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UNIVERSITY OF SAN DIEGO

San Diego

The Effects of Density Gradients on the Distribution and Behavior of Copepods

A thesis submitted in partial satisfaction of the requirements for the degree of

Master of Science in Marine Science

By
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2018

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2018

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ACKNOWLEDGEMENTS

First, I must thank my advisor, teacher, and committee chair, Dr. Jennifer Prairie, who has given me a gift of a lifetime of fulfilling my dream of becoming a marine scientist in collaborating on her research in plankton ecology. She has not only been an amazing mentor but has also led me on an academic odyssey in experiencing the ocean in not only beauty but mathematically observing it in its natural chaotic splendor. Her foresight, patience, and willingness to share her knowledge and experiences was truly a gift and a gift I will always hold on to and return forward.

As with my committee chair, my co-chairs, Moira Décima and Nathalie Reyns, are truly rockstars of plankton ecology. As plankton experts, they have guided me and have given this thesis the definition and structure that it deserves. My time through the knick and the knack of the ups and downs through strenuous amounts of isolated time in the laboratory would not have been as rewarding without the assistance of undergraduate students Katie Ghiorso and Grace Cawley, as well the entire Prairie Lab research team whose previous research, with the funding of NSF, has provided the foundation for this following study.

The completion of this project would not have been possible without the USD Environmental and Ocean Sciences faculty, staff, and graduate students. Without the assistance of Liz E Baker, my supervisor and graduate mother throughout these four years, I would literally still be frazzled, anxious and lost. Her guidance and aid throughout my career at USD was much needed. In addition, without the help of the USD staff, especially Russ Harrell, Soroya

Rowley, and Keith Fink, this research would still probably be in the process of just a written proposal. I would like to at this time give a shout-out to Sarah Gray, my teacher and graduate mentor, who told me during my first semester that if I wanted to succeed in science I needed to stop trying to perfect everything. It's the game of the scientist to fail and, in times of improbable success, fail again. To end this all, I would love to thank my family for believing in me and being my backbone, as well as my grandmother Judy Hudson who, after many failed attempts during childhood, finally taught me how to read. Thank you.

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ABSTRACT

Observations of fine-scale behavioral dynamics of zooplankton may shed insight into much larger-scale ecosystem patterns and phenomena. Some zooplankton, such as copepods, are known to aggregate near density gradients; however, it is not clear the extent to which density gradients alone affect copepod distribution and behavior, since these gradients are often associated with phytoplankton layers. In this study, we investigated the behavior of *Calanus pacificus* in response to salinity gradients through laboratory experiments observed with high-resolution video and analyzed using computational techniques. Image data were collected using two cameras recording continuously for a duration of 30 minutes or more, allowing us to construct 3D swimming paths of copepods. In stratified treatments, we observed a decrease in the swimming velocity of some copepods as they transitioned through the density gradient. In addition, in the treatment with the strongest density gradient, we found that jump frequency was significantly increased for copepods when they were in the middle of the tank, in the vicinity of the density gradient. However, when averaging copepod tracks throughout the entire tank, we found no consistent differences in behavioral properties, including velocity, jump frequency, and net-to-gross displacement ratio (NGDR), between the stratified treatments and the control treatment without a density gradient. Our results indicate that physical cues from density gradients elicited some behavioral responses from copepods; however, the observed behavioral responses in our experiments did not result in aggregations, indicating that chemical cues may be important for these aggregations to occur.

CHAPTER 1:

LITERATURE REVIEW AND INTRODUCTION

1.1 Plankton Ecology Across Scales

Plankton ecology is an interdisciplinary field that focuses on how plankton interact with their environment and other organisms (Prairie et al. 2012). A major theme from previous work, in addition to my thesis, is how planktonic ecosystem dynamics are connected across spatial scales. Approaching plankton ecology in the context of multiple scales will allow for a better understanding of how diverse plankton taxa interact with their fluid environment. In this study, I investigate small-scale planktonic processes (cms to meters) by observing the individual distribution and behavior of a species of zooplankton in response to density gradients in the laboratory. The broader goal of this work is to understand how these small-scale processes may affect pelagic food webs on much larger scales. I begin by outlining large-scale processes in plankton ecology and descend towards the microscale. The definitions of scale I will use throughout this thesis are: the mesoscale (10s - 100s of kms), submesoscale (1 - 10 kms), fine-scale (meters), and microscale (cms or less).

Global primary production can be measured at the submesoscale and mesoscale in the form of satellite images that reveal heterogeneous distributions of phytoplankton blooms (displayed as chlorophyll *a* concentrations). Within the Southern California Bight (SCB), spatially and temporally complex patterns involving phytoplankton bloom dynamics can result in variations in fluxes of

carbon to higher trophic levels and to the sediment (Eppley and Peterson 1979, Behrenfeld et al. 2006). These continental shelf ecosystems are globally dominant zones of oceanic primary production, with the SCB ecosystem contributing up to 5% of global production (Santoro et al. 2010). From this shelf ecosystem, a greater surface chlorophyll concentration and a more elevated subsurface chlorophyll maximum occur nearshore compared to offshore (Lucas et al. 2011, Goodman et al. 2012). This discrepancy is a result of fine-scale geological and physical processes that directly influence plankton community composition, abundance, and biomass.

Lucas et al. (2011) proposed that water column variability between the productive nearshore and the oligotrophic offshore region of the SCB is mainly driven by fine-scale internal tides. The dominant internal tide in this area is the semidiurnal tide, which can force isopycnal displacements greater than 15 m (Lerczak et al. 2003), thus affecting nitrate fluxes from below the euphotic zone offshore to inshore (Lucas et al. 2011). Changes to nutrient availability contribute to the observed productive nearshore dominance of larger diatoms and picoeukaryotes, with the oligotrophic offshore regions dominated by the smaller cyanobacteria, haptophytes, and some dinoflagellates (Lucas et al. 2011). These fine-scale internal waves are a function of larger-scale local dynamics such as internal tide energy and cross-shore isopycnal tilt driven by the local alongshore winds (Lucas et al. 2011).

Fine-scale internal waves have also been attributed to fine-scale patchiness in planktonic distributions and can affect plankton physiology and dynamics at

microscales (Shanks 1983, Lennert-Cody and Franks 1999). For example, fine-scale aggregations of plankton have been observed as a result of interactions between microscale phytoplankton swimming behavior and internal wave motion (Lennert-Cody and Franks 1999, 2002). In addition, internal waves can change the ratio of fluorescence to chlorophyll *a* in phytoplankton as they move vertically and thus experience varying light levels (Lennert-Cody and Franks 2002, Prairie et al. 2012).

Phytoplankton serve as the primary food source for many zooplankton, in addition to larval stages of both benthic and pelagic organisms that occupy these habitats of the inner continental shelves (Wieters et al. 2003). The size structure and carbon production of phytoplankton communities can also impact higher trophic levels, including commercially important fisheries, by regulating zooplankton size structure (Turner 2004, Rykaczewski and Checkley 2008). As described in relation to phytoplankton abundance and composition of the SCB, spatial scales at all magnitudes contribute to the ecological efficiency of the nearshore environment (Goodman et al 2012, Prairie et al. 2012). However, in order to fully understand carbon export and pelagic food web dynamics of nearshore environments, we also need to consider smaller-scale variations in plankton processes that can occur in the vertical dimension; in particular, we will discuss thin layers as an important example of fine-scale vertical patchiness in planktonic ecosystems.

