Vertical Distribution of *Pisaster ochraceus* larvae in a halocline in the presence and absence of a food patch

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Abstract

Thin phytoplankton layers are characterized by dense concentration of cells a few centimeters to several meters thick at haloclines. They extend horizontally for kilometers and can persist for days. These dense concentrations of phytoplankton have the potential to influence the behavior, feeding success, and predation by higher trophic levels. This study looked at changes in the vertical distribution and persistence of two algae *Isochrysis galbana* and *Rhodomonas* sp. at concentrations of 12,000-66,000 cells/ml, at and below a halocline in the presence and absence of a predator *Pisaster ochraceus* larvae. Three treatments with two replicates per treatment were set up, columns with, a) algae at the halocline + larvae, b) algae at the halocline – larvae, c) no algae + larvae in halocline. In each case, approximately 100 larvae were gravity fed to the bottom of each column. Patch width was measured 1, 3, and 6 hour after introduction into the columns. Patch width for *Rhodomonas* sp. remained near one cm for six hours while patch width for *I. galbana* increased to two cm in six hrs. For both algal species, cell concentration at the halocline declined significantly in the presence of *Pisaster* larvae. A greater decrease in cell concentration was observed for *I. galbana* when the food was at the bottom of the column rather than at the halocline. This was observed in the presence and absence of larvae. This indicates that for *I. galbana*, the decline in algal cell concentration at the bottom of the column might be due to cell movement towards the halocline. Interestingly, larvae remained around the halocline in the presence or absence of food for 6 hours. Living in stratified layers where thin phytoplankton layers might exist can be advantageous for *P. ochraceus* larvae by providing them with resources to feed, grow, and metamorphose but might also expose them to predation by higher trophic levels.

Key Words: halocline, thin phytoplankton layers, algae, salinity, larvae

Introduction

Climate has consequences on marine ecosystems and the social and economic systems that rely on them. (Harley et al, 2006). As global temperatures continue to increase, glaciers melt and discharge from rivers increase causing environmental changes in the ocean waters. These environmental changes have caused a decline in species diversity in marine ecosystems (Harley et al, 2006).

In coastal marine ecosystems, salinity is one of the most important environmental determinants of organismal performance and can affect the abundance and distribution of marine invertebrates (Kinne 1971). Research shows that salinity can vary between 15 and 20 ‰ (parts per 1000) in surface waters and between 30 ‰ and 35 ‰ in bottom waters (Khangankar et al. 2011; Sutherland et al. 2011). In the Pacific Northwest salinity fluctuates throughout the year mainly due to, acceleration of Artic ice melt, changes in precipitation patterns, and an increase in storm frequency (Harley et al. 2006; Yamamoto-Kawai et al 2009). Extreme highs or lows in salinity can have negative effects on marine invertebrates and can result in reduced performance, morphological changes and even death (Harley 2009). Several marine species have been affected by these trends found in the Puget Sound (Vasquez & Young 1996).

The San Juan Islands are located north of the Strait of Juan de Fuca and south of the Strait of Georgia in the Pacific Northwest. More than 19 rivers discharge fresh water into the Georgia Strait, (the Fraser River discharging the most), which mixes with saline waters from the Pacific Ocean leading to a stratified water column (Sutherland et al. 2011). Previous Studies have shown that invertebrate larvae congregate in or below haloclines, preferring more saline waters (Metaxas & Young 1998b). Found within the distinctive layers of saline waters are thin phytoplankton layers that can be advantageous for growth, reproduction, and food consumption of invertebrate larvae (Cheriton et al. 2009).

Thin phytoplankton layers are commonly found in coastal ecosystems and can be maintained by different physical and biological processes (McManus 2012). These layers usually have a width of less than five meters and can remain in the water for hours, days, and weeks (McManus 2012). Thin phytoplankton layers differ from other aggregations because they are a fraction of a meter in vertical thickness and have a stronger vertical concentration of phytoplankton, opposed to deep chlorophyll maxima aggregations with weak gradients in phytoplankton concentration (Durham& Stocker 2012). Several factors such as density and quality of the food patch can cause different behavioral changes in marine invertebrates (Burdett-Coutts & Metaxas 2004). Previous studies have shown that a dense algal patch of 10,000 cells/ml, similar to an algal bloom, makes it complicated for larvae to travel through it (Metaxas & Young 1998a). Algal species that make up these thin phytoplankton layers react to predators present in the same environment by changing their swimming patterns to avoid being eaten.

