

Distribution of the dinoflagellate *Alexandrium catenella* cysts in sediment cores from  
Discovery Bay, Washington: Possible relationship to climatic variation

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## Non-Technical Summary

Outbreaks of Paralytic Shellfish Poisoning (PSP) resulting in beach closures in the Pacific Northwest were first documented, in Sequim Bay and Discovery Bay, Washington in the late 1950's (Trainer et. al 2003). *Alexandrium catenella* is a dinoflagellate that produces toxin associated with PSP in Pacific Northwest waters. A resting form this dinoflagellate is a cyst that sinks to the bottom and is buried in the sediments. A large bloom of *A. catenella* occurred in Puget Sound in 1978 (Trainer et. al, 2003); this bloom coincided with an unusually dry and warm shift in climate known as a Pacific Decadal Oscillation (PDO).

The purpose of this research was to determine whether resting cysts found in sediments collected in Penn Cove and Discovery Bay appear during the positive stages of a Pacific Decadal Oscillation. The primary task will be to measure the quantities of *A. catellena* cysts in a sediment core and determine if cysts appear during climatic shift events identified in the 1940's, 1950's and 1970's (Wooster and Fluharty 1985, Mantua et al. 1997 and Trainer et al. 2003). A link between climate variation and cyst occurrence can be an applicable method for predicting future PSP events.

Sediment cores were collected using a spade box core and a kasten core on 20 and 22 March 2007 in Penn Cove and Discovery Bay on board the *R/V Thomas G. Thompson*. Time constraints allowed the analysis of only the Discovery Bay core; the Penn Cove sediments were archived for future study. X-radiograph images of the spade box core and kasten core samples show a homogeneous composition of fine grain material. Using  $^{210}\text{Pb}$  geochronology techniques a sedimentation rate of  $0.31 \text{ cm yr}^{-1}$  was determined. *A. catenlla* cysts were detected below 3 cm in the sediment and showed nearly consistent concentrations between 5 cm and 20 cm ( $125 \text{ cysts ml}^{-1}$  to  $425 \text{ cysts ml}^{-1}$ ). Samples observed at 10 cm increments after 30 cm did not show *A. catenella* cysts. A time scale created using the sediment accumulation rates places the cysts disappearance in the Discovery Bay core between 1975 and 1943, a time interval encompassing the 1978 *A. catenella* bloom in Puget Sound, and PDO climate shift events in the late 1950's and 1970's.

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## Abstract

*Alexandrium catenella* has been identified as a toxin producing dinoflagellate associated with Paralytic Shellfish Poisoning (PSP) in the Pacific Northwest. Documented beach closures related to PSP in this area began in the 1950's including Discovery Bay, Washington. A large bloom of *A. catenella* occurred in 1978 during a cyclic changes in local climate patterns known as the Pacific Decadal Oscillations (PDO). The hypothesis of this study tested the extent to which the resting cyst stage of *A. catenella* was found at increasing depth in the sediments, and its possible association with local climatic changes. In March 2007, sediment cores were collected at Penn Cove and Discovery Bay. Analysis of only the Discovery Bay core included X-radiographs,  $^{210}\text{Pb}$  geochronology, and *A. catenella* cyst abundance counts. The sediment accumulation rate of the fine grain Discovery Bay sediment was  $0.31\text{ cm y}^{-1}$ . Porosity determinations down the core showed little change in the sediment grain structure with increasing depth. Cysts of two dinoflagellate species, *A. catenella* and *Scrippsiella trochoidea*, were identified in the upper 30 cm in the core. A time scale determined from  $^{210}\text{Pb}$  geochronology and sediment accumulation determinations placed *A. catenella* cysts first appearance prior to 1975, closely matching a result obtained from a study in Sequim Bay in 2005. The presence of two species of cysts in the late 1970's and possibly the late 1950's during two climatic events suggest a correlation with cyst presence and climatic shift, although no cysts were found in samples deeper in the core.

## Introduction

Paralytic Shellfish Poisoning (PSP) is an acute illness striking people who eat shellfish that consume toxin producing dinoflagellates. The dinoflagellate *Alexandrium catenella* has been identified as the primary causative organism of PSP on the West Coast (Trainer et al. 2003). *A. catenella* is mainly associated with the colder temperate waters in coastal regions of the Pacific Ocean (Fukuyo 1985). It is found along the West Coast of the United States including Alaska, Chile, Australia, Tasmania and Japan in the Pacific and along western South Africa in the South Atlantic (Fukuyo 1985). Changes in environmental conditions cause *A. catenella* to form resting cysts that deposit on the bottom sediments. If bottom sediments accumulate quickly, the resting cyst will become trapped in the sediment thereby indicating level of cyst abundance over time. This study examined the occurrence of *A. catenella* resting cysts in the bottom sediments that were deposited in years prior to formal documentation of PSP outbreaks. The objectives of this study were 1) to determine if *A. catenella* cysts could be found with increased depth of the bottom sediments; 2) to determine if *A. catenella* cysts are present in sediments dated prior to documented PSP outbreaks; 3) to relate *A. catenella* cysts occurrence with local climatic changes; 4) to see if *A. catenella* cyst can be found during pre-industrial times. If a correlation exists between cyst abundance, climatic conditions, or the chronology of PSP outbreaks, it would provide useful information in understanding future PSP outbreaks. To pursue the objectives near surface and sub-surface sediments were collected using both a spade box core and kasten core. Sediments were collected at one location each in Discovery Bay and Penn Cove, Washington (Fig.1), using the shipboard capabilities *R/V Thomas G. Thompson* during the week of 19-23 March 2007.

A PSP monitoring program for shellfish was re-established in Washington State in 1957 to include all species of commercial shellfish in northern Puget Sound and the outer coast (Trainer et al. 2003). That same year shellfish beach closures caused by PSP began in Sequim Bay and Discovery Bay. In the 1970's shellfish beach closures had to be enforced in northern Puget Sound. A large scale bloom of *A. catenella* occurred in the central Whidbey basin near Holmes Harbor (Fig. 1) in 1978 and may have been motivated by global climatic events (Trainer et al. 2003). In 1977 a large shift to a positive Pacific Decadal Oscillation was observed (Trainer et al. 2003). Decadal intervals in climate and local environmental parameters were observed to vary approximately every 20 years (Ebbesmeyer et al. 1995).

The waxing and waning of the oscillations are known as positive and negative phases. The positive phases are warm and characterized by decreased precipitation. Major changes in the marine ecological community in the Puget Sound region have been correlated with PDO phase changes (Hare and Mantura 2000). Comparisons of temperature, pressure, tree ring and salmon catch data showed climatic shifts in the North Pacific that occurred in the 1920's and 1940's (Mantua et al. 1997). The climate changes during the positive phase would lead to decreased river runoff. This is marked by an enhancement of overall phytoplankton productivity related to changes in the upper ocean mixed layer depths and temperature in Puget Sound (Trainer et al. 2003). In addition to local decadal oscillations, climatic influences from strong El Nino events occurred in the 1957, 1972 and 1982 (Wooster and Fluharty 1985). El Nino events have durations of 6 to 18 months (Moore et al. unpubl.). The last positive phase of the PDO event occurred from 1977 to 1999 (Frances and Hare 1994). A smaller positive phase lasting only a few years occurred between 1957 and 1960.

The current understanding of PSP outbreaks comes from shellfish toxin measurements and not from the study of the ecology of the dinoflagellate *A. catenella* (Horner et al. 1997). For reasons not presently understood, when changes in ambient environmental conditions become unfavorable, *A. catenella* will form a resting cyst that sinks to the bottom. The *A. catenella* cysts are ellipsoidal in shape with rounded edges. Their lengths range from 38 $\mu$ m to 56  $\mu$ m and widths range from 23 $\mu$ m to 32  $\mu$ m (Fukuyo 1985).

*A. catenella* cysts have been found in surface layer sediments in Puget Sound (Greengrove, C, unpubl.), but little is known about the historical occurrence of *A. catenella* cysts in the sediment. Sediment accumulation rates in Puget Sound vary from 0.04 to 2.4 cm  $y^{-1}$  (Carpenter et al. 1985). If accumulation rates in areas of Puget Sound are high enough they will bury resting cysts and form a record of cyst activity. Dinoflagellate cysts buried in the sediments have been used to show changes in assemblage composition on the scale of tens to hundreds of years (Dale et al. 2001).

This study focuses on the occurrence of the dinoflagellate *A. catenella* cysts in the bottom sediments in Discovery Bay and Penn Cove, Washington. Sediments collected in Penn Cove will be retained in archive for future study. The goal was to determine if cysts are present in the sediments in years prior to the formal documentation (1957) of *A. catenella* blooms (Cox A. unpubl.). A secondary goal was to provide insight into the history of resting

cysts buried in the sediment, and discuss environmental variables that may influence *A. catenella* cyst abundance.  $^{210}\text{Pb}$  geochronology was used to provide age estimates for cyst bearing horizons in the sediment cores and to determine if the cysts were detectable between industrial and pre-anthropogenic periods in Puget Sound. Industrialization began in the Puget Sound region in the mid 1800's (Chasen 1981). In 1858, the Maynard Mill was established on the southwestern shoreline and is presently abandoned (JCHC, 1966). The former mill operation may have had impacts on the water quality of the bay. By using sediment accumulation rates *A. catenella* cysts buried in the sediments can be compared chronologically with periods on climatic change from Pacific Decadal Oscillations or El Nino effects.

