Assessing the State of Pteropod Shells and Ocean Acidification in Washington Marine Waters

By Jessamyn Johnson

PEF 2014

Contact: jessamyn.a.johnson@gmail.com

Key words: Ocean acidification, pteropods, aragonite undersaturation, shell dissolution, Washington Coast, Puget Sound

Abstract:

The relationship between pteropod shell dissolution and waters undersaturated with aragonite has been well documented (Bednarsek et al 2012). Low aragonite saturation levels decrease the availability of carbonate ions in the water column, causing aragonite to dissolve out of pteropod shell structures. The onset of ocean acidification has decreased the aragonite saturation states across the globe. While studies along the Washington coast have documented widespread pteropod dissolution during peak upwelling season, the state of pteropods during other seasons is unknown (Bednarsek et al 2014). Pteropods exposed to pCO_2 levels that exist within the Puget Sound have experienced dissolution in situ, but there has been no study done to date that examines the state of pteropods in the Puget Sound (Busch et al 2014). This study found widespread pteropod dissolution off the coast of Washington during the downwelling season with saturated surface waters, suggesting that even partial undersaturation of the water column can have deleterious effects on pteropod shells. Two stations examined from within the Puget Sound found severe dissolution in all pteropod samples and highly undersaturated waters, pointing towards conditions that are unsuitable for pteropods within the Puget Sound. While it is outside of this scope of this study to definitively conclude that ocean acidification is driving this, it is likely contributing to these damaging states.

Introduction:

In the past 250 years, with the onset of the industrial era, there have been revolutionary changes in production and technology that have had many unintended consequences. First and foremost, there has been a drastic increase in the input of anthropogenic carbon into the atmosphere due to accelerated deforestation, and fossil fuel use. Since the beginning of the industrial era, atmospheric carbon dioxide has risen from 279 ppm to 395 ppm (this measurement is from October 2014) (IPCC). Seventy-five percent of this is due to anthropogenic activity. (IPCC) This is an unprecedented change in the composition of the atmosphere: a rise in atmospheric carbon of this magnitude has not occurred in the past 650,000 years (IPCC). So we can only begin to predict what the consequences will be. This increase in carbon dioxide has not only been absorbed by the atmosphere, but by our global oceans as well. It is estimated that the ocean has absorbed one quarter of all anthropogenic carbon released into our atmosphere. (Sabine, Feely 2007). The ocean has been one of the largest sinks for industrial carbon. As of 2008, NASA estimates that the ocean holds 150 million tons of anthropogenic carbon.

This large input of carbon dioxide into the atmosphere impacts the chemistry of the oceans. When carbon dioxide is absorbed by the surface of the ocean, reactions occur that change the chemical characteristics of the seawater. One of the most critical products of this is an increase in hydrogen ions in the ocean, driving down the pH. Since the beginning of the industrial era the pH of global surface waters has decreased by 0.1, which may not seem significant, but considering that pH is in log scale, this is a ten-fold decrease (Orr et al 2005). By the end of the century the pH is estimated to drop from pre-industrial era value of 8.2 to 7.8, this would be a 150% increase in ocean acidity (Feeley et al 2009). This shift in the chemistry of the ocean, due to anthropogenic carbon dioxide, and other reactions is defined as ocean acidification.

Decreases in the pH of seawater can negatively impact organisms in the ocean, disrupting internal pH and testing the limits of the organism abilities to buffer a much higher internal pH (Fabry et al 2008). The response of organisms to decreases in pH is variable, with some organisms showing no or little impact, and others even benefiting from this change. However, low pH in seawater has been correlated with mortality, poor larval development, decreased fertility, and metabolic suppression across ecosystems (Fabry et al 2008).

Another serious consequence of increasing carbon dioxide concentration in the seawater is the reduction of carbonate ions. This decrease limits the availability of calcium carbonate, a key mineral that is used by calcifying organisms such as oysters, cocolithophores and pteropds. There are two main polymorphs of calcium carbonate, calcite and aragonite. Aragonite is 50% more soluble then calcite and is therefore more vulnerable to changes in saturation state. The saturation state of aragonite is defined as $[Ca^{2+}]*[C0_3^{2-}]/K'_{sp}$. With K'_{sp} varying with respect to temperature, salinity, and pressure. As carbonate ion concentration is reduced or increased, the saturation state of aragonite will decrease or increase accordingly. The saturation state of aragonite is typically referred to as Ω, and describes the thermodynamic favorability of aragonite. When the saturation state of Aragonite is above 1, then the precipitation of aragonite is thermodynamically favored; this is the preferred state for calcifying organisms, as it allows them to create calcified structures with less energy expenditure. When Ωaragonite is less then 1, the dissolution of aragonite is thermodynamically favored over calcification and the waters become corrosive, causing precipitated aragonite to dissolve. There are other factors involved in the dissolution of calcified shells such as the extent of the organic covering over the shell, and the mineral structure of the shell so that dissolution of aragonite incorporated into shells may not necessarily happen when saturation state dips below 1 (Fabry et al. 2008). Dissolution can be dangerous for shelled organisms, since it can leave their shells fragile and prone to breakage, leaving them vulnerable to predators. Feeley et al 2012 found that aragonite horizon (where the aragonite saturation drops below one in the water column) has been shoaling in the Pacific Ocean by 1-2m/yr for the last decade. Plankton living in the upper water column will be interacting with corrosive waters on an increasing rate as this continues.

Pteropods, a holoplanktonic gastropod, with a thin fragile shell, are very vulnerable to low aragonite saturation states. In a study published in 2014, Bednarsek et al demonstrates a strong relationship between low aragonite saturation states and the dissolution of pteropod shells sampled from the coast of Washington, Oregon and California. When the water column was 100% undersaturated with aragonite, 90% of sampled pteropod shells showed signs of dissolution. Busch et al 2014 found that shell dissolution began in situ when pteropods were exposed to aragonite saturation and pCO_2 states currently found within Puget Sound. Pteropod abundance in Vancouver Bay has declined in the last decade, and the pteropod *Clione sp* that preys on them has increased (Mackas 2012).

Pteropods play an important role in ecosystems across the globe. They are a key part of trophic transfer linking lower and upper trophic levels (Whitehead et al 2001). Because pteropods are nutrient rich, and can be very abundant, they are a critical food source for salmon, and other economically important organisms. During peak season, they can comprise up to 45% of the diet of pink salmon in the Gulf of Alaska. (Aydin et al 2005). As well as compromising an important part of the food web, they play key roles in the carbon cycle. Pteropods can help to sequester carbon on the ocean floor, as their shells sink when they die (Tsurumi et al 2005).

Washington marine waters have been affected by anthropogenic carbon output from both global and local sources. In the winter, Puget Sound and coastal waters are often undersaturated with respect to aragonite. During the summers, because of high productivity, the surface waters are primarily oversaturated with aragonite (Feeley et al 2012). However, coastal upwelling off the coast during the summer can cause the cold, saline, under saturated waters to reach the surface (Feeley et al 2008). These waters can enter into the Salish Sea via the Strait of Juan de Fuca (Moore et al 2008). Through strong tidal mixing and interactions with bathymetric sills, these waters can be mixed throughout the water column inside the Salish Sea (Moore et al 2008). Due to freshwater input and mixing above the sills these waters can reflux and stay in the system for an extended period of time (Rucklehaus et al 2007). Water within the Salish Sea is also affected by local carbon dioxide output, urban and agricultural runoff (Feeley et al 2010). Hypoxic events within restricted, narrow portions of the Salish Sea such as Hood Canal can also exacerbate ocean acidification conditions (Newton et al 2002). Both the coast of Washington and the Puget Sound have shown measurable declines in Ωaragonite. Harris et al 2010 found that coastal surface waters are undersaturated with respect to aragonite 30% of the time, as opposed to preindustrial waters that were under saturated 10% of the time. Feely et al 2010 found a decrease of surface aragonite saturation states in the Puget Sound that is estimated to be between 0.09-.33 since the industrial revolution. It is clear that the Salish Sea and coastal waters of Washington are changing due to ocean acidification. Pteropods are not only a valuable species to this ecosystem, but could also be an indicator species for potential effects that ocean acidification might have on the plankton community here. There have been no studies to date that have evaluated the status of pteropods within the Salish Sea. The primary objective of this study is to assess the relationship between the state of pteropod shells and the aragonite saturation states from various stations around the Salish Sea.

Methods:

Biological Sampling:

Sampling Locations:

Samples were taken from three biological stations during two different PRISM cruises. The locations ranged from the coast of Washington, through the Puget Sound and south into Hood canal. The aim of this study is to provide a broad scope of the state of Pteropds within the Salish Sea. One sample was taken during the first cruise and three from the second. The first sample was taken on October 24 at station P381, Lat 47.969 Lon. -124.9492, off the coast of Washington near La Push. The second cruise took place three days after the end of the first. The first sample was taken on October 30, from station P4 Lat. 48.2422 Lon. -122.5533, near Skagit Bay. The second sample was taken on October 30, from station P12 Lat. 47.4253, Lon. -123.1083, in the southern end of Hood Canal.

