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Survey of dinoflagellate cysts in Barkley Sound, Vancouver Island, Canada.

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NONTECHNICAL SUMMARY

The objective of this study was to examine how groups of dormant, spore-like structures produced by dinoflagellates called 'cysts' are distributed in the sediments of an estuary known as Barkley Sound, Vancouver Island, Canada. Samples were collected between 22 January and 3 February 2013 aboard the University of Washington *R/V Thomas G. Thompson*. Barkley Sound was divided into three areas of interest: Effingham Inlet, Imperial Eagle Channel, and Uchucklesit Inlet. The three regions differed greatly in the number of cysts observed, their diversity, and other characteristics. The number of cysts in Effingham Inlet and Imperial Eagle Channel was greater in less restricted areas and lower in confined areas. The total abundance within Uchucklesit Inlet was greatest in the head and least near the entrance; opposite of the other two regions. Cysts of toxic dinoflagellate genera were found at almost every station in varying concentrations. These differences are related to the unique bathymetry and current patterns of each region.

ABSTRACT

This study investigates the differences in concentrations and diversity of dinoflagellate cysts within the shallow sediments of Barkley Sound, Vancouver Island, British Columbia. Sampling took place between 22 January and 3 February 2013 aboard the University of Washington *R/V Thomas G. Thompson*. A comparison of the dinoflagellate cyst assemblages among the basins was accomplished through the use of sediment cores, stains, and epifluorescence microscopy. Stations surveyed in Barkley Sound included samples from Effingham Inlet, Imperial Eagle Channel, and Uchucklesit Inlet. The three regions varied greatly in terms of the abundance, diversity, and evenness of the cyst assemblages. The abundance of cysts in Effingham Inlet and Imperial Eagle Channel was greater in less restricted areas and lower in restricted areas. The abundance within Uchucklesit Inlet was greatest in the head and least near the entrance; opposite of the other two regions. Cysts of toxic dinoflagellates *Alexandrium, Lingulodinium, Operculodinium,* and *Spiniferites* were found at almost every station in varying concentrations. Variations in the composition of assemblages of dinoflagellate cysts within these basins have been attributed to differing circulation patterns affecting the distributions and diversity of the dinoflagellate cyst assemblages.

Primary production in the ocean constitutes the base of the global food chain. Plankton blooms are responsible for much of the photosynthesis that occurs on Earth and are also a major food source for larger organisms in the ocean. Within these blooms are the plankton known as 'dinoflagellates', and they fill several important roles within their communities relating to primary production as well as carbon transport. Many species are autotrophic, some are heterotrophic, and still others are mixotrophic (Taylor et al., 2008). The species that are capable of autotrophy contribute directly to carbon uptake and oxygen production. The heterotrophic species prey on other microbes and contribute to respiration and carbon excretion. Mixotrophs are capable of both autotrophy and heterotrophy depending on whether sufficient prey is available (Taylor, 1987). The primary controls on dinoflagellate blooms include nutrient concentrations, temperature, and the depth of the water column as well as geographical and bathymetric factors. Dinoflagellate productivity tends to be highest in relatively shallow water with strong stratification (Taylor, 1987).

Of the 2,000 extant species of dinoflagellates, 13-16% of them have periods in their lifecycles where they become non-motile and produce specialized, thickened cell walls around themselves creating spore-like structures known as ‘cysts’ (Head, 1996). Various factors such as nutrient depletion, temperature changes, and sexual reproduction can lead to the development of cysts (Pfiester and Anderson, 1987; Matsuoka and Fukuyo, 2000; Nagai et al., 2009). Dispersion of these structures has been connected to seasonal temperature changes, upwelling, oceanic current influence, and even climate change (Aksu et al., 1989; Powell et al., 1990; Head and Wrenn, 1992). Once produced, cysts settle to the bottom of the basin and become buried by sediments; these areas are known as ‘seed beds (Tyler et al., 1982).’ The cysts can stay buried from months to years before emerging from their protective coverings and rejoining the plankton community when conditions have returned to their previous state or the cyst’s mandatory dormancy period is over (Matsuoka and Fukuyo, 2000). The seasonal subtraction and addition of these predators and producers can affect the grazing, growth, and primary production rates of the plankton assemblages in these areas and therefore, the local and global carbon cycles. The study of dinoflagellate cysts is vital to the understanding of the role of these structures as a means to perpetuate dinoflagellate populations and expand their distributions in oligotrophic conditions (Matsuoka and Fukuyo, 2000).

Some species of dinoflagellates produce toxins that can contribute to fish mortality or become concentrated in shellfish tissues which can be deadly to humans who consume them (Tappan, 1980; Taylor, 1987; Taylor and Harrison, 2002). There are several genera of cyst-forming dinoflagellates common to the west coast of North America that have been linked to various types of toxicity. *Alexandrium catenella* has been associated with paralytic shellfish poisoning (PSP) (Trainer et al., 2003). *Protoceratium reticulatum*, referred to as *Operculodinium centrocarpum* in cyst form (Matsuoka and Fukuyo, 2000), has been associated with diarrhetic shellfish poisoning (DSP) (Paz et. al., 2008). The dinoflagellates *Lingulodinium machaerohorum (Lingulodinium polyedrum* in its motile form) and *Gonyaulax spinifera (Spiniferites)* are two additional dinoflagellates that produce cysts and have been connected to DSP (Matsuoka and Fukuyo, 2000; Paz et. al., 2008). Concentrations of these genera in sediments are representative of past blooms and could help to predict future toxic blooms.