## Methods

In order to determine the *A. catenella* cyst occurrence in surface and subsurface sediments, sediment cores were taken in Discovery Bay and Penn Cove, Washington (Fig. 1), using both a spade box core and a kasten core. Coring occurred onboard the *R/V Thomas G. Thompson* during the week of 19-23 March 2007. Sampling locations were selected based on the fine grain silty sand sediment characteristics (Roberts R. 1974) and the document history of PSP bloom activity in these areas (Trainer et al. 2003). A one-meter long sediment core, depending on the extent of surface layer bioturbation, would potentially date back approximately 100 years. A Sequim Bay sedimentation rate of  $0.6 \text{ cm y}^{-1}$  was assumed for planning the field sampling program in Discovery Bay (Cox A. unpubl.). Due to time constraints only the Discovery Bay cores were analyzed. The cores collected at both sites were processed (sub-sampled) on-board the vessel. A 3.5 kHz sub-bottom profiler on board the *R/V Thomas G. Thompson* was used to provide an overview of the seabed features and to confirm general sediment characteristics at the selected sites (Fig. 2).

## Sediment Cores

Sediment cores were collected from Discovery Bay and Penn Cove using a spade box core and a 3-meter long kasten core (Fig. 3, Table 1). The location information for each sampling station appears in Table 1. The spade box core was used first at each location to obtain a undisturbed surface layer and representative sample to a depth of about 60 cm. Once onboard the spade core was sub-sampled using a 15.24 cm diameter PVC core liner tube inserted vertically into the box (Fig. 3). The kasten core was then deployed to collect a

sample of the bottom sediments to a depth of 3 m. The Penn Cove cores were taken on 20 March 2007 at a water depth of 29.6 m was retained for archive. Sediments from the spade core sub-sample and kasten core from each location were processed immediately after sampling. The sub-sampled spade Core PVC liner was placed on a threaded sample extruding device that allowed the sample to be incremented at 1-cm or 2-cm intervals. The upper 15-cm of the spade core sample was sectioned at 1-cm intervals. 2-cm intervals and were collected for the remainder of the spade core sample. Samples were placed in Whirl-Paks, labeled and stored at 4° C. The kasten core sediments were sampled at 2-cm intervals for the first 100 cm. The remainder of the core was sampled in 2-cm sections 5-cm apart. The individual sampling sections were partitioned using stainless steel plates and the sediments were collected using stainless steel spatulas. The sub-sampling of the spade core and the partitioning of the kasten core sediments is shown in Fig 3.

X-ray radiographs were taken of the sediments collected by the spade core and the kasten core at the Discovery Bay sampling location. The X-ray radiograph images of the sediments were used to identify features in the depositional material such as inter-bedding, methane pockets or anthropogenic debris. In addition, an X-radiograph image for archive was taken of the spade core sample collected at the Penn Cove location. To obtain an X-ray radiograph of the sediment core a 60 cm x 12 cm x 2.5 cm plexi-glass photograph sample tray was inserted into the retrieved spade core sample. X-ray radiograph images of the kasten core were collected by first removing the sample barrel cover plates and then carefully placing 30 cm x 12 cm x 2.5 cm trays end to end along the entire length of the core sample. To retain the sample in the respective X-ray radiograph trays, plastic slider sections were inserted on the lower portion of each tray along guide slots. Labeled trays were stored in the field at 4 °C before being processed. The X-ray radiographs of each core section were obtained using a Medison Medical Corp, Model 1020 Diagnostic X-Ray Unit. X-rays were transmitted through the sediment sample and received on a FlashScan 35 Flat Panel Imaging System. FlashScan software was used for the capture and display of the images retrieved from the X-ray sensors located on the flat panel. The processed X-ray image was exported to Adobe Photoshop software for image enhancement and individual X-ray segments were linked to form a continuous representation of each core.

## Geochronology

$^{210}\text{Pb}$  is a decay product of  $^{238}\text{U}$ , having a half life of 22.3 y. Decay of  $^{226}\text{Ra}$  in marine sediments contributes to background or support levels of  $^{210}\text{Pb}$ . Excess  $^{210}\text{Pb}$  activity in marine sediments originates largely from  $^{210}\text{Pb}$  fallout produced by  $^{222}\text{Rn}$  in the atmosphere which enters the ocean and is preferentially absorbed to particles that sink to the bottom. The excess  $^{210}\text{Pb}$  profiles can be used to calculate rates of bioturbation and sediment accumulation ( Nittrouer et al. 1979). Activities of  $^{210}\text{Pb}$  were determined by measuring the alpha decay of its granddaughter,  $^{210}\text{Po}$ , assuming secular equilibrium between  $^{210}\text{Pb}$  and  $^{210}\text{Po}$ .

Each 1-cm or 2-cm interval from the sediment core selected for analysis was weighed wet, dried at 60° C for 24 hours and reweighed to determine water loss. The sample was then finely ground using a mortar and pestal. For each sample interval 5 grams of sediment was spiked with  $^{209}\text{Po}$  as a yield indicator. The sample was next digested in 10 ml of 15.8N  $\text{HNO}_3$  to separate the  $^{209}\text{Po}$  from other alpha-emitting radionuclides. The sample was dried under heat, then 10 ml of 6N HCl was added until dryness was again achieved. The sample was then treated with 5 ml of 6 N HCl and the sediment slurry was rinsed into a clean centrifuge tube and filled to a volume of 35 ml by adding 0.3 N HCl. The sample was centrifuged for a period of 10 minutes and the resultant supernatant was poured into pre-cleaned glass plating jars. The sediment in the base of the centrifuge tubes was re-suspended in 0.3 N HCl to a volume of 35 ml and process is repeated twice. Pre-cleaned silver discs were suspended in the plating jars containing the supernatant and the  $^{209}\text{Pb}$  and  $^{210}\text{Pb}$  in solution was plated via spontaneous electro-deposition onto the silver discs. The plating process occurs for no longer than 24 hours in order for a uniform distribution of polonium suitable for alpha spectrometry. The alpha decay of  $^{209}\text{Pb}$  and  $^{210}\text{Pb}$  isotopes on the silver discs was measured using a silicon surface barrier detector (Rotec Alpha Spectroscopy Workstation, model Octet Plus and EG&G Ortec Alpha Spectroscopy Workstation, model Octete PC both with ULTRA series detector model BU-017-450-100). Activities of  $^{210}\text{Pb}$  were calculated by ratio of the peak area of  $^{210}\text{Pb}$  to  $^{209}\text{Pb}$ .

## Cyst Counting

*Alexandrium catenella* cysts can be distinguished microscopically by their oval shape and the presence of double cell walls using a 16X objective under normal light. To prepare a

sample for viewing under the microscope the sediment samples were sonicated to remove collected mucus and sediment particles and sieved and were then stained with primuline (Yamaguchi et al. 1995). The primuline stained sediment samples were counted using epifluorescence illumination. Initially, the sediment sample representing a selected 1-cm or 2-cm interval was homogenized and 5 ml of sediment was diluted to 25 ml using distilled water. A 10 ml sample volume was extracted and sonicated using a Sonics and Materials Vibra Cell sonicator with a model CV26 Micro-Tip. The sonicated sample was sieved through both a 90  $\mu\text{m}$  and 20  $\mu\text{m}$  Nitex screen, treated with 10% paraformaldehyde and methanol prior to staining with primuline. After the staining procedure the sample was diluted to 10 ml. The sample was agitated to re-suspend the sediment and a 1 ml sample was transferred onto a Sedgewick-Rafter counting chamber. In most cases, the sample was diluted in a ratio of 1:10 or 1:15 using distilled water due to the amount of fine grain sediment in the sample.

The sample in the Sedgewick-Rafter counting chamber was placed on a Ziess standard microscope that was equipped with an epifluorescence condenser, an exciter filter, chromatic beam splitter and a barrier filter. The sample was observed through a 16X magnification objective. For each sediment core sample interval selected for counting, the Sedgewick-Rafter counting chamber was used twice to determine counting variation. The epifluorescence illuminated microscope and an example of a cell wall stained *A. catenella* is shown in Fig. 4. Counts for cysts using the epifluorescent microscope were performed at 1 cm intervals for the first 10 cm of the Spade core sample and at 2 cm intervals from 10 cm through 100 cm spaced at 10 cm intervals of the kasten core sample. The spade core sampling technique has a lower potential of sample disturbance in the upper layer compared to the faster lowering rate of the kasten core. Cyst counts obtained from depths greater than 10 cm were taken from the kasten Core, where the sample intervals are 2 cm..