Sampling Methods:

Samples were collected using a double-sided bongo net with a mesh size of 335µm. The net was towed using an oblique tow method where the net was lowered to approximately 10m above the bottom and was towed at approximately 1-3 knots at 15m intervals for 2-5min until the surface was reached. (Tow time and depth intervals depended on time of day in order to take advantage of the plankton's diel migratory pattern). This technique was used to vertically integrate the top 100m of the water column. The samples were then placed in 500ml jars and 100ml of 95% ethanol was added for preservation and to prevent dissolution.

Sample Processing:

1) Shell preparation:

After the completion of the cruises, samples were then taken back to Friday Harbor Laboratories. From each sample, 20-30 pteropods were chosen for analysis. When samples were originally placed in ethanol, minerals from the seawater precipitated out onto the shell surface. I used a series of treatments to rid the shell samples of these precipitated minerals along with any bacteria or detritus that may remain on the shell. Samples were placed in a baths of 70% ethanol, and then transferred to a bath of 50% ethanol for 2-3 minutes each. The shells were then rinsed in short DI rinses for up to 5 minutes. Samples were then placed into two consecutive 6% hydrogen peroxide rinses for 20 minutes. Finally, shells were rinsed in DI 2-3 times for a total of 5 minutes. It is necessary to remove the organic layer that stretches across the shell, as it can impede scanning electron microscope (SEM) analysis. To prevent this, the organic layer was partially removed using a 1% KOH solution for 2 hours and then rinsed thoroughly with DI water. Samples were then allowed to dry. Samples were carefully mounted on SEM stubs coated with conductive tape using small fine brushes so as not to damage the now fragile shells and set inside a desiccator to dry for a minimum of 12 hours. Special care was taken to ensure the dorsal ventral side of the shell was facing upwards so that the whorls of the shell were visible. Samples were sputter coated with gold panadium for 25 seconds at 35amp.

2) SEM analysis:

Samples were then taken to the Scanning Electron Microscope JEOL-500 for analysis. Ten to twenty shells were chosen for examination. Any shells with mechanical damage or damage that was not attributable to corrosion were removed from the analysis. Pictures of each sample were taken with focus on the overall dissolution of the shell (approximate surface area coverage) and the severity of damage (how far the dissolution had eaten away into the mineral structure of the shell). To quantify the state of dissolution, the method outlined in Bednarsek et al 2012 was used, where the severity and extent of dissolution was typified into three states, type I type II and type III. Type I being the least, with evidence of increased porousness, some exposure of the aragonite layer and no prominent dissolution across the shell. Type II with dissolution covering increasing areas of the shell, dissolution of the prismatic layer, and areas where the cross lamellar layer is visible. Type III is the most severe category with extensive prismatic dissolution and dissolution of the cross-lamellar layer. Photos taken on the SEM were analyzed for percent surface area with dissolution on the primary whorl of the pteropod using image J software.

Ocean Chemistry

Survey data:

Water column data was measured using the CTD aboard the Thomas G. Thompson research vessel. The CTD sampled from 10m above the bottom to the surface. CTD data was processed using Seabird, Searun and Excel programs. Skagit River discharge data was taken from the USGS water survey site. Sea Surface temperature data were taken from the climatology NVS on NANOOS.

Analysis of Water Samples:

ΩAragonite was estimated for each station using two sets of equations. The first set of equations was designed to be used off the coast of Washington (Juranek et al 2009). This equation uses temperature, salinity and oxygen to estimate dissolved inorganic carbon and total alkalinity. Dissolved inorganic carbon and total alkalinity from this equation are then used to calculate Ωaragonite using CO2SYS, a seawater alkalinity calculator. The total percentage of 0-100m that is under saturated with respect to aragonite is then calculated from this value. While the equation created by Juranek et al has been established as accurate for the coast of Washington, the accuracy of the equation within the Puget Sound was unknown. In order to verify the accuracy of this equation, measured total alkalinity and dissolved inorganic carbon data from 2011 and 2012 from the San Juan Channel was used (Sullivan, 2012). Both total alkalinity and dissolved inorganic carbon estimates were proved to be fairly accurate with an R^2 value of .9 and .8

respectively when compared against measured data. However, pH, pCO_2 and Ωaragonite proved to be inaccurate with R^2 values of .3, .3 and .1. While the Ωaragonite values were unpredictable, the percent undersaturated value for the top 100m of the water column proved to be fairly accurate, with a 5% difference between measured and estimated values.

Results:

P4:

Skagit Bay, near where P4 is located, receives the largest freshwater input of any bay in the Puget Sound from the Skagit River (Yang et al, 2009). At station P4 100% of the water column was under saturated with respect to aragonite. Average dissolved inorganic carbon and total alkalinity for the entire water column was 2067.62 µmol/kg and 2034.56 µmol/kg respectively. Average temperature, salinity, fluorescence and oxygen values were 11.91 C°, 29.64 PSU, 1.71 FU and 2.59 mg/L. Fluorescence values for the first 10m, were moderately high with an average of 6.48 FU, indicating the possibility of a bloom. Salinity values for the first 10 m of the water column were especially low, with an average of 25.90 PSU. The water column was very stratified, with a decrease in salinity from the 0m to 20m of 4.0 PSU. This is also evident for TA and DIC with DIC increasing as much as 300 µmol/kg between the surface and 50m. For the month of October, the discharge from the mouth of the Skagit River oscillated between $200f^3/s$ to 1,000 f^3/s higher than the decadal average.

Ten pteropod shells were sampled from this site. One hundred percent of the samples had type II dissolution. Forty percent of the shells had type III dissolution. All shells sampled showed evidence of type I dissolution. The relationship between undersaturated waters and shell dissolution was significant with a p value of <.0001.

Dissolution was mainly concentrated on primoviscous, or primary whorl of the pteropods, however type III dissolution was almost exclusively along the outer edges of the shell. Modeled results (Bednarsek et al 2014) created using data off the coast of Washington estimated 100% of the population with type II dissolution, with a range of 17%-100%. On the primary whorl, 17% of the surface area showed type II dissolution, with a standard deviation of 9%. The relationship between surface area of the shell with dissolution and undersaturated waters was not significant.

P12:

At Station P12, 100% of the top 100m of the water column was undersaturated with respect to aragonite. Dissolved inorganic carbon, and total alkalinity averages were 2112.01 µmol/kg and 2062.12 µmol/kg respectively. Average temperature, salinity, fluorescence and oxygen values were 10.46 C°, 30.33 PSU, 1.65 FU and 2.20 mg/L. Oxygen values between 10 and 30m were 1.36 mg/L. Fluorescence levels for the top ten meters was an average of 10.05 (FU). In 2011 and 2010 aragonite saturation values for Hood Canal were an average of 0.6 during October (Reum 2014).

Ten pteropod shells were sampled from this site. One hundred percent of pteropods sampled had type II dissolution. Thirty percent of samples showed type III dissolution. All shells sampled showed wide spread evidence of type I dissolution across the entire shell. Percent shell dissolution and undersaturated waters had a significant relationship with a p value of < .0001 Dissolution was once again mostly concentrated on the primary whorl of the shell. Modeled results estimated 100% of the population with type II dissolution, with a range of 17%-100%. On the primary whorl, 21% of the surface area was covered with type II dissolution with a standard deviation of 4%. Percent surface area on the first whorl with dissolution and percent undersaturated waters did not have a significant relationship.

P381:

At Station P381 24% of the water column was undersaturated with respect to aragonite. The average Ωargonite value for the top 100m was 1.32. The aragonite saturation horizon was at 66m, with the lowest Ωaragonite at 0.77. The highest Ωaragonite value was at the surface at 1.66. Dissolved inorganic carbon and total alkalinity averages were, 2100.32 µmol/kg and 2181.03 µmol/kg respectively. Temperature, salinity, fluorescence and oxygen values were 13.24 C°, 32.20 PSU, 1.12 FU and 4.88 mg\L. The temperature for October was 3 C° above the decadal average (NANOOS). The average temperature for the top 10m was 15.44 C°.

Fifteen Pteropod shells were sampled from the site. Seventy percent showed type II dissolution. Thirteen percent showed type III dissolution present. Type I dissolution was evident on all shells, and typically widespread across the shells surface. Modeled results estimated 30% of the pteropods showing type II dissolution, with a possible range of 10%-60%. Percent of pteropods with shell dissolution and % undersaturated waters had a significant relationship with a p value of .0001. On the primary whorl, 31% of the surface area was covered with type II dissolution, with a standard deviation of 23%. The % of the primary whorl with shell dissolution did not have a significant relationship with % undersaturated waters.

