Report to the Legislature

Assessment of Potential Health Impacts of Virus Discharge from Cruise Ships to Shellfish Growing Areas in Puget Sound

November 2007

Washington State Department of Health
Division of Environmental Health
Office of Shellfish and Water Protection

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DOH Pub 332-062

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ACKNOWLEDGEMENTS

We would like to thank all those stakeholders who supplied thoughtful comment to draft versions of both the UW study and this report.

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Quantitative Assessment of Acceptable Levels of Virus Discharge from Cruise Ships in Puget Sound

Executive Summary

Since 1999, the number of large cruise ships calling on the Port of Seattle has increased from 6 port calls with 6,615 passengers to more than 190 port calls with more than 750,000 passengers (http://www.portseattle.org/seaport/cruise/). Large cruise ships are essentially floating resorts, with populations (passengers and crew) between about 2,000 and 4,000. In 2004, the Department of Ecology entered into a Memorandum of Understanding (MOU) with the North West Cruise Ship Association (NWCA) that prohibits wastewater discharges into Puget Sound from member ships (typically large cruise ships) unless they have advanced wastewater treatment systems (AWTS). With these advanced wastewater treatment systems and upon approval by the Department of Ecology (Ecology), members of the North West Cruise Ship Association may continually discharge treated wastewater while transiting Washington waters, including while docked at the Port of Seattle.

AWTS can effectively remove bacteria but may not eliminate viruses that cause illnesses. This is of concern because fecal coliform bacteria are the standard indicators of contamination used to gauge the effectiveness of the removal of pathogens by wastewater treatment plants. Viruses such as norovirus are much more difficult to culture and quantify. However, it is widely recognized that viruses are the major cause of food borne illness from consumption of bivalve shellfish (oysters, clams, and mussels).

Cruise ships discharge their wastewater to surface waters at shallow depths, passing many shellfish growing areas on their way in and out of Puget Sound. There is little information on how well vessel treatment worked in limiting discharge of viruses near shellfish growing areas. The Centers for Disease Control and Prevention (CDC) reports that, since 2000, eighteen (18) norovirus (or presumptive norovirus) outbreaks have occurred among passengers and crew on large cruise vessels in the Pacific Northwest.

In response to these concerns, the State legislature budgeted $100,000 for a study on potential human health impacts from virus discharges from large passenger vessels. The Washington State Department of Health (DOH) contracted with the University of Washington (UW) to undertake this study. Their results indicate that, when AWTS are fully functional, viral discharges from large cruise ships should not cause illness through shellfish. However, if the treatment systems malfunction, virus discharges from cruise ships may reach some shellfish beds at levels that may lead to illness. It is therefore of utmost importance for AWTS to be working at optimal levels at all times that wastewater is being discharged. UW dilution models show there is little time when an upset (AWTS malfunction) occurs to notify growers in the most sensitive areas prior to discharge reaching growing beds. The main focus of the recommendations is therefore to limit the risk of an unacceptable discharge reaching these areas. Recommendations include:

- No discharges should occur within 0.5 nautical miles of bivalve shellfish beds that are recreationally harvested or commercially approved to harvest.
- Cruise ships should withhold discharge when a system upset occurs.
- DOH should be notified immediately in the event of an AWTS upset.
- A small passenger ship study should be done to assess potential impacts of these vessels.
- The Department of Ecology should revise their ‘Criteria for Sewage Works Design’ to address a minimum UV dosage for virus inactivation.

Introduction

The Department of Ecology (Ecology) currently has a Memorandum of Understanding (MOU) with members of the Northwest Cruise Ship Association (NWCA) that prohibits wastewater discharges into Puget Sound from member ships (typically large cruise ships) unless they have advanced wastewater treatment systems. With these advanced wastewater treatment systems (AWTS) and upon approval by Ecology, members of the NWCA may continually discharge treated wastewater while transiting Washington waters. AWTS normally employ an integrated system of enhanced aerobic digestion and low-pressure membrane filtration to treat wastewater, followed by UV disinfection. The filters used by advanced treatment systems after secondary treatment can effectively remove bacteria but may not eliminate certain viruses like the norovirus. Fecal coliform bacteria tests of water and shellfish are therefore no longer a reliable indicator of the effectiveness of disinfection after secondary treatment.

Large passenger vessels not covered under the MOU are required to follow U.S. Coast Guard requirements (under Section 312 of the Clean Water Act) to discharge from a certified operable marine sanitation device (MSD) within three nautical miles offshore within U.S. territorial waters. These vessels also follow more stringent Cruise Line International Association (CLIA) guidelines. CLIA guidelines specify all vessels process sewage through a MSD and discharge only when the ship is more than 4 miles from shore and when the ship is traveling at a speed of at least 6 knots. All vessels have holding tanks to allow them to store effluent if needed.

Appendix A shows the ship lanes and approved shellfish growing beds in areas of most concern (Admiralty Inlet and the waters off north Kitsap County).

The federal National Shellfish Sanitation Program (NSSP) lays out the requirements for the harvest of commercial shellfish. The NSSP requires that any state that exports shellfish establish a "closure zone" adjacent to each wastewater treatment plant outfall. The closure zone takes into consideration a possible interruption in the effectiveness of treatment or disinfection of the sewage being discharged. Because passenger ships traveling through Puget Sound pass numerous shellfish beds, the NSSP requires that the potential contamination of shellfish beds by discharges from such ships be assessed. In 2005, the legislature appropriated $100,000 to the Department of Health (DOH) to undertake this study.[1]

The Virus Study

On May 25, 2005, DOH and Ecology held a meeting with numerous stakeholders. An outline of study options was presented at that meeting and comments requested. As a result, DOH contracted with the University of Washington (UW) to study the impact of norovirus in discharges from large passenger ships.

The work that the University of Washington completed includes assessing:

- Estimation of virus discharge (*How many viruses may escape from a ship?*)
- Dilution from ship to shoreline (*How do currents and ship-speed dilute discharge?*)

[1] During the 2005 session, HB 1415 was introduced that would have formalized the MOU into law. Although HB 1415 did not pass, it directed that a study be done that addressed the impact of viruses on shellfish.

- Uptake and retention of viral particles by shellfish *(If the viruses reach shellfish, how many will accumulate in shellfish and how long might they be retained?*)
- Risk of disease *(If viruses reach shellfish, what is the risk of human illness from consuming the shellfish?*)

UW Study Results

Estimation of virus discharge *(How many viruses may escape from a ship?*)

A review of the literature found a large variation in viruses shed by individuals. Symptomatic individuals shed anywhere from 10^4 to 10^{10} viruses per milliliter (ml) of stool, with up to 5000 ml of stool per day (mode of 1500 ml). Asymptomatic shedding is estimated to range from 10^0 to 10^6 viruses/ml of stool.

Because of the wide variations in shedding, just a few individuals with high shedding rates may discharge as many viruses as many individuals with lower discharge rates. Therefore, virus discharge rates between an outbreak and non-outbreak condition form more of a continuum than two distinct ranges of virus discharge. CDC uses a threshold of 3% of passengers falling ill for an outbreak investigation, so this was used as the cutoff in comparing the 'outbreak' versus the 'non-outbreak' condition. Asymptomatic shedding was ignored in the calculation of risk for transient modeling as being small compared to symptomatic shedding. However, for calculation of background risk asymptomatic shedding was estimated at 1% of the ship population.