Oceanography and Geology

Barkley Sound is a bay on the southwestern coast of Vancouver Island, British Columbia, Canada (Fig. 1). The main channels receive frequent input from the Pacific Ocean (Emswiler, 2010). Nutrient regimes in Barkley Sound depend on upwelling events in the summer months that are tied to oscillations between two atmospheric cells known as the Aleutian Low and the North Pacific High (Kendrew and Kerr, 1955; Patterson et al., 1995). These systems control local wind patterns which influence the oceanic current patterns that affect Barkley Sound. Ekman transport causes upwelling events on this coast during the summer months, which lead to large phytoplankton blooms that contain dinoflagellates (Patterson et al. 2000). Many of these bloom events occur within the channels and confined inlets of Barkley Sound and contribute to its seed beds.

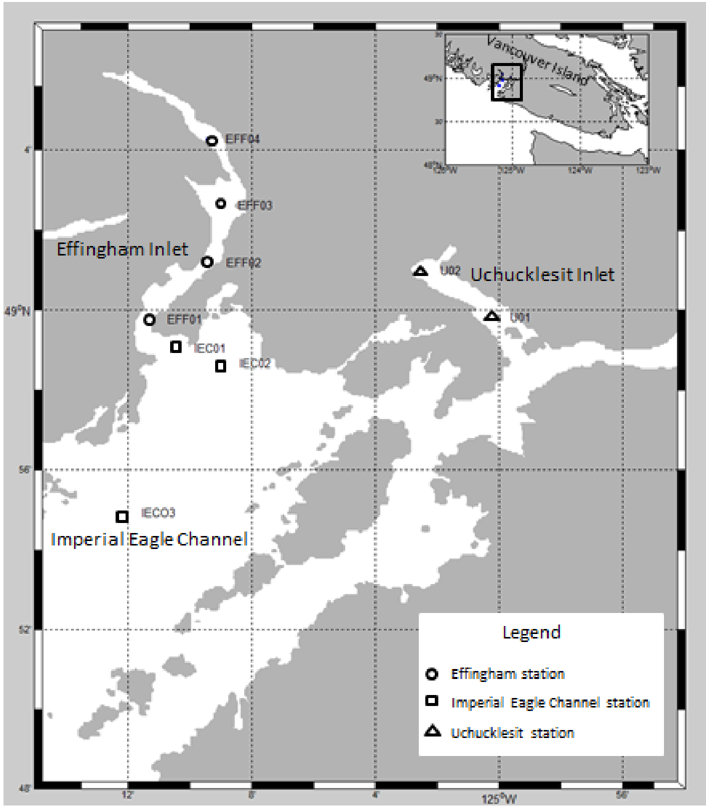


Figure 1. A map of the stations surveyed by this study in Barkley Sound (inset).

Barkley Sound is part of a fjord system composed of volcanic rock that create steep, hard slopes that are conducive to the accumulation of sediments that become concentrated in the bottom of the inlets and channels. Additionally the Effingham River and Henderson Lake drain into Effingham Inlet and Uchucklesit Inlet, respectively, and contribute sediment and fresh water to the region as well as many other smaller streams. The majority of sediments found in Effingham Inlet and Uchucklesit Inlet are composed of thin, brown mud near the heads of the inlets and compact, grey mud near the mouths. The sediments taken from Imperial Eagle Channel were more like the sediments found in the entrances of the inlets with less organic matter.

Sills present in the area have a profound influence on the movements of currents, sediments, and biota throughout the sound. Effingham Inlet receives the least influence from oceanic current input because it is separated by a sill at 65m depth from Imperial Eagle Channel (Fig. 2) (Patterson et al., 2000; Ingall et al., 2005). The presence of a second sill (45m depth) at the entrance of the inner basin restricts the flow of the inlet further from the main channels of Barkley Sound (Fig. 2). Imperial Eagle Channel is the deepest of the three regions of interest; it has no sills and is open to the Pacific Ocean (Fig.1). It receives frequent sea water incursions from oceanic currents (Emswiler, 2010). Uchucklesit Inlet is not open to the ocean, but does have a sill at roughly 45m depth near its entrance. It is shallower near the head, becoming deeper toward the entrance (Fig. 3). Based on these observations, it is reasonable to assume that this previously unstudied inlet receives more circulation that Effingham Inlet, but less than Imperial Eagle Channel. The unique bathymetry of each region can affect the characteristics of the water in each region.

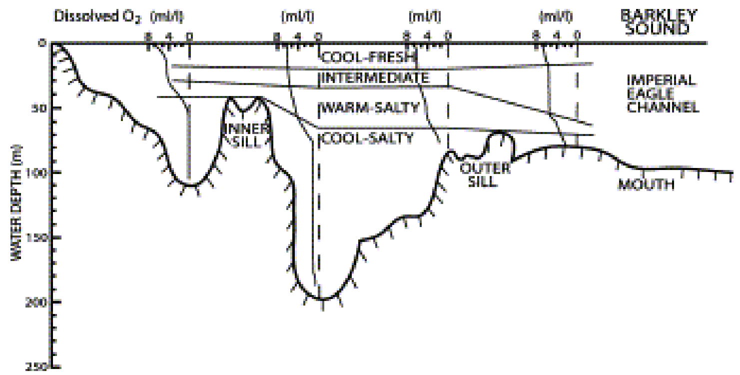


Figure 2. A cross section of Effingham Inlet depicting stratification, salinity, and depth with oxygen profiles at selected location (Kumar and Patterson, 2000).

Several of the areas surveyed near sills have histories of anoxic conditions (Patterson et. al., 2000; Ingall et al., 2005). Under suboxic or anoxic conditions, laminations or thin layers can be formed by the settling sediments. Areas with less defined or no laminations have been related to well oxygenated conditions (Ingall et al., 2005). Relatively high levels of homogeneity of the assemblages within the layers in recent sediments may be indicative of bioturbation activity at sites that have oxygenated bottom water. The sedimentation rate in the region can vary between 0.5 and 1.0cm yr^{-1} and so the top layers are representative of present conditions (Patterson et al., 2000; Ingall et al., 2005). Cysts found in these layers were believed to have been deposited recently.