Discussion:

The significant relationship between pteropod shell dissolution and undersaturated waters suggests that ocean acidification is contributing to less favorable conditions for the pteropod population in Washington marine waters. Previous studies have shown a decline of aragonite saturation state since the beginning of the industrial era due to anthropogenic carbon in this area. Off the coast of Washington and in the Puget Sound it is estimated that Ωaragonite has decreased of .5 (Feely et al 2008,) and .2 (Harris et al 2013) respectively. This study found severe shell dissolution at all stations sampled, with 70- 100% of Pteropods showing type II dissolution, and 13% - 40% with type III dissolution. While undersaturated waters were present throughout all samples, the conditions driving this state were unique at each station, giving a broad scope of the forces behind low aragonite conditions.

Station P4, located near Skagit Bay in the Whidbey basin, was heavily influenced by freshwater input from the Skagit R. During the month of October, when samples were taken, the discharge from the Skagit River into the bay was unseasonably high. High inputs of freshwater can dilute the alkalinity of seawater. The total alkalinity values from P4 are the lowest of the three stations. These low alkalinity values contributed to the low aragonite saturation of the water column, which was 100% undersaturated over the entire depth. Dissolved inorganic carbon values were the lowest of the three stations suggesting that this is a less influential driver of the low aragonite saturation states for this station. A phytoplankton bloom stimulated by nutrient input from the Skagit River, indicated by relatively high fluorescence values in the first 10m of the water column, may explain the low DIC values. While the surface water undersaturation is likely due to high levels of Skagit River discharge, undersaturation in the deeper water is likely due to respiration of phytoplankton and detritus from the river input. Whether this status is typical of the area in fall is not known, due to the highy anomalous river input which was 158% greater then the decadal mean (USGS).

Conditions at Hood Canal were similar to those in the Whidbey basin. A station P12, 100% of the water was undersatured with aragonite. Hood Canal often experiences low oxygen conditions that lead to hypoxia as a result of respiration stimulated by large phytoplankton blooms (Newton et al 2002). Fluorescence values indicate a late in the season bloom that likely contributed to the low oxygen conditions and high inorganic carbon conditions. This was likely the driver of low Ωaragonite values. Historical data suggests that these waters may be chronically under saturated during this season.

Station P381 is located off the coast of Washington near La Push. While this area has been previously sampled in other studies (Bednarsek et al 2014), sampling only took place during peak upwelling periods (August-September). This is the first study to look at pteropods in the down welling season. The seasonal transition to downwelling, brought surface waters in from the Pacific Ocean, normally warmer than upwelled waters, and which were, in fall 2014 anomalously warm onto the coast. Average surface temperatures for the Washington coast were 3 C° higher then the decadal average in the month of October. This likely contributed to the saturated waters of the sampling station. The water column here was only 24% undersatured with aragonite. While the majority of the water column was saturated, pteropods would have still been interacting with undersaturated waters because they are diel vertical migrators that regularly reach depths as low as 100m.

Across all three stations, pteropod dissolution was widespread throughout the samples. It is likely that shell dissolution is effecting large portions of the pteropod populations. Off the coast of Washington, the least severe dissolution states of three stations were seen, as well as the least amount of type II and type III dissolution. While the cross-lamellar layer was evident in the type III dissolution there, only the structure immediately at the surface was damaged, with very little intrusion into the rest of the mineral structure. In type II dissolution, much less of the prismatic layer was dissolved. Samples may show less severe dissolution then the other stations because a lower percentage of the water column is under saturated and it is likely those waters are less undersaturated then other two stations, as this station has the highest total alkalinity values, and the some of the lowest dissolved inorganic carbon values.

Stations P4 and P12 showed similar states of dissolution. This makes sense as both stations had 100% undersaturation. The extent of dissolution of the pteropods collected at P4 and P12 was very severe with type II dissolution intruding far into the prismatic layer and wide spread amongst all the samples. Type III dissolution also had a large impact on the state of the shells, covering a large portion of the outer shell, evidenced by the shortening and breakage of the cross lamellar layers. In several of the samples at both stations, the intrusion of the mineral layer was so severe that holes began to form within the shell. With such severe structural damage, the pteropod shell structure is less compact and more fragile than it would be in its intact state. Type II and III dissolution mostly occurred on the outer ridges and the primary whorl of the shell, contributing to the observation (PC: Bednarsek) that these areas are structurally the most vulnerable to dissolution.

While P4 and P12 had very similar results, there were some key differences between the two. P4 had more severe dissolution, with 10% more of the samples effected by type III dissolution then P12. One possibility behind this is that pteropods at P12 have begun to adapt to chronically undersaturated conditions. While it is not clear if pteropods can repair areas of their shell that have been damaged by dissolution, there is some evidence that pteropods may increase calcification to thicken their shells in low aragonite saturation states (Lishka et al 2012). On one of the pteropods sampled from P12 there appears to be a thickening of the organic layer across the first whorl where extensive dissolution had taken place. On the rest of the shell, the organic layer had been successfully removed, however in this particular area, a very thick organic layer remained. It is possible that pteropods could thicken their organic coating to help protect the mineral structure of their shell from dissolution, much in the same way they would increase calcification. This was also observed in two of the shells from P381, suggesting pteropods here may also be defending themselves from undersatured waters. Both station P381 and P12 have waters that have been documented to be undersatured at least part of the time on an annual basis (Bednarsek et al 2014, Reum et al 2014). No such studies have been done at P4, and it seems that one of the great drivers of undersaturation there may be a seasonal anamoly. It is possible that pteropods when exposed to regular undersaturation may build defenses for themselves, while in areas were extreme undersaturation is not the norm the pteropod population may not have these defenses. Before any conclusions can be made on this point, a more thorough analysis of pteropods at each station would be needed (samples sizes for this study were fairly small between 10-17) and further analysis of Ωaragonite conditions at P4 are needed.

Using data collected off the Washington coast during August, Bednarsek et al fitted a model to the percent of the water column undersaturated with aragonite that would estimate the portion of pteropods with type II and III dissolution. While the methods used to examine pteropods for this study differed slightly from the methodology outlined in Bednarsek et al 2014 due to limited time and equipment, this equation was used to verify the accuracy of this approach. At station P381, which was off the coast of Washington, the model was expected to have the best fit, as this is where it was designed to be used. However, the results differed fairly substantially, with the results being 10% greater then the highest possible value as predicted by the equation. Variance may be due to seasonal differences since this model was creating using data for the summer season, the severity of undersaturation (e.g. how far below 1 Ωaragonite is) or the effect of previous understaturation of the water column. The dissolution seen here may also be a relic from the upwelling season which typically creates very undersaturated water conditions, lasting June through Septembe. These samples were taken during late October, so pteropod samples would likely have experienced long periods of upwelling. The results at station P4, and P12 however, were exactly the same as the predicted values. This suggests that this model may be a good fit for use within the Pugest Sound. More data points, with a range of values (since P4 and P12 have the same %undersaturated and %shell dissolution) are needed to further test the accuracy of the equation within the Puget Sound.

Identifying dissolution as type I, II and III was a qualitative analysis done by typifying the extent of the mineral dissolution into the shell structure. In order to further investigate the extent of dissolution quantitatively, measures of the surface area covered by type II and type III were taken. These measures were only done on the primary whorl of the shell, because this is where most of the dissolution took place, and because of time constraints on this project. There was no significant relationship between the % surface area with dissolution and % undersaturated waters. There was also no significant relationship between % of surface area with dissolution and % of pteropods with dissolution. In fact, the station with the lowest % of pteropods with dissolution, and the least severe dissolution, P381, had the greatest % surface area with dissolution. The station with the most severe dissolution, P4, had the least % surface area with dissolution. The sample size for these measurements is very small, and more data would likely be needed in order to detect any significant trends.

If dissolution of pteropods is affecting such a large portion of pteropod populations, this may pose a threat to the pteropod population on the Washington coast and within the Puget Sound. Shell dissolution can weaken the overall structure of the shell, and cause pteropods to be more vulnerable to predators and may also negatively affect reproductive success (Bednarsek et al 2014). Dissolution is not the only affect that undersaturated waters can have on pteropods, and when combined with shell fragility, may put pteropods at risk. Studies have demonstrated a decrease in metabolism with levels of pCO_2 that cause Ωaragonite undersaturation (Seibel et al 2012). Undersaturated waters may also cost pteropods energetically as more of their energy buget goes to calcification. (Wood et al 2008), In combination with other factors, such as temperature and fresh water input, high pCO_2 levels consistent with low aragonite levels can cause pteropod mortality (Lischka et al 2012, Manno et al 2012). This is of particular significance to this study as these pteropods experienced unusually high temperatures and low salinity conditions, as well as unersaturated waters.

Past studies have documented the state of pteropods on the coast of Washington (Bednarsek et al 2014). However, this study newly demonstrates extensive pteropod dissolution even outside of peak upwelling season. The dissolution on the Washington Coast was not as severe as the dissolution found within the Puget Sound, but was still more severe then expected for the conditions the pteropods where found in. Cleary, more studies are needed to better assess pteropod dissolution off the coast of Washington throughout the seasons.