## Results

### Sediment Cores

Sediment collection intervals for the Discovery Bay  $^{210}\text{Pb}$  radio-isotope and *A. catenella* procedures are shown in Table 2. The spade box core samples were used for analysis of the upper sediments because the potential for sample disturbance is low compared to the kasten core sampling technique.

The X-ray radiographs of the Discovery Bay spade box core sediment sample is presented in Fig. 5 and a mosaic of the individual kasten core X-ray radiograph images is shown in Fig. 6. The X-ray radiograph images show a fairly homogeneous fine grain sediment with intermittent shell debris. The upper 20 cm of the spade box core image appears to be mixed or loosely compacted. The lower layers of the core are compacted with cracking or void spaces indicating methane in the lower sediments. A layer of undisturbed fine-grained sediment can be seen in a 4 cm wide band starting 150 cm from the sediment surface.

#### <sup>210</sup>Pb Geochronology

The activities of <sup>210</sup>Pb and sediment porosity for the selected depth increments are shown in Table 3. A profile of <sup>210</sup>Pb activity and associated sediment porosity is shown in Fig 7. The logarithmic representation of the alpha decay (Fig. 7) shows a mixed layer in the upper 20 cm of the core. This is denoted by the activity values greater than 10 dpm g<sup>-1</sup> that may be attributed to biological reworking of the sediments. The activity values ranged 10.97 dpm g<sup>-1</sup> to 0.73 dpm g<sup>-1</sup>. A background (supported) activity was found below 50 cm and an average value of 0.74 dpm y<sup>-1</sup> and was applied to the sediment accumulation rate calculation. An average background activity for Puget Sound (Lavelle et al.1986) is 0.5 dpm y<sup>-1</sup>.

The porosity in the Discovery Bay core sediment decreased from 91% at the surface to 79% at 131 cm. The porosity calculation first involved the determination of the water fraction ( X = wet sediment weight- dry sediment weight). The porosity equation

$$\phi \text{ (porosity)} = (X/p_w)/((X/p_w)+(1-X/p_s)), \quad (1)$$

Where  $p_w$  = density of water ~1.01 g cm<sup>-3</sup> and  $p_s$  = density of the sediment ~ 2.65 g cm<sup>-3</sup>.

The sediment <sup>210</sup>Pb accumulation rate (cm y<sup>-1</sup>) used the following equation:

$$\text{Accumulation Rate (cm y}^{-1}\text{)} = (\lambda z)/(\ln(C_o/C_z)). \quad (2)$$

$C_o$  is an activity found high in the profile,  $C_z$  is the activity found lower in the profile, and  $z$  is the vertical depth between  $C_o$  and  $C_z$ . The half life for <sup>210</sup>Pb is ~22.3 y, therefore  $\lambda = 0.693/(22.3 \text{ y})$  that equals ~0.031 y<sup>-1</sup>. The total <sup>210</sup>Pb activity from the water column and the background <sup>210</sup>Pb activity are from decayed <sup>226</sup>Rn in the seabed. The background activity of 0.74 dpm y<sup>-1</sup> was subtracted from both the  $C_o$  and  $C_z$  and then using the accumulation rate formula a rate of 0.31 cm y<sup>-1</sup> was calculated.

## Cyst Counts

Two species of dinoflagellate cysts were commonly observed in the Discovery Bay core sediments, *Alexandrium catenella* and *Scrippsiella trochoidea*. Both species were observed at the similar depth intervals, though *Scrippsiella* had a substantial presence in the upper 2-4 cm. Table 4 shows the dinoflagellate cysts counts in the number of cysts per milliliter for *A. catenella* and *Scrippsiella trochoidea*. *A. catenella* cysts did not appear until the 4 cm sediment interval. Between 5 cm and 21 cm *A. catenella* ranged from 175 cysts ml<sup>-1</sup> to 425 cysts ml<sup>-1</sup>. *S. trochoidea* reached peak values of 3060 cysts ml<sup>-1</sup> between 2 cm - 3 cm and decreased steadily with depth. No cysts were detected below 30 cm. Neither cyst species was detected in the 0 cm -1 cm layer. A measured cyst profiles for Discovery Bay is represented in Fig. 8. Microscope images of *A. catellena* and *S. trochoidea* under epifluorescent illumination are presented in Fig. 9. Sediment intervals at 150 cm and 160 cm were selected to determine cyst occurrence in an undisturbed sediment layer suspected to represent offsite material related to local climate fluctuations. These samples were dated using the <sup>210</sup>Pb geochronology calculated sediment accumulation rate (0.31 cm y<sup>-1</sup>) to around 1550. Cyst counts at both sediment intervals showed no activity. A cyst count performed at the bottom of the core (240 cm- 242 cm) showed no cyst activity in a pre-anthropogenic environment that was dated to approximately 1297.

## Discussion

The sediments recovered from the spade box core and the kasten core were fine grain silt that was loosely compacted in the upper layers and more compact deeper in the core. Maps of the surface sediments in Discovery Bay (Roberts 1974) depict the area that was selected for sampling as a muddy sand region bordered by sandy muds. Discovery Bay is a small and shallow inlet that tends not to flush well and water parcels have long residence times (Strickland 1983). Throughout the length of the kasten core large void spaces and cracks in the sediment were observed (Fig. 6). The caused is presumed to be a redox reaction resulting in the oxidation of sedimentary organic matter known as methanogenesis (James 2005). The <sup>210</sup>Pb geochronology of the core show the upper 20 cm to be well mixed. The cyst profiles within this mixing zone are the result of bioturbation and particle mixing due to physical processes. Other biological factors affecting the cyst occurrence in this mixing dominated region are germination and mortality of the cysts (Keafer et al. 1992). Analysis of

the spade core show the majority of cyst occurrence in the sediment mixing zone. *A. catenella* show only slight variations in cyst counts at each depth interval where cysts were seen. This uniform distribution was likely the result of individual cysts being mixed back to the surface layer and also being buried deeper in the core. In areas where biological mixing dominates over sediment accumulation, pulse input model results indicate the cysts deposited on the surface layer will be mixed rapidly and within a year a large fraction of the population will be mixed to depths exceeding 10 cm or greater (Keafer et al. 1992). *A. catenella* was not observed in the upper 4 cm and the lack of cysts at this level may be due to germination of the cysts that were either transported to the surface or recently deposited from the water column. Low levels of oxygen found a few centimeters deeper in the sediment will inhibit the germination of a cyst. Cysts found in these levels will remain in the sediment until continued mixing can bring it to the oxygenated surface layer (Anderson et al. 1987). In 2006, a large bloom of *A. catenella* was observed Puget Sound wide and may have included germination of cysts from the Discovery Bay sediments leading to the observed absence of cysts in the upper layer (Horner R. personal comm.).

*Scripsiella trochoidea* is a dinoflagellate common in the Pacific Northwest (Tomas, C 1996 ). It is a cosmopolitan neritic and estuarine species. The *S. trochoidea* cyst does not produce PSP related toxins. High occurrence of this cyst occurred between 2 cm and 4 cm and it was presumed these high cyst counts may be from recent deposition following a bloom (Table 4). The pattern of cyst occurrence with depth was similar to *A. catenella* with the exception of greater *S. trochoidea* counts and a its first appearance at 2 cm. The vertical profiles of the cysts that show peak abundances near the surface to relatively few at depth varies with the species of cyst (Keafer et al. 1992). These patterns are dependent on the timing of cyst deposition, and the eventual effects of germination, mortality, bioturbation, sediment burial and surface erosion. The mortality rate also varies with species during burial. Eventually, the deeply buried species will die, where they may or may not be preserved as a fossil record (Dale et al. 1976). Both species were not observed at the 40 cm depth or deeper indicating cyst mortality due to anoxic conditions or complete absence of either cyst species at the time when this layer was deposited. Observations at the sediment intervals below 30 cm did not reveal cysts in varying stages of decomposition or in fossilized states.

A sediment accumulation rate of  $0.31 \text{ cm y}^{-1}$  was determined for the Discovery Bay sediments using  $^{210}\text{Pb}$  geochronology. The base of the mixed layer also denotes the top of the region where  $^{210}\text{Pb}$  radioactive decay begins. This boundary was used to refer to the present time and the sediment intervals below this point represent the years in the past (Nittrouer et al. 1979). Assuming that biological mixing dominates over sediment accumulation the sediments at the based of the mixed layer will represent the present. A measure of the residence time of a particle in the mixed layer is calculated by dividing the sediment mixed layer by the sediment accumulation rate. A cyst deposited at the sediment surface can vary from immediate transport to the base of the mixing zone to a maximum residence time in this zone of 64 years. For this study sediment dates represent the youngest age probable, and the 20 cm depth represents the year 2006. The derived year for the last occurrence of *A. catenella* at 30 cm is 1975. The next sediment interval sampled (40 cm) where no cysts were found was derived to be 1943.