The availability of adequate disinfection on a continuous basis is a key factor in limiting the amount of virus discharge from a ship. UV disinfection used by AWTS provides a 4 log (or 99.99%) reduction in the numbers of viruses discharged, while all the treatment preceding disinfection is estimated to provide between 2.5 to 4 log virus reductions depending on the type of system employed.[*] If AWTS are working properly, the median concentration of viruses in treated effluent is estimated at less than 10 viruses per liter under both outbreak and non-outbreak conditions. At this discharge virus concentration, and with dilution factors described below, no public health concerns are expected.

Dilution from ship to shoreline (*How do currents and ship-speed dilute discharge?*)

Two types of dilution are commonly recognized for calculating the total dilution of a wastewater effluent to a water of concern. Initial (or near field) mixing includes the combined effects of turbulence (the difference in velocity and direction), buoyancy (salinity and temperature differences between the effluent and receiving water), and stratification (depth and the amount of density difference in the receiving water) between the outfall and ambient water. Far field

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[*] The type of AWTS employed by individual cruise ships for the 2007 cruise season can be accessed on the Ecology website:
http://www.ecy.wa.gov/programs/wq/wastewater/cruise_mou/Washington%20discharge%20status%206-19-07.pdf

*A general description of each type of AWTS employed by large cruise ships is provided in the State of Alaska's Large Commercial Passenger Vessel Wastewater Discharge General Permit Information Sheet:
http://www.dec.state.ak.us/water/cruise_ships/pdfs/GP%20Information%20Sheet.pdf

dilution occurs after initial dilution where the effects of ambient conditions (currents, tides, winds, bathymetry) predominate. Near field dilution effects are normally much greater than far field dilution effects.

Cruise ships outfalls are different than stationary wastewater treatment plant (WWTP) outfalls. Since cruise ship outfalls move they have the potential to affect a wider area. Cruise ship outfalls discharge at shallow depths when compared with stationary WWTPs, also increasing their relative potential to impact shellfish beds in more shallow intertidal zones. Many stationary WWTP outfalls are deep and take advantage of water stratification to minimize their impacts to nearby shellfish growing areas. Modeling areas of potential impact from cruise ships is more difficult as currents and water depths vary along the shipping lanes over very short spans.

On the positive side, cruise ship speed and use of propellers provides more active initial dilution compared with stationary WWTPs that rely on passive diffusers and buoyancy differences to promote turbulent mixing. In addition, the waste stream of cruise ships is not as vulnerable to the variations in waste flows due to storms and industrial processes as stationary WWTPs. These differences are summarized in Table 1.

Table 1 – Comparison Cruise Ship and Stationary WWTP discharges

	Cruise Ship AWTS	**Conventional WWTP**
Outfall location	Moving, shallow	Stationary, deep
Waste stream	Consistent	Variable
Method of dilution	Propellers, motion	Diffusers, buoyancy differences

Near field dilution factors were culled from previous studies by EPA on cruise ships. In a Florida study examining the near-field dilution of wastewater effluent for passenger vessels using tracing dyes and fluorimetry, EPA reported average near-field dilution factors ranging from 1:195,000 to 1:643,000. Minimum dilution factor estimates ranged from 1:27,700 to 1:48,200. For the purposes of the UW study, a range of near-field dilution factors from 1:30,000 to 1:200,000 was used.

To estimate far field dilution and transport of possible discharges from a cruise ship in Puget Sound, UW used a three-dimensional hydrodynamic model, based upon the Princeton Ocean Model (POM). This model is maintained by the Puget Sound Marine Environmental Modeling (PSMEM) partnership (http://www.psmem.org). UW first modeled the hits along the entire Puget Sound shoreline from particle releases from the cruise ship track. Then modeling was done on the most adverse locations (where dilutions would be expected to be the most minimal). The model predicted that the highest levels of particles that hit landfall did so in the general areas of Admiralty Bay and Useless Bay in Island County. Conversely, particles released in the eastern Strait of Juan de Fuca do not tend to move into Puget Sound at the surface. The average far field dilution factor in the most adverse case locations was about 100:1, but could be as low as 45:1 (at Point Jefferson in Kitsap County).

Uptake and retention of viral particles by shellfish *(If the viruses reach shellfish, how much will accumulate in shellfish and how long might they be retained?*)

Bivalve shellfish (oysters, clams, mussels) are filter feeders and will concentrate pathogenic organisms as well as other toxins. The rate of uptake and elimination of viruses varies by the species of shellfish and virus, temperature, and other water quality factors. Bivalve shellfish eliminate viruses at much slower rates than fecal coliform bacteria. Thus, many viral outbreaks have occurred from consumption of shellfish from growing areas that meet bacteriological standards. Several previous studies showed that bivalve shellfish can bioaccumulate viruses in the range of 3 to 1000 times the viral concentration in the overlying water. This range of values was used in the risk model.

Risk of disease *(If viruses reach shellfish, what is the risk of human illness from consuming the shellfish?*)

The risk of disease is based on two main factors, the dose and the response to the dose. The dose is a function of water quality, bioaccumulation of viruses in the shellfish, and the shellfish consumption rate. Water quality is estimated from the virus discharge rate from the ship (which in turn is calculated from the estimated viral shedding rates and wastewater treatment effectiveness) and the dilution factors (both near and far field) from ship to shore. A range of values for bioaccumulation rates and dose response data were taken from several previous studies. CDC reports that as few as 10 viral particles may be sufficient to infect an individual, and 'recent evidence also suggests that susceptibility to infection may be genetically determined, with people of blood group O being at greatest risk for severe infection'.

Shellfish consumption was based on a study done for the Suquamish tribe. Suquamish shellfish consumption rates are much higher than the general population, and many of their members live and harvest in the areas most vulnerable to a potential virus discharge from a cruise ship. UW used dilution rates in Suquamish harvest areas in the model. For the purposes of calculating annual risk, only oyster consumption was used as oysters are normally eaten raw or minimally cooked.

In general, consumption of raw or undercooked shellfish is known to increase risk of food borne illness. The DOH Food Safety Program requires consumer advisories on menus to advise people of this, particularly those that have certain medical conditions. The US FDA estimates cases of norovirus gastro-enteritis related to seafood consumption at some 100,000 cases per year, and the UW study speculates this figure may be as high as 276,000 cases per year. Based on this number, the UW estimates the existing norovirus illness risk from eating raw oysters at greater than 1 in 1000. In the absence of a recognized acceptable risk level for shellfish the UW uses an 'acceptable risk' of one additional illness per 10,000 people per year, based on an EPA standard for microbial risk from drinking water, in calculating acceptable levels of virus in the water column overlying shellfish beds. The UW 'acceptable risk' is therefore an order of magnitude more conservative than the observed baseline risk from eating raw oysters.

Related Studies

Small Passenger Ships

DOH obtained a small grant ($5000) from the Puget Sound Action Team to investigate the potential impacts of smaller passenger ships discharging near harvestable shellfish. An intern from UW collected information on small passenger ships in the summer of 2006, but was hampered by a lack of response from these companies for requests for information. Twelve companies employing more than 35 vessels were identified. Small passenger vessels are defined as having capacity of less than 250 passengers.

The Cruise Ship MOU bans discharge in Washington waters except for vessels with AWTS. Small passenger vessels are not party to this MOU and they have less sophisticated treatment (such as maceration and chlorination), with little or no holding capacity or other reliability features such as alarms with automatic shutdown capability. Vessel discharges are not regulated by the State; only the U.S. Coast Guard currently has authority to regulate their discharges. Smaller cruise ships dock in many areas of Puget Sound and can potentially move closer to shellfish growing areas than larger cruise ships.

