 and 48 h of our survivorship experiment. We chose this time period because survivorship was not significantly affected and there were no differences in copepod density between our treatments but copepods had enough time to feed that we could detect measurable differences in rates of phytoplankton removal. Just prior to the addition of animals, a 20 mL sample was removed from experimental beakers and preserved in Lugol’s solution. The next day, copepods were removed from beakers and another 20 mL sample was removed and preserved in Lugol’s solution. To determine copepod ingestion rate, the number of cells removed were divided by the number of surviving copepods in the beaker and the number of hours incubated (24 h).

Visual counts were performed using replicate 300 μl-1mL preserved samples, so that a minimum of 500 cells were counted for each subsample. The ingestion rate of each algal species was calculated from the formula described by Frost (1972).

$$I = \frac{(C_1 - C_2)V}{N} \times 24$$

Where $C_1$ is the cell concentration in the beaker just prior to adding copepods, $C_2$ is the cell concentration immediately after copepod removal, $V$ is the cell biovolume and $N$ is the number of live copepods at the end of the 24 h period. To control for animals that died during the incubation, we assumed that animals died halfway through the experimental period. We ran copepod-free controls with mixtures of *Alexandrium fundyense* and *Rhodomonas lens* prior to grazing experiments to determine any changes in phytoplankton cell density due to growth or settling. We found no significant change in cell density over a 24 h period for either species ($A. fundyense$, $p = 0.96$, $n = 4$; $R. lens$, 63
\[ p = 0.68, \ n = 4, \text{ two-tailed t-test} \]. Therefore, we did not include a growth parameter in our ingestion equation.

**Egg production and hatching success experiments.** To determine the effects of *Alexandrium fundyense* on copepod fecundity, we incubated three male and three female *Temora longicornis* in 250 mL beakers containing an ‘egg basket’ \((n = 4)\). Egg baskets consisted of a 5.5 cm diameter and 10 cm height plexiglass tube with 160 \(\mu\)m mesh attached to the bottom and their purpose was to create a false bottom in which copepod eggs could pass through, separating copepods from their eggs and thus preventing egg cannibalism (Prince et al. 2006). To control for previous mating and feeding history, males and females were separately incubated for 24 h in filtered seawater prior to experiments. Copepods were individually added to beakers containing one of the following treatments: 100\% *Rhodomonas lens*, 80\% *Alexandrium fundyense* + 20\% *R. lens*, low food *R. lens* control and filtered seawater control (described in detail above). Beakers were covered with parafilm and placed in a 14°C incubator and exposed to a 14:10 h light:dark cycle. Every 24-25 h, egg baskets containing copepods were moved to new beakers with fresh phytoplankton. Copepods that were lying on the bottom of the egg basket were visually inspected under a dissecting microscope. Dead copepods were discarded. Experiments were conducted for 3 d.

We maintained a 1:1 sex ratio within our treatments to control for any bias in hatching success due to either fertilization limitation or mate competition. Therefore, if a female died during the incubation we removed a male from that replicate. In contrast, dead males were replaced with a new male from a supplemental stock culture that had
been incubated in the appropriate treatment since the beginning of the experiment. Experiments were run for 3 days.

After moving copepods to new beakers, we gently transferred the eggs to a petri dish and counted them immediately. Petri dishes were covered to prevent evaporation and placed in a 14°C incubator for 48 h to allow eggs enough time to hatch. After 48 h, we added a few drops of acetic acid to the petri dishes to kill and stain nauplii (making them easier to count) and counted the number of nauplii. For the purpose of analysis, we pooled the total number of eggs and nauplii produced female\(^{-1}\) replicate\(^{-1}\) from 48 h and 72 h time period (excluding the 0-24 h time period) to ensure that copepods had enough time to assimilate ingested phytoplankton (Mauchline 1998).

**Statistical analysis.** Mean swimming speed and net:gross displacement ratio violated normality assumptions, so we used a Boxcox transformation to facilitate parametric comparisons (Crawley 2005). We used Welch’s \(t\)-test to assess differences between mean swimming speeds and net:gross displacement ratios. We determined that these parameters did not differ between trial dates \((p > 0.16, \text{ ANOVA})\), therefore, we pooled trajectories across trial dates (Kramer et al. 2011). Therefore our analysis is based on a total of 41 trajectories consisting of 11, 619 successive animal positions for 100% *Rhodomonas lens* treatment and 43 trajectories (11, 562 positions) for *Alexandrium fundyense: R. lens* treatment.