In Sequim Bay located immediately west of Discovery Bay, *A. catenella* was observed in a sediment core collected on 16 March 2005 using a souter box core (Cox A. unpubl.). A 1- meter long sediment core was collected and *A. catenella* cysts ranging from 330 to 1381 cysts  $\text{g}^{-1}$  dry weight were detected throughout the entire sample. The bottom of the Sequim Bay core was dated back to 1976 using  $^{210}\text{Pb}$  geochronology techniques. In 2005 during a survey of Quartermaster Harbor, *A. catenella* cysts were dated in a sediment core to approximately 1955 (Horner R. unpubl.). The difference in depths of cyst activity between the two neighboring embayments suggests Sequim bay has a recurrent seeding from the cyst bed (Cox, A. unpubl.).

The cores from Discovery Bay, Sequim Bay and Quartermaster Harbor all indicate the presence of *A. catenella* in the late 1970's. Cyst presence in the 1950's is confirmed in each core except Discovery Bay. Due to time constraints on this project, an inspection of the sediment layer between 30 cm and 40 cm could not be accomplished. Because  $125 \text{ cysts ml}^{-1}$  occur at the 30 cm depth, It could be proposed that cyst activity may occur at a slightly deeper interval past 30 cm.

The Shellfish closures that began in 1957 in Sequim and Discovery Bays and encompassed the San Juan Islands and Lumni Bay by 1958. The elevated *A. catanella* cyst counts in Sequim and Discovery Bay in the late 1970's appeared to coincide with the record

*A. catenella* bloom in Holmes Harbor in 1978 (Trainer et al. 2003). This time period is within the positive phase stage of the 1977 -1999 PDO climatic shift. The shellfish closures in 1957 and 1958 appear to coincide with El Nino events in 1957 and 1958 (Wooster et al, 1985). The Quartermaster Harbor sediment core show *A. catenella* cyst activity in the late 1950's and the Discovery Bay and Sequim Bay cores do not have data representing this time period. Based on this information a correlation with the El Nino event in the 1950's cannot be established. No correlation was found with the positive phase of PDO events in the 1920's and 1940's. The 10 cm intervals selected for sample analysis dated the 50 cm and 60 cm samples around 1943 and 1911 and no cysts were detected.

A unique undisturbed layer of sediments different from the surrounding horizons between 150 cm and 160 cm had no cysts observed. This layer was dated between 430 years and 460 years ago from the present. The sediment accumulation rate of  $0.31 \text{ cm y}^{-1}$  was based on  $^{210}\text{Pb}$  geochronology techniques that were accurate for 100 to 150 years from the present, meaning, dates older than this are assumed. The 150 cm to 160 cm sample interval was also representative of pre-industrial sediments. The source of the undisturbed layer could not be determined. Potential sediment sources include two creeks that discharge into the south end of Discovery Bay or by catastrophic events (volcanic, terrestrial slumping).

A sample collected from the bottom of the core was dated back from the present time to around 700 years to 765 years ago. Although no *A. catenella* cysts were observed in the sample, other phytoplankton species (mainly diatoms) and a pine pollen grain named *Pinaceae* were well present (Fig. 10).

## Conclusion

*Alexandrium catenella* cysts were detected in the sediments of Discovery Bay, and were not found at the upper 4 cm or below 30 cm in the sediments. The absence of *A. catenella* in the upper surface layers may be attributed to germination of cysts during a phytoplankton bloom that occurred in 2006. The profiles of *A. catenella* and *S. trochoidea* found in the upper layer sediments can vary by the cyst species, the timing of cyst deposition and bioturbation, germination, mortality and sediment burial. The residence time of *A. catenella* and *S. trochoidea* located in the 20 cm mixing zone can vary in age between the present time and 64 years. Assuming bioturbation dominates local sedimentation rates then sediment located at the base of the 20 cm mixing zone represented the present time when

$^{210}\text{Pb}$  geochronology sediment dating. *A. catenella* cysts were not detected in samples analyzed greater than 30 cm. The youngest date for the 30 cm layer was 1975. *A. catenella* cyst activity was also observed in Sequim Bay and Quartermaster Harbor during the late 1970's which corresponds with the 1977 to 1999 positive PDO shift. A correlation with the 1957 to 1958 El Nino climatic event could not be established due to a lack of data at the sediment depths associated with this climatic event. No correlation could be made with PDO events in the 1920's and 1940's, either due to no *A. catenella* cyst present or the spacing of samples that missed the sediment intervals representing the year of the PDO event. Two samples collected at 150 cm and 160 cm to characterize the undisturbed sedimentary features possibly formed during a climatic shift revealed no cyst activity. This feature also occurred during pre-industrial events in Discovery Bay. *A. catenella* was absent in a sample collected at the base of the sediment core dated to 1290 that contained remain of some phytoplankton species and pine pollen grains. No fossilized cysts were observed in the sediment core samples. The absence below 30 cm suggest that *A. catenella* cysts were not present in Discovery Bay until the last 70 years. If further investigations were planned in Discovery Bay, it would be useful to count cysts and conduct  $^{210}\text{Pb}$  geochronology a sediment core at 1 cm intervals down the core until *A. catenella* does goes to 0 cysts  $\text{ml}^{-1}$ . Analysis of the archive core collected in Penn Cove for *A. catenella* and  $^{210}\text{Pb}$  geochronology is also recommended.

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Table 1. Location coordinates of sediment core stations in Penn Cove and Discovery Bay. Positions are referenced to North American Datum (NAD) 1983.

Station	Latitude	Location	Depth (meters)	Spade Core Length (cm)	Kasten Core Length (cm)
Penn Cove	48° 14.27' N	122° 39.00' W	30	55	295
Discovery Bay	48° 02.00' N	122° 51.00' W	48	57	256

Table 2. Summary of Discovery Bay sediment sample depth intervals used in  $^{210}\text{Pb}$  radioisotope analysis and Alexandrium catenella cyst counting.

Depth (cm)	Sample Interval (cm)	Sample Source	$^{210}\text{Pb}$ Analysis	Cyst Counting
0-10	1	spade core	X	X
10-15	1	spade core	X	
15-25	2	spade core	X	
10	2	kasten core	X	X
20	2	kasten core	X	X
30	2	kasten core	X	X
40	2	kasten core	X	X
50	2	kasten core	X	X
60	2	kasten core	X	X
70	2	kasten core	X	
80	2	kasten core	X	
90	2	kasten core	X	
100	2	kasten core	X	
110	2	kasten core	X	
120	2	kasten core	X	
130	2	kasten core	X	
140	2	kasten core	X	
150	2	kasten core	X	X
160	2	kasten core	X	X
170	2	kasten core	X	
180	2	kasten core	X	
190	2	kasten core	X	
200	2	kasten core	X	
240	2	kasten core		X

Table 3.  $^{210}\text{Pb}$  Activity ( $\text{dpm g}^{-1}$ ) and sediment porosity at selected depths in the Discovery Bay sediment cores. The depth represents the mid-point for each sample interval.

Depth (cm)	Activity ( $\text{dpm g}^{-1}$ )	Porosity (%)	Depth (cm)	Activity ( $\text{dpm g}^{-1}$ )	Porosity (%)
0.5	10.97	91	24	7.23	84
1.5	13.78	91	31	3.06	80
2.5	13.78	90	41	1.72	80
3.5	12.34	90	51	0.79	81
4.5	16.9	91	61	0.76	82
5.5	12.2	92	71	0.88	81
6.5	11.12	90	81	0.68	80
7.5	19.23	89	91	0.79	81
8.5	17.92	88	101	0.8	81
9.5	14.73	88	111	0.87	80
10.5	12.1	88	121	0.81	80
11.5	13.49	87	131	0.52	79
12.5	10.59	87	141	0.68	80
13.5	12.84	86	151	0.67	79
14.5	15.14	86	161	0.73	82
16	10.3	86	171	0.66	82
18	10.49	84	181	0.72	80
20	7.38	84	191	0.75	80
22	6.83	83	201	0.73	79

Table 4. Dinoflagellate cyst counts with depth in Discovery Bay sediments. The depth represents the mid-point of each sample interval. The 0 cm to 10 cm depths was sampled at 1 cm intervals and the 20 cm to 60 cm, 150 cm, 160 cm and 240 cm were sampled at 2 cm intervals.

Depth (cm)	<i>Alexandrium catenella</i> (cysts ml <sup>-1</sup> )	<i>Scrippsiella trochoidea</i> (cysts ml <sup>-1</sup> )
.5	0	0
1.5	0	75
2.5	0	3600
3.5	0	2063
4.5	175	950
5.5	300	575
6.5	325	950
7.5	300	500
8.5	350	800
9.5	425	325
10.5	325	600
21	400	350
31	125	50
41	0	0
51	0	0
61	0	0
151	0	0
162	0	0
241	0	0

## Figure Legend

Fig. 1 Map of Puget Sound, Washington showing the embayment of Discovery Bay and Penn Cove selected for sediment core sampling. The general locations of the sediment core sites are shown by solid circles. Exact sampling locations are given in Table 1. This project was focused on the occurrence of *A. catanella* cysts in Discovery Bay sediments; the coring collected in Penn Cove was retained as an archive for future study.