Pisaster ochraceus is a keystone species in the Pacific Northwest that feeds on a variety of phytoplankton during the larval stage. Studies on *P. ochraceus* interacting with thin phytoplankton layers are unknown. Lab studies have used *Dunaliella tertiolecta, Rhodomonas lens*, *Isochrysis galbana*, and other algae to look at larval growth (Pia et al. 2012). The adult *P. ochraceus* feeds mainly on *Mytilus califorrnicus* and is responsible for sustaining much of the local diversity in rocky intertidal communities (Strathmann

1971). A decline in *P. ochraceus* may cause a problematic increase in other marine species (e.g. *Mytilus califorrnicus)* and cause an exclusion of other intertidal macroinvertebrates. *P. ochraceus* larvae that come across a thin phytoplankton layer in a halocline can benefit from a steady source of food that can help its growth and development to metamorphosis. Studying the behavior of *P. ochraceus* in haloclines with food patches will give us an understanding of their behavior within thin phytoplankton layers.

The purpose of the study is to investigate larval behavior of *P. ochraceus* in food patches at different placements within haloclines. The food patch consisted of two different types of algae*, Rhodomonas sp*, and *Isochrysis galbana,* at a concentration similar to an algal bloom. Previous studies have shown that *Rhodomonas sp.* is preferred by many echinoderm larvae (Schiopu et al 2006). It is more nutritious and easily digested (Schiopu et al 2006). Both species of algae vary in size, shape, and motility. *Rhodomonas sp.* is bigger in size in comparison to *Isochrysis galbana (*Pedrotti &Fenaux 1993). *Rhodomonas sp.* and *Isochrysis galbana* both have advantages that allow them to survive in marine ecosystems where predators are present. The three main questions for this research were

1. Does the presence or absence of larvae have an effect on algal cell concentration when placed below and in a halocline?
2. How does the vertical distribution of *P. ochraceus* larvae differ when algae are placed at and below the halocline?
3. Does the type of algae determine the vertical position of *P. ochraceus* larvae?

We tested the hypothesis that when larvae are present in a halocline with a food patch, there will be a greater change in algal cell concentration of *Rhodomonas sp.* than *Isochrysis galbana* because the former alga is more nutritious and easily digested. We also tested the hypothesis that when algae are placed below or at the halocline, majority of the larvae will stay within the food patch for the duration of the experiment. This study first looked at the behavior of algae cultured at 24.5 ‰ and 30 ‰ in a halocline at 1,3, and 6 hrs. in the presence and absence of *P. ochraceus larvae*. We then looked at *P. ochraceus* larvae and their movement within the halocline in relation to the different food patches.

Methods

Species Collection and Fertilization

Eight adult *Pisaster ochraceus* were collected from the rocky intertidal during low tide at Friday Harbor Laboratories, San Juan Island, Washington, USA. Four days after capture seven *P. ochraceus* were injected with 2 ml of 1-methyladenine to induce spawning and gametes were collected and viewed using light microscopy. Fertilized eggs were measured (ranging in size from 144.3-177.6 μm) and placed in jars containing 2000 ml of 0.45μm of filtered seawater.

Larval rearing

Two days after fertilization**,** six jars containing 31‰ 0.45μm filtered seawater were prepared and placed in a sea table (78.7x129.5x16.5 cm) which had fresh seawater pumped in from Friday Harbor (water temperature ranged between 11-13°C.) The total number of swimming embryos (all in the gastrula stage) was counted and approximately 2,000 embryos were placed in each of the six jars. A system of swinging paddles were placed in the cultured jars, which allowed the algae and larvae to stay suspended in the water column.