An assessment of small passenger ship discharges by the State of Alaska published in 2004 found that small ship stationary effluent does pose some risk to the marine environment. They conclude that "Due to the high concentration of fecal coliform, the effluent from some small ships may pose a risk to human health in areas where aquatic life is harvested for raw consumption." The State of Alaska has consequently given these ships a timetable to meet effluent quality standards of the larger cruise ships. This is an area that needs further investigation.

Virus Test Kits

DOH has assembled virus sampling kits to be placed on large passenger ships traveling Puget Sound in order to evaluate the effectiveness of onboard wastewater treatment systems during a norovirus outbreak. These test kits were underutilized by members of the NWCA in 2006 and 2007, but should be used in the future to get a better idea of the levels of virus shedding and effectiveness of wastewater treatment under outbreak and non-outbreak conditions.

Discussion

National Shellfish Sanitation Program (NSSP)

FDA guidance states the purpose of a prohibited zones for effluents is two-fold, both related to the time required for pollutants to travel from the source of pollution, through the prohibited area, to the shellfish growing areas:

1. Ensure the public health is protected by preventing pathogen contamination of shellfish waters for normally operating treatment works. Mixing and dilution occur as the pathogens traverse the prohibited area and the pathogen concentrations are decreased accordingly. The

objective is achieved by defining the prohibited area so that pathogen concentrations beyond the prohibited boundary are at acceptable levels under normal operating conditions.

2. Provide the time necessary for notification to cease harvesting in the shellfish growing waters following a malfunction in the wastewater treatment works. The prohibited area will be defined to provide for both objectives. However, the controlling one will be the situation requiring the greater distance from the outfall.

The UW study reports show that that total dilution factors from ship to shore meet the NSSP criteria above. However, assumptions of viral loading are much greater than normal due to the 'institutional effect' of a confined population in close proximity on a cruise ship. That, coupled with the short times (about one hour) that particles from ships can reach the shore if discharged under adverse conditions, leave some doubt that the second criteria above can be met. Stringent requirements to prevent unacceptable discharges from cruise ships are therefore needed to avoid a wider prohibited zone that may impact currently approved shellfish growing areas.

The UW Study

The UW study goes into detail on how the results of the study were limited by the quality of the data used to develop and run the models. Much of it will not be repeated here, unless it relates to the recommendations.

Their results indicate that viral discharges from large cruise ships should have no significant impacts on shellfish beds when AWTS are working well. However, under upset conditions (such as loss of disinfection), virus discharges from cruise ships may reach some shellfish beds at levels that may lead to illness. Two factors need to be taken into consideration that may add some conservatism to the risk model. First, an assumption is made that all oysters consumed were from areas most readily impacted by a cruise ship discharge upset event. The cruise season (April to September) also does not match closely with times that most oysters are harvested.

Second, a discussion of the probability that an upset condition might occur was not included in the analysis. Many cruise ships have added reliability factors (alarms with automatic shutdown capability, capacity for effluent storage, back-up chlorine disinfection) that were not taken into consideration. The probability of an upset event happening in the same general location on an annual basis is expected to be low.

Wider Issues.

One of the major issues in establishing a closure zone based on viral risk is that there is no established viral indicator standard from which to base a sanitary line. The UW study used a water quality standard for norovirus based on an additional annual risk of one additional illness per 10,000 people. This risk translates into a threshold concentration of about one virus in 10,000 liters in the water overlying shellfish beds. However, this concentration of concern for norovirus cannot practically be measured and acceptance of such a standard would need to go through a very rigorous national review. Also, whatever came out of that process would need to apply to stationary WWTPs as well. Many stationary WWTPs are upgrading to AWTS technology used on cruise ships, and current discharge permits issued by the Department of Ecology are geared towards controlling bacterial (not viral) risks. Many viruses (like Norovirus) require higher UV doses to have a similar level of inactivation for fecal coliform bacteria.

Currently, the MOU is a voluntary agreement because the Department of Ecology has no formal regulatory authority over cruise ships. The U.S. Environmental Protection Agency (EPA) and the U.S. Coast guard were both given authority to regulate Marine Sanitation Devices (MSDs – the term used for onboard waste treatment) by the Clean Water Act, Section 312. EPA establishes MSD performance standards, while the Coast Guard is responsible for regulating MSD design, construction, installation, certification and enforcement consistent with EPA's standards. This section of the Clean Water Act has recently been overturned (see http://www.epa.gov/owow/invasive_species/ballast_water.html for details), and EPA is scheduled to come out with a final rule in September 2008 which may allow individual states to develop their own regulations with respect to cruise ships and other vessels.

Recommendations

As discussed earlier, when AWTS are fully functional, viral discharges from large cruise ships should not cause illness through shellfish. However, if they malfunction, virus discharges from cruise ships may reach some shellfish beds at levels that may lead to illness. As the dilution models show, there is very little time when an upset condition occurs to notify growers in the most sensitive areas. The main focus of the recommendations is therefore to limit the risk of an unacceptable discharge reaching these areas.

- *No discharges should occur within 0.5 nautical miles of bivalve shellfish beds that are recreationally harvested or commercially approved to harvest..* DOH concurs with the Alaska Department of Environmental Conservation and their Science Advisory Panel when they wrote: "Prohibiting discharges within 0.5 mile of shellfish beds aids the protection of human health. Because there are many chemicals, (e.g. drugs and endocrine disruptors) and possibly viruses discharged in all effluents, including those from advanced wastewater treatment systems, the panel recommends minimizing these elements from reaching shellfish that could be consumed by humans." This distance is also consistent with values used by the DOH for prohibited zones around wastewater outfalls for similar populations prior to the widespread use of computer modeling software. As the maps in the Appendix show, ship lanes maintain a 0.5 nautical mile distance of all currently approved growing areas save two portions of the Kingston growing area. As the ship lanes are one mile wide in each direction, it should be feasible for vessels to maintain a 0.5 nautical mile distance from these areas as well.
- *Cruise ships should withhold discharge with a system upset occurs.* The current MOU has a passage allowing discharge within 1 mile of berth if "documentation of system design demonstrates the AWTS can be automatically shut down if monitoring of treated effluent indicates a system upset; or documentation that demonstrates that operational controls exist to insure system shut down if monitoring of treated effluent indicates a system upset.". These requirements should be applied to all cruise ships that discharge in Washington waters. The definition of 'upset' should be amended to include a loss of disinfection below levels effective for four log (99.99%) inactivation of norovirus.
- *DOH should be notified immediately in the event of an AWTS upset.* This is consistent with NPDES permit conditions of stationary WWTP near shellfish growing areas.
- *A small passenger ship study should be conducted to assess potential impacts of these vessels.* DOH concurs with this recommendation in Ecology's '2006 Assessment of Cruise Ship Environmental Impacts in Washington'.

- *The Department of Ecology should revise their 'Criteria for Sewage Works Design' to address a minimum UV dosage for virus inactivation.* FDA guidance specifies "During the disinfection process, instrumentation is needed to monitor and gage the U.V. light intensity, lamp/bank status and water level. The disinfection system itself requires (1) monitoring of the status of banks to make sure they will be activated, (2) monitoring of U.V. intensity in the banks and water level in the U.V. banks. The monitors should be connected to alarms to warn when critical limits established in the performance standards are not met."

Conclusion

The UW study findings indicate that viral discharges from large cruise ships should have no significant impacts on shellfish beds when AWTS are functioning well. However, under upset conditions (e.g. loss of disinfection), virus discharges from cruise ships may reach some shellfish beds at levels that may lead to illness. Because there is no established viral indicator standard, and AWTS reliability factors are not considered in the risk assessment analysis, DOH recommends that cruise ships maintain a distance of 0.5 miles from known shellfish growing areas. A review of disinfection reliability of all cruise ships under the MOU should also be done to minimize the potential for an unacceptable discharge in the proximity of shellfish growing areas.

