Spring time abundance and community structure of dinoflagellates in Barkley Sound, BC

Ashley Leung
University of Washington
School of Oceanography
Box 357940
1503 NE Boat St, Seattle, Washington 98195
Email: leunga@u.washington.edu

Non-technical summary

Dinoflagellates are a type of phytoplankton that are part of the base of the marine food chain. Dinoflagellates are most abundant during late summer when nutrients are low. When high numbers of dinoflagellates occur, they can sometimes cause harmful algal blooms (HABs) which have problematic effects on local economies. Dinoflagellates that produce HABs usually carry some sort of toxin which gets passed to filter feeders such as clams and oysters when eaten. The toxins can then be passed to humans if the contaminated shellfish is consumed. Due to their potential harmful affect, it is important to understand community composition and abundance of dinoflagellate blooms. Dinoflagellate community composition and abundance were determined in Trevor Channel and Effingham Inlet in Barkley Sound, BC from 20-22nd March 2010. Samples were taken using net tows at the surface and whole water was collected from Niskin bottles at 5m. Samples were analyzed by concentrating 50ml of surface water down to 2ml and cell abundances determined by counting the number of cells using an inverted microscope. Dinoflagellate abundance was much greater at the head of Effingham Inlet due to the two sills that shelter it from any offshore transport. Only one potentially harmful species was found within Barkley Sound. However, abundance of this species was very low and only sporadically found within Effingham Inlet and Trevor Channel meaning that it may always be present but in low numbers.

Acknowledgments

I would like to thank all of the editors of this manuscript, Colleen Durkin and Rick Keil for answering all of my questions and helping me analyze and understand my data. I would also like to thank Rita Horner for listening to my ideas and helping me put them all together. I am very grateful for Kathy Newell for assisting me in all my lab work and analysis along with helping me put my paper together. A special thank you to Kira Rombeau and Carly Moreno for allowing me to use their diatom data, Riley Linder and Rachel Emswiler for their CTD data, and Diana Haring for graciously collecting my water samples during the second leg. Thank you to the UW School of Oceanography for funding my trip to Bamfield and providing me with this experience.

Abstract

Certain species of dinoflagellates have been known to cause harmful algal blooms. The patterns of their blooms must be monitored in order to help prevent the harmful toxins from causing damage to wildlife and humans. Barkley Sound is an area on the west coast of Vancouver Island, BC that contains oyster and fish farms. Daily cruises were conducted between 20-22nd March 2010 in order to determine the abundance and community composition of dinoflagellates. Net tows were taken at the surface along with whole water at 5m from Trevor Channel and Effingham Inlet. Samples were analyzed by concentrating 50ml of surface water to 2ml and cell abundances were determined by counting the number of cells using an inverted microscope. Effingham Inlet was less saline and contained the highest abundance of dinoflagellates than Trevor Channel which was exposed to offshore transport. *Protoperidinium* species (spp.), *Scrippsiella* sp., *Ceratium* spp., *Heterocapsa* sp. and *Dinophysis* sp. were identified. Diversity was compared at each station using the Shannon-Wiener Diversity Index. The most diverse station was located in Trevor Channel. It was dominated by diatoms and no dinoflagellates were present. A potential toxin producing dinoflagellate, *Dinophysis* sp., was identified during this study. Past research shows that harmful species usually occur in this area during fall (Taylor and Haigh 1996). However, this study examined dinoflagellate community composition in the spring and the highest abundance was found at the head of Effingham Inlet meaning that potentially harmful species may always be present in low abundances.

Introduction

Harmful algal blooms (HABs) have been increasing in frequency all around the world (Hallegraeff 1993). There are various types of harmful algal blooms and each has its own consequences. For example, toxic poisoning is the most common effect of HABs. In some severe cases, brain damage can occur (Hallegraeff 1993). Others can cause Diarrhetic Shellfish Poisoning (DSP), Amnesic Shellfish Poisoning (ASP), Neurotoxic Shellfish Poisoning (NSP) and Paralytic Shellfish Poisoning (PSP) (Hallegraeff 1993). These are all harmful to humans and are usually transferred by eating contaminated shellfish. There are also algal species that affect only the fish that eat them by clogging their gills (Hallegraeff 1993). This creates major fish kills and can potentially affect local economies. HABs can destroy these economies by contaminating fisheries, which are the main source of protein and monetary value for low income areas (Lembeye 2008). There are also some blooms that are not actually toxic but just produce water discoloration. Learning how these organisms adapt to local ecosystems is key to predicting when they will occur.