Fig. 2 3.5 kHz sub-bottom profiles for Penn Cove and Discovery Bay. The sediments in both sampling area are a loose to compact silt. The darker shading of the sub-surface sediment indicated in profile (b) for Discovery Bay made be attributed to a greater abundance of methane in the sub-surface sediments. The white rectangular shapes represent the sample collection location.

Fig. 3 Photograph of spade box core and kasten core samples showing the sub-sampling procedure. Photo A shows the sub-sampling of the spade core sample box. The rectangular shape on the left is the X-ray radiograph tray, a 15.24 cm diameter PVC sub-sampling core in the center and a 7.62 cm core tube on the right (not used). Photo B is the kasten core barrel with some cover plates removed showing the compact fine grain sediment. The stainless still plates were used to separate the individual sample intervals. The color chart was used for a field description of the sediment color.

Fig. 4 Photo A shows the of Ziess microscope with an epifluoresent illumination source used in counting the dinoflagellate cysts. Photo B shows *Alexandrium catenella* with primuline stained cell walls that was illuminated by the epifluoresent light source. The cyst was viewed under a 16X objective.

Fig. 5 An X-ray radiograph image of the spade core sediment sample collected in Discovery Bay. The depth of the sediment mixing zone is shown along with sampled depth intervals that depict approximate year that the sediment layer first appeared using  $^{210}\text{Pb}$  geochronology and sediment accumulation rate of  $0.31 \text{ cm y}^{-1}$ .

Fig. 6 Presented in this figure is a mosaic of 30 cm long X-ray radiograph trays aligned to represent the entire kasten core collected in Discovery Bay. The arrows represent the actual depth interval of the sediment measured from the sediment surface down to the bottom of the core. The sediments at the bottom of each core tray are compressed when a sample retaining plate was inserted from the bottom of each tray. The feature at 150 cm down the core was a relatively undisturbed layer unique from the surrounding sediments that could potentially indicate a fine grain material introduced from elevated river discharge or non-local marine source. The dates indicated in the figure are estimated using the sediment accumulation rate of  $0.31 \text{ cm y}^{-1}$  determined by  $^{210}\text{Pb}$  geochronology techniques.  $^{210}\text{Pb}$  geochronology is accurate for approximately 150 years from the present and dating beyond that can only be an estimate.

Fig. 7 Profile of  $^{210}\text{Pb}$  activity and sediment porosity from the Discovery Bay sediment core. The porosity presented in percent units (blue line) shows a slight decrease in particle void space to approximately 30 cm. The  $^{210}\text{Pb}$  activity (red line) shows a mixed layer depth of 20 cm. The region of active decay is the slope situated between 20 cm and 50 cm. The average background value (supported activity) of  $0.74 \text{ dpm y}^{-1}$  occurs below 50 cm.

Fig. 8 Counts of dinoflagellate cysts (A.) *Alexandrium catenella* and (B.) *Scrippsiella trochoidea* in the Discovery Bay sediment core. The cyst count units are  $\# \text{ cysts ml}^{-1}$ . The sediment mixing zone caused by physical and biological processes occurs between the surface and 20 cm. Both *A. catenella* and *S. trochoidea* were not detected at depths greater than 30 cm.

Fig. 9 Photos of *Alexandrium catenella* (a) and *Scrippsiella trochoidea* (b). The cysts in the photos have their cell walls stained with primulin and were detected using epifluorescence illumination and viewed under a 16X microscope objective.

Fig. 10 Photo of a pine pollen grain *Pinaceae* observed in a sediment sample collected at 240 cm. An estimated date of this interval using  $^{210}\text{Pb}$  geochronology derived sediment accumulation rate ( $0.31 \text{ cm y}^{-1}$ ) was approximately 700 to 765 years from the present time.

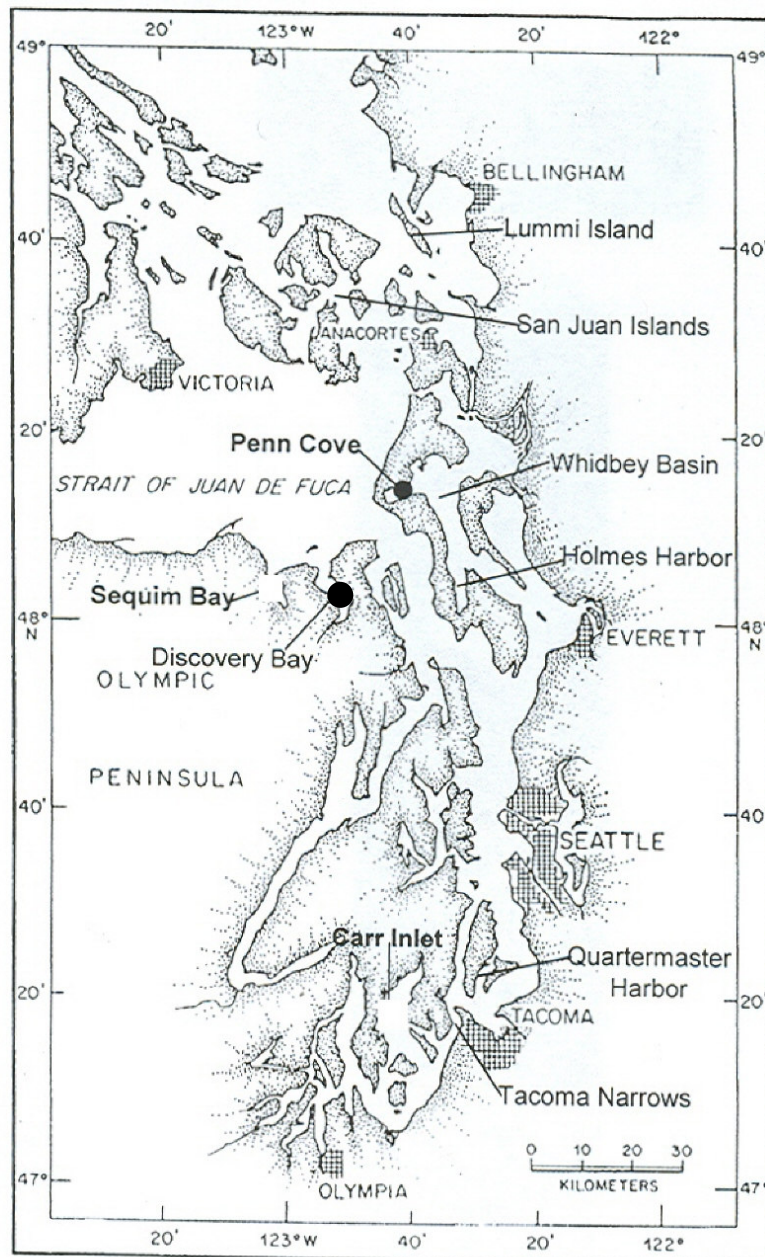
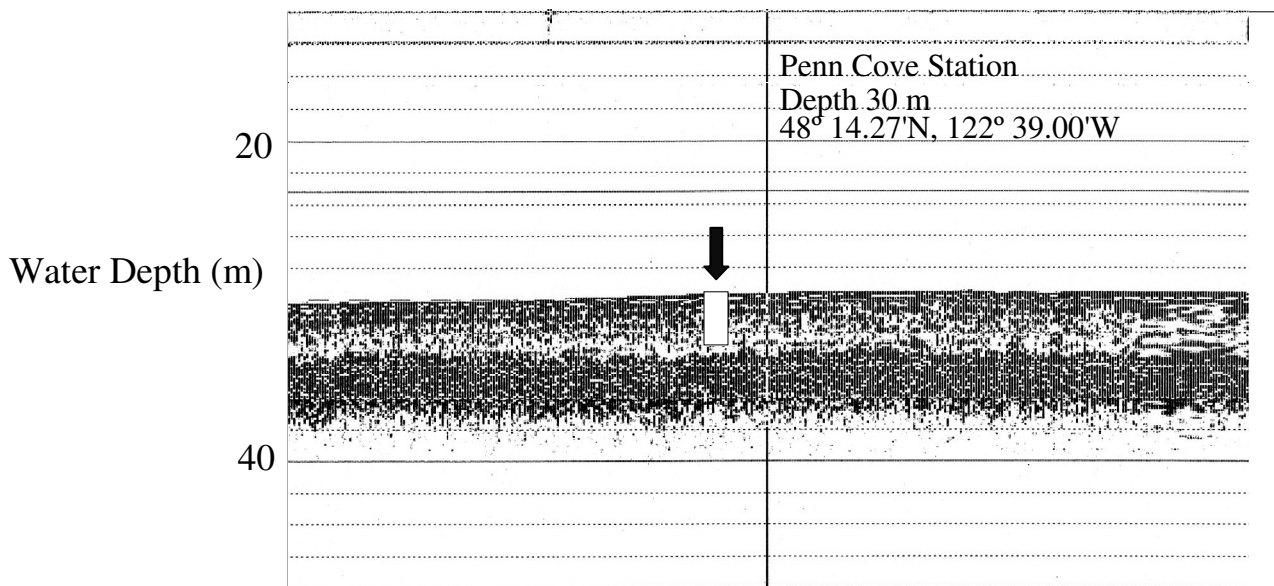
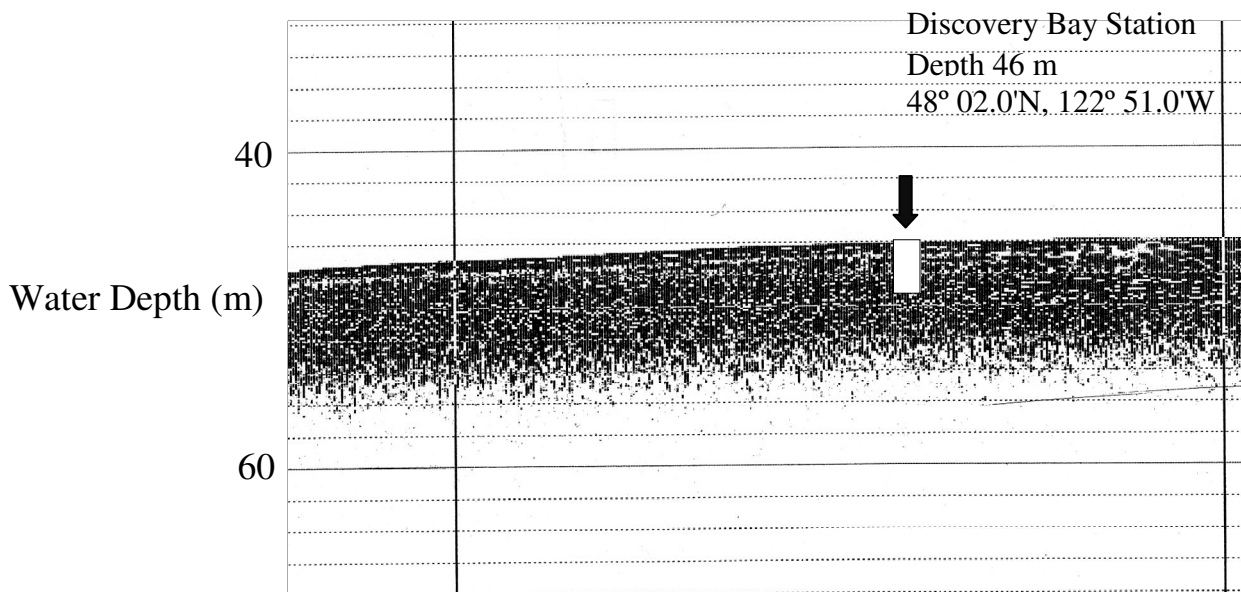