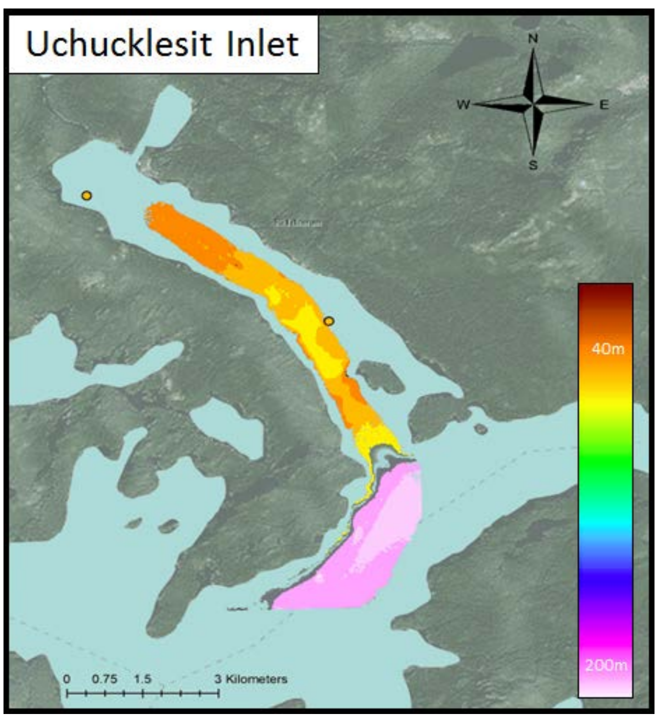


Figure 3. A bathymetric map of Uchucklesit Inlet (Rembold, 2013).

Previous Studies

There have been several similar studies conducted previously. These studies include a record of seasonal climate change indicators related to past El Niño events and red tide events from sediments in Sannich Inlet (Mudie, 1998a, b). Heusser (1983) conducted a palynological study of two sediment cores, also from Sannich Inlet, investigating the abundance of dinoflagellate cysts over the last 12kyr. Surveys of cyst distributions in the shallow sediments of Effingham Inlet have been undertaken by Kumar and Patterson in 2000 and 2002. Recently, a study by Radi et al. (2007) investigated relationships between dinoflagellate cyst assemblages and environmental parameters such as sea-surface temperature and salinity in several inlets of Barkley Sound.

Objectives

The study of the diversity and abundance of the dinoflagellate seed beds in Barkley Sound can help to define their roles in local food webs as well as both global and local carbon cycles as producers and consumers. Knowing which toxic genera are present, their spatial distribution, and abundance is essential to predicting and preparing for the results of these microorganisms' toxicity. Additionally, studying these seed beds may allow toxic bloom events to be more closely monitored and their detrimental effects limited. The objective of this study is to observe the distribution of dinoflagellate cysts in Barkley Sound, specifically within Effingham Inlet, Uchucklesit Inlet, and Imperial Eagle Channel. This survey also investigates the relationships between cyst distribution and various environmental influences that could affect them such as nutrient and oxygen concentrations, current input, and bathymetric factors.

METHODS

Sample collection

In order to examine differences in the spatial distributions and diversity of the dinoflagellate cyst assemblages in Effingham Inlet, Imperial Eagle Channel, and Uchucklesit Inlet a survey of the shallow sediments was conducted. The samples were collected between 26 January and 2 February, 2013 aboard the *R/V Thompson*. Sediment samples were taken using an Ocean Instruments MC200 sediment multicorer with coring cylinders 1m long and 10cm in diameter. The sediment was sliced off in 2.5cm increments to obtain the top 5cm of each core. Each layer was halved and each half stored in sediment bags in a dark cooler during transit back to a laboratory at the University of Washington for analysis.

Sample Preparation

Concentration and staining of the cysts followed a version of the method described by Yamaguchi et al. (1995) that has been modified by Kirsten Feifel, a biological oceanography graduate student at the University of Washington. A 5cc subsample of raw sediment was taken from the top and bottom layer samples from each core. The sediment was diluted in deionized water, sonicated, and sequentially sieved through 90µm and 20µm nitex screens. The concentrated cysts were fixed using a 10% formaldehyde solution then stored in pure methanol for 48 hours to remove organic materials that could conceal the cysts during the counting phase. Finally, the concentrated cysts were stained with Primuline fluorescent stain and suspended in 5mL of deionized water (Yamaguchi et al., 1995; modified by Kirsten Feifel).

Cyst Counting

In order to enumerate the cysts, 1mL of stained sample from each layer sample was pipetted into a Sedgwick-rafter counting chamber for analysis. Visualization of the cysts was done using a Zeiss Axiovert 35 inverted microscope with epifluorescence. A mercury bulb power source, blue (420-490nm) light excitation filter, and a 330-380nm band pass excitation filter (for UV excitation) was used to activate the Primuline stain applied to the cysts. The samples were viewed under 1200x magnification. The counting chamber was carefully scanned for cysts and a fraction of the total chamber area was enumerated for each sample. All samples were counted 3 times. Genera were identified based on genus-specific morphological features (Wall and Dale, 1968; Hallegreff, Anderson, and Cembella, 1995; Matsuoka and Fukuyo, 2000). Observed cysts were identified and tallied, generating genus rosters as well as total cyst abundance for each sample. Many of the cysts identified were photographed using a Nikon micrograph camera system and Q-Capture micrograph imaging software for later visual inspection and record keeping. The abundance, as well as the Shannon Diversity Index, Shannon Equitability Index, Simpson's Dominance Index, and richness for each basin were calculated.