Dinoflagellates are primary producers that contribute to the base of the marine food chain. Their abundance varies with the seasons and is usually greatest during the summer and fall months. Abundance can also vary with nutrient and light levels. Limiting nutrients such as nitrogen and phosphorus are often the greatest factor in determining the composition of dinoflagellate populations. Blooms usually occur during the summer when nutrient levels are low (Anderson et al. 2008). Dinoflagellates generally do better when there are low silica concentrations because there is less competition with diatoms (Moncheva et al. 2001). Learning what causes these blooms is vital to coastal and fishing communities because certain dinoflagellates can have negative affects on the physical and economic health of these communities.

Mixotrophy is a unique element that specific dinoflagellates use to obtain nutrients such as the *Dinophysis* species (sp.) and *Scrippsiella* sp. (Burkholder et al. 2008). This means that through photosynthesis, they can convert inorganic carbon into organic carbon if there is no organic carbon available for them to use (Burkholder et al. 2008). They also have the option to assimilate organic carbon from the water and incorporate it into their cells like a heterotroph (Burkholder et al. 2008). Mixotrophy allows them to have an advantage over other organisms that are strictly phototrophs or heterotrophs because they can capitalize on the resources available to sustain a large bloom. Mixotrophs are generally more efficient in the way they assimilate nutrients which helps them survive during periods with very low nutrient concentrations (Anderson et al. 2008). Alternatively, some genera, like *Protoperidinium* spp. are non-photosynthetic organisms that use heterotrophy as their mode of obtaining carbon. *Protoperidinium* spp. has been known to eat diatoms, bacteria and occasionally other dinoflagellates (Jeong and Latz 1994). If conditions are not optimal for them to appear, certain dinoflagellates can rest cysts in the sediments and begin to grow when conditions such as higher temperatures occur (Kumar and Patterson 2002).

The objective of this research was to determine the community structure and abundance of dinoflagellates in Barkley Sound, BC during early spring in order to determine if potentially harmful dinoflagellates were present. Barkley Sound is located on the west coast of Vancouver Island, BC where there are many oyster and fish farms. Trevor Channel is narrow, deep and exposed to the offshore environment (Taylor and Haigh 1996). The channel is also influenced by upwelling of deep, nutrient-rich waters from the Pacific Ocean and freshwater input from Alberni Inlet (Pickard 1963). Effingham Inlet is composed of two basins and contains two sills that trap high quantities of sediment and influences the flow of water (Kumar and Patterson 2002). Effingham River flows into the head of the inlet while only a small creek flows into the outer basin. The inlet is considered a low runoff fjord and is depleted of surface nutrients required for diatom growth (Pickard 1963). It is hypothesized that dinoflagellates will be more abundant in enclosed waters such as Effingham Inlet due to the unfavorable conditions in Trevor Channel during this time of year.

Methods

Field work:

Samples were collected between 20-22nd March 2010. Cruises left Bamfield Marine Station on Vancouver Island, BC (Fig 1) at 8am and returned by 4pm each day. Samples were collected from stations throughout Trevor Channel (including a coastal station) and Effingham Inlet (Fig 1). Stations were located in the center of the channel and inlet/ Net tows were taken at the surface of all stations with a 20µm mesh net. Whole water samples were taken at 5m from