Cruise ships (with their moving outfalls) and AWTS systems (which filter most bacteria but not viruses) present new challenges for the DOH. The Model Ordinance that DOH OSWP must use to classify growing areas does not have guidance with regards to assessment of either moving outfalls or viral risk. The study of AWTS discharges has implications for stationary WWTPs, future efforts on water quality restoration such as the Puget Sound 2020 plan and creates a precedent that may impact future development of the NSSP Model Ordinance.

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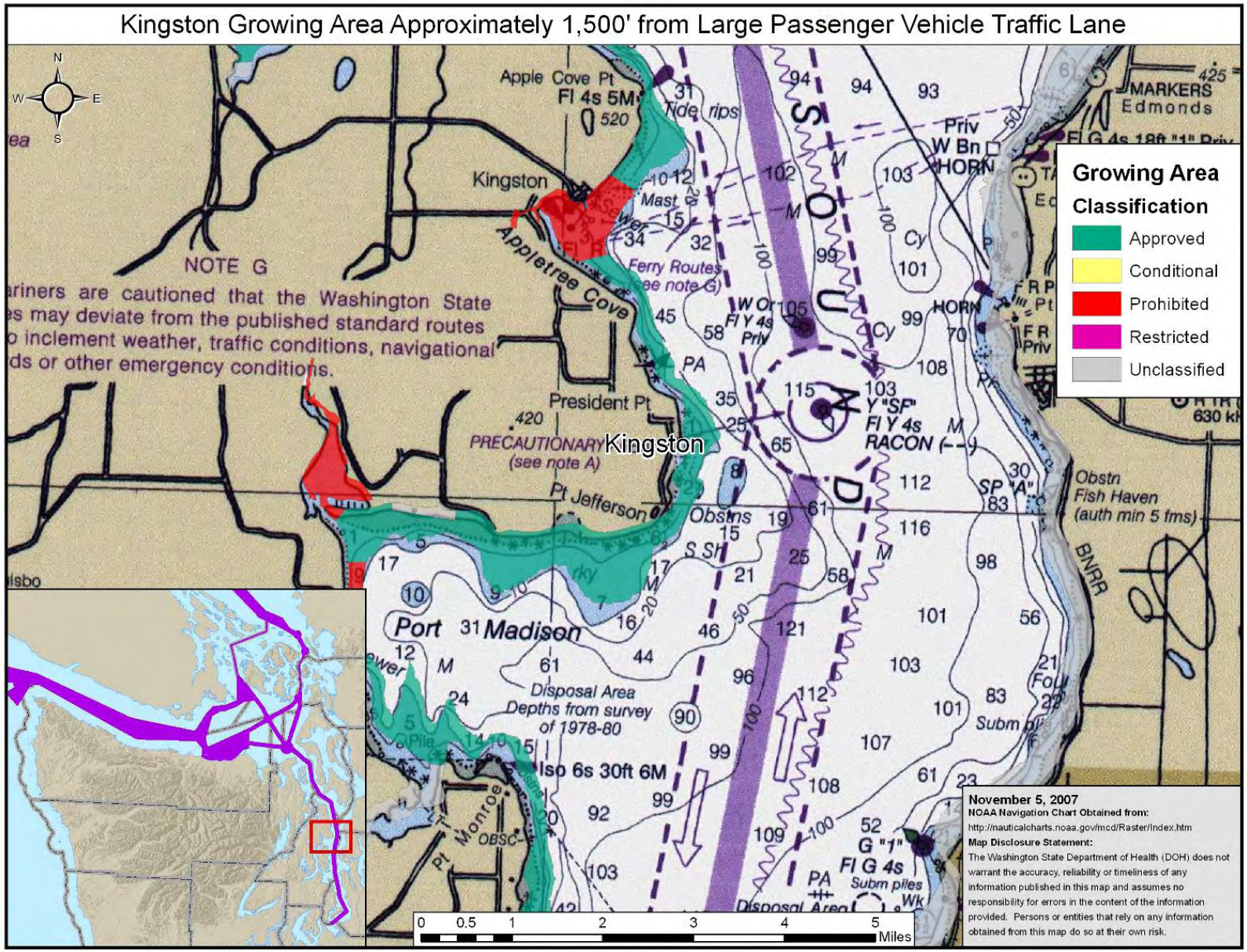

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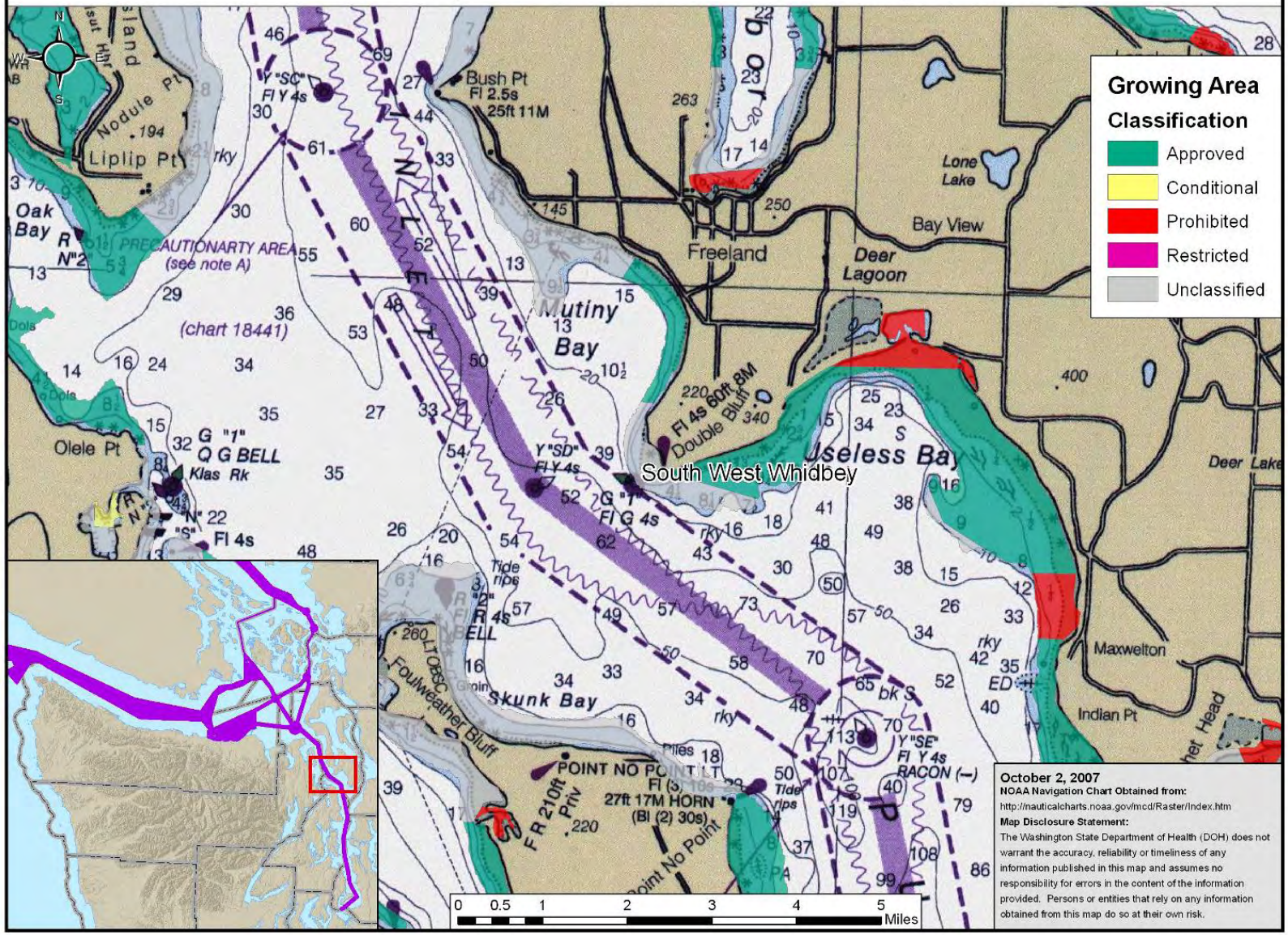
South West Whidbey Growing Area Intersection with Large Passenger Vehicle Traffic Lane