RESULTS

Barkley Sound

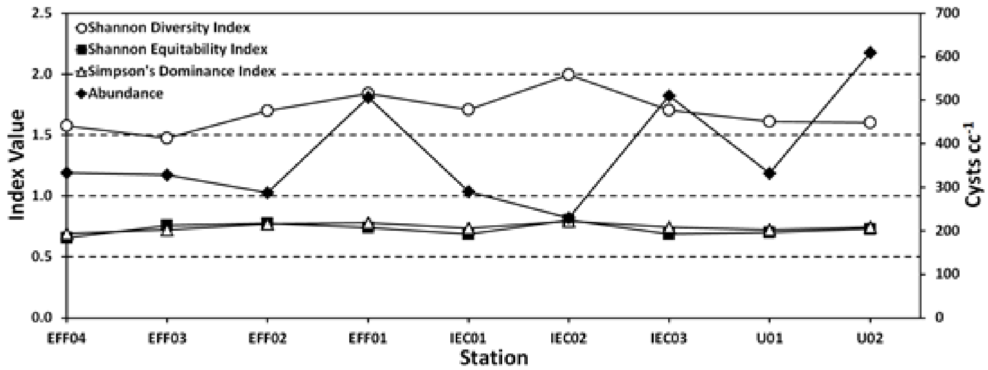
A total of 16 different genera were identified during this survey. Total concentrations and proportions for each station and genus may be found in Appendix 1-a and 1-b. *Alexandrium*, *Islandium*, *Operculodinium*, *Scrippsiella*, and *Spiniferites* were present at every station. Cysts of *Alexandrium*, a toxic dinoflagellate, were present in the highest abundances at stations U02, EFF02, EFF03 and IEC01. The lowest concentrations of this genus were found at stations EFF04, IEC02, U01, and IEC03 (Fig. 1; Fig. 4c). *Islandium* was observed in its highest abundances at IEC03, EFF01, and U01; stations with the lowest abundance included IEC01 and EFF03 (Fig. 1). Cysts of *Operculodinium*, another toxic dinoflagellate, were observed in all samples, across all stations. The highest concentrations were observed at U02, EFF04, and EFF01. The lowest abundances were found at IEC03 and U01 (Fig. 1; Fig. 4c). *Scrippsiella* cyst concentrations were highest at IEC03 with the lowest at U01. The highest abundances of *Spiniferites* were found at IEC03, U02, EFF01, and U01; the lowest were observed at EFF04 and IEC02. The total abundances of cysts in Effingham Inlet and Imperial Eagle Channel were greater in unrestricted areas and lower in confined areas. The total abundance within Uchucklesit Inlet was greatest in the head and least near the entrance; opposite of the other two regions (Table 1).

Table 1: The sampling depth, total abundance (cyst cc^{-1}), number of genera present (S), the Shannon Diversity Index (H), the Shannon Equitability Index (E_H), Simpson's Dominance Index (D), and the number of toxic genera present out of four possible (P_h) for each station.

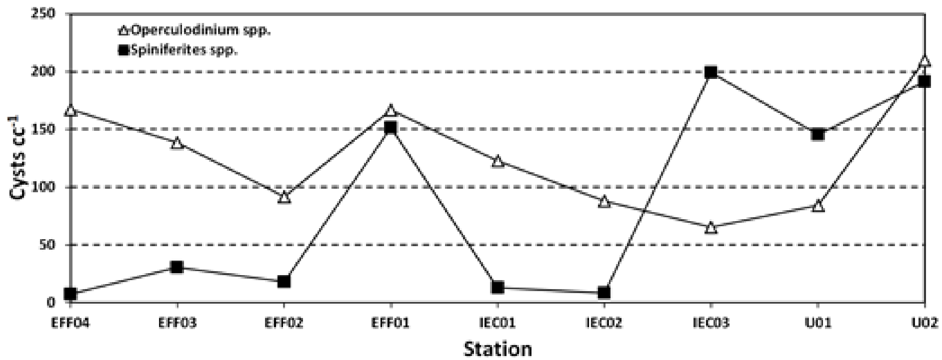
Station	Depth (m)	Abundance	S	H	E_H	D	P_h
EFF01	89	506	12	1.84	0.74	0.78	4
EFF02	117	288	9	1.70	0.77	0.77	4
EFF03	202	329	7	1.47	0.76	0.72	3
EFF04	70	334	11	1.57	0.66	0.69	4
IEC01	86	290	12	1.71	0.69	0.73	4
IEC02	94	229	12	2.00	0.80	0.80	4
IEC03	103	510	12	1.71	0.69	0.74	4
U01	76.9	332	10	1.61	0.70	0.72	4
U02	58	609	9	1.60	0.73	0.75	4

There were also genera that were not present in every sample. *Ataxiodinium* was found only at U01 and IEC01. *Cotadinium* was found at IEC01, EFF01, EFF03, and EFF04. Cysts of the toxic dinoflagellate *Lingulodinium* were found at every station except for EFF03 (Fig. 4c). *Polykrikos* was also found everywhere but EFF03 and *Pentapharsodinium* only at EFF01. *Brigantedinium* was identified only at IEC02. *Protoperidinium* was found at U02, IEC03, EFF01, and EFF02. *Quinquecuspis* was found at all stations except for U02, U01, and EFF03. *Pyxidiniopsis* was found only at IEC03, IEC02, and EFF04. *Selenopemphix* was found at all stations except U02 and EFF02. *Votadinium* was found at all stations except those in Effingham Inlet.