Fig. 1



A.

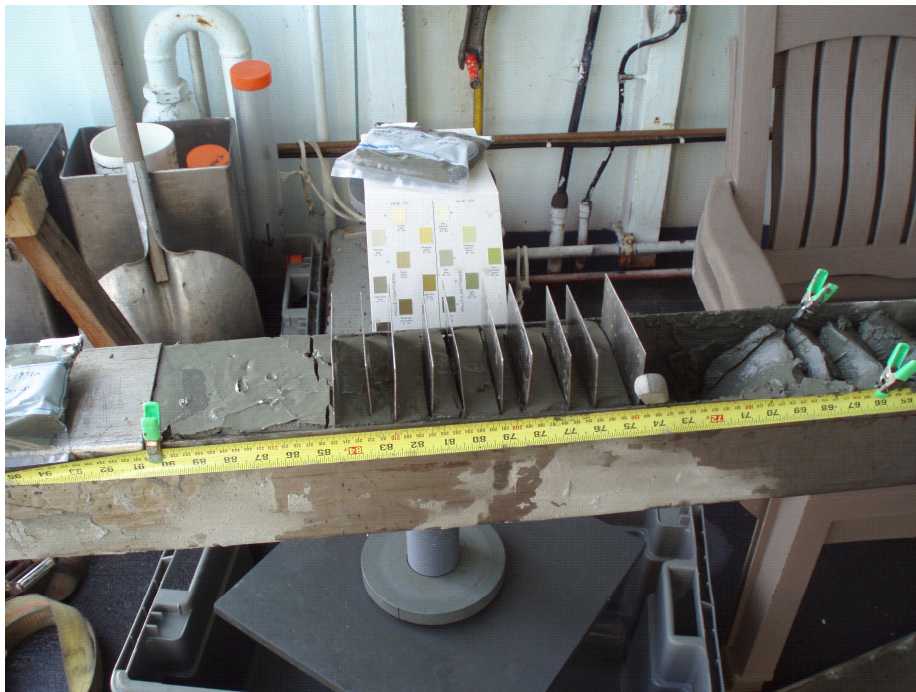


B.

Fig. 2



A.

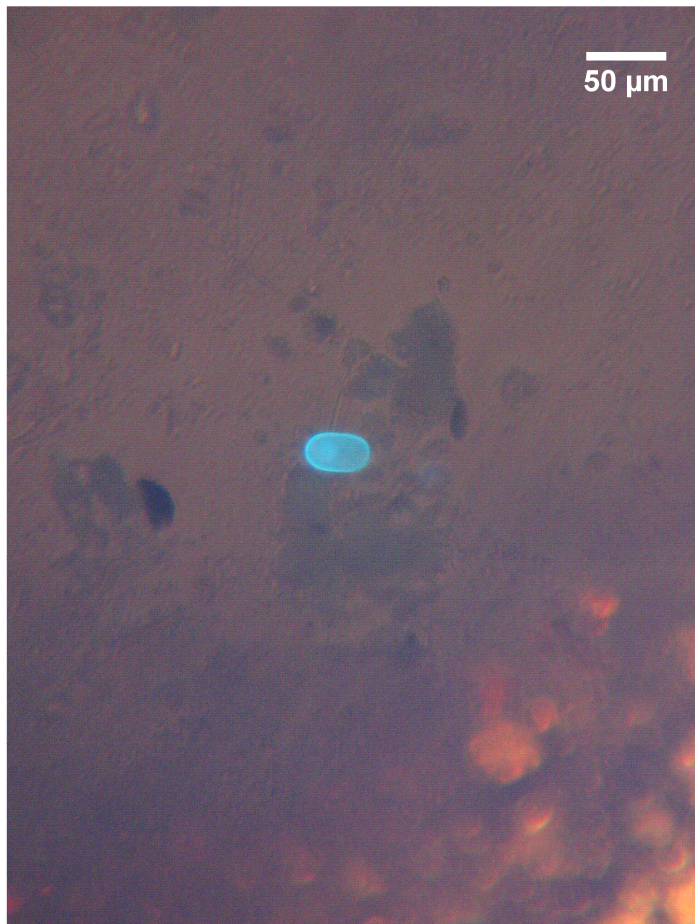


B.

Fig 3.