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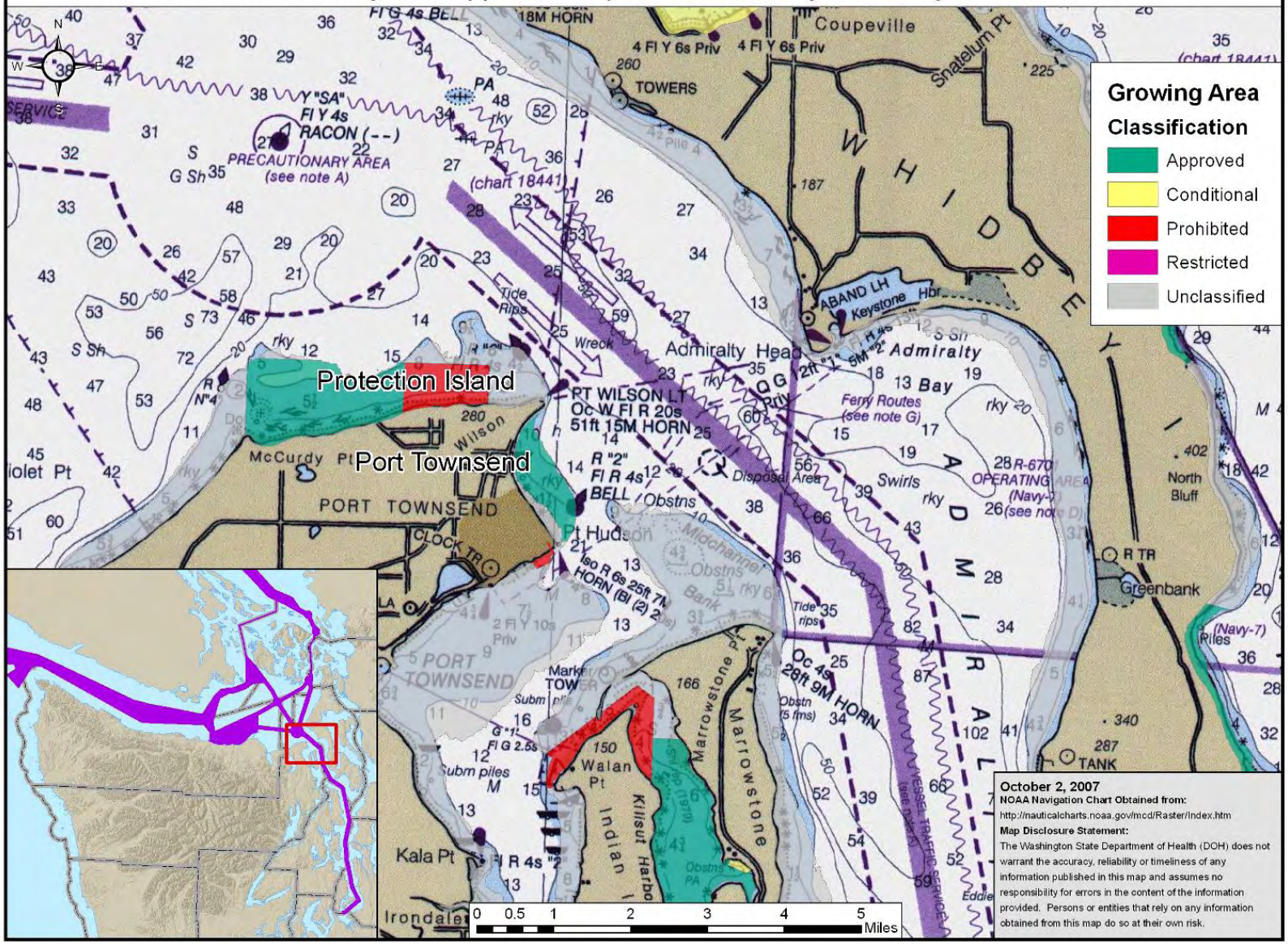
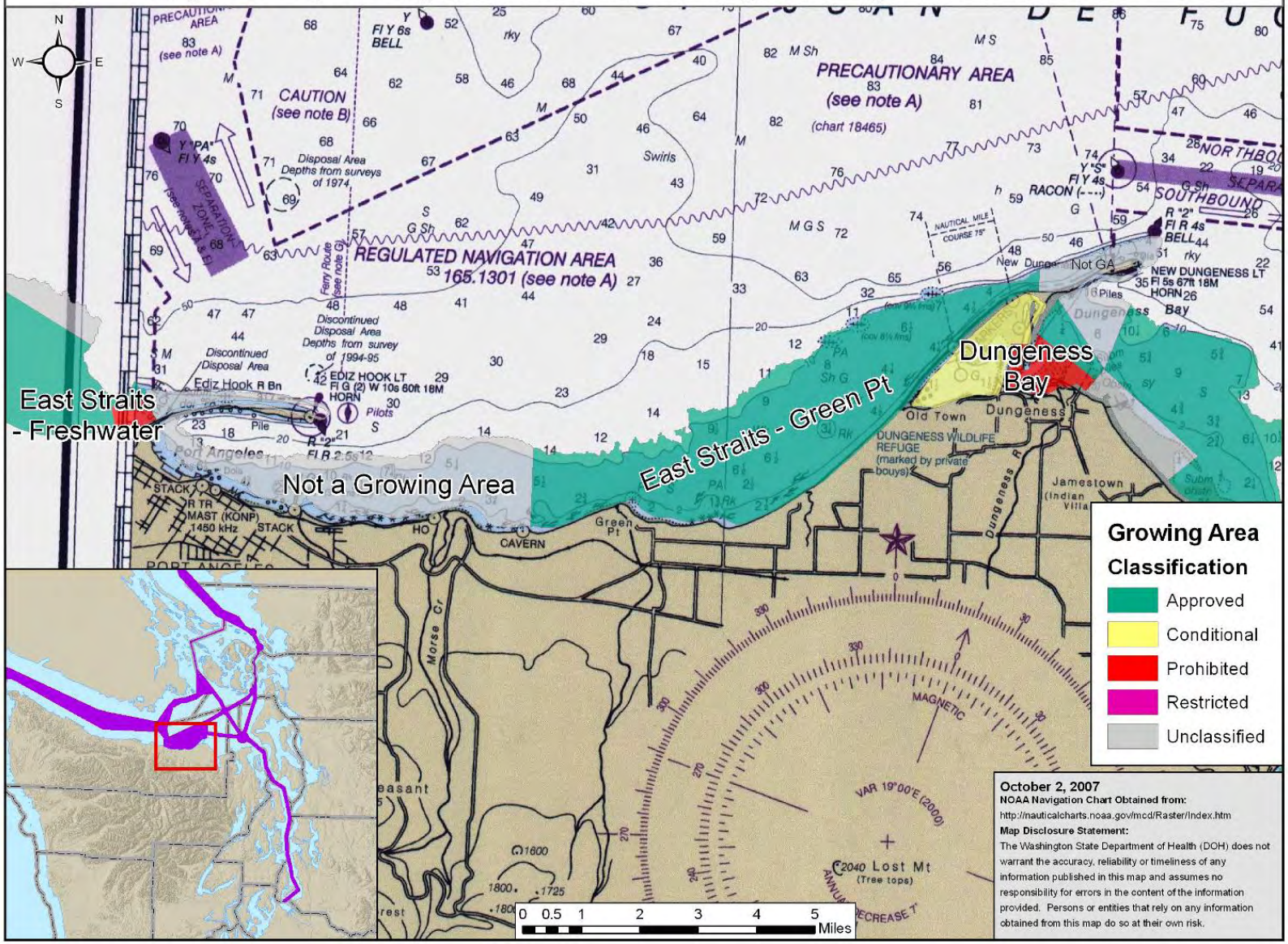
Protection Island Growing Area Approximately 1,000' from Large Passenger Vehicle Traffic Lane