1.2 Fine-Scale Vertical Patchiness: Thin Layers

One of the main problems foraging zooplankton face when trying to find and capture phytoplankton and other prey is that these food sources are diluted in a huge volume of fluid, and zooplankton need to be efficient enough to clear large volumes over short times in order to gain enough nutrition to survive (Kiørboe 2011). One of the reasons nearshore planktonic ecosystems can attain high levels of diversity and productivity lies in the fact that plankton can be concentrated in fine-scale patches near the pycnocline (i.e. sharp density gradient). In fact, 75% of phytoplankton biomass can be concentrated in one or a few of these well-defined regions known as planktonic thin layers (Holliday et al. 2003). Without these highly concentrated prey patches, it has been suggested that zooplankton would not be able to maintain observed population sizes (Mullin and Brooks 1976).

Thin layers are a relatively recent focus in plankton ecology and have only been observed in the last few decades due to advances in optical and acoustic technology (Cowles et al. 1993, Dekshenieks et al. 2001, McManus et al. 2003, Prairie 2012, True 2014). Thin layers represent regions with concentrations of phytoplankton 1.5 to 3 times higher than ambient regions above or below and can last for hours to a few days; they are defined to have thicknesses of a few meters or less and maximum horizontal extents reaching up to kilometers (Alldredge et al. 2002, McManus et al. 2003, Prairie et al. 2012). Compared to their ambient surroundings, these layers are likely “hot spots” of microbial degradation and remineralization, and potentially sites of elevated trophic interactions (Alldredge et al. 2002, Prairie et al. 2012). Despite the characterization of thin layers as “hot

spots”, grazing rates within thin layers have only been quantified in a few studies and thus the role of thin layers in larger-scale trophic dynamics are still poorly understood (Woodson et al. 2007b, Menden-Deuer and Fredrickson 2010).

1.3 Zooplankton: Calanoid Copepods

Zooplankton represent a diverse group of organisms that spans multiple taxa, morphologies, and sizes (from micron-sized flagellates to meter-sized gelatinous organisms) (Kiørboe 2011). These planktonic organisms are defined as animals that are passively advected and spatially distributed in their fluid environment by horizontal currents with velocities that exceed their own swimming capabilities (Mackas et al. 1985, Huber and Lorke 2011). The notion of plankton as passive particles does not hold true when considering their vertical distribution, since vertical currents are much slower than horizontal currents and thus zooplankton have the ability to swim against them (Gallager et al. 2004). With 11,500 known species (Blaxter et. al 1998), copepods are the most abundant metazoans in the ocean (Townsend et al. 1994, Longhurst 1995, Turner 2004) and can often dominate secondary production (Landry 1977, Bautista and Harris 1992, Valdés et al. 2017).

As an abundant planktonic crustacean, copepods play a major role in transferring energy through the marine food web (Landry 1977, Valdés et al. 2017). Planktonic copepods utilize their small size, mostly transparent body, and diel vertical migration to hide themselves from predators, which include larval fish and carnivorous zooplankton, such as ctenophores, chaetognaths, medusae,

and larger copepods (Tiselius and Jonsson 1990, Verity and Smetacek 1996, Turner 2004). In part, copepod diversity and abundance is due to various feeding and reproductive strategies (Turner 2004).

Once copepods reach sexual maturity and reproduce, the new generation will start their life cycle as an egg. After hatching, copepod larvae will develop through six naupliar stages (N1– N6) until becoming a copepodite. After five copepodite moltings (C1– C5), the final adult stage is reached and molting ceases. In our study, we examined the behavior of late-stage copepodites (C4 and C5 stages) and adult copepods of the calanoid copepod, *Calanus pacificus*.

Calanoid copepods mainly display one or two types of swimming behaviors: 1) the use of the cephalic appendages and maxillipeds for slow movement, position maintenance, and filter feeding (Tiselius and Jonsson 1990, Paffenhöfer 1998), and 2) the use of the thoracic limbs for fast movement, which is seen with rapid bursts of one or more short jumps (Fields and Yen 1997, Lewis et al. 2006). Some species of copepods are known for their ability to travel relatively large distances in short time periods by jumping, whether it be for capturing a prey or escaping a predator (Tiselius and Jonsson 1990, Verity and Smetacek 1996, Lewis et al. 2006).

To locate and capture their prey, zooplankton display a diverse multitude of behaviors to find food (Kiørboe 2010). One feeding mode displayed by some calanoid copepods, suspension feeding, involves creating feeding currents using their feeding appendages to entrain and capture phytoplankton (Saiz and Kiørboe 1995). When a feeding current is not being used, some copepods can display an

ambush predator behavior by stealthily sinking in the water column waiting to capture prey (Tiselius and Jonsson 1990). At other times, they can display cruising behavior by actively swimming towards their prey (Tiselius and Jonsson 1990).

1.4 Cue Hierarchy

Planktonic spatial patterns were historically characterized in relation to large-scale physical forcings, such as studies describing sharp density gradients serving as a physical barrier for vertically migrating plankton (Harder 1968, Hamner 1988). More recently, attention has pivoted towards the importance of small-scale physical or chemical cues and their role in eliciting behaviors, which ultimately influence larger-scale community structure and population dynamics (Franks 1995, Gallagher et al. 2004, Woodson et al. 2005).

Research on mechanoreceptors and chemoreceptors using modern high-speed imaging has revealed the abilities of zooplankton, including most copepod species, to remotely sense particles (Price 1988, Tiselius and Jonsson 1990). The copepod's antennae serve as the primary organs for remotely sensing mechanical and chemical environmental stimuli (Paffenhofer and Lewis 1990, Bundy and Paffenhofer 1993, Verity and Smetacek 1996, Lewis et al. 2006), although chemosensors are also distributed along the cephalic appendages (Paffenhofer 1998). Copepod antennae have fine hair projections called setae that are able to sense hydrodynamic signals, which will elicit specific neurophysiological responses based on the deformation rate (Kjørboe 2011). Hence, feeding studies

have shown that copepods are able to recognize dissolved substances, food particles, potential predators, and mates without actually making direct physical contact with the stimulus (Poulet and Ouellet 1982, Buskey 1984, Price and Paffenhofer 1985, Huntley et al. 1986, Schultz and Kiørboe 2009).

Zooplankton, including copepods, can aggregate toward phytoplankton thin layers, which are usually associated with sharp density gradients (Tiselius et al. 1994, Menden-Deuer 2008, Möller et al. 2012). One important open question revolves around cue hierarchy, or the assessment of which type of cue, physical or chemical, is more important in triggering a behavioral response (True 2014). Understanding cue hierarchy when studying behavior associated with density gradients is tricky, since phytoplankton layers co-occur with density gradients. Thus, it is unclear the extent to which density gradients alone can cause changes in zooplankton behavior that lead to aggregations. In our study, we observed copepod behavior in the presence of density gradients alone to isolate the effect of this physical cue. The objective of this study is to determine how sharp density gradients affect the small-scale vertical distribution and behavior of *Calanus pacificus* using high-resolution stereoscopic imaging.

1.5 Questions and Hypotheses

There is still a lot unknown about the mechanisms underlying the behavior of zooplankton allowing them to aggregate in thin layers (Woodson et al. 2007a, Woodson et al. 2007b). There is a gap in the knowledge on the specific role, if any, that density gradients play in affecting zooplankton behavior and

distribution. To answer this question, our study used a novel experimental design testing copepod behavior in response to three different density gradient treatments. Our objective was to determine how sharp density gradients affect the small-scale vertical distribution and behavior of *Calanus pacificus* in the laboratory using high-resolution stereoscopic imaging. Broader implications for this work include the effects on larger-scale processes such as pelagic food webs, trophic links, and carbon export in nearshore environments. The questions that were addressed through this study, along with the accompanying null hypotheses, are:

1. Is there a significant difference in the vertical distribution of copepods in the absence of a density gradient compared to those in a weak density gradient or a strong density gradient?