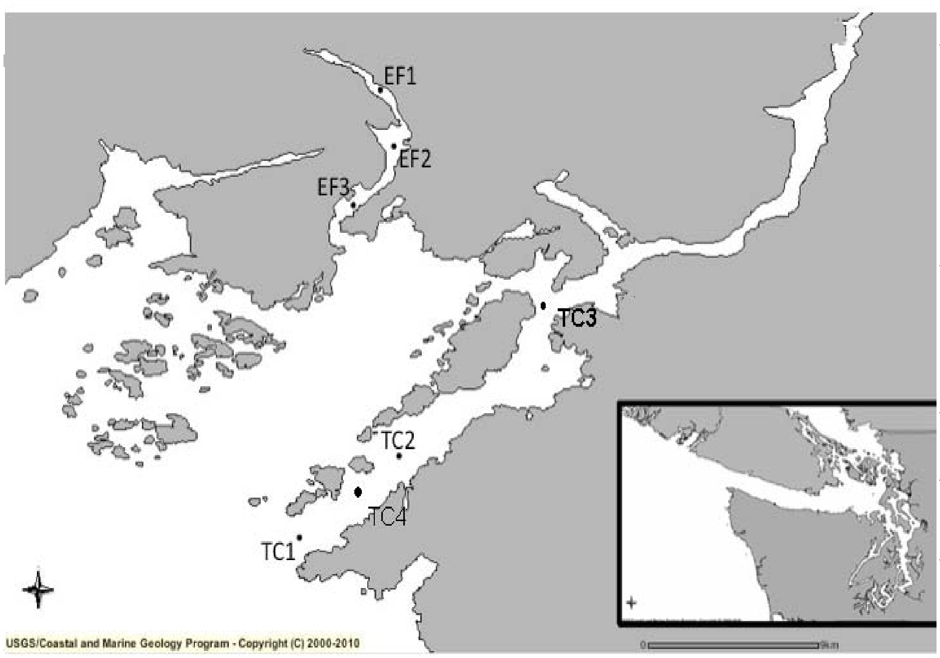


Fig 1: Map of Vancouver Island, BC. Inset: Map of all stations within Barkley Sound, BC. Map courtesy of Diana Haring.

a Niskin bottle and used to fill a 125ml jar. Water samples for nutrient analysis were filtered through a syringe with a 0.2µm filter. Nutrient samples were frozen and later analyzed at the University of Washington Marine Chemistry Lab.

Lab work:

All net tow samples and whole water were preserved with 1% formalin. Dinoflagellates were identified at each station using microscopy. Whole water samples were placed into a 50ml Utermohl settling chamber and settled on a slide down to 2 ml. After a 24hr settling period, cells were counted on each slide once across through the center at 312.5x magnification using a Zeiss inverted microscope equipped with phase contrast illumination. A vertical transect was also counted at 120.5x magnification. The dinoflagellate abundance at each station was calculated by relating the volume settled to the actual volume of the entire sample. Diatom community structure and abundances were determined using the same method by Kira Rombeau and Carly Moreno (2010). Phytoplankton diversity was also determined using the Shannon-Wiener Diversity Index (Zar 1984).

Results

Phytoplankton Composition

Five genera of dinoflagellates were identified in samples collected throughout Barkley Sound. The *Protoperidinium* spp. was seen at every station except for TC2 located in Trevor Channel adjacent to

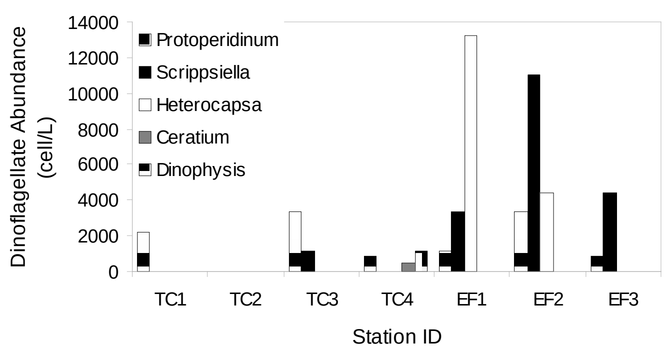


Fig 2: Dinoflagellate abundance at all stations throughout Barkley Sound.

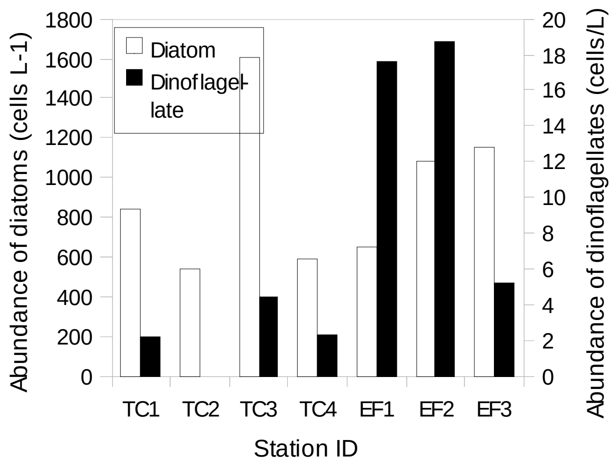
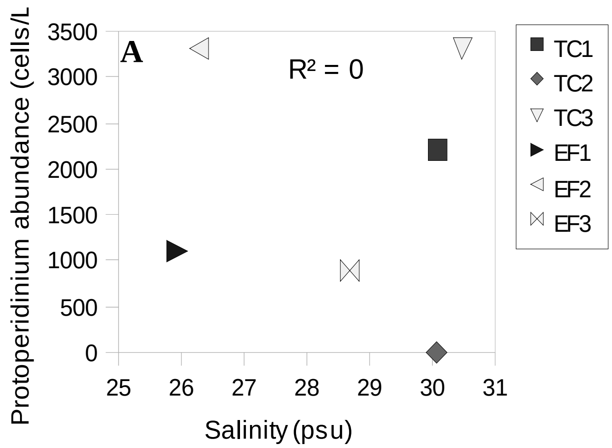