Another new contribution of this study is assessment of Petropolis in the Salish Sea While the status of pteropods throughout the Puget Sound has yet to be thoroughly studied, it is clear that parts of the Sound are not favorable pteropod habitat. The damage done to the pteropod shells by dissolution at stations P12 and P4 could be affecting the population's ability to sustain itself in the Puget Sound. Further studies are needed to fully analyze the extent of the damage being done. The results found here may also be an important indicator of the health of the rest of the zooplankton population in the Puget Sound. How much ocean acidification is already and will continue to have a detrimental effect on this ecosystem is not well known but these results show that deleterious affects from corrosive waters are occurring now. Ocean acidification is not the only stressor to the zooplankton community in the Salish Sea; combined with other climate-change related stressors, such as high temperature and low oxygen, these conditions pose a very real threat to the vitality to the Puget Sound ecosystem.

Acknowledgements:

Many thanks to the entire PEF crew, we all worked as a great team and none of this would have been possible without you. Special thanks to Gabriella Zayas for help with equation analysis, glad you were there with me for the many hours of brainstorming we did. Thanks to our professors Matt Baker and Breck Tyler, with special thanks to Jan Newton for being a fantastic advisor and an inspiration. Thanks to our fantastic TA Rebecca Guenther for rescuing me whenever I was stuck, and providing constant support to every apprentice when we needed it. Thanks to Connie Sullivan, and Simone Alin for their help with aragonite data and the use of Simone's equations. Many thanks to Nina Bednarsek for her invaluable advice on pteropods as well as providing me with data and materials needed for pteropod analysis. I truly could not have done this project without you. Thanks to Adam Summers for the use of his SEM. Thanks to the crews of the Centennial and the Thomas G. Thompson R.V., I learned so much from all of you and enjoyed my time on all those great cruises.

Citations:

Aydin, K. Y., McFarlane, G. A., King, J. R., Megrey, B. A., & Myers, K. W. (2005). Linking oceanic food webs to coastal production and growth rates of Pacific salmon (Oncorhynchus spp.), using models on three scales. Deep-Sea Res PT II, 52:757-780. doi:http://dx.doi.org/10.1016/j.dsr2.2004.12.017

Bednaršek, N., Tarling, G. A., Bakker, D. C., Fielding, S., Cohen, A., Kuzirian, A., McCorkle, D., Lézé, B. and Montagna, R. (2012), Description and quantification of pteropod shell dissolution: a sensitive bioindicator of ocean acidification. Glob Change Biol, 18: 2378–2388. doi: 10.1111/j.1365-2486.2012.02668.x

Bednarsˇek N, Feely RA, Reum JCP, Peterson B, Menkel J, Alin SR, Hales B. (2014) Limacina helicina shell dissolution as an indicator of declining habitat suitability due to ocean acidification in the California CurrentEcosystem. Proc. R. Soc. B. 20140123.
http://dx.doi.org/10.1098/rspb.2014.0123

Busch DS, Maher M, Thibodeau P, McElhany P (2014) Shell Condition and Survival of Puget Sound Pteropods Are Impaired by Ocean Acidification Conditions. PLoS ONE 9(8): e105884. doi: 10.1371/journal.pone.0105884

Fabry V. J., Seibel B. A., Feely R. A, and Orr J. C. (2008) Impacts of ocean acidification on marine fauna and ecosystem processes ICES J. Mar. Sci. 65 (3): 414-432 doi:10.1093/icesjms/fsn048

FAQ 7.1 ¡ ¡Are the Increases in Atmospheric Carbon Dioxide and Other Greenhouse Gases During the Industrial Era Caused by Human Activities?¡ IPCC Fourth Assessment Report: Climate Change 2007 http://www.ipcc.ch/publications_and_data/ar4/wg1/en/faq-7-1.html

Feely, R. A., Alin, S. R., Newton, J., Sabine, C. L., Warner, M., Devol, A., Krembs, C., et al. (2010). The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. Estuar, Coast and Shelf S, 88(4), 442-449. doi:http://dx.doi.org/10.1016/j.ecss.2010.05.004

Feeley R. A., Klinger T., Newton J.A., Chadsey M. (2012). Scientific Summary of Ocean Acidification in Washington State Marine Waters. NOAA OAR Special Report.

Feely R. A., Sabine C.L, Byrne R. H., Millero F.J, Andrew G. Dickson, Wanninkhof, R. Murata A., Miller L.A.,and Greeleym D.(2012) Decadal changes in the aragonite and calcite saturation state of the Pacific Ocean. Global Biogeochem Cy, 26, GB3001, doi:10.1029/2011GB004157

Harris, K. E., DeGrandpre, M. D., & Hales, B. (2013). Aragonite saturation state dynamics in a coastal upwelling zone. Geophy Res Lett, 40(11), 2720-2725. doi:10.1002/grl.50460

Human and Natural Drivers of Climate Change. IPCC Fourth Assessment Report: Climate Change 2007 http://www.ipcc.ch/publications_and_data/ar4/wg1/en/spmsspm-human-and.html

Lischka S., Budenbender J., Boxhammer T., and Riebesel U. (2010) Impact of ocean acidification and elevated temperatures on early juveniles of the polar shelled pteropod Limacina helicina: mortality,shell degradation, and shell growth. Biogeosciences, 8, 919–932, www.biogeosciences.net/8/919/2011/doi:10.5194/bg-8-919-2011.

Mackas D. and Galbraith M. (2012). Pteropod time-series from the NE Pacific ICES J. Mar. Sci. 69 (3): 448-459 first published online October 11, 2011 doi:10.1093/icesjms/fsr163

Manno, C., Morata, N., & Primicerio, R. (2012). Limacina retroversa's response to combined effects of ocean acidification and sea water freshening. Estuarine, Coastal and Shelf Science, 113(0), 163-171. doi:http://dx.doi.org/10.1016/j.ecss.2012.07.019

Moore S.K., Mantua N. J., Kellogg J. P., and Newton J. (2008). Local and large-scale climate forcing of Puget Sound oceanographic properties on seasonal to interdecadal timescales. Limnol. Oceanogr.,
53(5), 2008, 1746–1758 doi: http://andrewsforest.oregonstate.edu/pubs/pdf/pub4542.pdf

Newton J.A., Albertson S.L.,Van Voorhis K., Maloy C., Siegel E., Washington State Marine Water Quality in 1998 Through 2000 Publication #02-03-056 Washington State Department of Ecology, Environmental Assessment Program, Olympia, Washington (2002)

NVS Climatology, Sea Surface Temperature Anomaly. NANOOS. http://nvs.nanoos.org/Climatology

Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., et al. (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature, 437(7059), 681-686. Doi: http://dx.doi.org/10.1038/nature04095

Ruckelshaus MH, McClure MM. 2007. Sound science: synthesizing ecological and socioeconomic information about the Puget Sound ecosystem. US Department of Commerce, National Oceanic and Atmospheric Administration, Seattle, Washington. 93 pp.

Sabine C. L., Feely R.A.. The oceanic sink for carbon dioxide D. Reay, N. Hewitt, J. Grace, K. Smith (Eds.), Greenhouse Gas Sinks, CABI Publishing, Oxfordshire, UK (2007), pp. 31–49

Seibel, B. A., Maas, A. E., & Dierssen, H. M. (2012). Energetic plasticity underlies a variable response to ocean acidification in the pteropod, Limacina helicina antarctica. *PloS one*, *7*(4), e30464.

Sullivan, CA. 2012. Carborn Chemistry in the San Juan Channel: Characterizaion and Suggestions for Mitigation. Dissertation, University of Washington.

Tsurumi, M., Mackas, D. L., Whitney, F. A., DiBacco, C., Galbraith, M. D., & Wong, C. S. (2005). Pteropods, eddies, carbon flux, and climate variability in the Alaska Gyre. Deep-Sea Res Pt II: Topical Studies in Oceanography, 52(7–8), 1037-1053. doi:http://dx.doi.org/10.1016/j.dsr2.2005.02.005

TS.2.1.1 Changes in Atmospheric Carbon Dioxide, Methane and Nitrous Oxide, IPCC Fourth Assessment Report: Climate Change 2007
http://www.ipcc.ch/publications_and_data/ar4/wg1/en/tssts-2-1-1.html

USGS 12200500 Skagit River Discharge. National Water Information System, USGS. http://waterdata.usgs.gov/usa/nwis/uv?site_no=12200500

Whitehead, K., Karentz, D., & Hedges, J. (2001). Mycosporine-like amino acids (MAAs) in phytoplankton, a herbivorous pteropod (Limacina helicina), and its pteropod predator (Clione antarctica) in McMurdo Bay, Antarctica. Mar Biol, 139(5), 1013-1019 doi:10.1007/s002270100654

Wood, H. L., Spicer, J. I., & Widdicombe, S. (2008). Ocean acidification may increase calcification rates, but at a cost. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1644), 1767-1773.