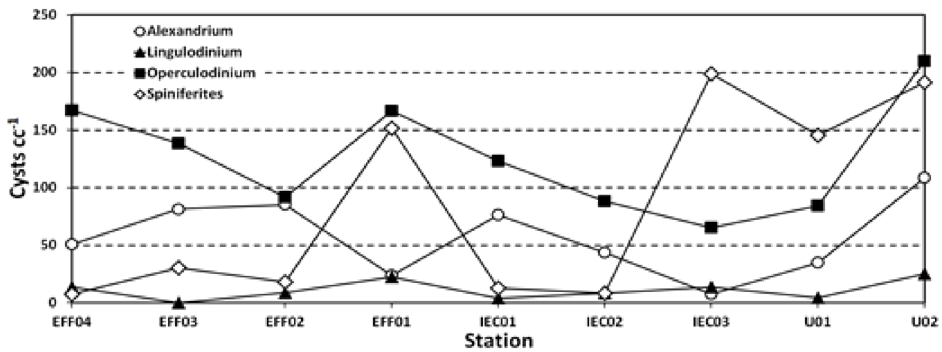
There were two major groupings of cysts observed. 'Ornamented' cysts have processes extending outward from the body of the cyst; 'unornamented', or smooth-walled cysts, do not have any processes (Matsuoka and Fukuyo, 2000). The genera *Islandium, Spiniferites* (Fig. 5C), and *Operculodinium* (Fig. 5B) were the most common ornamented cysts and made up 59.7% of the total abundance for all stations. The other 'ornamented' cysts, *Lingulodinium, Votadinium* (Fig. 5D), and *Polykrikos* accounted for only 7.3% of the total abundance. Cysts without ornamentation observed during this survey include *Ataxiodinium, Brigantedinium, Cotadinium, Pentapharsodinium, Quinquecuspis,* and *Pyxidiniopsis.* They made up 5.9% of the total abundance for all stations. The most common unornamented cysts, *Alexandrium* (Fig. 5A) and *Scrippsiella* accounted for 27.1% of the total abundance for all stations.



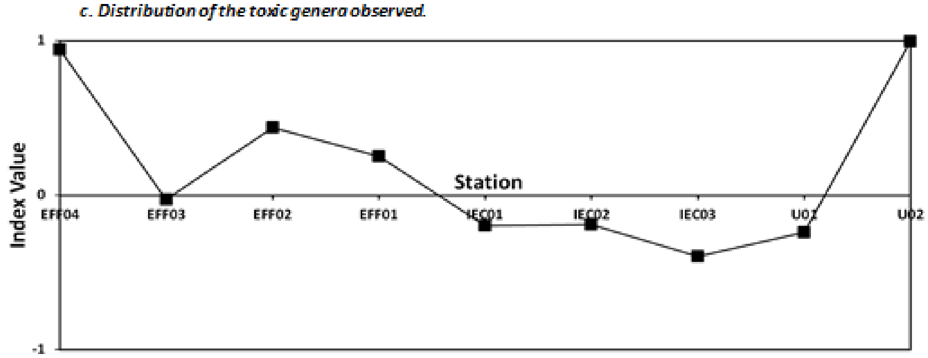
a. Variation of cyst diversity, equitability, dominance, and abundance (cysts cc-1).



b. Distribution of Operculodinium spp. and Spiniferites spp.



c. Distribution of the toxic genera observed.



d. Homogeneity vs. Heterogeneity of sample sediment layers.

Figure 4, (a) Variation of diversity, equitability, and dominance among the stations. (b) Distribution of Operculodinium spp. and Spiniferites spp. (cysts cc^{-1}). (c) Distribution of toxic genera observed (cysts cc^{-1}). (d) Homogeneity of the layers of sediment: a value of zero indicates homogeneity (no difference between layers); a value of one or negative one indicates heterogeneity (one layer has more cysts than the other).

Two of the most commonly observed genera in this survey have been connected to specific environmental conditions. High concentrations of *Spiniferites* (Fig. 5C) are considered an indicator of oceanic influence, while high concentrations of *Operculodinium* (Fig. 5B) are typical of inlet-based conditions (Kumar and Patterson, 2002). For a listing of their concentrations according to station, see Appendix 1-a.The highest concentrations of *Spiniferites* were observed at IEC03, U01, U02, and EFF01; these stations are situated at the mouths of inlets or channels (except U02) and so receive the most input from the Pacific Ocean. Concentrations of this genus typically became lower as the stations became more restricted further into each inlet or channel. The lowest concentrations were found at EFF04 at the head of Effingham Inlet (Fig. 1; Fig. 4b). Concentrations of *Operculodinium* were observed to be lowest at inlet and channel mouths and become greater toward the heads of the inlets. The highest concentrations were found at EFF04, EFF01, and U02; the lowest was observed at IEC03, the station closest to the entrance of Barkley Sound (Fig. 1).

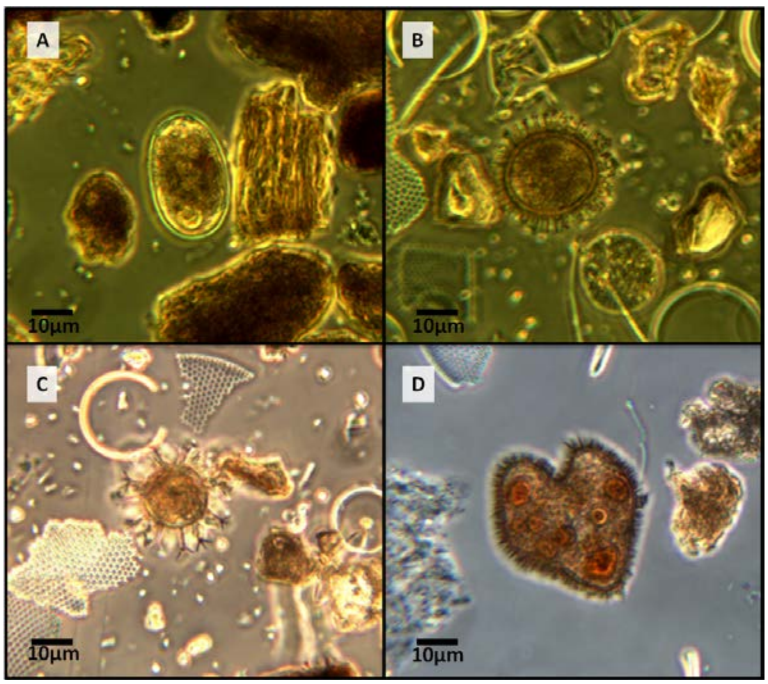


Figure 5. (A) Alexandrium cyst; (B) Operculodinium cyst; (C) Spiniferites cyst; (D) Votadinium cyst.