Fig 3- Dinoflagellate abundance was compared to diatom abundance by station.

Bamfield. *Scrippsiella* sp. was found at all stations throughout Effingham Inlet. *Heterocapsa* sp. was only found in Effingham Inlet. *Ceratium* spp. and *Dinophysis* sp. were only found at station TC4 (Fig 2). Diatom abundance was greater than dinoflagellate abundances at all stations. However, out of the three stations sampled in Effingham Inlet, EF1 had the lowest diatom abundance but there was an increase in dinoflagellate abundance (Fig 3). The dinoflagellate and diatom community had opposite trends as samples moved closer to the head of Effingham Inlet. There was a high abundance of diatoms at the mouth of the inlet and low abundances of dinoflagellates. Going towards the head, dinoflagellate abundances began increasing while diatom abundances decreased. The diatom community was dominated by *Skeletonema costatum* (Taylor and Haigh 1996) which was abundant at every station. According to the Shannon-Wiener Diversity Index, which measures biodiversity, station TC2 was the most diverse while TC4 was the least diverse throughout Barkley Sound (Table 1).

Physical Water Properties

Salinity in Trevor Channel was much higher than in Effingham Inlet. For example, TC1 was closest to the coast and had a salinity level of 30.72psu at the surface. EF1 was at the head of Effingham Inlet and had a salinity value of 28.67psu at the surface. *Protoperidinium* spp. were abundant in Trevor Channel where salinity was higher (Fig 4A). The abundance of photosynthetic dinoflagellates (*Scrippsiella* sp., *Ceratium* spp., *Heterocapsa* sp. and *Dinophysis* sp.) was correlated (R^2 value of 0.32) with less saline waters and were therefore more prevalent in Effingham Inlet (Fig 4B). Temperature at the surface was just under 9°C at all stations. Silica, phosphate, and nitrogen concentrations were also highest in Trevor Channel. Dinoflagellate abundances were much higher in regions with low silica concentrations (R^2 value 0.74) (Fig 5A). Dinoflagellates were also abundant when phosphate and nitrogen ($[NO_3]+[NO_2]+[NH_4]$) concentrations were low (R^2 value of 0.63 and 0.6 respectively) (Fig 5B,4C).



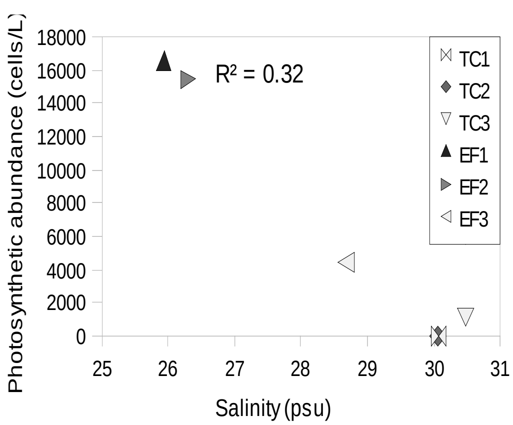


Fig 4: (A) *Protoperidinium* spp. and (B) photosynthetic dinoflagellate abundance compared to salinity levels at each station. Salinity values provided by Rachel Emswiler (2010).

Table 1: Shannon-Wiener Diversity Index of total phytoplankton at all stations. Diatom taxa identified by Kira Rombeau and Carly Moreno (2010).