The survivorship of individual copepods between treatments was compared using a Cox proportional hazard model with mixed effects. The beaker holding the copepods was considered a random effect. The number of eggs produced female\(^{-1}\) between phytoplankton treatments were analyzed using an analysis of variance (ANOVA). To
determine differences in percent hatching success, we fit a generalized linear mixed model (GLM) with a logit-link function with a quasibinomial distribution (to account for significant overdispersion).

Differences in total ingestion rate between the 100% *Rhodomonas lens* and the 80% *Alexandrium fundyense* + 20% *R. lens* treatment were compared using a Welch’s *t*-test. Differences in the *R. lens* ingestion rate between the low food *R. lens* control and the 80% *A. fundyense* + 20% *R. lens* were also compared using a Welch’s *t*-test. All statistical tests were conducted using R (R version 2.14.1).

**Results**

After being exposed to phytoplankton treatments for 2 h, followed by 15 h of consuming non-HAB food, copepods from the 80:20 *Alexandrium fundyense: Rhodomonas lens* treatment swam significantly faster than those from the 100% *R lens* treatment (Fig. 3.1A; *t* = -3.7, df = 77, *p*-value = 0.0004, Welch’s *t*-test).

![Figure 3.1](image)

**Figure 3.1.** Effects of ingesting *Alexandrium fundyense* on the swimming speed (A) and net:gross displacement ratio (B) of *Temora longicornis* 15 h after exposure (mean ± s.e.). Significant differences were determined using a Welch’s *t*-test to compare mean swimming speed, (*t* = -3.7, df = 79, *p*-value = 0.0004) and net:gross displacement ratio (*t* = -2.7, df = 77, *p*-value = 0.008).
Additionally, copepods from the treatment containing *A. fundyense* exhibited more directional persistence in their swimming motion, as indicated by significantly larger net:gross displacement ratios (Fig. 3.1B; \( t = -2.7, \text{df} = 77, \text{p-value} = 0.008, \) Welch’s \( t \)-test) and a larger correlation time scale (Figs. 3.2 & 3.3).

Figure. 3.2. Three dimensional depictions of 10 s swimming paths of *Temora longicornis* after ingesting either *Rhodomonas lens* (A) or 80:20 mixture of *Alexandrium fundyense: Rhodomonas lens* (B). Copepods were incubated in treatments for 2 h and then fed nutritional food for 15 h prior to behavioral recordings.
Figure 3.3. Root-mean square distance travelled (RMS) of *Temora longicornis* swimming paths after ingesting either *Rhodomonas lens* (A) or 80:20 *Alexandrium fundyense* + *R. lens* (B). Taylor’s equation was fit to the data to estimate the correlation time scale. The estimated correlation time scale is located at the intersection of the dashed lines. Note the different y-axis scale.

Figure 3.4. Average number of surviving *Temora longicornis* incubated in either 100% *Rhodomonas lens*, 80:20 *Alexandrium fundyense* + *R. lens*, low food *R. lens* control (20% *RI*) or filtered seawater (starved control). The total number of survivors at the end of 4 d was compared using an analysis of variance, ANOVA (\(F_{3,36} = 1.41, p = 0.26\)).
The average number of survivors did not differ among phytoplankton treatments (Fig. 3.4; $F_{3,36} = 1.41, p = 0.26$, ANOVA). Furthermore, there was no difference between the amount of total food ingested by copepods when offered 80:20 *Alexandrium fundyense* : *Rhodomonas lens* versus 100% *R. lens* (Fig. 3.5; $t = -0.77$, df = 5.4, $p = 0.47$, Welch’s *t*-test). Additionally, *Temora longicornis* consumed similar amounts of *R. lens* when offered the low food *R. lens* control versus 20% *R. lens* + 80% *A. fundyense* (Fig. 3.5; $t = -0.45$, df = 4.8, $p = 0.67$, Welch’s *t*-test) indicating that *T. longicornis* did not discriminate against or become incapacitated by ingesting *A. fundyense*.