Five days after fertilization, larvae were fed a diet consisting of *Dunaliella tertiolecta, Isochrysis galbana,* and *Rhodomonas sp*. These algal cultures were reared under fluorescent light and used during the exponential phase**.** To determine the concentration of algae needed to feed larvae, cell counts were made each week. (Table. 1)

Rotation of the cultured jars in the sea table (so that jars experienced all possible conditions in the sea table) and water changes took place once a week. Seawater was drained from the cultured jars to 650 ml. Fresh 0.45µm filtered seawater was added to each jar totaling a volume of 2150 ml. Ninety nine percent of the embryos were in the bipinnaria stage (four weeks after fertilization) and developed into the brachiolaria stage over eight weeks.

Experimental Design

Three treatments with two replicates per treatment were set up, columns with *Rhodomonas sp* at the halocline with larvae, *Rhodomonas sp* at the halocline without larvae, larvae without algae (control) in a halocline (Figure 1). In each case, approximately 100 larvae were gravity fed to the bottom of each column. The experiment was repeated using *Isochrysis galbana.* The three treatments were also repeated for algae cultured at 30 ‰. These conditions were used because 20 ‰ seawater at the surface and 30 ‰ seawater at the bottom are characteristics found in the Puget Sound (Khangaonkar et al. 2011). The biovolume of each algal species remained constant throughout the experiment. Each algal species varied in size and to increase visibility within the columns, the volume of each species added was multiplied by a consistent factor. Algal concentrations varied between 12,000-66,000 cells/ ml mimicking an algal bloom. The columns were under a black tarp for the duration of the experiment eliminating natural light.

Halocline Setup

All six columns were filled with 20 ‰ FSW 15 cm from the bottom. The controls were prepared by gravity feeding higher saline water of 30 ‰ at a slow rate to 40 cm from the bottom of the cylinder. Two clamps per tube controlled the flow of the water and algal species into the columns. For the haloclines with a food patch at 24.5 ‰, each species of algae was gravity fed into the cylinder (at a slow rate to avoid mixing) below the low salinity water. Following the food patch, higher salinity water of 30 ‰ was gravity fed into the water column to 40 cm from the bottom. This method was repeated for the food patch below the halocline at 30 ‰. The haloclines with a food patch reared in 30 ‰ seawater, were created by gravity feeding 30 ‰ seawater below the 20 ‰ seawater. Algae were gravity fed lastly to the bottom of the water column.

After one and six hour, salinity was recorded using the three way valves on the side of the columns. The columns were removed from the sea table one at a time for observation. The widths of the algal patches were measured at 1, 3, and 6 hour after introduction into the columns. Samples of algal cells were also counted before and after the experiment to note the concentrations.

Part A: The behavior of algae in the presence and absence of larvae in a halocline

Isochrysis galbana and Rhodomonas sp were reared in 30‰ and 24.5 ‰ seawater under fluorescent light. The haloclines were set up in clear acrylic cylinders (45 cm height, 9 cm diameter). The seawater at 20‰ and 30 ‰ was prepared the day before the experiment and placed in a sea table (89cm x 31.5cm x 59cm) with running seawater at a temperature of (11-13°C). On the day of the experiments, the water was drained from the sea table. The acrylic columns were placed in the sea table when water was completely drained. When the columns and haloclines were established, water was pumped back into the sea table to a height of 23cm from the bottom. This allowed the columns in the sea table to stay at temperatures relative to the water around Friday Harbor.

Part B*: Larval behavior with a food patch and halocline present*

While observing the behavior of algal patches in a halocline, the behavior of larvae was also observed. One hour after introduction of the larvae, each column was removed from the sea table and placed in the counting chamber for observation, which was a dark tank preventing natural light. To enhance visibility, a fluorescent light was placed behind the columns to count the larvae. The larvae were counted in 5cm increments (from the top of the column to the bottom) for 30 seconds each. This was repeated for three-hour time intervals for each treatment. Following this, the water columns were placed back in the sea table.

Data Analysis

The Welch Test was used to determine whether algal cell concentration varied with treatment as data was not normally distributed and the variances were not equal. Chi-squared test were used to determine whether the proportion of larvae above, at and below the halocline differed among treatments. Jmp 9.0 was used to analyze all data.

Results

Part A: Behavior of algae in the presence and absence of larvae Algae

Algae at the halocline

When algal patches were at and below the halocline, patch width for *Rhodomonas sp*. remained the same (1cm) for the 6 hour time interval whereas *Isochrysis galbana* patch width increased from 1cm to 2 cm over the 6 hrs. (Figure 2).