Yang, Z., & Khangaonkar, T. (2009). Modeling tidal circulation and stratification in Skagit River estuary using an unstructured grid ocean model. Ocean Modelling, 28(1–3)

Figures and Tables:

Figure 1: Map of sample Station

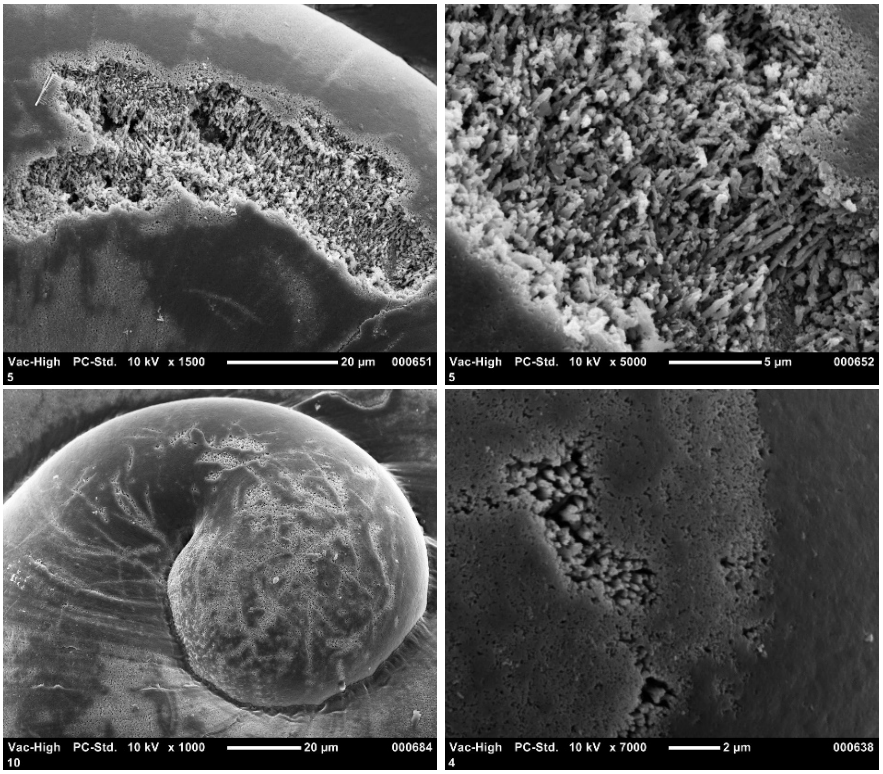
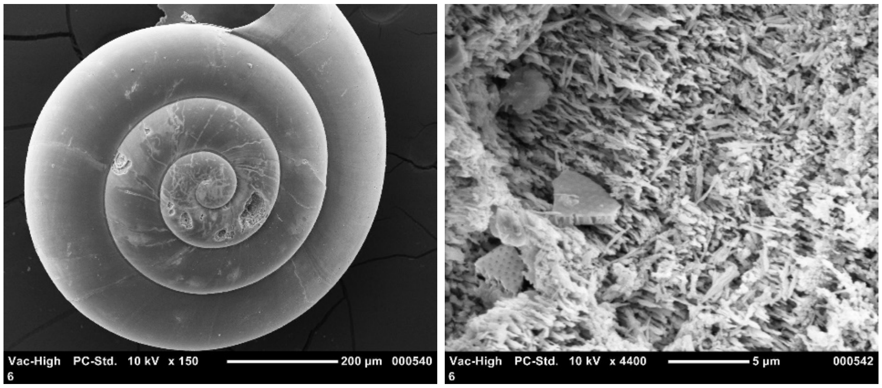


Figure 2: Representative photos of pteropod samples from station P4: Top: Type III dissolution with severe, deep intrusion into the cross lamellar layer. Bottom: Type II dissolution: photo on the bottom right shows the top of the prismatic layer, and gaps where it has dissolved.



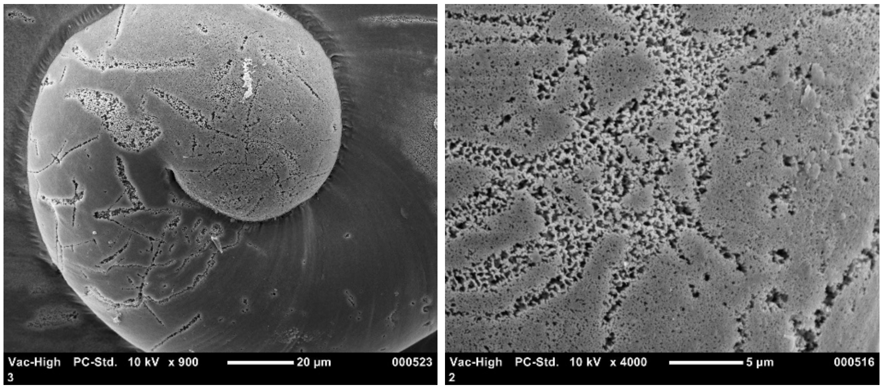


Figure 3: Representative photos of pteropod samples from station P12. Extensive Type III dissolution on the top two photos. Type II dissolution evident on the bottom photos.

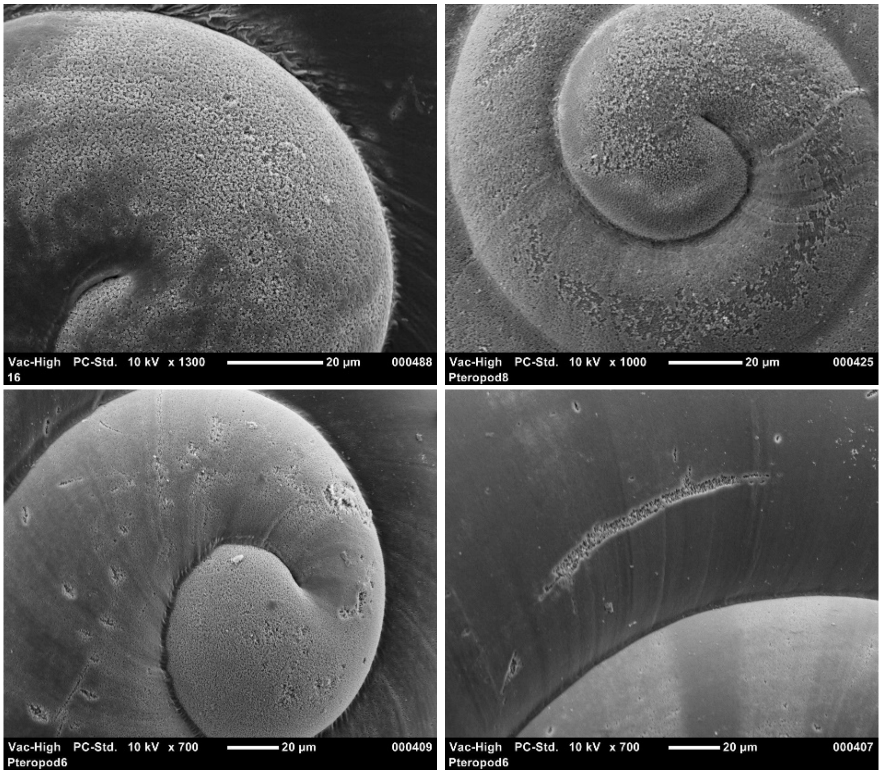


Figure 4: Representative pteropod photos from sample P381. While more of the surface area is covered with dissolution, corrosion appears less extensive, with less intrusion into the cross-lamellar layer. Bottom two photos representative of type Ii dissolution found at this station.

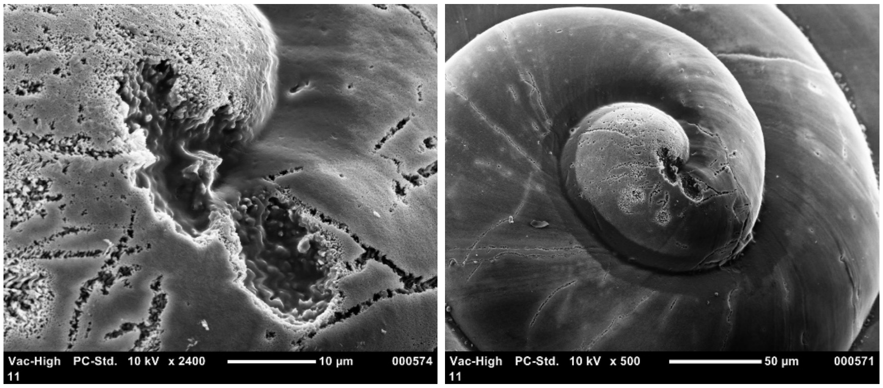


Figure 5: Thickened organic layering that could potentially be the pteropods protective reaction to dissolution