![Graph showing the biomass of phytoplankton ingested by Temora longicornis over a 24 h period.](Image)

**Figure 3.5.** The biomass of phytoplankton ingested by *Temora longicornis* over a 24 h period when 6 individuals were incubated in either 100% *Rhodomonas lens*, low food *R. lens* control (20% *Rl*) or 80% *Alexandrium fundyense* + 20% *R. lens*. The total amount of food consumed between the 100% *R. lens* and 80:20 *A. fundyense* + *R. lens* mixture was compared using a Welch’s *t*-test. The amount of *R. lens* consumed between the 20% *R. lens* treatment and the 80:20 *A. fundyense* + *R. lens* mixture was also compared using a Welch’s *t*-test. There were no significant differences detected.

There was no difference in copepod egg production among the different food treatments (Fig. 3.5A; $F_{3,12} = 1.34, p = 0.31$, ANOVA). Egg hatching success was only significantly affected by the starved treatment (Fig. 3.5B; $t = -2.3$, df = 12, $p = 0.04$,
GLM, quasibinomial). Both samples of our stock cultures contained PSTs (as verified by ELISA and HPLC). As detected by HPLC, our stock culture contained 2.9 pg total saxitoxin equivalents cell$^{-1}$. The carbon: nitrogen ratios for *Rhodomonas lens* and *Alexandrium fundyense* were $7.16 \pm 0.44$ and $5.04 \pm 0.17$ (mean ± s.e., $n = 2$) respectively.

![Graph A](image1.png)  ![Graph B](image2.png)

Fig. 3.6. The number of eggs produced female$^{-1}$ (A) and the percent hatching success (B) of *Temora longicornis* while incubated in either 100% *Rhodomonas lens*, 80:20 *Alexandrium fundyense* + *R. lens*, low food *R. lens* control (20% RI) or filtered seawater (Starved). The total number of eggs female$^{-1}$ was analyzed using an analysis of variance, ANOVA ($F_{3,12} = 1.34, p = 0.31$). Percent hatching was analyzed using a generalized linear model with a quasibinomial distribution and logit-link function ($t = -2.3$, df = 12, $p = 0.04$). Different lower case letters indicate differences at the 0.05 alpha level.

**Discussion**

To assess the effects of a 2 h exposure of *Temora longicornis* to *Alexandrium fundyense*, the following factors were examined: copepod swimming speed and swimming behavior after 15 h of depuration, copepod survival after 4 days exposure to HABs, copepod ingestion and food preferences after 1 day HAB exposure, egg production rates and hatching success after 2-3 days. Of these factors, the only significant effect was on the copepod’s swimming behavior. After being exposed to *A. fundyense*
for 2 h and then allowed to depurate for 15 h, the long lasting effects of feeding on HABs were in the copepod swimming behavior. *Temora longicornis* increased their swimming velocity by 24% which leads to an 24-54% increase in the theoretical encounter rate with visual and rheotactic predators, respectively (Gerritsen and Strickler 1977; Visser 2007). Furthermore, copepods increased the directional persistence of their swimming path which also leads to increased encounter rates (Visser and Kjørboe 2006). The actual mechanism responsible for this behavioral change is unknown.

Copepods exhibit straight and fast swimming motility when searching for a food patch (Buskey 1984) or in response to starvation (van Duren and Videler 1996). Therefore, a potential hypothesis is that *T. longicornis* were more hunger-motivated after ingesting *Alexandrium fundyense* due to feeding incapacitation or nutritional inadequacy. However, our ingestion experiments indicate that *T. longicornis* readily feed on *A. fundyense* (Fig. 3.5) and do not become incapacitated. Our carbon: nitrogen ratio test suggests that *Alexandrium fundyense* may be of lower nutritional quality than *Rhodomonas lens* due to its higher carbon: nitrogen ratio 7.16 ± 0.44 and 5.04 ± 0.17 (mean ± s.e., n = 2) respectively (no statistical analysis was performed due to low sample size). Nonetheless, to control for the effects of nutritional deficiency, we fed *Temora longicornis* the nutritional food, *Tetraselmis spp.* for 15 h at non-limiting quantities following treatment and control incubations. If *A. fundyense* interfered with food assimilation, this could still lead to *T. longicornis* being more food-motivated. However, if assimilation efficiency was affected by nutritional inadequacy we would expect an increased ingestion rate in our *A. fundyense* treatments due to compensatory feeding (Cruz-Rivera and Hay 2000, Prince et al. 2006). There was no significant difference
between total ingestion rates in the 80:20 *A. fundyense: Rhodomonas lens* versus the 100% *R. lens* treatment. Therefore, it is unlikely that the behavioral change observed is due to differences in hunger levels between our treatments.