A



B

Fig. 4

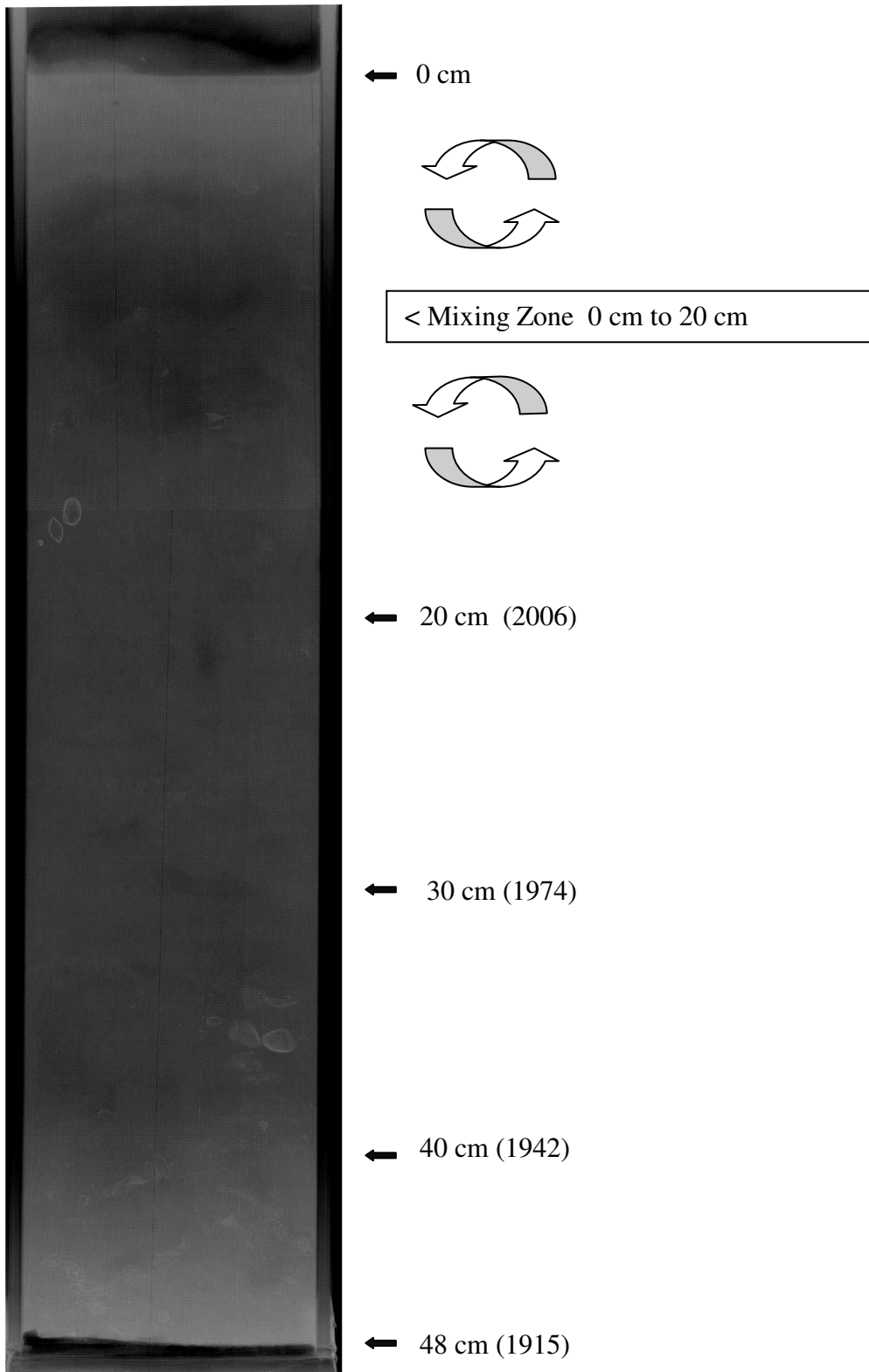


Fig. 5

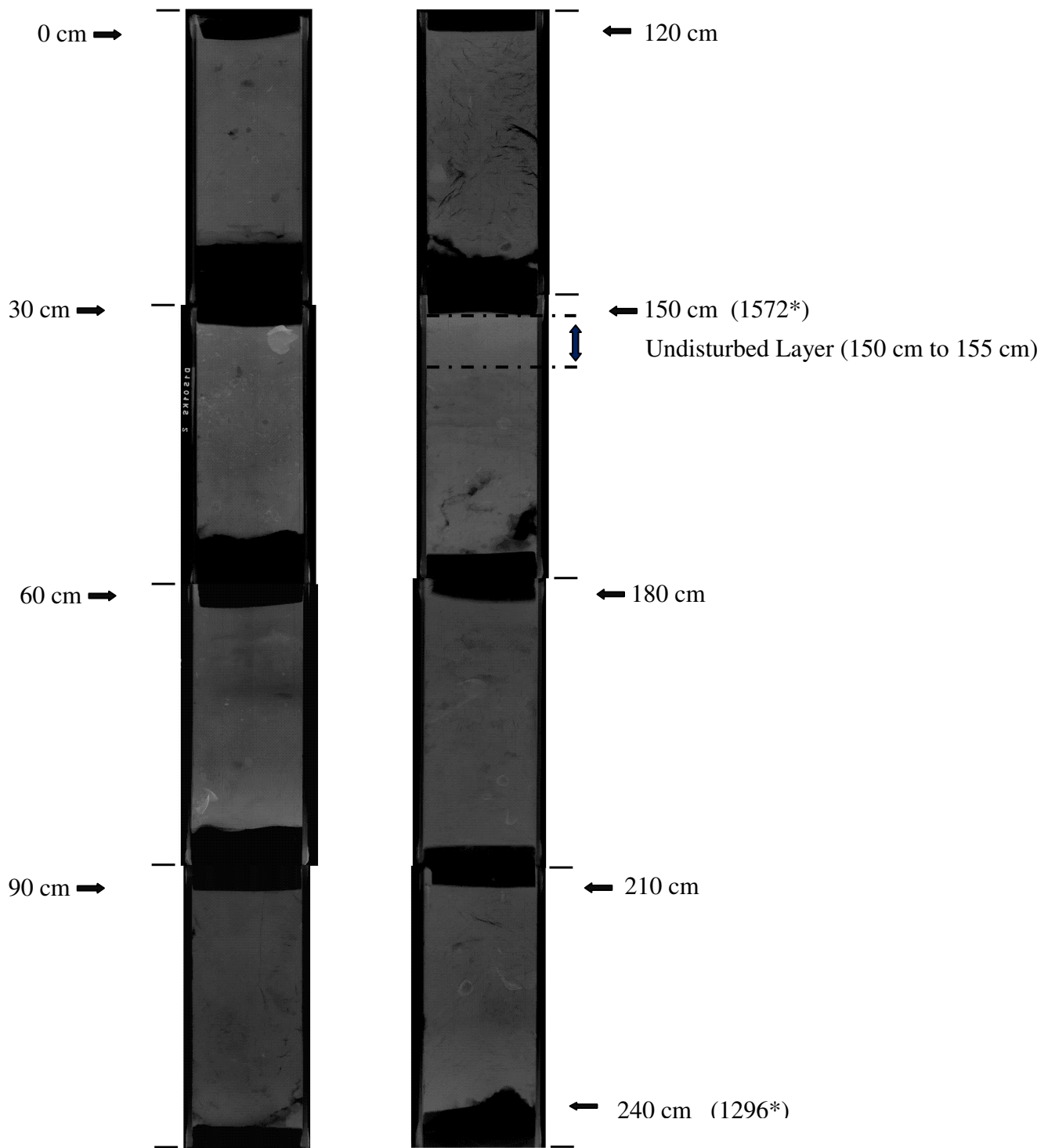


Fig. 6

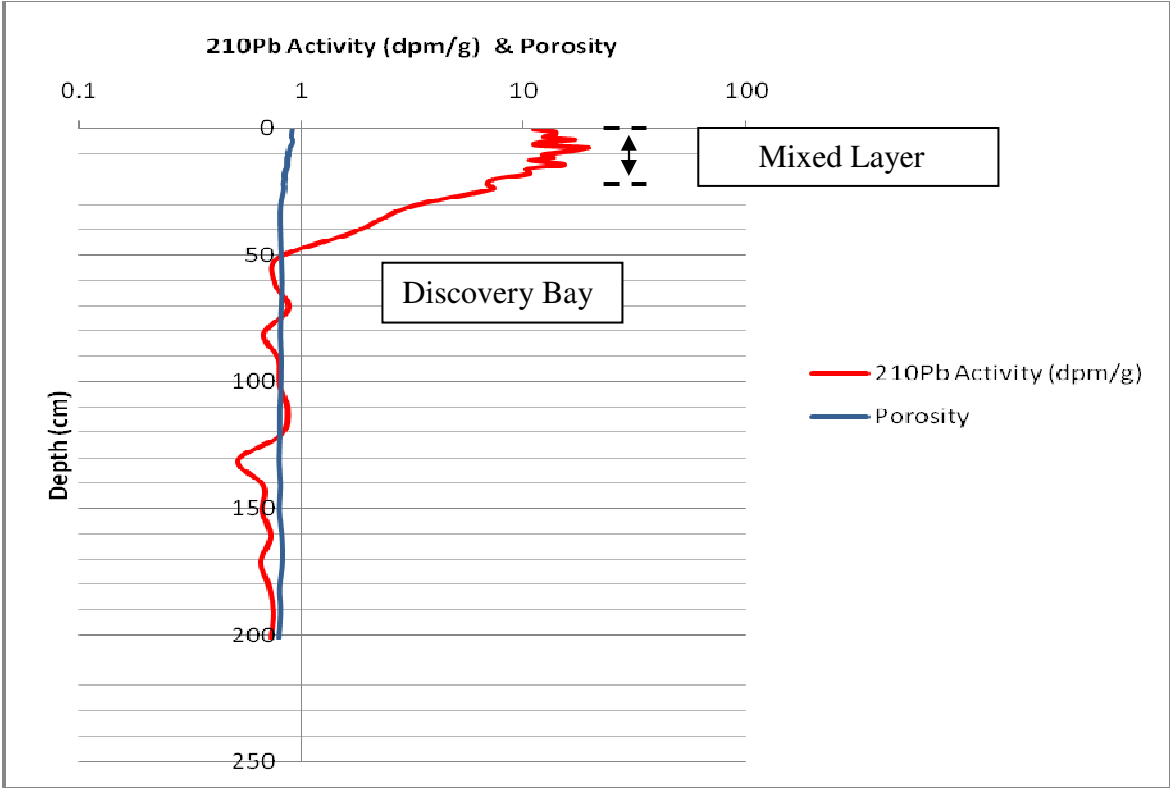
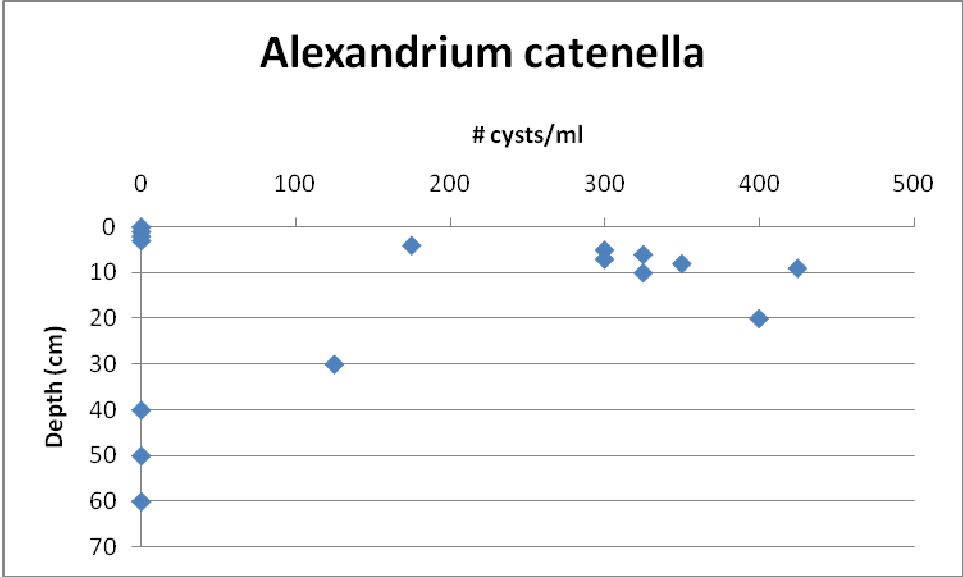
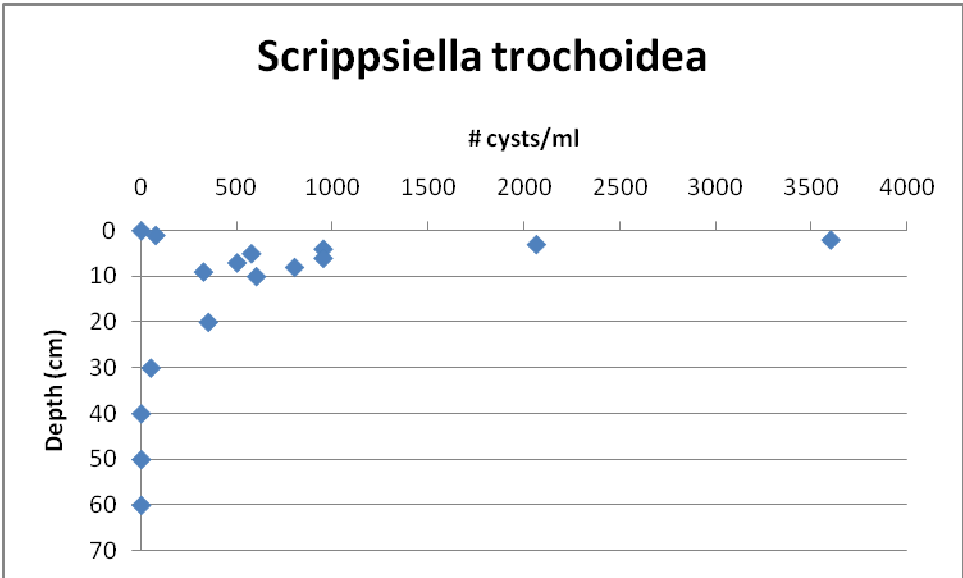