When larvae were absent, the average change in cell concentration for *Isochrysis galbana* was 6.3 10^4 cells/ml/hr and the change in cell concentration for *Rhodomonas sp*. was 2.15*10^5 cells/ml/hr. When larvae were present (n=64-81, the number of larvae in the halocline), the average change in cell concentration for *Isochrysis galbana* was 1.4*10^5 cells/ml/hr and the change in cell concentration for *Rhodomonas sp* was 2.3*10^5 cells/ml/hr. A greater change in algal cell concentration was observed for *Rhodomonas* sp. than for *Isochrysis galbana.* There was a significant decrease in algal cell concentration for *Rhodomonas sp.* compared to *Isochrysis galbana* (Figure 3)**.** The sample size was too small to analyze the data statistically so the experiment with the algal species cultured at 24.5 ‰ was repeated (Figure 4). Using the Welch test, there was a significant difference between the change in algal cell concentration in the presence and absence of larvae (P<0.0063).

Algae at the bottom of the columns

When larvae were present (n= 49-61)*, Isochrysis galbana* had an average change in cell concentration of 3.7*10^5 cells/ml/hr and *Rhodomonas sp.* had an average change in cell concentration of 5.9*10^4 cells/ml/hr. When larvae were absent*, Isochrysis galbana* average change in concentration was 3.5*10^5 cells/ml/hr and *Rhodomonas sp.*

2.9*10^4 cells/ml/hr (Figure 5). Only a slight decrease in algal cell concentration was observed for *Isochrysis galbana* or *Rhodomonas sp.* when placed below the halocline.

Part B: *Larval behavior with a food patch and halocline present*

The majority of larvae congregated around the halocline whether food was present or absent. For all three treatments, the salinity remained constant throughout the 6 hour time period. Larvae remained between 15-20 cm in each column with *Isochrysis galbana* and *Rhodomonas* sp. present. When both species of algae was at the halocline, a significant amount of larvae were in the halocline for the duration of the experiment. For the control, a small but significant amount of larvae moved to the top of the column by six hour with majority of the larvae staying within the halocline (Figure 6). When both algal species were at the halocline there was a significant difference in the proportion of larvae for all treatments (Pearson: 1hr: $p< 0.0001$, 3hr: $p< 0.0001$,6hr: 0.0021).

When the food patch was placed at the bottom of the column, a small but significant amount of larvae stayed below the halocline for the first three hours and a significant amount of larvae were at the halocline (Figure 7). When algal species were placed below the halocline, there was also a significant difference in the proportion of larvae in each treatment. (Pearson Chi-squared = 1hr: $p<0.0001$, 3hr: $p <0.0003$, 6hr:$p < 0.0001$)

Discussion

Algal behavior in haloclines

My results supported my hypothesis. When larvae were introduced into the columns, there was a greater change in algal cell concentration of *Rhodomonas sp*. than

Isochrysis galbana at the halocline. This trend may have occurred due to the fact that previous studies showed marine invertebrate larvae prefer *Rhodomonas sp.* to other species of algae (Seixas et al., 2009).

The algal patch of *Rhodomonas sp*. remained at one cm and *Isochrysis galbana* spread to two cm during the duration of the experiment. Previous studies have shown that algal species tend to behave differently in a halocline when larvae are present or absent. Menden-Deuer & Grunbaum (2006) study showed when predators are present, algal species behaved differently. Swimming velocities in phytoplankton varies widely with species. Both algal species swim through the water column escaping from their predators using flagellae. Studies have also shown that algal species can detect a near by predator and change their direction of swimming (Guasto et al. 2012). Vertical migration of these algae allows them to move to heights and depths where predation risk is low (Ryan et al. 2010).