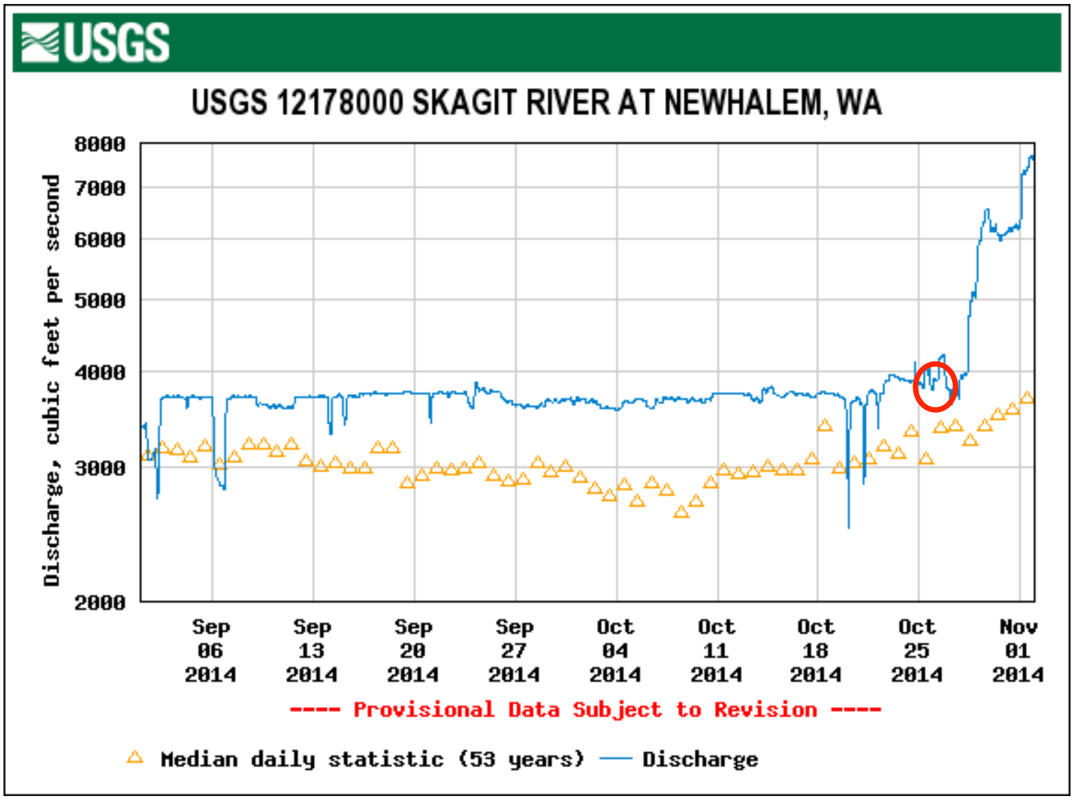


Figure 6: USGS discharge from the Skagit River mouth, for September and October 2014. Time period in which sample taken from P4 circled in red.

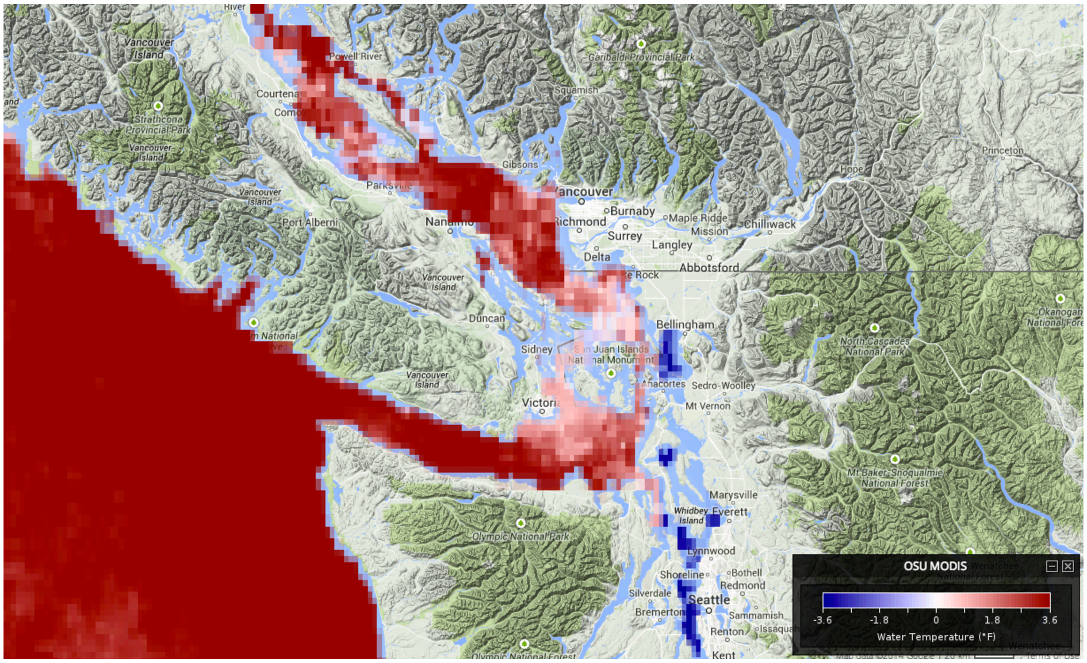


Figure 7: SST anomaly data for the coast of Washington for the month of October (data taken from NANOOS)