	Abundance of genera per station (cells L^{-1}) reported in thosands						
Phytoplankton Taxa	TC1	TC2	TC3	TC4	EF1	EF2	EF3
Asterionellopsis	8.8	26.0	35.0				
Bacillaria					5.5		
Basteriastrum				4.4	22.0	33.0	47.0
Chaetoceros spp	6.2		20.8		1.6		
Corethron							
Cylindrotheca	9.9	4.4	6.6	3.3	2.2	5.5	14.3
Ditylum						1.1	
Navicula					0.6		
Pleurosigma	1.1	1.1					
Psuedo-nitzschia	7.7	24.2	36.4		4.4	19.8	35.3
Rhisozolenia						1.1	
Skeletonema	793.7	441.6	1451.7	563.0	607.2	986.9	996.9
Thalassionema spp.	5.5	23.1	6.6	1.1	5.5	4.4	32.0
Thalassiosira spp.	11.0	15.4	49.6	19.8	1.6	26.4	23.1
Protoperidinum spp.	2.2		3.3	0.8	1.1	3.3	0.8
Scrippsiella sp.			1.1		3.3	11.0	4.4
Heterocapsa sp.					13.2	4.4	
Ceratium spp.				0.4			
Dinophysis sp.				1.1			
Total # genera	9.0	7.0	9.0	8.0	12.0	11.0	8.0
Total abundance	846.1	535.8	1611.1	593.9	668.2	1096.9	1153.8
Shannon Wiener Index Value	**0.51**	**0.74**	**0.49**	**0.23**	**0.33**	**0.51**	**0.62**

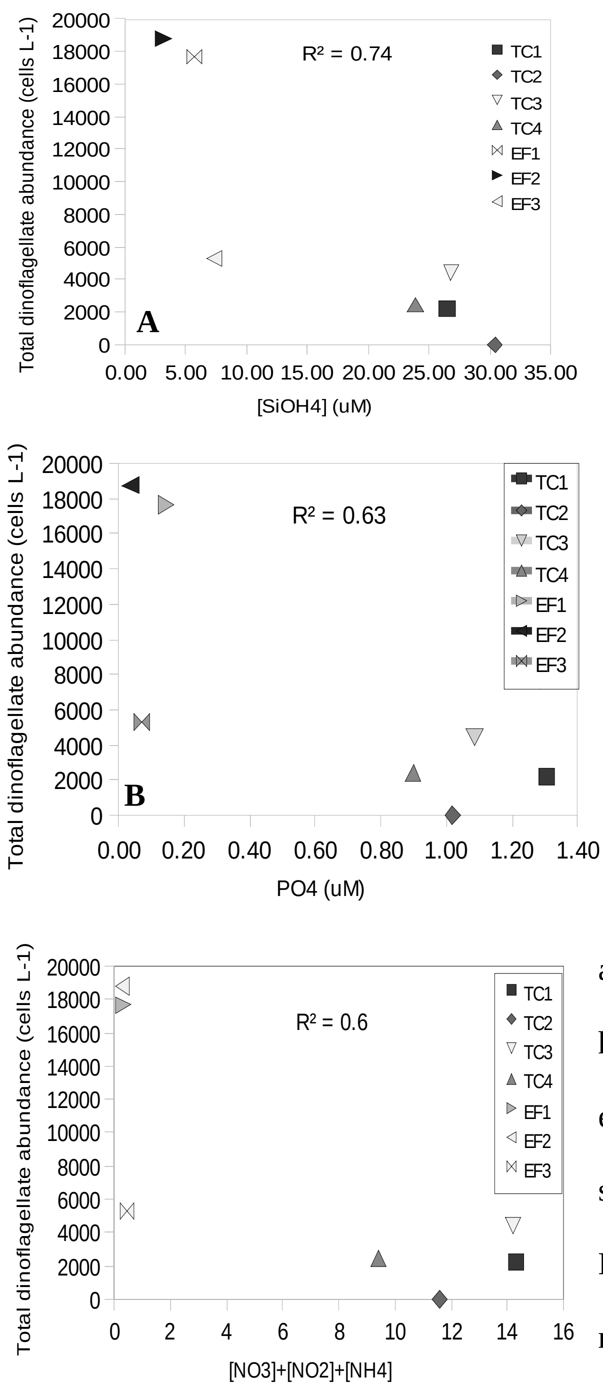


Fig 5A- Total dinoflagellate abundance compared to (A) silicic acid concentrations, (B) phosphate concentrations and (C) nitrogen concentrations at each station.