Effingham Inlet

The samples from station EFF01 (Fig. 1) were taken from a depth of 89m and had a total abundance of 506 cysts cc^{-1}, a total of 12 distinct genera present (richness), and a diversity value of 1.84. It was the highest in the region for these characteristics. Its equitability value was 0.74, which was the second lowest for the inlet. It was dominated by *Spiniferites* and *Operculodinium* making up ~30% of the total abundance respectively. All four of the genera capable of producing toxins were present at this site. The highest concentration of *Spiniferites* (151 cysts cc^{-1}) in the region was observed here. The two layers of sediment collected here were moderately heterogeneous (Table 1; Fig. 4a-d).

The location of EFF02 is further up the inlet's channel, just beyond the bend, near the entrance (Fig. 1). Samples were taken from a depth of 117m. It had the lowest regional abundance at 288 cysts cc^{-1}, 9 distinct genera present, and a diversity value of 1.70. It was only slightly more equitable than EFF01 and was dominated by *Alexandrium* and *Operculodinium*, each of which accounted for ~30% of the total abundance. Again, all four toxic genera were present and the highest concentration of *Alexandrium* (85 cysts cc^{-1}) in the region was observed at this station. The top 5cm of sediment exhibited medium heterogeneity (Table 1; Fig. 4a-d).

Located in the inner basin of Effingham Inlet is station EFF03 (Fig. 1). Samples collected from 202m exhibited a total abundance of 329 cysts cc^{-1}, a total of seven distinct genera present, and a diversity value of 1.47. Its equitability value was 0.76, a 0.01 difference from EFF02. This station was dominated by *Operculodinium, Alexandrium,* and *Scrippsiella* which accounted for 42%, 25%, and 17% of the total abundance. *Lingulodinium*, *Spiniferites* and *Operculodinium* were the only three of the potentially toxic genera that were present here. The sediments were nearly homogeneous (Table 1; Fig. 4a-d).

EFF04 is located in the inner basin of Effingham Inlet (Fig.1). The samples were taken from a depth of 70m. The total abundance for this station was 334 cysts cc^{-1}, it had a richness of 11, and its diversity value was 1.57. The value computed for equitability was 0.66; the lowest in the inlet. The station was dominated by *Operculodinium* which constituted 50% of the total abundance. All four of the toxic genera were observed at this site. The sediment layers were heterogeneous (Table 1; Fig. 4a-d).

Imperial Eagle Channel

Station IEC01 is located just outside of the mouth of Effingham Inlet on the channel side of the sill (Fig. 1). The samples here were taken from a depth of 86m and had a total abundance of 290 cysts cc^{-1}, a richness value of 12, and a diversity index of 1.71. The equitability value associated with this station is 0.69 and it is dominated by *Operculodinium* and *Alexandrium* which constituted 42% and 26% of the total abundance respectively. All four toxic genera were observed in the samples from this site. The sediments were moderately heterogeneous (Table 1; Fig. 4a-d).

The second station in Imperial Eagle Channel, IEC02, is located just east of IEC01 (Fig. 1). Samples were collected from a depth of 94m. It had a total abundance of 229 cysts cc^{-1}, the lowest in the region. It had a diversity value of 2.00 and an equitability value of 0.8, making it the most diverse and even station in Imperial Eagle Channel. It was also the most dominated, with a dominance value of 0.8. *Operculodinium* constituted the highest proportion of the total abundance at 38.4%. In terms of richness, 12 genera were observed. This station contained all four toxic genera and its sediments were the least heterogeneous of the stations in this region (Table 1; Fig. 4a-d).

The final station in Imperial Eagle Channel, IEC03, was located in the main channel near the entrance to Barkley Sound and samples were gathered from a depth of 103m (Fig. 1; Table 1). It had the highest regional abundance at 510 cysts cc^{-1}. Its values associated with diversity, equitability, dominance, and richness were nearly identical to that of IEC02 (Table 1). The most dominant genus was *Spiniferites*, constituting 39% of the total abundance. *Scrippsiella* and *Operculodinium* were also dominant genera, but made up only 28.7% and 12.8% of the total abundance, respectively. All four toxic genera were observed. The sediments collected from this station were the least homogeneous of the stations in Imperial Eagle Channel (Table 1; Fig. 4a-d).

Uchucklesit Inlet

Station U01 is located in the mouth of Uchucklesit Inlet, just inside the entrance to Alberni Inlet (Fig. 1). Samples from this station were collected from a depth of 76.9m (Table 1). It had a total abundance of 332 cysts cc^{-1}, a diversity value of 1.61, and a 10 genera present. Its equitability value was 0.70 and it was dominated by *Spiniferites* and *Operculodinium*, which constituted 43.8% and 25.4% of the station's abundance. All four toxic genera were present at this station. The sediments collected were moderately heterogeneous (Table 1; Fig. 4a-d).

The other station from this Inlet, U02, was located near the head and samples were collected from 58m depth (Fig. 1). Its abundance was nearly double that of U01 at 609 cysts cc^{-1}, its diversity value was 1.60, and 9 genera were identified. The equitability value for this station was 0.73 and it was dominated by *Spiniferites* and *Operculodinium*, which made up 31.4% and

34.5% of the total abundance for this station (Appendix 1-b). All four of the toxic genera were identified in the samples. The sediment layers collected here were completely heterogeneous (Fig. 4a-d).