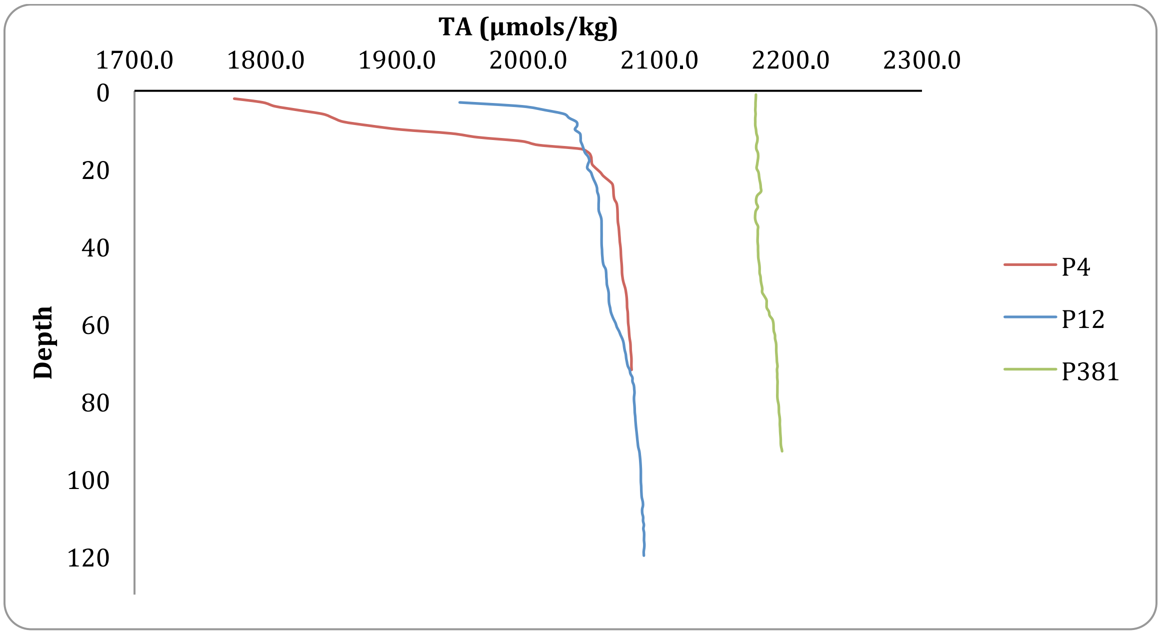


Figure 8: Total Akalinity deoth profiles for Stations P4, P12, and P381

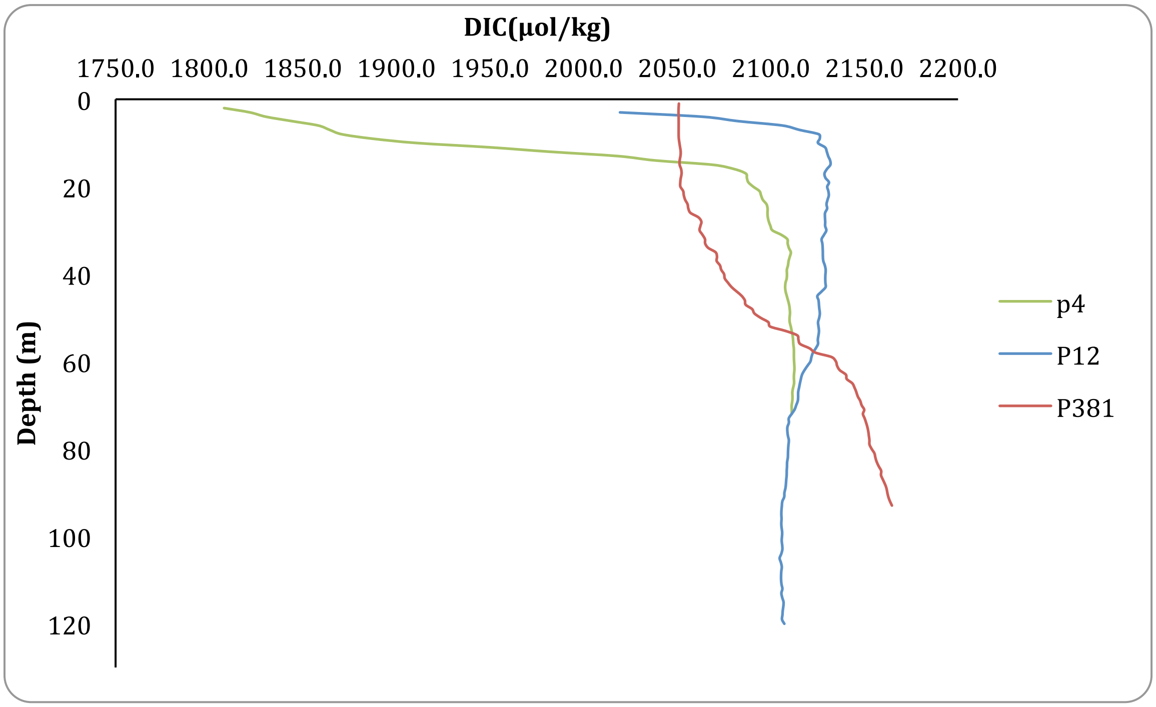


Figure 9: Dissolved Inorganic carbon depth profliles for P4, P12, P381

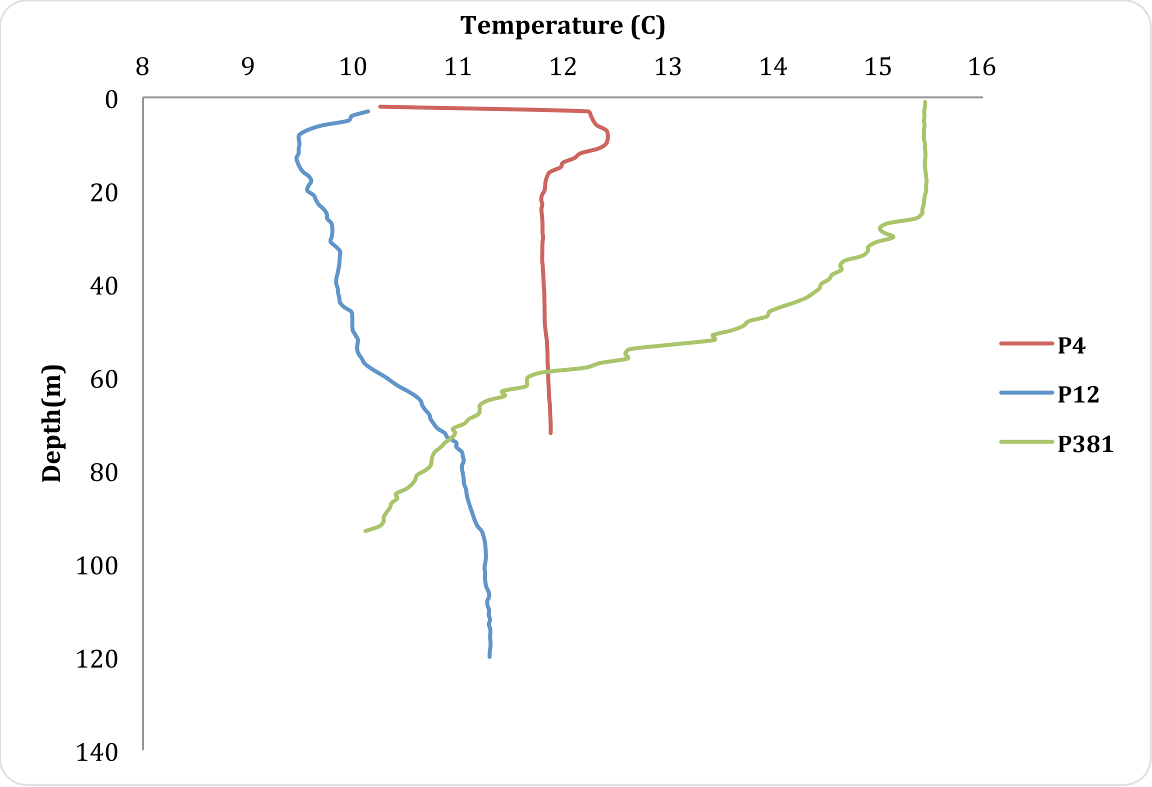


Figure 10: Temperature deoth profiles for P4, P12, and P381

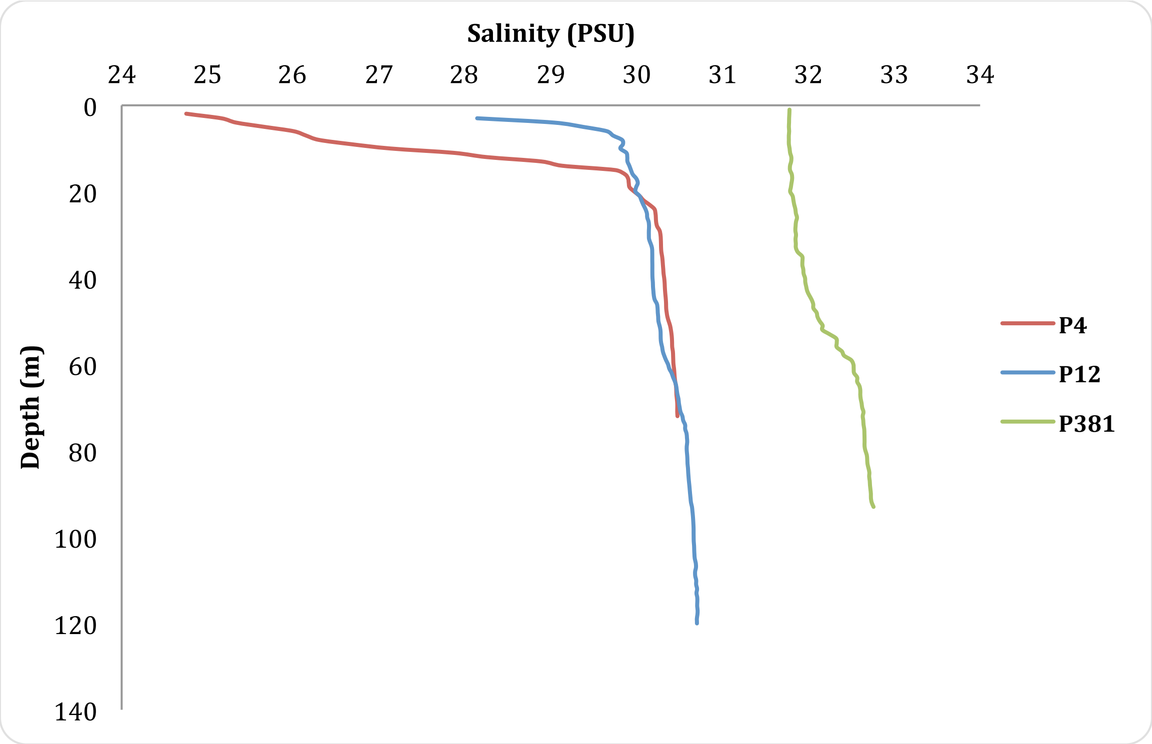


Figure 11: Salinity depth profiles for sample stations P4, P12, P381

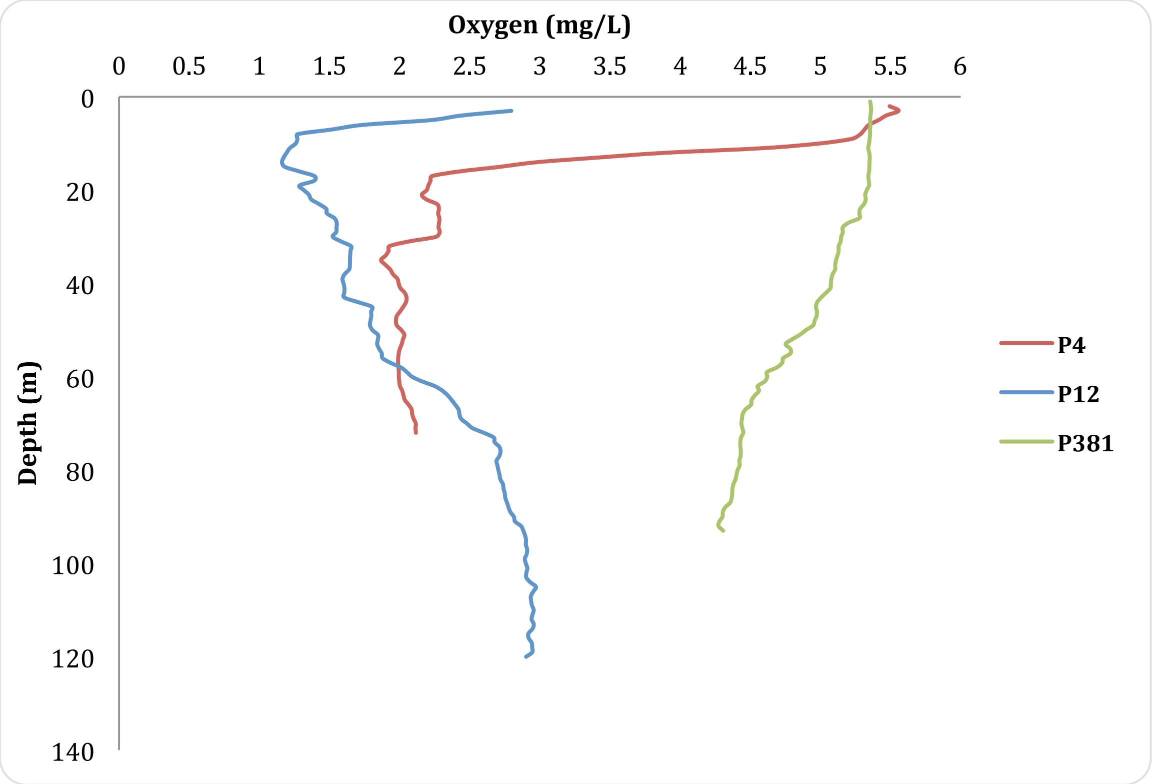


Figure 12: Oxygen depth profiles for sample stations: P4, P12, P381

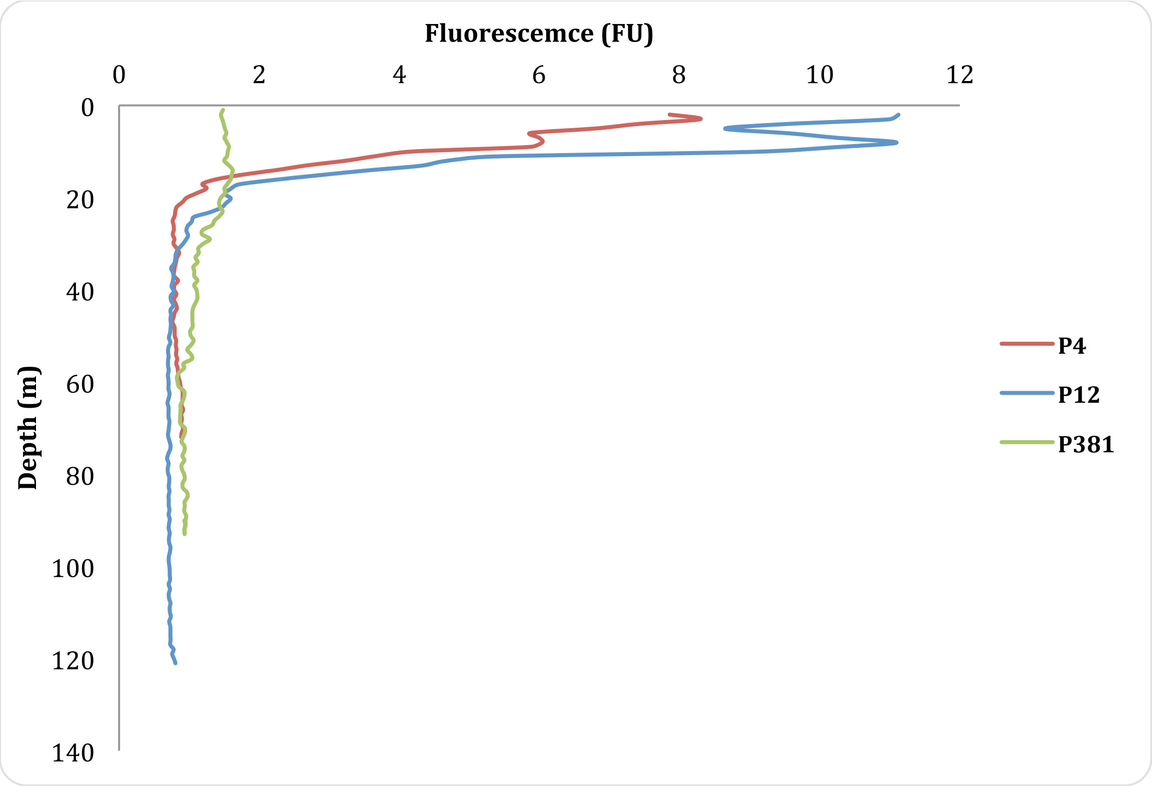


Figure 13: Fluorescence depth profiles for sample stations P4, P12, P381

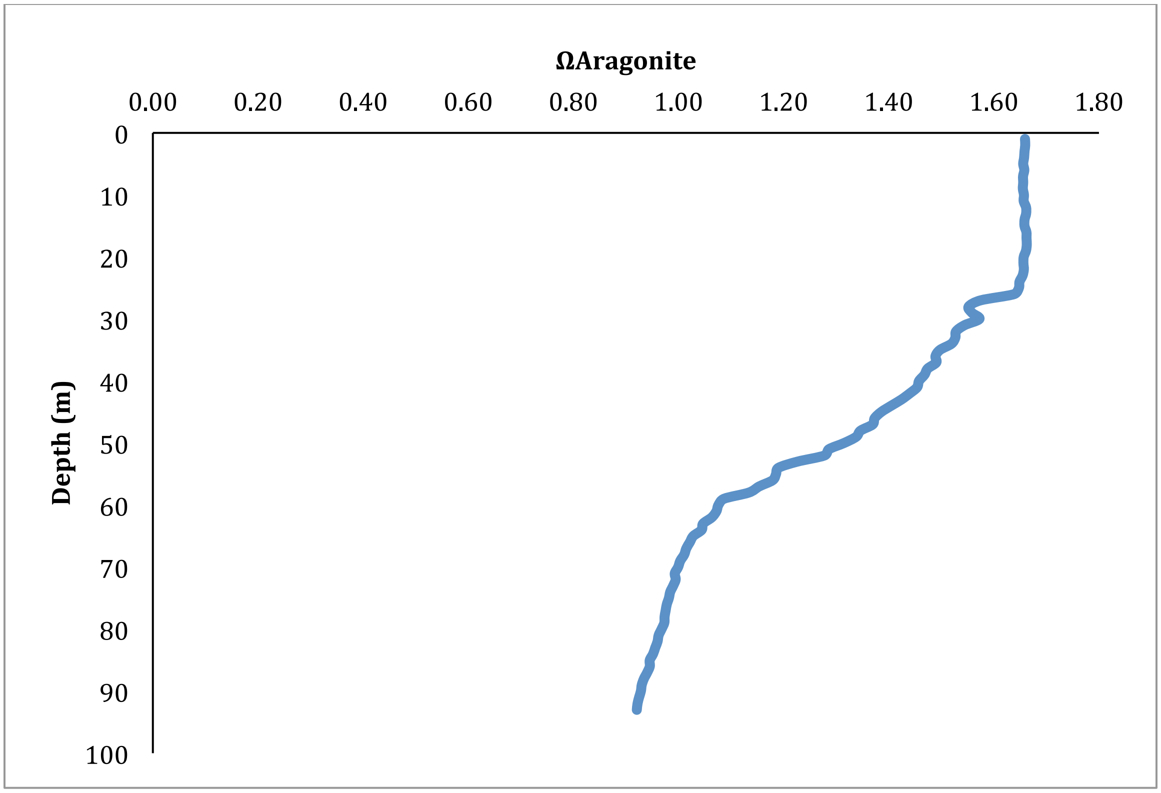


Figure 14: ΩAragonite for Station P381

Station	%Undersaturated Waters	% Pteropods with Type II dissolution	Modeled result for % pteropods with type II dissolution	Range	P value
P4	100	100	100%	17-100%	<.001
P12	100	100	100%	17-100%	<.001
p381	24	70	30%	10-60%	<.001

Table 1: Percent under saturated waters, with percent pteropods with type II dissolution, both measured and modeled for each station

Station	Sample size	Avg. % dissolved area	Std	P value
P4	3.00	0.17	0.09	.204
P12	3.00	0.21	0.04	.204
P381	3.00	0.31	0.23	.204

Table 2: Average surface area of the primary whorl that is affected by type II dissolution

Station	**Avg. Temp (C°)**	**Avg. Salinity (PSU)**	**Avg. Fluorescence (FU)**	**Avg. (Oxygen mg/L)**	**Avg. DIC (umol/kg)**	**Avg. TA (umol\kg)**	**Avg.Ω Argonite**
P4	11.91	29.64	1.71	2.59	2067.62	2034.56	N/A
P12	10.46	30.33	1.65	2.20	2112.01	2062.12	N/A
P381	13.24	32.20	1.12	4.88	2100.32	2181.03	1.32

Table 3: Average Temperature, Salinity, Oxygen, DIC, TA, and Aragonite for each sampling station.