- H_0 : There is no significant difference in the vertical distribution of copepods regardless of density gradient.

2. Are there significant differences in swimming behavior properties, including velocity, turning behavior, and jump frequency, with respect to density gradient (none, weak, and strong)?

- H_0 : There is no significant difference in the swimming behavior properties of copepods, including velocity, turning behavior, and jump frequency, regardless of density gradient.

CHAPTER 2:

THE EFFECTS OF DENSITY GRADIENTS ON THE DISTRIBUTION AND BEHAVIOR OF COPEPODS

2.1 Introduction

Copepods, one of the primary consumers of phytoplankton and the most abundant metazoans in the ocean, play an important role in the transport of carbon to higher trophic levels in marine ecosystems (Townsend et al. 1994, Longhurst 1995, Turner 2004). At high food abundances, populations of some coastal species can increase rapidly and at times dominate secondary production (Landry 1977, Bautista and Harris 1992, Valdés et al. 2017). For example, off southern California, *Calanus pacificus*, the species of focus in this study, usually represents less than a third of total zooplankton biomass, but may account for more than 80% of biomass at the apex of a phytoplankton bloom (Landry 1981). Given the important role of copepods in trophic dynamics, it is essential to learn more about their spatial distribution, and the chemical and physical cues that govern it. Previous studies have demonstrated that a wide range of biological and environmental factors affect zooplankton behavior and distribution, including behavior driven by foraging (Tiselius and Jonsson 1990, Tiselius 1992, Tiselius et al. 1997, Menden-Deuer and Grünbaum 2006), predator avoidance (Fields and Yen 1997, Kiørboe et al. 1999), and position maintenance (Genin et al. 2005, Woodson et al. 2005, Seuront 2006, Woodson et al. 2007b).

Sharp density gradients, or sharp increases in density as a function of depth (caused by discontinuities in salinity or temperature), are common

occurrences in coastal waters and can lead to zooplankton aggregations (Harder 1968, Mackas and Louttit 1988, Holliday et al. 1998). Physical cues from density gradients can potentially elicit behavioral responses allowing copepods to maintain position in the water column or signal the depth of diel vertical migrations (Lance 1962, Bochdansky and Bollens 2004, Woodson et al. 2007b). Field studies have provided evidence of associations between zooplankton and density gradients in natural environments; for example, Tiselius (1994) found elevated concentrations of zooplankton within pycnoclines, with adult copepods displaying the highest variations in their vertical distributions. Using a 3D video plankton recorder mounted onto an ROV, Gallagher et al. (2004) also observed planktonic aggregations, including patches of *Calanus* spp., near the thermocline. In a lab study, Harder (1968) also found certain zooplankton aggregate to density gradients. More recent experimental studies have examined the small-scale behavior underlying zooplankton aggregations related to changes in salinity or density. Seuront (2006) found that different salinities altered the swimming activity of a calanoid copepod, *Eurytemora affinis*, and others have observed copepods responding to physical gradients, allowing them to find and remain within stratified regions of the water column (Bochdansky and Bollens 2004, Woodson et al. 2007a). Although these studies have demonstrated that density gradients may be important in shaping copepod behavior in some cases, the mechanisms of copepod aggregation at density gradients remains poorly understood (Woodson et al. 2005).

Planktonic vertical spatial patterns were historically characterized in relation to physical forcings on the scale of the water column, such as sharp density gradients serving as a physical barrier for migrating plankton (Harder 1968, Hamner 1988). More recently, attention has pivoted towards the importance of small-scale (on the scale of cms to meters) physical or chemical cues and their role in eliciting behaviors, which ultimately influences larger-scale community structure and population dynamics (Franks 1995, Gallager et al. 2004, Woodson et al. 2005). One of the main questions revolves around cue hierarchy, or the assessment of which type of cue, physical or chemical, is more important in triggering a behavioral response (Woodson et al. 2007a, True 2014).

Understanding cue hierarchy when studying behavior associated with density gradients is tricky, since sharp density gradients in the field (characterized here as regions where buoyancy frequency, N , exceeds $\sim 0.01 \text{ s}^{-1}$) (Prairie et al. 2010) are often associated with food patches (Menden-Deuer 2008), thus presenting a problem when trying to distinguish between the role of physical and chemical cues.

Phytoplanktonic thin layers are regions where phytoplankton concentration is enhanced by many times background levels, within vertical extents of meters or less (McManus et al. 2003). These thin layers represent hot spots of enhanced trophic interactions, as suggested by observations of zooplankton, including copepods, aggregating near these layers (McManus et al. 2003, Menden-Deuer 2008, Möller et al. 2012). Laboratory studies have found that zooplankton, including some copepods, can successfully find and remain

within these regions of high food concentrations by orientating horizontally, increasing turn frequency, decreasing velocity, and altering jump frequency (Tiselius 1992, Menden-Deuer and Grünbaum 2006). However, given that these phytoplankton layers often co-occur with density gradients, it is unclear the extent to which density gradients alone can cause changes in zooplankton behavior that lead to aggregations.

The objective of this study is to determine how sharp density gradients affect the small-scale vertical distribution and behavior of *Calanus pacificus*. Copepods were observed in laboratory experiments using stereoscopic imaging, comparing copepod behavior in three treatments: a control without a density gradient and two density gradients of differing strengths. Here, we present experimental results to address two questions: 1) Is there a difference in the vertical distribution of copepods in the presence of a density gradient compared to no density gradient? and 2) Is there a difference in copepod behavioral properties, including velocity, net-to-gross displacement ratio (NGDR), and jump frequency, in the presence of a density gradient compared to no density gradient? Since behavior controls distributions of organisms in the ocean at the scale of meters, understanding the mechanisms driving copepod behavior may aid in understanding larger-scale distribution patterns and planktonic food-web dynamics (Castro et al. 1991, Tiselius et al. 1994, Folt and Burns 1999, Prairie et al. 2012, Valdés et al. 2017).

2.2 Methods

During the summer of 2017, three sets of experiments were conducted on August 1, September 6, and September 9 to observe the effect of density gradients, of differing strengths, on the vertical distribution and behavior of *Calanus pacificus*. Each experiment consisted of three treatments: a control (without a density gradient), a tank with a weak gradient (with the density of the top layer about 0.0020 kg/m³ less than that of the bottom layer), and a tank with a strong gradient (with the density of the top layer about 0.0040 kg/m³ less than that of the bottom layer) (Figure 1). Hereafter, the terms “weak density gradient” and “strong density gradient” refer to the relative strengths of the density gradients in our experiments, and are not used in relation to the strength of density gradients found in the field (as described later in the Discussion).

2.2.1 Field collection

C. pacificus was chosen for our experiments since it is a common local species and its relatively large body size can be easily detected with our cameras. *C. pacificus* was collected off a boat near Scripps Canyon in La Jolla, CA (32° 51' 23.8" N, 117° 16' 00.1" W) 6-7 days before each experiment. With a 300 µm mesh plankton net (0.5 m diameter mouth), 5-6 oblique tows were taken per sampling trip at a depth of at least 40 m and for a duration of 3 to 5 minutes. After each tow, the content of the cod end was emptied into two plastic buckets filled with filtered seawater and transferred into a cooler layered with ice.