When both species of algae were placed at the bottom of the columns, *Isochrysis galbana* had a greater change in algal cell concentration in comparison to *Rhodomonas sp.* I believe this trend occurred because the algae could disperse easily throughout the low salinity water (30 ‰) and not have to cross any halocline barriers. This may support the fact that *Isochrysis galbana* is a better swimmer than *Rhodomonas sp.*

Larval Behavior with food patches in haloclines

Many planktonic predators alter their swimming patterns in response to external factors, including the presence of prey. Menden-Deuer (2006) showed the geometry movement of phytoplankton in the absence of a predator appeared less complex than when a predator was present in the same environment (Menden-Deur and Grunbaum

2006). I rejected my hypothesis on the vertical distribution of larvae in haloclines in the presence and absence of a food patch. When algae was present below and at the halocline majority of the larvae congregated around the halocline one hour after introduction and remained there for six hours. In the absence of algae, majority of the larvae swam to the halocline after 1 hour and a small proportion moved to the top of the column at six hour.

When the algae were placed at the bottom of the column, a few larvae stayed below the halocline for three hours but by the six hour, majority had moved to the halocline. This trend may have occurred because larvae might have fed on the algae during the first 30 minutes and moved to the halocline shortly after, however; observations took place one hour after introduction.

This study showed that a significant amount of larvae preferred being in the halocline when a food patch was present. If a food patch persists in saline waters that impose stress on *P. ochraceus* larvae, the larvae will still be able to obtain food and return to the halocline. Previous studies in the lab have shown that phytoplankton also aggregate within these haloclines indicating that swimming to these salinity gradients are widespread (Durham &Stocker 2012).

Trends show that there is a major change in algal cell concentration when there are larvae present, primarily for *Rhodomonas sp*. We witnessed these thin phytoplankton layers persisting for several hours and naturally separating over time. This study also showed that majority of *P. ochraceus* larva*e* stayed around the halocline whether there was food present or not. Being around these thin phytoplankton layers in haloclines can be advantageous to their reproduction, growth, and can lead to an increase in their food consumption.

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References

Burdett-Coutts, V., Metaxas,A.,2004. The effect of the quality of food patches on vertical distribution of the sea urchins *Lytechinus variegatus* (Lamarck) and *Strongylocentrotus droebachiensis* (Muller).J.Exp.Mar.Biol.Ecol.308,221-236.

Cheriton OM, MA McManus, MT Stacey, JV Steinbuck, and JP Ryan.. 2009. Physical and biological controls on the maintenance and dissipation of a thin phytoplankton layer. Marine Ecology Progress Series. Vol. 378: 55–69.

Durham, W. M., & Stocker, R. (2012). Thin Phytoplankton Layers: Characteristics, Mechanisms, and Consequences. *Annual Review of Marine Science*, *4*(1), 177–207.

Guasto JS, Rusconi R, Stocker R. 2012. Fluid mechanics of planktonic microorganisms. *Annu. Rev. Fluid Mech.* 44:1–28

Harley, C.D.G., Hughes, A., Randall, Hultgren, M., Kristin, Miner, G., Benjamin, Sorte, J.B., Cascade, Thornber, S., Carol, Rodriguez, L., Tomanek, Williams, L., Susan (2006). The impacts of climate change in coastal marine systems. *Ecology Letters,* (2006) 9: 228-241

Khangaonkar, T., Yang, Z., Kim, T. et al. (2011). Tidally averaged circulation in Puget Sound sub-basins: Comparison of historical data, analytical model, and numerical model. *Est. Coast. shelf Sci*. **93**, 305-319

Kinne, O. (1971). Salinity. In Kinne O. (Ed.) Marine Ecology Environmental Factors. Part 2, Vol. 1. Wiley- Interscience, London, pp. 821-995

McManus MA, Woodson CB. 2012. Plankton distribution and ocean dispersal. J Exp Biol 215:1008–16.

Metaxas A, Young, C.M., 1998a. Responses of echinoid larvae to food patches of different algal densities. Mar.Biol.130,433-445

Metaxas A, Young CM, 1998b. The effect of a dense food patch on larval response to haloclines. In : Mooi R, Telford M (eds) Echinoderms: San Fransisco. AA Blakema, Rotterdam (In press)

Menden-Deuer, S., & Grunbaum, D. (2006). Individual foraging behaviors and population distributions of a planktonic predator aggregating to phytoplankton thin layers, *51*(1), 109–116.

Pia, T. S., Johnson, T., & George, S. B. (2012). Salinity-induced morphological changes in Pisaster ochraceus (Echinodermata: Asteroidea) larvae. *Journal of Plankton Research*, *34*(7), 590–601.