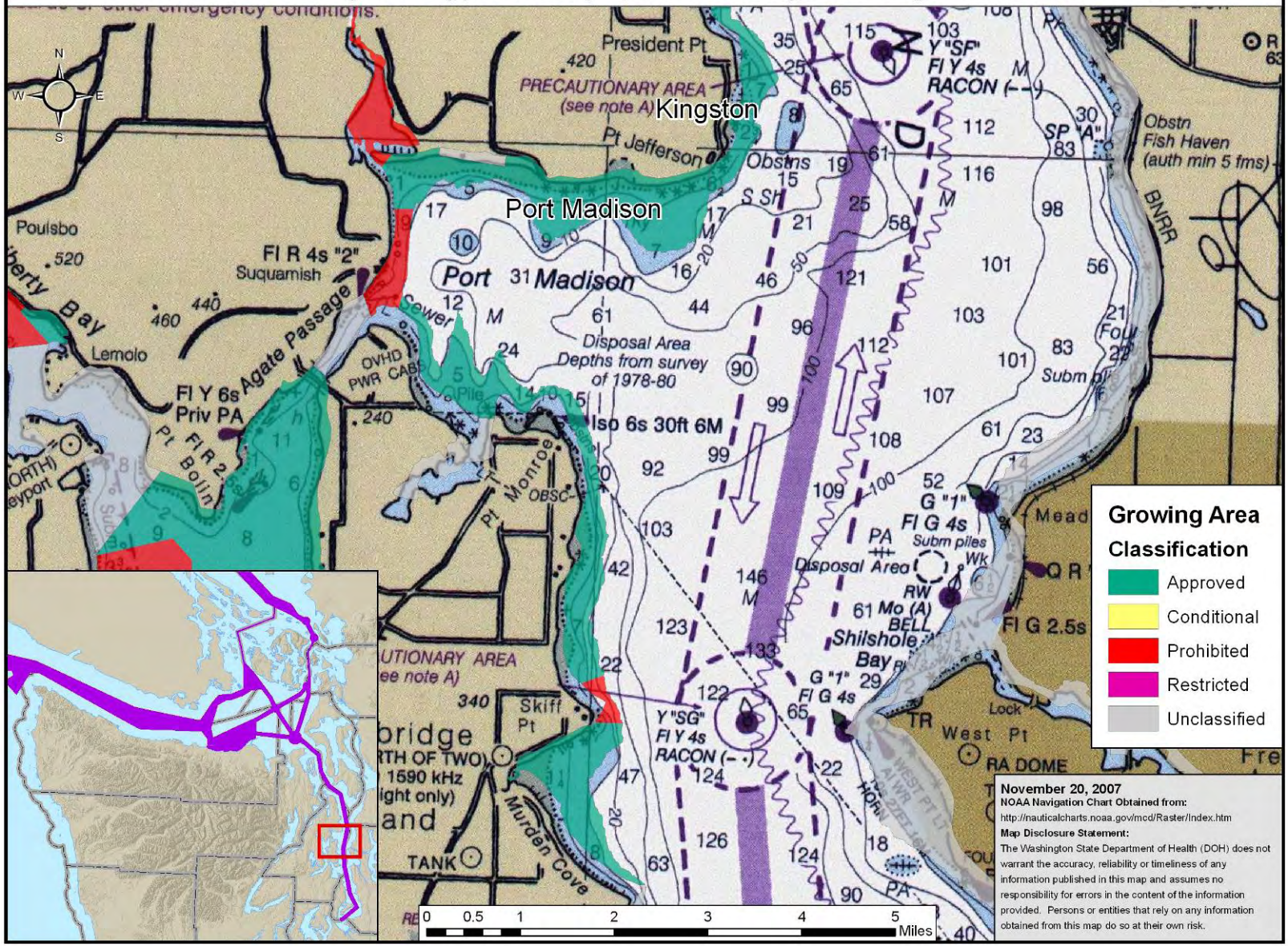


Washington State Department of Health

Dungeness Bay Growing Area Intersection with Large Passenger Vehicle Traffic Lane



Port Madison Growing Area Approximately 2,300' from Large Passenger Vehicle Traffic Lane



Washington State Department of Health

Final Report (Revised)

Quantitative Assessment of Acceptable Levels of Virus Discharge from Cruise Ships in Puget Sound

11/13/2007

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EXECUTIVE SUMMARY

Admiralty Inlet (AI) and Puget Sound (PS) are valuable resources for the harvest of shellfish by commercial fishermen, native tribes, and recreational shellfishers. These resources are potentially impacted by the Washington cruise line industry. In the last several years, the cruise line industry has grown rapidly, creating additional stresses on shellfish growing waters. Cruise ships are essentially floating cities, and their waste discharge must be considered a wastewater outfall. Shellfish are filter feeders and may bioaccumulate human enteric viruses introduced into their growing waters with discharges of human waste. These bioaccumulated viruses may be concentrated in the digestive diverticula of the shellfish and pose a risk to consumers if shellfish are consumed raw or minimally cooked. Unfortunately, most wastewater treatment processes were not designed for removal of enteric viruses. Although many treatment processes do significantly reduce effluent levels of virus, viruses may not be fully eliminated. Consequently, treated wastewater discharges from cruise ships in PS may, under upset conditions, contain significant levels of Norovirus and other enteric viruses. The levels of virus in discharged waste have not been adequately assessed, nor have an acceptable level of virus discharge been determined. Deterministic and stochastic models were developed to estimate the risk associated with shellfish harvested from water of a particular quality, and to estimate the impact of large cruise vessel wastewater discharges on water quality in shellfish growing areas within the AI and PS. The results of the proposed study identify knowledge gaps and areas in need of further research to better ascertain the risks from consumption of shellfish that are contaminated by wastewater discharges.

INTRODUCTION AND BACKGROUND

Puget Sound Description

Puget Sound (PS) is the second largest estuary in the U.S. Parts of PS can be described as a large fjord, due to the relatively narrow width of water between its shorelines. The width of the inlet between shorelines along portions of Admiralty Inlet (AI) and PS is less than four miles.

Although relatively well flushed, PS waters are not pristine. The PS corridor in Western Washington has seen large population growth (over 40 percent since 1980) and nearly two-thirds of the state's 6 million people reside around the shores of PS (PSAT, 2003a). Unfortunately, one of the major ecological impacts of the population growth is increased human wastewater discharges. Currently, more than 100 wastewater treatment plants (WWTPs) impact the waters of PS (Vasconcelos, 2002). In addition to WWTPs, a large number of non-point contamination sources may contribute significantly to the human fecal loading of PS waters, including: Land application of biosolids; On-site wastewater treatment systems; and Random discharges (e.g. shore-based fishermen) (Anonymous, 1996). Additionally, mobile point sources, such as large passenger or military vessels, and small vessels (e.g. fishing boats, private boats, day cruise vessels, etc.), may contribute significantly to fecal loading (Determan, 2003).