Samples were sorted in the lab to isolate late-stage copepodites (C4 and

C5 stages) and adults of *C. pacificus*. Copepods were maintained with regular water changes in an incubator in the dark at 18°C until the experiment and fed a mixed diet of *Thalassiosira weissflogii* and haptophytes (*Tisochrysis sp.* and *Pavlova sp.*).

2.2.2 Experimental setup

Prior to each experiment, top layer fluids were mixed for each of the three treatments by diluting filtered seawater with DI water. Densities of top layer fluids were measured immediately before each experiment using a handheld density meter (DMA 35, Anton Paar) and were kept roughly consistent between experiments (Table 1). Copepods were starved for 24 hours prior to each experiment by transferring 20-25 *C. pacificus* individuals (late-stage copepodites and adults) into a 1 L beaker and acclimated to the top layer fluid for each treatment. Each beaker was wrapped in aluminum foil to maintain darkness and kept at room temperature. Copepods were inspected to ensure a normal swimming behavior before being transferred to the experimental tank.

The experimental tank had a square base (10 cm × 10 cm) and a height of 50 cm (Figure 1). The non-stratified control treatment was set up by pouring ~5 L of filtered seawater (hereafter referred to as bottom layer fluid) into the tank. For the stratified treatments, density gradients were established by filling the tank with ~2.5 L of bottom layer fluid, followed by 2.5 L of less dense top layer fluid. Top layer fluid was slowly pumped on top of the bottom layer fluid through a diffuser. The diffuser (initially soaked with top layer fluid) acted as a buffer to

establish a sharp density transition between the top layer and bottom layer fluid by minimizing mixing (as described in Prairie et al. 2013, 2015).

With completion of tank setup, 15 - 17 copepods were chosen for each treatment (see Table 1) to avoid substantial wall effects within our tank volume of ~5 L (as described in Dodson et al. 1997, Michalec et al. 2012, 2013). These copepods were transferred into a 50 mL beaker by pipette.

Once image recording had initiated, copepods were slowly poured into the top of the tank, being careful not to disturb the density gradient. For each treatment, copepods were observed in the tank using two high-resolution cameras (Grasshopper3 4.1 MP Mono USB3 Vision, Pt. Grey) set up perpendicularly to one another to allow for 3D imaging (Figure 2). To image without visible light, a 730 nanometer near-infrared light-emitting diode (M730L4 730 nm, 515 mW Mounted LED, Thorlabs), collimated using a Fresnel Lens, illuminated the tank from below through an acrylic panel installed in the table supporting the experimental tank. Preliminary experiments demonstrated that this light source caused no noticeable heat effects within the tank. Each treatment was recorded for ~30-35 minutes (Table 1) at 12 frames s⁻¹. The field of view of each camera was approximately ~30 cm x ~10 cm (with the horizontal section cropped to the width of the tank), allowing observation of copepods ~15 cm above and below the center of the tank.

To ensure that each density gradient persisted throughout each experiment and had a consistent thickness between experiments, vertical profiles of conductivity and temperature were taken after each treatment using a conductivity

probe (MSCTI, Precision Measurement Engineering, Encinitas, CA).

2.2.3 Data analysis

For all analyses, depth was defined relative to the middle of the density gradient (that is, a depth of 0 cm indicates the depth of the density gradient); this was determined as the depth of the average density (between the top and bottom layer) as measured by the conductivity probe (Appendix A). Thus, negative depth values indicate positions above the density gradient, and positive depth values indicate positions below the density gradient. For control treatments (without a density gradient), the middle depth was chosen as the averaged middle depth from the stratified treatments from each corresponding experiment (Appendix A).

Quantifying zooplankton swimming behavior was done by reconstructing copepod tracks in both 2D and 3D using MATLAB. Copepods were first identified in images in both cameras as objects above a specified brightness threshold and size, and positions of copepods were recorded. Copepod tracks were then assembled (for each camera separately) based on previously developed particle tracking methods (see Guezennec et al. 1994). Briefly, this was accomplished by minimizing a combination of the distance and change in velocity between individual copepods in neighboring frames. However, we allowed for a larger maximum displacement between images in our analyses compared to traditional particle tracking with passive particles to account for copepod jumps. Position was linearly converted from pixels to cms using the measured dimensions of the field of view of the camera (from images of a ruler taken

immediately after each treatment concluded). Corresponding tracks from each camera that aligned in the z-axis (vertical direction) were then combined to reconstruct 3D copepod tracks. In some cases, 2D tracks were not able to be combined because the copepod was not visible in both cameras concurrently (particularly when copepods were near a wall or not in the center of the tank and thus out of focus in one camera). Because of this, fewer 3D tracks were available to analyze than 2D tracks (number of 2D tracks and 3D tracks given in Table 2), so some behavioral characteristics were analyzed from 2D tracks from one camera (see below).

Copepod trajectories were used to plot vertical positions of individual copepod tracks within the tank as a function of time to illustrate patterns in vertical distribution between the three treatments. Since copepods in all treatments initially descended to the bottom of the tank within the first several minutes, the number of tracks that ascended back into the field of view after 500 s, as well as those that reentered the middle layer containing the density gradient (defined as the region between -2.5 and 2.5 cm from the middle depth), were also quantified.

Several behavioral properties were calculated from copepod tracks: overall and vertical velocity, NGDR, and jump frequency. Only 2D and 3D tracks over 10 s in duration were used for analyses since systematic differences in behavioral properties were found for tracks shorter than 10 s. Vertical velocity and jump frequency were calculated from 2D tracks since these properties are calculated

based on the z-coordinate only, and the 2D tracks provided higher sample size. Overall velocity and NGDR were calculated from 3D tracks.

Overall velocity was calculated by dividing the copepod's distance between sequential images by the time between images. Because we expected the density gradient might affect copepod swimming direction relative to the vertical axis, vertical velocity (using only the distance travelled in the vertical direction) was also calculated. For both overall and vertical velocity, the average velocity per copepod track was used for statistical analyses.

To quantify the tortuosity of copepod tracks (that is, the curvature of the swimming trajectories), NGDR was calculated throughout the duration of each copepod track as defined by Buskey (1984):

$$NGDR = \frac{\text{net distance travelled}}{\text{gross distance travelled}} \quad (1)$$

The cumulative NGDR value was then used for each copepod track for statistical analyses. Values of NGDR lie between 0 and 1, where values closer to 0 indicate swimming trajectories with greater turn frequencies, and values closer to 1 indicate relatively linear copepod swimming paths (Buskey 1984, Dur et al. 2011).

We calculated jump frequency for each copepod track by dividing the number of copepod jumps by the total time of the copepod track. Copepod jumps were defined as times when the copepod travelled upwards at a rate greater than 0.34 cm/s. This definition was loosely based on Tiselius and Jonsson (1990), who defined a copepod jump as vertical movements longer than 1 body length within 0.08 seconds. A Shapiro-Wilk test was conducted for each behavioral property to

test for normality. Behavioral properties that were normally distributed, overall and vertical velocity, were compared between treatments using Analysis of Variance (ANOVA). NGDR and jump frequency were not normally distributed and were compared between treatments using the non-parametric Kruskal–Wallis test, since this test does not assume a normal distribution.

Lastly, to compare copepod behavior at different vertical regions within the tanks, copepod tracks were identified in three depth bins (each with a thickness of 5 cm), defined as top (-10 to -5 cm), middle (-2.5 to 2.5 cm), and bottom (5 to 10 cm) regions. Total number of 2D and 3D tracks in each of these depth bins for each treatment (with all three experiments combined) are given in Table 3. Behavioral properties were quantified for tracks residing within these depth bins, and then compared between regions for each treatment using an ANOVA (overall and vertical velocity) or a Kruskal–Wallis test (NGDR and jump frequency), combining tracks from all experiments. Throughout the analyses, a significance level of $\alpha=0.01$ was used to reduce Type I error given the large number of ANOVAs and Kruskal-Wallis tests run, although it is noted in the few cases where tests resulted in p-values between 0.01 and 0.05.