The *Alexandrium fundyense* strain used in this experiment contains saxitoxins. Saxitoxins can block sodium channels and interfere with nerve function, leading to paralysis or death in vertebrates (Hartshorne and Catterall 1981, Geraci 1989). Furthermore, saxitoxins can have detrimental effects leading to increased mortality in copepods (Avery and Dam 2007). This begs the question as to whether copepods were suffering saxitoxin-induced health effects after ingesting *A. fundyense*, which could explain the observed behavioral changes. Typically, when copepods are negatively affected by saxitoxins they exhibit signs of incapacitation and paralysis that are consistent with typical sodium-channel blocking events (Hartshorne and Catterell 1981; Ives 1987). *Temora longicornis* swimming faster does not concure with this mechanism. It is possible that the behavioral effects we see are due to copepods that have recovered from a mild sodium-channel blocking event (i.e. too mild to detect ingestion or fitness differences). However, preliminary results suggest that *T. longicornis* does not exhibit slower swimming speeds immediately after exiting *A. fundyense* blooms compared to control blooms (unpublished observation).

It is also possible that *Temora longicornis* were unaffected by the saxitoxins and the behavioral effects were not saxitoxin-mediated. *Temora longicornis* co-occurs with *Alexandrium fundyense*. Some co-occurring populations of *Acartia hudsonica* are unaffected by saxitoxins (Colin and Dam 2003) and can even experience positive effects demonstrated through increased survivorship, ingestion and egg production rates (Avery
personal communication). The mechanism responsible for saxitoxin-resistance in *Acartia hudsonica* is a simple genetic mutation in a sodium channel protein that reduces the binding affinity of the saxitoxins (Chen 2010). Therefore, it is possible that *T. longicornis* were unaffected by the saxitoxins and that the behavioral effects are due to another chemical compound(s). It is important to note that phytoplankton compounds responsible for large-scale economic and human-health consequences do not necessarily mediate interactions between phytoplankton and their competitors (Prince et al. 2008) or grazers (Ger et al. 2010). This is shown to be true for *Alexandrium sp.* as well; they have detrimental effects on competitors (Fistarol et al. 2004) and grazers (Tillman and John 2002) that are independent of saxitoxin content (but see Selander et al. 2006).

Another hypothesis is that *Alexandrium fundyense* possesses a chemical compound(s) that causes *Temora longicornis* to increase their metabolic rate or oxygen consumption which subsequently causes them to increase their swimming speed. Hassett (2003) found that *T. longicornis* respiration rate was not affected by ingesting *A. fundyense*. However, our experiments were not designed to measure changes in metabolism or oxygen consumption so we do not know whether this could have played a role in the behavioral changes we observed.

Copepods having increased encounter rates with predators after ingesting HAB species could have important consequences for bloom maintenance and the fate of toxins in food webs. However, for this mechanism to feasible in our system, we make a very important assumption here; that predation rate is primarily mediated through increases in encounter rate. If ingesting *Alexandrium fundyense* affects the outcome of these encounters (i.e. by affecting escape behavior), then this will likely affect the magnitude of
these effects. In order to determine the magnitude of grazer removal, we would have to determine how or if ingesting *A. fundyense* affects *Temora longicornis* escape behavior. Furthermore, it is important to note that swimming straighter and faster likely leads to increased encounter rates with potential mates. Therefore, it is difficult to predict what the overall effect of HAB-induced behavioral changes would be on *Temora longicornis* population dynamics.