Viruses in Wastewater

Routine monitoring of pathogens is not feasible, due to the shear number of potential analytes, lack or limitations of available methods, time, and cost. As a result fecal indicator bacteria (e.g. fecal coliforms, *E. coli*, *Enterococci*, etc.) have traditionally been relied on as an indicator of fecal contamination and the possible presence of pathogens. Their occurrence, however, is frequently poorly correlated with the occurrence of pathogenic organisms, particularly enteric viruses.

There are >200 types of viruses that may be shed in feces. These viruses differ significantly in size and composition from indicator bacteria. It is well described that many of these viruses are more resistant to conventional wastewater treatment than most indicator bacteria and may be discharged in greater amounts than typically estimated by consideration of the type of treatment alone (Vasconcelos, 2002). Further, virus particles are more persistent in the environment than most bacterial species, and may persist in seawater for extended periods of time (Nasser et al., 2003).

Perhaps one of the more significant enteric viruse types present in conventional wastewater are the noroviruses. Noroviruses (NVs) are non-enveloped, 27-nm by 32-nm spherical particles. These particles are comprised of a single-stranded, positive sense ribonucleic acid (RNA) genome surrounded by a protein capsid. NV infection causes gastroenteritis with a common duration of illness of 1 to 3 days (Kapikian and Chanock, 1985). Noroviruses may be divided into five to seven distinct genogroups, at least three of which infect humans. Each genogroup contains numerous genotypes. Severity and duration of health effects of these viruses may vary by genotype or strain, and may also be host dependent (Lindesmith et al., 2003; Readford et al., 2004; Lindesmith et al., 2005; Hutson et al., 2005; Le Pendu et al., 2006).

NVs are estimated to be the leading cause of food-borne illness attributable to a known agent (Evans et al., 1998; Deneen et al., 2000). The number of estimated annual cases of foodborne NV gastroenteritis is 9,200,000 (Mead et al., 1999). Numerous foodborne outbreaks of NVs are well described and a wide range of foods have been implicated as the contaminated vehicle, including shellfish (in particular oysters). Three percent of reported foodborne outbreaks between 1998 and

2000 were attributable to NV in oysters (Widdowson et al., 2005). Applying the observed 3% of NV outbreaks from oysters to the estimated number of foodborne cases suggests that as many as 276,000 oyster related cases of NV might be expected per year in the United States.

Puget Sound Cruise Industry

In the last several years, the cruise industry in Washington has grown rapidly. Since 1999, the number of large passenger vessels calling on the port of Seattle has increased from 6 port calls with 6,615 passengers to more than 190 port calls with more than 750,000 passengers (including crew members) (http://www.portseattle.org/seaport/cruise/). However the Port of Seattle counts a passenger twice during a round-trip cruise: once upon departure and a second time upon return. In narrow regions of PS, passenger ships corridors are relatively close to shoreline in these areas (much less than two miles, taking the width of the corridor into account). As a result, large passenger vessels are potentially a significant source of fecal contamination to PS (Jankowiak et al., 2004).

Most large passenger vessels making port calls in PS have advanced wastewater treatment systems (AWTS) on board for treatment of black and gray waters. These systems typically offer very good treatment and are based on biological treatment, filtration and disinfection. Other large vessels (e.g. cargo ships, Navy vessels, etc.) and smaller vessels (e.g. day cruises, private vessels, etc.) more likely have Type I or Type II Marine Sanitation Devices (MSDs), depending on their size. These systems offer treatment based on maceration and disinfection and must meet specific fecal coliform (FC) effluent standards. Treatment and disinfection of ship wastewater by various advanced wastewater treatment systems, and even well-operated Type II MSDs, can achieve a good kill or removal of FCs. However, smaller microorganisms, such as enteric viruses (especially if unattached to solids), can pass through MSDs and even pores of AWTS membranes into the effluent. The United States Environmental Protection Agency (EPA) recognizes that advanced wastewater treatment systems that utilize membranes remove bacteria but do not remove all viruses (EPA/625/R-04/108). Additionally, many enteric viruses are relatively resistant to disinfection by chlorination or UV radiation (compared to FCs).

Under the 2004 memorandum of understanding (MOU) with the State of Washington, vessels with Washington State Department of Ecology (Ecology) approved AWTS are allowed to discharge waste if ≥1 nautical mile of berth and moving at ≥6 knots. Only vessels owned by Northwest Cruiseship Association (NWCA) members that are making a call at a port in Washington are subject to the MOU. Restrictions on discharges from other vessels are less well controlled. NWCA vessels make up the majority of large cruise vessel port calls in Washington.

Additionally under the 2004 MOU, a cruise ship subject to the agreement may discharge less than one mile from berth if its AWST meets more stringent treatment and monitoring standards. In 2006 four NWCA vessels were approved for continuous discharge at or within 1 nautical mile of berth. An additional seven vessels were approved by Ecology for discharge if ≥1 nautical mile of berth and moving at ≥6 knots. For the 2007 cruise season, 4 NWCA cruise ships received approval by Ecology to discharge at or within one nautical mile from berth. These same vessels are the only ones approved by Ecology for discharge in Washington waters subject to the MOU. NWCA cruise ships without approved AWTS are not allowed by the MOU to discharge treated wastewaters in the Sound.

Puget Sound Shellfish Industry

Washington State is the leading producer of farmed bivalve shellfish in the United States. Washington shellfish also provide an important sport fishing resource, with an estimated quarter of a million people harvesting shellfish from PS's beaches annually (Office of Shellfish and Water Protection, 2006). The annual recreational harvest alone is estimated at 700,000 pounds of clams and 900,000 pounds of oysters. PS is one of the richest shellfish growing areas in Washington. It serves as an important resource for commercial, tribal, and recreational shellfish harvests.

Several shellfish harvesting areas, commercial and recreational, are located on shorelines on both sides of the ship corridor in Puget Sound and Admiralty Inlet, and are potentially impacted by discharges from vessels in the corridor. These growing areas contain subtidal geoducks, public recreational beaches, tribal harvest areas, and intertidal commercial shellfish beds. For example, shellfish growing areas are located along the west shoreline of Whidbey Island, the northeast shoreline of the Kitsap Peninsula, and near Port Townsend.

The quality of shellfish is dependent upon many factors, but clean growing water is one of the most important. It is the responsibility of the Division of Environmental Health's Office of Shellfish and Water Protection (OSWP) in the Washington State Department of Health (DOH) to protect public health by classifying shellfish growing waters and establishing shellfish closure zones to prevent the consumption of potentially contaminated shellfish. Currently classification of growing waters and establishment of closure zones are based on FC concentrations and known discharges. Unfortunately, the relationship between FC concentrations and levels of enteric pathogens, particularly enteric viruses, is questionable. Studies in other locations have documented that FCs are inadequate as indicators of enteric viruses in shellfish and water (Robertson et al., 1983; Shiaris et al., 1987; Dore et al., 2000; Dore et al., 2003). Newer wastewater treatment technologies, such as membrane bioreactors, may exacerbate the inadequacies of the FC standard as they are very good at removing bacterial contamination but as a whole have not been adequately examined for viral removal/inactivation.