2.3 Results

Conductivity meter profiles confirmed that our density gradients persisted throughout each experiment and remained relatively consistent for Experiments 1 and 3 (Figure 3 and Appendix B). However, in Experiment 2 conductivity profiles

indicated that there was not as large a difference as expected between the weak and strong density gradient (Appendix B).

A few general patterns can be observed from the copepod tracks (Figures 4 and 5). Upon entry into the water column, copepods initially descended in the tank, with most reaching the bottom of the field of view by 500 s (Figures 4 and 5). Secondly, the slopes of some individual copepod tracks appear to decrease as the copepods approached the density gradient (at a depth of around 0 cm) in the stratified treatments, indicating that the copepods were slowing down near the density gradient; this increased time transitioning through the gradient region was more noticeable in the stronger density gradient treatment (Figure 5 B, C, E, F, H, I). In addition, in the weak and strong density gradient treatments, copepods appear to be jumping more frequently within the middle region of the tank during their descent, although this is more noticeable in some experiments and treatments (Figure 5 B, C, E, F, H, I). Lastly, after ~500 s, more copepods were observed ascending in the water column, as well as returning to the region of the density gradient, in the stratified treatments compared to the control (Figure 4 and Table 4). In the control treatment (for all experiments combined), 12 out of a total 84 tracks (14%) represented copepods ascending back into the field of view after the initial descent, compared to 32 out of a total 87 tracks (37%) in the weak gradient and 45 out of a total 105 tracks (43%) in the strong gradient. However, there was high variability in the percentage of copepods ascending back into the field of view when comparing experiments separately (Table 4). The stratified treatments also had nearly 2 times more copepods ascending back into the region defined as

the density gradient than the control (Table 4). Throughout all experiments, the majority of copepods stayed close to the bottom of the tank, outside the field of view.

No consistent pattern on the effect of stratification on overall velocity was observed across experiments; in Experiments 1 and 2, copepods in the weak density gradient treatment had the highest average overall velocity, but in Experiment 3, the highest average overall velocity was observed in the control treatment (Figure 6 A, B, C). In all experiments, differences in overall velocity between treatments were not significant (ANOVA, $p > 0.01$). Average vertical velocity (Figure 6 D, E, F) in both Experiments 1 and 2 was lower in the stratified treatments than in the control treatment; however, these differences were again not significant between treatments (ANOVA, $p > 0.01$), and the highest average vertical velocity in Experiment 3 was found in the weak density gradient (also not significant).

No consistent pattern was observed for NGDR in response to a weak or strong density gradient (compared to the control), and no significant difference was found between treatments for NGDR for all experiments (Kruskal-Wallis test, $p > 0.01$) (Figure 7). In addition, no consistent pattern or significant difference in average jump frequency was found between treatments for all experiments (Kruskal-Wallis test, $p > 0.01$, although Experiment 3 had a p-value between 0.01 and 0.05) (Figure 8).

Behavioral properties were also compared between vertical regions of the experimental tank to determine if there was any effect from proximity to the

density gradient (Figure 9). No significant differences were found in overall velocity, vertical velocity, and NGDR between top, middle, and bottom regions (ANOVA and Kruskal-Wallis test, $p > 0.01$, although Experiment 2 for overall velocity had a p-value between 0.01 and 0.05). Overall velocity (Figure 9 A, B, C) was not related to the proximity to the density gradient, since the middle and bottom regions had similar average velocities with average velocities in the top region more variable. Vertical velocity (Figure 9 D, E, F) was lowest in the middle region for the stratified treatments (< 0.20 cm/s) consistent with the observed decrease in slope of the trajectories near the density gradient in Figure 5. However, these differences were not significantly different from the other regions of the tank. NGDR (Figure 9 G, H, I) also displayed no consistent pattern in how the curvature of the copepods' swimming trajectories varied in relation to region of the tank. Unlike the other behavioral properties, jump frequency was significantly different between regions of the tank (Kruskal-Wallis test, $p < 0.01$, Figure 9 J, K, L). Jump frequency increased sequentially between top, middle, and bottom regions for the control and the weak density gradient treatment (Figure 9 J, K). However, in the strong density gradient treatment, average jump frequency was highest in the middle of the tank, suggesting a possible behavioral response to the density gradient (Figure 9 L).

2.4 Discussion

In this study, we used laboratory experiments to observe copepod behavior in the presence of density gradients, allowing us to isolate the effects of this

physical cue and gain a better understanding of its role in shaping the distribution of copepods in the coastal ocean. This is important since the specific mechanisms and behaviors driving this patchiness is still poorly understood, as biological and physical factors in natural environments often vary together spatially and temporally in ways that confound the direct effects of any specific biological or physical cue. In addition, by using high-resolution stereoscopic imaging, we directly analyzed small-scale individual copepod movements in 3D and inferred how this fine-scale behavior may affect planktonic trophic dynamics on much larger scales. Our experimental methods allowed us to answer two main questions: 1) Do density gradients cause a change in the vertical distribution of copepods? and 2) Do density gradients cause a change in copepod behavioral properties?

In addressing the first question regarding the vertical distribution of copepods, we did not observe aggregations near the density gradient in any of our experiments, unlike the observations of some previous studies (Tiselius et al. 1994, Menden-Deuer and Grünbaum 2006, Möller et al. 2012). However, compared to the control, a decrease in the slope of vertical trajectories was observed as some copepods approached and entered the density gradient for the weak and strong density gradient treatments, with this being more noticeable in the stronger density gradient treatment. Although this observed decrease in velocity as copepods crossed the density gradient could indicate a behavioral response, it could also be the result of fluid entrapment around these copepods, since copepod trajectories appeared to resemble the trajectories of aggregates

sinking through density gradients (as shown in Prairie et al. 2015). Thus, these density gradients may have served as physical barriers (particularly given the strength of our density gradients relative to those in natural environments), slowing down or inhibiting initial passage of these descending copepods.

The discrepancy between the apparent decreases in velocity for some copepods as they crossed the density gradient and the lack of significant differences in overall or vertical velocity between treatments may have been caused by averaging all behavioral properties throughout the entire tank and across the entire duration of the experiment. This is important to consider since the density gradient only occupied a small region of our tank and thus may not have affected copepod behavior in other parts of the tank. Because of this, we decided to additionally analyze behavioral properties in top, middle, and bottom regions of the tank for each treatment to specifically quantify the impact of the density gradient (with copepod tracks in the middle layer) compared to the regions without a density gradient (top and bottom regions).

Even after comparing behavioral properties in different regions of the tank separately, jump frequency was the only behavioral property to vary significantly with proximity to the density gradient. Although a difference in jump frequency between regions of the tank was found for every treatment, in the control and weak density gradient, average jump frequency was similar for the middle and bottom regions, and much lower in the top of the tank. This reduced jump frequency observed in the top region could be a result of inhibited movement of the copepods due to an initial shock after they were introduced to the tank. In the

strong density gradient, jump frequency was significantly higher in the middle region, suggesting that copepods elicited a behavioral response when passing through the gradient, and in line with the observation of more frequent jumps near the density gradient. This increase in jump frequency in response to a density gradient was previously found in Tiselius (1992), who suggested that the higher observed jump frequency in the presence of a food layer (concurrent with a density gradient) may be correlated with feeding bouts. However, both Tiselius (1992) and Menden-Deuer and Grünbaum (2006) also observed other substantial changes to behavioral properties when zooplankton found and remained in food layers (that co-occurred with density gradients), such as decreased velocity, horizontal orientation, and decreased turn frequency. We did not observe such behavioral changes, suggesting that the lack of a food layer in our experiments impacted behavioral responses. Thus, this may indicate the importance of chemical stimuli over (or in addition to) physical stimuli in determining the ability of certain zooplankton to aggregate.