Discussion

Dinoflagellate composition was dominated by *Protoperidinium* spp. and *Scrippsiella* sp. Few dinoflagellates were found in Trevor Channel but many of them were found at stations EF1 and EF2 with the exception of *Protoperidinium* spp. EF1 was at the head of Effingham Inlet and close to Effingham River. The salinity in this region was lower than all other stations. Silicic acid concentrations in Effingham Inlet were also lower than all the stations in Trevor Channel. Less competition with diatoms allows dinoflagellates to develop and use all the resources possibly allowing for a higher diversity (Kumar and Patterson 2002). For example, diatom and dinoflagellate abundances showed opposite trends going from station EF3 to EF1. Diatom abundance decreased from the mouth to the head of the inlet while dinoflagellate abundances increased. Previous studies in this area have determined that salinity levels and proximity to the open ocean and river mouths determine the distribution of dinoflagellate assemblages (Kumar and Patterson 2002). These results are in contrast to the study done by Kumar and Patterson in March, 2002. When dinoflagellate cyst assemblages were examined in Effingham Inlet, their results showed that there was a higher abundance and diversity in the outer basin when compared to the inner basin. They concluded that certain dinoflagellates prefer higher salinity. However, since they were working with cysts, a definite correlation cannot be made comparing vegetative cells. Tidal currents, winds and estuarine circulation would need to be taken into account for the distribution of cysts. Differences in species throughout the spring and summer could also change.

Protoperidinium spp. were the only dinoflagellate present throughout Trevor Channel except at TC2. It is the only genus identified that is non-photosynthetic. The abundance patterns of the dinoflagellates are consistent with the research done by Kumar and Patterson (2002). Since there was a high abundance of diatoms in Trevor Channel, *Protoperidinium* spp. could live in the high salinity environment and have enough diatoms to eat. Even though there was no correlation between salinity and *Protoperidinium* spp., they can still use diatoms as a energy source. As a result, the competition between *Protoperidinium* spp. and diatoms is unique and not always negative as it would be with photosynthetic dinoflagellates. Being a heterotroph can give *Protoperidinium* spp. an advantage over photosynthetic dinoflagellates because they do not depend on nutrient concentrations and ample sunlight to fuel them. As long as there are enough diatoms and bacteria to eat, *Protoperidinium* spp. can grow thus possibly explaining why they were observed in six out of the seven stations while photosynthetic dinoflagellates were not. However, the beginning of spring does not allow all types of dinoflagellates to bloom since there may not be enough light (Aktan et al. 2005). A shift in the dominance of different dinoflagellates could occur as the season progresses.

As the spring and summer seasons progress, nutrients concentrations tend to decrease as they get used up (Anderson et al. 2008). Dinoflagellates usually do better in an environment with lower nutrient concentrations because there is less of a competition with diatoms. There was a strong correlation of high dinoflagellate abundance and low nutrient concentrations found in this study. When abundances were compared to silicic acid concentrations, the dinoflagellate population decreased dramatically as concentrations increased to more than 5µm. Diatoms depend on silicic acid to build their silica shells (Hay et al. 2003). If there is a decrease in silicic acid, diatom abundance will decrease giving dinoflagellates the chance they need to grow. There was also a strong correlation between dinoflagellate abundance with nitrogen and phosphorus concentrations. Again, as nutrient concentrations decreased, dinoflagellate abundances increased showing that they do better when diatom blooms are not occurring.

Only one genus of potentially toxic producing dinoflagellates was seen in the samples. The *Dinophysis* sp. was found at one station in Effingham Inlet and one station in Trevor Channel. Taylor and Haigh (1996) reported that there were 14 harmful species of dinoflagellates identified in Barkley Sound but these observations were made during fall. The identification of a toxic species present in the study suggests that they may always be present but in low abundances. Physical properties of the water such as low silicic acid concentrations and salinity might be a key factor to help predict when an onset of dinoflagellate blooms will occur. It is important to monitor the rise in abundances of potentially harmful dinoflagellates so that conditions in which HABs are successful can be better understood.

Shannon-Wiener Diversity Index

When the presence of diatoms and dinoflagellates was compared, diatoms were clearly more abundant in the net tows and whole water samples. The diversity index shows that TC2 has the most diverse population of total phytoplankton. However, this station also did not have any dinoflagellates present. The dinoflagellates may not have had the resources to compete with the diatoms and were therefore absent at this station. The station with the least diversity was TC4 which also had the least amount of diatoms present. Accordingly, the dinoflagellate population was allowed to survive due to the lower competition rate with the diatoms. When percent of abundance was compared in a past study by Harris et al. (2009), dinoflagellates were not present in samples taken off the west coast of Vancouver Island. Abundance of both photosynthetic and heterotrophic dinoflagellates was not seen until July. The present study examined the dinoflagellates in more sheltered regions of Barkley Sound which may explain why they were seen earlier in the season. However, samples need to be taken over a longer time period in order for the results to be comparable to past studies. The seasonal pattern of dinoflagellates allows them to grow in areas that allow for optimal survival. Since diatoms are abundant in coastal areas during the spring, dinoflagellates grow in locations where diatoms are less abundant (Aktan et al. 2005).