Typically, the response variables measured in copepod-phytoplankton experiments are direct effects such as ingestion rate, survivorship and fecundity (but see Cohen et al. 2007, Amin and Båmstedt 2011). In our study, we did not find any effect of *Alexandrium fundyense* on *Temora longicornis* ingestion (Fig. 3.5). Our results indicate that *Temora longicornis* do not discriminate against or become incapacitated by ingesting *Alexandrium* cells. Additionally, ingesting *A. fundyense* did not decrease copepod survivorship over a 4 d period. Copepod mortality was 23-35% during the 4 d experiment with the greatest mortality occurring in the starved treatment. However, there were no significant differences between any of the treatments. Because there were no differences detected between even the starved and 100% *Rhodomonas lens* treatment, our results can be difficult to interpret. We cannot conclude whether or not long term exposure of *A. fundyense* could affect *T. longicornis* survivorship. However, we can conclude that the behavioral changes we observed in our experiments after 2 h exposure and 15 h depuration were not due to the *Temora longicornis* being ‘on the verge of death’ because they survived well when exposed to *A. fundyense* continuously for 4 d.

This research highlights the need to address the behavioral consequences of ingesting harmful phytoplankton species and how these behavioral changes can affect
encounters with predators. Future work aimed at determining the outcome of elevated encounters with predators is needed to fully determine the impact harmful algal species can have on grazers. Furthermore, this work can be extended to determine how soon after ingestion copepods become affected and how long-lasting these effects are.
CONCLUSIONS AND FUTURE DIRECTIONS

Summary

My thesis work explored the importance of behaviorally mediated interactions between marine copepods, their resources and predators, as well as the consequences of these interactions for the productivity of copepod populations. In Chapter 2, I conducted a field survey to explore the prevalence of fertilization limitation within two dominant copepod species *Temora longicornis* and *Eurytemora herdmani*. Results from this study suggest that both species are reproductively limited by low fertilization success in the field. Factors that correlated with fertilization success span population, community and ecosystem level factors.

Field patterns revealed a correlative relationship between mysid predator abundance and *Eurytemora herdmani* mating success. To determine a causal relationship, I conducted controlled mating experiments in the presence of mysid predators and predator cues (Chapter 3). *Eurytemora herdmani* mating success and offspring production were significantly reduced not only by predators but also in the presence of predator cues when no actual predators were present.

In addition to the effects of predators on copepod behavior and mating, I explored how prey species (phytoplankton) can trigger behavioral changes in grazing copepods. Using filmed behavioral assays, I determined that *Temora longicornis* increase their swimming speed and the straightness of their swimming path after ingesting the harmful bloom forming algae, *Alexandrium fundyense*. These behavioral changes lead to a 24-54% increase in the theoretical encounter rate with ambush predators.
Conclusions

Results from this study suggest that copepod reproduction is limited by mating success in the field and the stage at which this limitation occurs is influenced by copepod life history. In this study, the free spawning, *Temora longicornis* laid unfertilized eggs in substantial proportions at certain times of the year. In contrast, the brooded clutches of *Eurytemora herdmani* consistently contained a high proportion of fertilized eggs, but there was a high prevalence of mature females without egg sacs at certain times of the year. This difference between the fertilization status of a free spawning and brooding species likely reflects the different costs associated with releasing versus carrying an unfertilized egg.

Current theories suggest that population level factors such as density and sex ratio limit the number of mated females in copepod populations. In contrast, results from this study suggest that ecosystem factors such as temperature and phytoplankton concentration explain the most variation in *Temora longicornis* fertilization success in the field. Furthermore, in addition to density and sex ratio, mysid predator abundance was associated with variation in *Eurytemora herdmani* mating success in the field.

Laboratory experiments revealed that the effect of mysid predators on *Eurytemora herdmani* mating success is behaviorally mediated. *Eurytemora herdmani* mate less frequently and with less success (in terms of spermatophore transfer) in the presence of a mysid predator or only a predator cue. Furthermore, these changes in mating behavior lead to a significant decrease in the number of offspring produced highlighting the importance of top-down forces in copepod behavior and productivity. Additionally, results from this study suggest that behaviorally mediated bottom-up forces are
important. Copepods that were exposed to harmful bloom phytoplankton altered their swimming behavior in a manner that would likely increase their probability of encountering predators.