2.4.1 Considerations and future research

Using lab experiments to observe copepod trajectories in the presence of stratification allowed us to isolate the role of this specific cue on small-scale copepod behavior. However, our experimental density gradients were much stronger than those found in nature (a consequence of the scale of our tank being much smaller than that of the water column in the field); the maximum buoyancy frequency (N) in our experiments ranged from 0.81-1.76 s⁻¹, where maximum

buoyancy frequency in stratified regions in coastal waters is typically around $\sim 0.01\text{-}0.04\text{ s}^{-1}$ (Gallagher et al. 2004, Prairie et al. 2010), and only reaches $\sim 0.1\text{ s}^{-1}$ even in highly stratified conditions (Alldredge et al. 2002). Since our density gradients were $\sim 40\text{-}80$ times stronger than those found in the field, copepods may not have been able to easily transition between the top and bottom layers (Woodson et al. 2005, 2007b), which could potentially explain the greater decrease in velocity observed when crossing the stronger density gradient compared to the weaker density gradient.

In addition, although our experimental setup was designed to test the effects of density gradients of two different strengths, the conductivity profiles from at least one experiment indicated that there may not have been a substantial difference between the weak and strong density gradient treatment (Experiment 2, see Appendix B). Moreover, in our experiments we specifically used changes in salinity to create our density gradients, and, since many pycnoclines in the coastal ocean are driven by changes in temperature, the behavioral changes we observed may not be fully representative of these situations. These observations should be considered in future work to separate the role of active zooplankton behavior and direct effects of the physical environment. Despite the fact that the density gradients in our experiments were at times variable, our results provide a conservative estimate of the potential effects of density gradients on copepod behavior since the density gradients in our experiments were stronger than those typically found in nature. Thus, the observed behavior in response to density

gradients should provide insight into the role of these physical features, in the absence of chemical cues, in eliciting zooplankton behavior.

Copepods were starved for 24 hours before the experiment to minimize level of satiation as a potential confounding variable; however, this may have affected copepod behavior in some ways that may not be representative of their behavior in natural environments. In particular, copepods remained near the bottom of the tank, outside the field of view, for the majority of all experiments and treatments, which could have been caused by either starvation or a lack of food within the tank. To investigate the effect of food availability on copepod behavior, we replicated the experimental setup for the control treatment (no density gradient), but with a low concentration of phytoplankton (3000 cells/mL of the species *Thalassiosira weissflogii*) mixed evenly within the tank. Copepod tracks (recorded only in 2D) are shown in Figure 10. In this experiment, copepods can be observed actively swimming within the field of view for the duration of the experiment (29.4 minutes), unlike the control experiments without food, in which copepods immediately descended down to the bottom of the tank when first entering from above (with the exception of control Experiment 2, Figures 4 and 5). This pilot experiment with phytoplankton suggests that the presence of food does have substantial effects on copepod behavior; the lower total swimming time in our experiments without phytoplankton could indicate that, without a food source, it was not worthwhile for copepods to expend excess energy. Thus, our experimental results may not fully reveal the effects of density gradients as physical cues for zooplankton behavior when considering the range of real-world

conditions. This should be considered in future studies in trying to understand the interactions between physical and biological cues in driving zooplankton behavior and small-scale distributions.

Morphologically and taxonomically, zooplankton, and even copepods, are very diverse. Although all zooplankton must find and capture food, their feeding mechanisms can vary widely (e.g., passive ambush feeding, cruise feeding, filter feeding), and thus the behaviors organisms exhibit and the cues triggering these behaviors are often quite different (Kiørboe 2011). Since our study focused on a single species of calanoid copepod, it will be important to test the effects of density gradients as physical cues for other copepods and zooplankton to gain a better understanding of the breadth of behaviors that may be exhibited and their implications for larger-scale pelagic ecology.

2.4.2 Implications and ecological context

Zooplankton, including copepods, aggregate toward fine-scale phytoplankton thin layers, which are usually associated with sharp density gradients (Tiselius et al. 1994, Menden-Deuer 2008, Möller et al. 2012). Much remains unknown about the mechanisms underlying the behavior of zooplankton allowing them to aggregate in these regions (Woodson et al. 2007b); one key to understanding this is determining which type of cue, physical or chemical, plays the most important role in eliciting relevant behavioral responses (Woodson et al. 2007a, True 2014). Investigating this cue hierarchy in the field is challenging because of the frequent co-occurrence of sharp density gradients with food

patches (Menden-Deuer 2008); this association between planktonic thin layers and density gradients is likely because pycnoclines represent regions of reduced mixing that allow these food patches to be maintained (Prairie et al. 2010). Previous lab studies of zooplankton behavior in food patches also featured a concurrent density gradient since phytoplankton layers can only be sustained in an experimental setting if the water column is stratified (Tiselius 1992, Menden-Deuer and Grünbaum 2006).

Unlike previous studies, we did not find significant changes in the majority of behavioral properties sampled in the presence of sharp stratification. Our results do indicate, however, that density gradients may have some behavioral effects on copepods, as seen with both increased jump frequency in the region of the density gradient and an apparent decrease in velocity as copepods descended through the density gradient. The lack of strong behavioral patterns could be a result of the absence of a food source in our experimental setup, since Menden-Deuer and Grünbaum (2006) found that density gradients may serve as the initial cue for finding and remaining within an area, but without a chemical stimulus there would be no zooplankton aggregations. Chemical cues may be more important than, or important in addition to, physical cues in controlling copepod behavior and distribution that may ultimately shape their spatial distribution in the coastal ocean on much larger scales. Our experiment with phytoplankton supports the idea that there may be interactions between physical and chemical cues in driving small-scale zooplankton behavior.

Overall, given that the observed behavioral changes in our experiments with density gradients alone were modest or, in many cases, not present, chemical cues may be the dominant mechanism driving copepod aggregations in phytoplankton layers associated with sharp density gradients, or at least chemical cues may be needed in addition to physical cues to trigger substantial behavioral responses.

This project is among the first to study physical cues alone, in the context of the effect of density gradients on the distribution and behavior of copepods. It is noteworthy that both chemical and physical cues may be needed to promote behavioral changes of *Calanus pacificus* that result in aggregations and larger-scale patchiness, even though a hierarchy might exist. Copepods contribute such a large part of both the biomass and abundance of primary consumers (Turner 2004) and thin layers may represent regions of enhanced grazing that are particularly important for how the flow of carbon occurs in pelagic ecosystems. Thus, the findings of this study are significant in that they provide insight into the role of density gradients in shaping behavior and distributions at these important oceanic features.

CHAPTER 2 TABLES AND FIGURES

Table 1. Experimental conditions for each experiment and treatment, including the number of copepods in the experimental tank, density and temperature of the top layer and bottom layer fluid (with only bottom layer for the control), and the duration of the experiment (min), calculated by taking the number of recorded frames and dividing by the recording rate (12 s^{-1}).