DISCUSSION

Effingham Inlet

The distributions of the dinoflagellate cysts in Effingham Inlet are most likely a product of current input, bathymetry, stratification of the water column, and bioturbation. Estuarine circulation patterns typical of well stratified fjords cause the fresh water layers near the surface to flow into the inlet and the cold, salty, dense water of the bottom layers to flow out of the inlet (Linder, 2010). As water enters the inlet, it must flow over the sill and introduces new dinoflagellate individuals to the environment. Once formed, dinoflagellate cysts begin to sink to the inlet floor and may be transported by the outflowing lower layers toward the mouth of the inlet. Due to the high stratification of this region, they are unable to clear the sill and become buried in the sediment just inside the inlet where station EFF01 is situated (Fig. 1).The high abundance and richness at this station are artifacts of this concentrating effect. *Operculodinium* was present in slightly higher concentrations than *Spiniferites*, suggesting a strong inlet based influence at this station (Fig. 4b). The moderately heterogeneous sediments collected suggest that some level of bioturbation may have occurred, mixing the sediments slightly along with any cysts present (Fig. 4d).

As water flows from the wider area near the entrance of Effingham Inlet toward the head, it must pass through a narrow passage that opens into the deep outer basin. Due to conservation of momentum, the currents in the passage are moving at a faster rate than that of the wider basins. Cysts being carried in these currents are less likely to sink to the bottom; similar to how silt sized sediment particles are unable to settle out in high velocity currents (Dale, 1976). Additionally, the increased depth of the passage could allow more cysts to be destroyed during the settling process. These environmental factors contribute to the low abundance of this station (Table 1). The level of heterogeneity of the shallow sediments (Fig. 4d) also reflect the current influence in that the relatively fast moving water keeps the bottom well oxygenated, making bioturbation a possibility. Additionally, the current may even interact with the sediment as the current moves through the channel.

Station EFF03 is located in the outer basin, the largest and deepest in Effingham Inlet (Fig. 1; Fig. 2). The inflowing water quickly spreads throughout the upper layers of the basin, also dispersing whatever dinoflagellates and/or cysts may have been entrained in the current. The stillness of the water in this basin allow for large plankton blooms to occur here. Dinoflagellate cysts formed in the upper layers must sink a long distance to reach the sediments of this basin, creating ample opportunity for them to be destroyed or consumed prior to deposition. These factors produce the low abundance and richness found here (Table 1). A history of anoxic conditions and the stillness of the lower water column preserve the laminations of the sediment, by retarding bioturbation and current influence of the sediments (Fig. 4d).

The inner basin of Effingham Inlet is much shallower and smaller in area than the outer basin (Fig. 1). Strong stratification and the restriction of flow caused by the height of the sill create a concentrating effect similar to that of the sill at the entrance to the inlet (Fig.3). The quiescent waters here are conducive to a patchy nutrient environment which leads to uneven distribution of plankton blooms and subsequently, cyst seed beds. The dominance of the genus *Operculodinium* over *Spiniferites* reflects the restrictive inlet conditions expected at this station (Fig. 4b). A history of near permanent anoxic conditions at the bottom of this basin restricts bioturbation and the lack of current influence leave the sediment layers completely heterogeneous (Fig. 4d).

Imperial Eagle Channel

The distributions of dinoflagellate cysts in Imperial Eagle Channel are mainly attributed to the influence of water circulation patterns that are a product of the local bathymetry. Station IEC03, is located nearest to the entrance of Barkley Sound (Fig. 1) and receives the most input from the Pacific Ocean which is evident in the observed characteristics. The large abundance of cysts at this station is most likely because there simply were a higher number of dinoflagellates that bloomed in this area due to the size of the channel and increased nutrient concentrations caused by upwelling events. Additionally, the distribution of cysts at this station likely reflects the patchy nature of large scale nutrient regimes, driving the equitability and diversity down (McManus et al., 2003). Concentrations of *Operculodinium* were much lower than that of *Spiniferites*, indicating a strong oceanic influence in this area (Fig. 4b).

Further into the channel, near the head of Imperial Eagle Channel, between the entrances to Effingham Inlet and Alberni Inlet, lies station IEC02 (Fig.1). The transition from a wide channel to a narrow inlet creates faster moving currents in this area that disperse the blooms that occur here more evenly; this is reflected in the high equitability of the seed beds. These combined influences create the most diverse cyst assemblage of any station surveyed during this study (Table 1). However, the overt dominance of *Operculodinium* is indicative of a change from oceanic to inlet based influence in this area (Fig. 4b).

The final station in Imperial Eagle Channel, IEC01, is located just outside the entrance of Effingham Inlet (Fig. 1). The upper layers of water flowing into the inlet carry dinoflagellate blooms into the inlet, but some individuals low in the column are unable to clear the sill. Once they encyst, they are deposited in the sediments on the channel side of the sill. The basin is shallower here, allowing more cysts to settle to the bottom than in deeper regions where they can be scavenged or destroyed before deposition. Additionally, this station had even higher concentrations of *Operculodinium* relative to *Spiniferites* than the previous station, which is related to its proximity to an inlet based ecosystem (Fig. 4b). Although characteristics such as abundance and diversity vary by station in this region, richness is the same for all three, most likely due to the higher level of mixing that occurs in the channel versus that of enclosed inlets (Fig. 4d).

Uchucklesit Inlet

The distribution of the cysts assemblages in Uchucklesit Inlet are different than what was expected based on the findings of this survey and that of previous studies of Effingham Inlet. The abundance was greatest in the head of the inlet; nearly double that of the mouth. In terms of diversity, equitability, and dominance, the two stations did not differ greatly (Fig.4a; Table 1). A likely cause of these characteristics is that more cysts were deposited in the head of the inlet because a greater number were able to settle out without being consumed or destroyed because of the shorter distance to the bottom. Additionally, it is likely that the sample collected at U02 was taken from an area that had a dense bloom caused by nutrient patchiness, causing a higher concentration of cysts to be deposited there.