**Unique contributions and applications**

This thesis provided the following unique contributions:

i) Conducted the first simultaneous assessment of the fertilization status of brooding and free spawning copepod species

ii) Modified an existing technique for rapidly assessing the fertilization status of copepod eggs from field-caught females

iii) Tested the hypothesis that population level factors explain variation in copepod mating success

iv) Uncovered a field pattern of *Eurytemora herdmani* mating success being reduced when there abundant males in the population

v) Provided the first evidence that mating behavior and offspring production of marine copepods is reduced in the presence of a predator cue (with no actual predator present)

vi) Discovered a biological mimic of the physical cue created by a mysid predator

vii) Subjected copepods to predator kairomones at realistic concentrations in field

viii) Conducted the first experimental test of the effects of ingesting *Alexandrium fundyense* on copepod swimming behavior

**Implications and future directions**

The discovery that reproduction in *Eurytemora herdmani* and *Temora longicornis* is limited by mating success in the field leads to many questions. How are other species
affected by mating success in the field? Does the pattern suggested by our data (i.e. brooding species having higher fertilization status than spawning species) hold up when more species are analyzed? Finally, could this phenomenon explain low hatching success in previous studies (Dam and Lopez 2003, Ianora et al. 2007, Maps et al. 2005)? To address these questions, the fertilization status of more copepod species spanning various geographic locations should be assessed to determine broad patterns. Moreover, useful insights could be gleaned from fertilization tests conducted during copepod productivity surveys in addition to the more commonly assessed metric of egg production rate.

The finding that *Temora longicornis* fertilization was affected primarily by temperature and food concentration brings up an obvious question as to whether or not these variables affect spermatophore production rate. Male mating capacity has been shown to limit copepod mating success in previous studies (Kiørboe 2007, Sichlau and Kiørboe 2011). However, compared to the multitude of studies conducted on the effects of food and temperature on egg production rates, the effect on spermatophore production rate has been rarely explored. In light of these results, future research addressing constraints on spermatophore production rate would yield valuable insights.

*Eurytemora herdmani* fertilization was negatively associated with high population densities and male-skewed populations. Therefore, this research contradicts current theories suggesting that fertilization is limited by the abundance of males and indicates that there may be a threshold point where too many males can be detrimental to the fertilization caused from mating too frequently or male-male competition (Arnqvist and Nilsson 2000; Parker 2006). This phenomenon has not been explored for marine copepods, likely due to the current tenet that populations are typically male-limited
(Kiørboe 2007). Therefore, my results call for research to determine how mating success is affected when there are too many males.

The effect of mysid predator cues reducing mating frequency and success leads to further questions. How do copepods respond to other predators such as fish? How do copepods respond to predation risk over longer time scales? For example, these results indicate that *Eurytemora herdmani* responds to mysid predator cues by reducing mating frequency. However, the question remains as to whether or not copepods would adapt to elevated predation risk by adopting less risky mating behavior (such as decreasing mating duration) as seen in some freshwater species (Jersabek et al. 2007). Therefore, raising *E. herdmani* generations under various predation regimes and measuring their mating behavior would provide useful insights into the plasticity of copepod mating under increased predation risk.

Sublethal effects of harmful algal bloom species on copepod behavior are not well understood, but results from this study suggest that ingesting harmful algae increase a copepod’s predation risk and therefore, has important consequences for bloom dynamics. These results bring up several questions. Are these patterns specific to the organisms investigated or indicative of broad patterns among copepod grazers and HAB species? Does this pattern occur between copepod grazers and allopatric HAB species? As the geographic range of most HAB species is expanding due to climate change and eutrophication, these questions become increasingly relevant to the health of coastal ecosystems.

Predation rate depends on both encounter rates between predators and prey as well as the prey’s escape ability. In this study, we found that ingesting the HAB species,
Alexandrium fundyense increases the theoretical encounter rate between grazing Temora longicornis and ambush predators. The question still remains as to whether or not ingesting A. fundyense alters the escape ability of T. longicornis. Experiments investigating the effects of A. fundyense ingestion on T. longicornis escape ability are necessary to fully elucidate the interactions between these three trophic levels (i.e. algae, copepod, ambush predator).

Together these studies demonstrate that interactions between copepods, their predators and prey alter copepod behavior and these behavioral changes can translate to reduced mating success and increased susceptibility to predation. Understanding the factors that contribute to birthrates and death rates is necessary for predicting population dynamics of any species. It is especially important to understand the population dynamics of marine copepods due to their basal trophic position in food webs and their importance to commercially harvested species (Runge 1988; Mauchline 1998).
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