Expt. Number	Treatment	# of copepods	Top fluid density (kg m ⁻³) and temperature (°C)	Bottom fluid density (kg m ⁻³) and temperature (°C)	Duration (min)
1	Control	16	1.024 kg m ⁻³ , 21.20 °C	1.024 kg m ⁻³ , 21.20 °C	34.80
	Weak Gradient	16	1.021 kg m ⁻³ , 21.00 °C	1.021 kg m ⁻³ , 21.40 °C	34.20
	Strong Gradient	16	1.019 kg m ⁻³ , 21.10 °C	1.023 kg m ⁻³ , 21.20 °C	34.20
2	Control	16	1.024 kg m ⁻³ , 21.10°C	1.024 kg m ⁻³ , 21.10°C	34.80
	Weak Gradient	16	1.022 kg m ⁻³ , 21.10 °C	1.024 kg m ⁻³ , 21.60 °C	34.20
	Strong Gradient	16	1.019 kg m ⁻³ , 21.00 °C	1.024 kg m ⁻³ , 21.60 °C	29.40
3	Control	15	1.024 kg m ⁻³ , 21.60 °C	1.024 kg m ⁻³ , 21.60 °C	29.40
	Weak Gradient	17	1.022 kg m ⁻³ , 20.90 °C	1.024 kg m ⁻³ , 21.60 °C	30.90
	Strong Gradient	16	1.020 kg m ⁻³ , 20.70 °C	1.024 kg m ⁻³ , 21.60 °C	30.60

Table 2. For each experiment and treatment, the total number of 2D and 3D tracks over 10 seconds in duration.

Experiment	Treatment	Total # 2D tracks	Total # 3D tracks
1	Control	24	17
	Weak Gradient	46	26
	Strong Gradient	37	26
2	Control	30	26
	Weak Gradient	21	19
	Strong Gradient	34	19
3	Control	23	14
	Weak Gradient	20	11
	Strong Gradient	34	11

Table 3. For each treatment (all experiments combined), the number of 2D and 3D tracks over 10 seconds in duration in each of the three depth bins of the tank (top, middle, and bottom).

	2D Tracks			3D Tracks		
Treatment	top	middle	bottom	top	middle	bottom
Control	13	14	16	33	35	34
A	5	16	20	17	33	41
B	4	17	17	15	48	35

Table 4. For each experiment and treatment (along with the total for all experiments combined), the total number of all 2D tracks are reported (>10 s in duration), along with the number (and percentage) of these tracks representing copepods that ascended back into the field of view after 500 s, and the number (and percentage) of tracks that entered into the region of the density gradient (defined as the middle bin) after 500 s.

Expt. #	Treatment	Total # of 2D tracks	# of tracks (and percentage) returning to field of view after 500 s	# of tracks (and percentage) in density gradient region after 500 s
1	Control	24	0 (0 %)	0 (0%)
	Weak Gradient	46	27 (59%)	13 (28%)
	Strong Gradient	37	16 (43%)	2 (5%)
2	Control	30	11 (37 %)	7 (23 %)
	Weak Gradient	21	5 (24 %)	0 (0 %)
	Strong Gradient	34	14 (41 %)	3 (9 %)
3	Control	30	1 (3 %)	0 (0 %)
	Weak Gradient	20	0 (0 %)	0 (0 %)
	Strong Gradient	34	15 (44 %)	9 (26 %)
TOTAL	Control	84	12 (14 %)	7 (8 %)
	Weak Gradient	87	32 (37 %)	13 (15 %)
	Strong Gradient	105	45 (43 %)	14 (13 %)

Figure 1. Schematic showing the three experimental treatments; water density is illustrated by shading and copepods are shown in orange.

Figure 2. A) Schematic of camera setup. B) An example of three 3D copepods tracks (shown in different colors) over a 40 s time period.

A

B

Figure 3. Example of salinity profiles as a function of depth (shown for Experiment 3 taken after experiment was completed) for the three treatments: control (A), weak density gradient (B), and strong density gradient (C).

Figure 4. All individual 2D copepod tracks (at least 10 seconds in duration) plotted in varying colors for each treatment for each experiment. Vertical location vs. time is plotted for each track for the entire duration of each experiment (30-35 minutes depending on experiment). (A-C) Copepod tracks for control, weak density gradient, and strong density gradient, respectively for Experiment 1. (D-F) Copepod tracks for control, weak density gradient and strong density gradient, respectively for Experiment 2. (G-I) Copepod tracks for control, weak density gradient and strong density gradient, respectively for Experiment 3. The dotted line represents the location of the density gradient (with the exception of the control group, where the dashed line represents the average location of the gradient in the other treatments).

Experiment 1

Experiment 2

Experiment 3

Figure 5. All individual 2D copepod tracks (at least 10 seconds in duration) plotted in varying colors for each treatment of each experiment. Vertical location vs. time is plotted for each track for the first 400 s (~6.7 mins) of each experiment. (A-C) Copepod tracks for control, weak density gradient, and strong density gradient, respectively for Experiment 1. (D-F) Copepod tracks for control, weak density gradient and strong density gradient, respectively for Experiment 2. (G-I) Copepod tracks for control, weak density gradient and strong density, respectively for Experiment 3. The dotted line represents the location of the density gradient (with the exception of the control group, where the dashed line represents the average location of the gradient in the other treatments).

Figure 6. (A-C) A comparison of mean overall velocity between treatments for Experiments 1, 2, and 3 respectively, and (D-F) a comparison of mean vertical velocity between treatments for Experiments 1, 2, and 3 respectively. Overall velocity was calculated from 3D copepod tracks of at least 10 seconds in duration, and vertical velocity was calculated from 2D copepod tracks of at least 10 seconds in duration (sample sizes given in Table 2). Error bars represent standard error. For all other experiments, no significant difference was found between treatments for overall velocity or vertical velocity (ANOVA, $p = 0.081$ (A), $p = 0.592$ (B), $p = 0.843$ (C), $p = 0.292$ (D), $p = 0.592$ (E), and $p = 0.999$ (F)).

Figure 7. (A-C) A comparison of mean net-to-gross displacement ratio (NGDR) between treatments for Experiments 1, 2, and 3 respectively. NGDR was calculated from all 3D copepod tracks of at least 10 seconds in duration (sample sizes given in Table 2). Error bars represent standard error. For all experiments, there were no significant differences between treatments (Kruskal-Wallis test, $p = 0.906$ (A), $p = 0.127$ (B), $p = 0.263$ (C)).

Figure 8. (A-C) A comparison of mean jump frequency between treatments for Experiments 1, 2, and 3 respectively. Jump frequencies were calculated from all 2D copepod tracks of at least 10 seconds in duration (sample sizes given in Table 2). Error bars represent standard error. For all experiments, there were no significant difference between treatments (Kruskal-Wallis test, $p = 0.655$ (A), $p = 0.525$ (B), $p = 0.032$ (C)).

Figure 9. A comparison of behavioral properties between top, middle, and bottom regions of the tank including overall velocity (A-C), vertical velocity (D-F), NGDR (G-I), and jump frequency (J-L), with data from all experiments combined for each treatment. Behavioral properties were quantified from 3D tracks (overall velocity and NGDR) or 2D tracks (vertical velocity and jump frequency) within 5 cm bins in top, middle, and bottom sections of the tank respectively (sample sizes given in Table 3). Error bars represent standard error. For each treatment, no significant difference was found between top, middle, and bottom regions for either overall velocity or vertical velocity (ANOVA, $p = 0.276$ (A), $p = 0.0497$ (B), $p = 0.0961$ (C), $p = 0.105$ (D), $p = 0.673$ (E) $p = 0.0629$ (F)). Additionally, for each treatment, no significant difference was found between top, middle, and bottom regions for NGDR (Kruskal-Wallis test, $p = 0.242$ (G), $p = 0.426$ (H), $p = 0.084$ (I) However, jump frequency was significantly different between regions of the tank for all treatments (Kruskal-Wallis test, $p < 0.0001$) (J-L)

Figure 10. All individual 2D copepod tracks (at least 10 seconds in duration) plotted in varying colors for the experiment with phytoplankton (and no density gradient). The dashed line represents the middle of the tank, comparable to the location of the density gradient in the stratified experiments.