While there is no historical data of anoxic conditions near the bottom anywhere in the inlet, the sediments in the head were completely heterogeneous, suggesting minimal bioturbation or mixing due to current influence (Fig. 4d). The concentrations of *Spiniferites* and *Operculodinium* in the head of the inlet were much higher than that of the station near the entrance, but the proportions of each were similar at both stations. This suggests that there is a balance between oceanic and local influences that affect the distributions of dinoflagellate cysts in Uchucklesit Inlet.

Toxic Dinoflagellate Cysts

The concentrations of the four genera associated with toxin production observed in the sediments of the stations surveyed for this study varied greatly within Barkley Sound (Fig. 4c). Station U02, in Uchucklesit Inlet had the highest concentration of *Alexandrium* and *Lingulodinium*, meaning that this area is at the highest risk for toxic blooms that could cause PSP. However, *Alexandrium* cysts were found in relatively high concentrations at nearly every station examined, meaning that a bloom could occur in any basin of the sound (Fig. 4c). Cysts of *Lingulodinium* were present in lower concentrations relative to that of other toxic genera, but its presence could add to the toxicity of the other, more prevalent genera.

Operculodinium was present at every station, usually in concentrations over 100 cysts cc^{-1} (Appendix 1-a). It was the most common genera overall and therefore poses the greatest risk for seeding toxic blooms, especially in restricted areas where its concentrations were highest (Fig. 4c). *Spiniferites* was found in the highest concentrations in areas that receive oceanic influence, such as inlet and channel entrances. Although they are not as abundant as *Operculodinium*, they are still present at every station, three of which have concentrations over 100 cysts cc^{-1}. The areas with high concentrations of *Operculodinium* and Spiniferites are at the greatest risk for blooms that could cause DSP. The presence of these toxic dinoflagellate cysts in the shallow sediments of Barkley Sound indicate that there have been potentially toxic blooms in the past and will most likely occur again in the near future.

CONCLUSIONS

1) The three regions surveyed for this study differed greatly in terms of abundance, diversity, and richness. The cause of the differences is likely the unique bathymetry and circulation patterns associated with each region.

2) *Operculodinium, Spiniferites, Alexandrium, Scrippsiella,* and *Islandium* were present at all stations and were found in the highest overall concentrations, in that order.

3) Diversity was generally greater in less restricted areas like the entrances to inlets or channels than more restricted areas like basins behind sills or separated from the main channels by distance.

4*)* The abundances of cysts in Effingham Inlet and Imperial Eagle Channel were greater in unrestricted areas and lower in restricted areas. The abundance within Uchucklesit Inlet was greatest in the head and least near the entrance; opposite of the other two regions.

5) Concentrations of *Operculodinium* were greatest in restricted, shallow basins that received the least influence from oceanic conditions. The highest concentrations of *Spiniferites* were found near the entrances of inlets and channels or areas that are more influenced by oceanic conditions.

6) The laminations in the sediments of restricted, shallow basins that have experienced suboxic or anoxic events were well preserved relative to the sediments of well oxygenated basins. The most likely causes for heterogeneity in the layers of a station are bioturbation and/or current influence.

7) Toxic genera have bloomed in the recent past in Barkley Sound and will most likely occur again in the near future when conditions are optimal.

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ng the concentrations of each genus according to station.

EFF04	EFF03	EFF02	EFFO1	IEC03	IEC02	IEC01	U0
50.63	81.38	85.22	23.89	7.70	43.58	76.20	108.
0.00	0.00	0.00	0.00	0.00	0.00	4.50	0.0
0.00	0.00	0.00	0.00	0.00	3.92	0.00	0.0
4.50	3.58	0.00	3.75	0.00	0.00	4.13	0.0
18.02	4.50	16.31	22.52	25.38	17.43	9.01	15.
13.51	0.00	9.01	22.52	13.51	8.53	4.02	25.
167.15	138.42	91.93	166.85	65.16	88.22	122.94	210.
0.00	0.00	0.00	4.50	0.00	0.00	0.00	0.0
4.17	0.00	9.19	37.58	4.50	17.05	13.14	28.
0.00	0.00	3.19	3.75	8.63	0.00	0.00	8.0
2.82	0.00	0.00	0.00	13.51	9.01	0.00	0.0
4.50	0.00	4.50	3.75	15.16	8.53	4.02	0.0
56.99	57.33	50.82	43.54	146.75	8.53	30.31	18.
4.50	13.43	0.00	22.52	7.75	8.53	4.50	0.0
7.32	30.51	18.20	151.71	199.13	8.53	12.77	191.
0.00	0.00	0.00	0.00	3.52	7.94	4.50	4.0
334.12	329.15	288.38	506.90	510.71	229.78	290.06	609.

e percentage (in decimal form) of the total abundance observed for each genus, over all

	EFF04	EFF03	EFF02	EFFO1	IEC03	IEC02	IE
	0.152	0.25	0.30	0.05	0.02	0.19	0.
	0.000	0.00	0.00	0.00	0.00	0.00	0.
	0.000	0.00	0.00	0.00	0.00	0.02	0.
	0.013	0.01	0.00	0.01	0.00	0.00	0.
	0.054	0.01	0.06	0.04	0.05	0.08	0.
	0.040	0.000	0.03	0.04	0.03	0.04	0.
	0.500	0.421	0.319	0.329	0.128	0.384	0.4
	0.000	0.000	0.000	0.009	0.000	0.000	0.0
	0.012	0.000	0.032	0.074	0.009	0.074	0.0
	0.000	0.000	0.011	0.007	0.017	0.000	0.0
	0.008	0.000	0.000	0.000	0.026	0.039	0.0
	0.013	0.000	0.016	0.007	0.030	0.037	0.0
	0.171	0.174	0.176	0.086	0.287	0.037	0.1
	0.013	0.041	0.000	0.044	0.015	0.037	0.0
	0.022	0.093	0.063	0.299	0.390	0.037	0.0
	0.000	0.000	0.000	0.000	0.007	0.035	0.0