Conclusion

1. Dinoflagellates were seen in higher abundances throughout Effingham Inlet than Trevor Channel and especially closer to the head. Dinoflagellate abundance was correlated with lower nutrient concentrations along with lower salinity.
2. *Protoperidinium* spp. was the only genus seen at six out of the seven stations. It is also

the only heterotrophic dinoflagellate observed.

3. Only one potentially harmful dinoflagellate genus was observed. *Dinophysis* sp. was seen at one station in both Trevor Channel and Effingham Inlet.
4. The most diverse station was TC2 which is located in the middle of Trevor Channel near Bamfield Marine Research Station. However, there was no dinoflagellates present at this station. Diversity was determined using the Shannon-Wiener Diversity Index. The least diverse station was TC4 which is located closer to the coast.
5. Spring time abundance and community composition of dinoflagellates need to be monitored in order to learn more about why harmful algal blooms occur and what conditions they prefer.

References

Aktan, Y., Tufekci, V., Tufekci, H., Aykulu, G. 2005. Distribution patterns, biomass estimates and diversity of phytoplankton in Izmit Bay (Turkey). Estuarine, Coastal and Shelf Science. **64:**372-384.

Anderson, DM., Burkholder, JM., Cochlan, WP., Glibert, PM., Gobler, CJ., Heil, CA., Kudela, RM., Parsons, ML., Rensel, J., Townsend, DW., Trainer, VL., Vargo, GA. 2008. Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. Harmful Algae. **8:** 39-53.

Burkholder, JM., Glibert, PM., Skelton, HM. 2008. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. Harmful Algae. **8:**77-93

Jeong, HJ., Latz, MI. 1994. Growth and grazing rates of the heterotrophic dinoflagellates *Protoperidinium* spp. on red tide dinoflagellates. Mar. Ecol. Prog. Ser. **106**:173-185.

Hallegraeff, GM. 1993. A review of harmful algal blooms and their apparent global increase. Phycologia. **32**:79-99.

Harris, SL., Varela, DE., Whitney, FW., Harrison, PJ. 2009. Nutrient and phytoplankton dynamics off the west coast of Vancouver Island during the 1997/98 ENSO event. Deep-Sea Research II. **56:**2487-2501.

Hay, MB., Pienitz, R., Thomson, RE. 2003. Distribution of diatom surface sediment assemblages within Effingham Inlet, a temperate fjord on the west coast of Vancouver Island (Canada). Marine Micropaleontology. **48:**291-320.

Kumar, A., Patterson, R.T., 2002. Dinoflagellate cyst assemblages from Effingham Inlet, Vancouver Island, British Columbia, Canada. Palaeogeogr. Palaeoclimatol. Palaeoecol.**180**:187-206.

Moncheva, S., Gotsis-Skretas, O., Pagou, K., Krastev, A. 2001. Phytoplankton blooms in Black Sea and Mediterranean coastal ecosystems subjected to anthropogenic eutrophication: similarities and differences. Estuarine,Coastal and Shelf Science. **53:**281-295.

Moreno, C. 2010. Diatom diversity and primary production in Barkley Sound and Effingham Inlet, Canada based on light microscopy. UW Undergraduate Senior Thesis, unpublished.

Pickard, GL. 1963. Oceanographic characteristics of inlets of Vancouver Island, British-Columbia. Journal of the Fisheries Research Board of Canada. **20:**1109-1144.

Rombeau, Kira. 2010. Diatom abundance and community structure in Barkley Sound, Vancouver Island, BC: significant impacts on primary productivity. UW Undergraduate Senior

Thesis, unpublished.

Taylor, F.J.R., Haigh, R., 1996. Spatial and temporal distributions of microplankton during the summers of 1992-1993 in Barkley Sound, British Columbia, with emphasis on harmful species. Can. J. Fish. Aquat. Sci. **53**:2310-2322.

Zar, JH. 1984. Biostatistical Analysis, second ed. Prentice-Hall Inc., Englewood Cliffs, NJ.