CHAPTER 3:

GENERAL THESIS CONCLUSIONS AND FUTURE DIRECTIONS

Through this project, we demonstrated that density gradients may play a partial role in eliciting behavior and shaping the vertical distribution of copepods, while a food source might be crucial for these behaviors to be sustained. Therefore, when studying plankton thin layers in the field, both physical and chemical cues should be examined together to understand the implications for larger-scale dynamics including carbon export and pelagic food web dynamics. It is important to understand the role each cue has in controlling behavior and distribution at an individual level, as well as their combined effects in shaping the nearshore environment.

Our study focused on one species of copepod, *C. pacificus*, but zooplankton represent a diverse group with individuals displaying a wide range of behaviors (Kiørboe 2011). Despite this limitation, since copepods are the most abundant metazoans in the ocean (Townsend et al. 1994, Longhurst 1995, Turner 2004), and often the dominant consumer during spring blooms in the Southern California Bight (Landry 1981), we hope that this research will provide a general understanding of how density gradients can act as physical cues to drive zooplankton behavior that can affect their distribution, and thus their role as grazers.

Previous studies have demonstrated that an inhibition of copepod growth was correlated with limited food sources, and copepods must continuously search

and locate food patches to efficiently feed (Mullin and Brooks 1976, Tiselius 1997, Woodson et al. 2007b). In laboratory studies, Tiselius (1992) and Menden-Deuer and Grünbaum (2006) observed how fast behavioral responses by zooplankton to prey patches can lead to advantageous feeding conditions for the individual. In fact, without these adaptations to exploit patchy ecosystems, zooplankton may not be able to sustain observed population sizes (Mullin and Brooks 1976, Davis et al. 1991).

One of the gaps of knowledge in previous literature is the impact of density gradients alone in affecting distribution and behavior of copepods. Previous studies observing zooplankton aggregation behaviors, including Tiselius (1992) and Menden-Deuer and Grünbaum (2006), used density gradients to create defined prey layers, and thus the effects of the physical and chemical cues could not be separately ascertained. A study by Harder (1968), on the other hand, observed a plethora of zooplankton, with the exception of copepods, and found almost all zooplankton aggregated at density gradients. Unlike these studies, our study focused solely on the effect of density gradients on copepod behavior. We observed no aggregations nor any significant differences between treatments in the majority of behavioral properties; significant differences in jump frequency were only found when comparing different regions of the tank. Menden-Deuer and Grünbaum (2006) observed that even though behavior may be altered due to physical cues, without a chemical stimulus (i.e. chemical exudates) there will be no overall aggregative effect. This could explain our results, since in nature zooplankton have to expend energy to find and capture their prey in a large, dilute

fluid volume (Kiørboe 2010, 2011). Thus, cue hierarchy could be an adaptation to save energy and thus increase survival.

To build on my study, investigating behavior and distribution of *C. pacificus* within the nearshore environment of the SBC may help inform the ecological impacts of individual foraging on larger scales (Woodson and McManus 2007, Durham and Stocker 2012, Prairie et al. 2012). Better understanding zooplankton behavior near density gradients is important since thin layers, which are associated with sharp density gradients, are known to occur in almost all marine environments, including estuaries (Donaghay et al. 1992), coastal shelves (Cowles and Desiderio 1993, McManus et al. 2003), fjords (Holliday et al. 1998, Dekshenieks et al. 2001), and open ocean waters (Bjornsen and Nielsen 1991). Since a major theme of plankton ecology is using a bio-physical multi-scaled approach, it is important to understand how small-scale processes such as aggregation at thin layers impact larger scales. These small-scale processes play an important role as “ecological engines” (True 2014) in driving the productivity of nearshore ecosystems and impacting larger-scale dynamics such as carbon export, pelagic food web dynamics, and the sustainability of fisheries.

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APPENDICES

APPENDIX A. For each experiment and treatment, the middle density (calculated from conductivity profile) is given, along with the depth below the surface where that middle density occurred. For the control treatment, middle depth was calculated as the average middle density depth of its corresponding experiment's weak density gradient and strong density gradient treatments. Maximum buoyancy frequency, N , calculated from the conductivity profiles, are also shown.

Expt. #	Treatment	Middle Density (kg m^{-3})	Depth below surface (cm) of middle density	Max Buoyancy Frequency, N (s^{-1})
1	Control	1.022 kg m^{-3}	22.25 cm	N/A
	Weak density gradient	1.022 kg m^{-3}	22.39 cm	1.186 s^{-1}
	Strong density gradient	1.021 kg m^{-3}	22.10 cm	1.762 s^{-1}
2	Control	1.021 kg m^{-3}	20.54 cm	N/A
	Weak density gradient	1.021 kg m^{-3}	19.79 cm	0.813 s^{-1}
	Strong density gradient	1.020 kg m^{-3} ,	21.30 cm	1.197 s^{-1}
3	Control	1.022 kg m^{-3}	23.80 cm	N/A
	Weak density gradient	1.022 kg m^{-3}	23.73 cm	1.028 s^{-1}
	Strong density gradient	1.021 kg m^{-3} ,	23.87 cm	1.468 s^{-1}

APPENDIX B. Profiles of salinity, temperature, and density vs. depth, measured by the conductivity probe after completion of experiment. (A-C) Profiles for control, weak density gradient, and strong density gradient respectively for Experiment 1. (D-E) Profiles for weak density gradient and strong density gradient respectively for Experiment 2. Profiles for the control of Experiment 2 were not taken. (F-H) Profiles for control, weak density gradient, and strong density gradient respectively for Experiment 3.

Experiment 1

Experiment 2

Experiment 3

APPENDIX C. All individual 3D copepod tracks (at least 10 seconds in duration) plotted in varying colors for each treatment of each experiment. Vertical location vs. time is plotted for each track for the entire duration of each experiment (30-35 minutes depending on experiment). (A-C) Copepod tracks for control, weak density gradient, and strong density gradient, respectively for Experiment 1. (D-F) Copepod tracks for control, weak density gradient, and strong density gradient, respectively for Experiment 2. (G-I) Copepod tracks for control, weak density gradient, and strong density gradient, respectively for Experiment 3. The dotted line represents the location of the density gradient (with the exception of the control group, where the dashed line represents the average location of the gradient in the other treatments).

Experiment 1

Experiment 2

Experiment 3

APPENDIX D. All individual 3D copepod tracks (at least 10 seconds in duration) plotted in varying colors for each treatment of each experiment. Vertical location vs. time is plotted for each track for the first 400 s (~6.7 mins) of each experiment. (A-C) Copepod tracks for control, weak density gradient, and strong density gradient respectively for Experiment 1. (D-F) Copepod tracks for control, weak density gradient, and strong density gradient respectively for Experiment 2. (G-I) Copepod tracks for control, weak density gradient, and strong density gradient respectively for Experiment 3. The dotted line represents the location of the density gradient (with the exception of the control group, where the dashed line represents the average location of the gradient in the other treatments).