Pedrotti, M.L., Fenaux, L., 1993. Effects of food diet on the survival, development and growth rates of two 259 cultured echinoplutei (Paracentrotus lividus and Arbacia lixula). Invertebr. Reprod. Dev. 24, 59–70.

Ryan JP, McManus MA, Sullivan JM. 2010. Interacting physical, chemical and biological forcing of phyto-plankton thin-layer variability in Monterey Bay, California. *Cont. Shelf Res.* 30:7–16

Schiopu, D., George, S. B., & Castell, J. (2006). Ingestion rates and dietary lipids affect growth and fatty acid composition of Dendraster excentricus larvae. *Journal of Experimental Marine Biology and Ecology*, *328*(1), 47–75.

Seixas, P., Coutinho, P., Ferreira, M., & Otero, A. (2009). Nutritional value of the cryptophyte Rhodomonas lens for Artemia sp. *Journal of Experimental Marine Biology and Ecology*, *381*(1), 1–9.

Strathmann, R. R. (1971). The feeding behavior of planktotrophic echinoderm larvae: mechanisms, regulation, and rates of suspension feeding. J. *Exp Mar. Biol. Ecol.* 6, 109-160

Sutherland, D. A., MacCrady,P., Banas,N.S. et al. (2011). A model of the Salish sea estuarine circulation. J. *Phys. Oceanogr.* **41**, 1125-1143

Vazquez, E., & Young, C. M. (1985). Responses of compound ascidian larvae to haloclines, (1951).

Yamamoto-Kawai, M., Mclaughlin, F. A., Carmack, E. C.et al. (2009). Aragonite undersaturation in the Artic Ocean: Effect of ocean acidification and sea ice melt. *Science* **326**, 1098-1100

Tables 1. Algae species, cell count, diameter, and concentrations fed to P. ochraceus larvae the first three weeks after fertilization.

Week 1

Algae	Cell Count	Diameter (μm)	Concentration (cells/ml)
Rhodomonas sp	12-54	8.34-16.68	1.23×10^6 -1.24×10^6
Dunaliella tertiolecta	22-44	8.34-13.9	2.68×10^6
Isochrysis galbana	31-55	5.56	8.0×10^6

Week 2

Algae	Cell Count	Diameter (μm)	Concentration (cells/ml)
Rhodomonas sp	32-54	8.34 - 16.68	1.24×10^6 – 3.15×10^6
Dunaliella tertiolecta	19-44	8.74 - 16.68	2.67×10^6 – 5.59×10^6
Isochrysis galbana	31-84	4.17 - 8.34	1.32×10^7 - 8.6×10^6

Week 3

Algae	Cell Count	Diameter (μm)	Concentration (cells/ml)
Rhodomonas sp	9-13	8.34 - 16.68	2.1×10^6
Dunaliella tertiolecta	5-9	8.74 - 16.68	3.04×10^6 – 6.5×10^6
Isochrysis galbana	36-56	4.17 - 8.34	2.25×10^7 - 3.57×10^7

Figure 1: Experimental design to observe two algae species (*Isochrysis galbana, Rhodomonas sp*) in the presence and absence of larvae and to look at the vertical distribution of *P. ochraceus* larvae in a halocline.

Figure 2: Change in patch width (cm) over the 6 hour time period for two different species of algae (*Isochrysis galbana, Rhodomonas sp.).*

Figure 3: Change in algal cell concentration over 6 hour time period at the halocline when *P. ochraceus* larvae are present and absent. N-value represents the amount of larvae present in the halocline.

Figure 4: Change in algal cell concentration over 6 hour time period at the halocline when *P. ochraceus* larvae are present and absent. N-value represents the amount of larvae present in the halocline.

Figure 5: Change in algal cell concentration over 6 hour time period below the halocline when *P. ochraceus* larvae are present and absent. N-value represents the amount of larvae present in the halocline.

Figure 6: Vertical distribution of *P. ochraceus* larvae (maintained in control 30ppt salinity) in haloclines over a six-hour time period in the presence or absence of a food patch at a halocline.

▭ = Algae

Figure 7: Vertical distribution of *P. ochraceus* larvae (maintained in control 30 ppt salinity) in haloclines over 6 hour time period with no algal patch present.

▭ = Algae