Fig. 7



A



B

Fig. 8

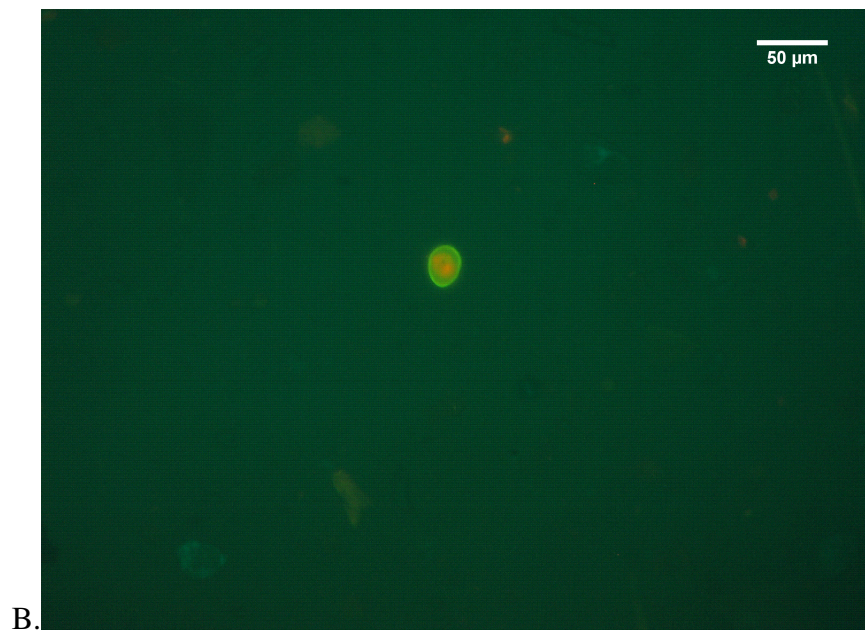
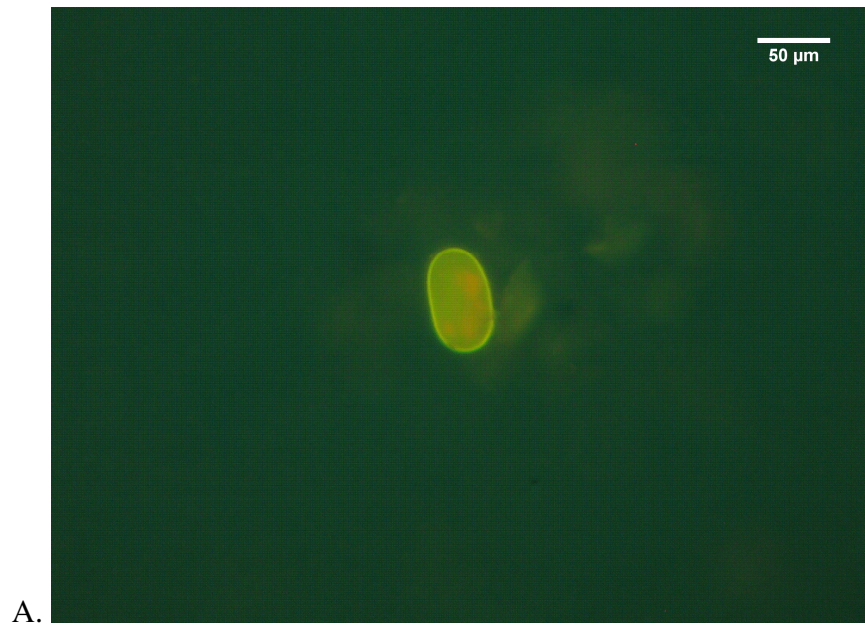


Fig. 9

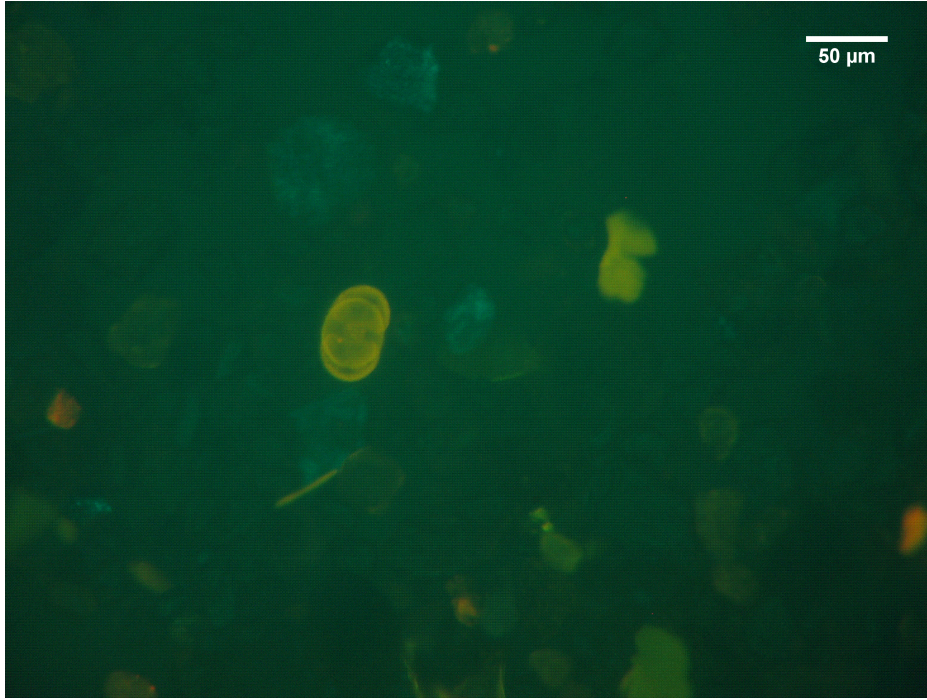


Fig. 10