Viruses in Shellfish

Shellfish (in particular bivalve molluscs) are filter feeders. During feeding, large quantities of water are pulled in through an incurrent siphon, and then passed through the body cavity over the gills. Cilia tracts present on the gills filter food particles out of the water, moving nutritious particles along the labial palps towards the mouth. Once food enters the mouth, it passes through a short esophagus to the stomach. Non-nutritive particles are moved to the exhalent chamber, where they are pushed out of the body through the exhalent siphon as pseudofeces. Filtration rate is thought to be under physiological control. However, both nutrient level and temperature are critical factors affecting the filtration rate of bivalves.

Particles 3 to 30 micron range are efficiently removed during bivalve feeding. Though much smaller, viruses are frequently adsorbed to particulate matter and may incidentally accumulate during filter feeding, resulting in tissue concentrations significantly greater than present in the water column. Viruses are also believed to selectively bind to shellfish tissues and mucus, thus resisting elimination processes (DiGirolamo et al., 1977; Schwab et al., 1998; Le Guyader et al., 2006).

Uptake and elimination of viruses may differ for different types of shellfish, though accumulation has been observed in various species of clams, mussels, and oysters. Some studies suggest that shellfish may demonstrate selective accumulation of particular pathogens and may vary seasonally (Burkhardt et al., 1992; Burkhardt and Calci, 2000). Additionally, multiple virus types have been reported in the same shellfish specimen or batch of shellfish (Sugieda, et al., 1996; Kingsley et al., 2002).

Studies have demonstrated depuration rates of viruses to be much slower than FCs and other bacteria (Dore and Lees, 1995; Schwab et al., 1998; Dore et al., 2000; Lees, 2000; Dore et al., 2003). Subsequently, significant numbers of shellfish that have been compliant with FC standards (growing water and tissue) have been shown to be contaminated with viruses. Several studies have reported no correlation between viral load and bacterial concentration in bivalves (Gerba et al., 1979; Jehl-Pietri et al., 1991; Romalde et al., 2002; Muniain-Mujika, et al., 2003). Environmental factors may play a significant role in the elimination of viruses from shellfish by depuration or relaying. Temperature has frequently been identified as an important factor (Lees, 2000). Similarly, water quality parameters and the level of initial contamination levels may play a role (Cook and Ellender, 1986). The bioaccumulation of viruses in shellfish combined with the tradition of eating shellfish raw or minimally cooked creates a potential health risk for consumers.

Shellfish-related Outbreaks

Worldwide foodborne disease from shellfish is a significant public health concern (Potasman et al., 2002). More than 400 outbreaks, totaling over 14,000 reported cases, of shellfish-borne illness have been reported in the United States (Rippey, 1994). Further, reported cases of shellfish-borne illness are estimated to represent the tip of the iceberg, because the vast majority of cases are thought to go unreported, as the most common associated illness is a relatively mild gastroenteritis (Mead et al., 1999). The total number of foodborne illnesses is estimated to be 38.6 million cases of illness per year in the United States (Mead et al., 1999). Shellfish are thought to be responsible for roughly 6% of total food-borne illness, or 4.5 million cases per year (Mead et al., 1999; Wallace et al., 1999).

Viruses are the leading cause of shellfish-borne outbreaks. Each year numerous outbreaks of shellfish associated viral gastroenteritis are reported worldwide (Gill et al., 1983; Morse et al., 1986; Truman, et al., 1987; Wanke and Guerrant, 1987; Sekine et al., 1989; Desenclos et al., 1991; Halliday et al., 1991; Anonymous, 1995; Chalmers and McMillan, 1995; Dowell et al., 1995; Le Guyader et al., 1996; Luthi et al., 1996; Dalton, 1997; Lipp and Rose, 1997; Stafford et al., 1997; Wallace et al., 1999; Berg et al., 2000; Conaty et al., 2000; Godoy et al., 2000; Shieh et al., 2000; Bosch et al., 2001; Kingsley et al., 2002; Potasman et al., 2002). Noroviruses are now clearly the predominate cause of gastroenteritis associated with shellfish consumption (Lees, 2000).

According to DOH's Foodborne Outbreak Database, fourteen outbreaks (two or more linked individual illnesses) associated with consumption of seafood have been documented in the State of Washington since 2001 (Dreitzler, 2005). Six of the eleven reported outbreaks were determined to be caused by noroviruses and were linked to consumption of raw oysters. One of the remaining outbreaks, though the etiologic agent was not determined, had symptomology consistent with norovirus infection, and another one of the remaining outbreaks was caused by norovirus from an unspecified seafood based salad. In addition, at least 37 other foodborne outbreaks were reported in the same period for which no particular food item could be implicated. Seventeen of these

outbreaks were conclusively linked to norovirus or other human Caliciviruses as the etiologic agent, still others were consistent with norovirus incubation and duration periods. Norovirus outbreaks of suspected foodborne origin are reportable to DOH, while individual cases are not. It is widely accepted that reported outbreaks represent only a small fraction of actual foodborne illness (Mead et al., 1999). In addition to reported and unreported illness, at least one batch of shellfish exported from PS was rejected by the importing country due to Polymerase Chain Reaction (PCR) detection of norovirus RNA (Woolrich, 2004).

PROBLEM STATEMENT

The OSWP is required by the National Shellfish Sanitation Program (NSSP) Model Ordinance (Chapter IV@.03E(5)) to classify an area as Prohibited for shellfish harvest if adjacent to a "sewage treatment plant outfall or any other point source outfall of public health significance." The large passenger vessels are essentially moving outfalls and as a result OSWP must determine a prohibited zone. The intent of the prohibited or closure zone is the protection of public health by prevention of harvesting and consumption of contaminated shellfish. This section in the NSSP also stipulates that factors such as wastewater dilution, dispersion and the location of shellfish resources in relation to the discharge be used to determine the size of the area to be classified as "Prohibited." Additionally, the OSWP assumes an upset condition when establishing prohibited zones. The upset condition typically assumes a failure of disinfection.

Since viruses are the leading cause of shellfish related illness in Washington, the OSWP is particularly concerned with the impact of potential discharge of human enteric viruses into marine waters from passenger vessels.

This study was commissioned to assess the impact on public health of large passenger vessels discharging enteric viruses (specifically noroviruses) near shellfish beds in PS along the shipping corridor, and to inform OSWP in their establishment of a prohibited zone and classification of other potentially impacted shellfish harvesting locations.

METHODS

Risk Framework Approach

This study utilized a basic quantitative microbial risk framework to assess the potential risk to public health of viral discharges from large passenger vessels impacting shellfish. The basic framework for quantitative microbial risk assessment includes four basic components: Hazard Identification/Problem Formulation, Exposure Assessment, Health Effects Assessment, and Risk Characterization.

The simplest approach to modeling involves deterministic models. The deterministic approach utilizes point estimates for each model parameter. This approach is usually the first step in modeling an exposure scenario, but is limited in terms of the information it can provide for the parameter(s) of interest (due to their also being point estimates). A more extensive approach to modeling is the use of a probabilistic model that incorporates distributional data into a Monte Carlo analysis. This type of stochastic approach allows the explicit definition of the uncertainty and variability components of each model parameter. In so doing, these models can yield more informative results in the form of distributions rather than single point estimates. The major difficulty of the probabilistic approach is that these models require far more data than deterministic

models. Due to the limited availability of desired data for the current analysis, the modeling approach utilized in this study was semi- probabilistic in nature.

The hazard identified in this study is the potential discharge of viruses, in particular noroviruses, from large cruise vessels. The basic problem formulated in this study is depicted in Figure 1. Briefly, viruses are discharged in the effluent from a large passenger vessel. The viruses are then diluted due to the movement of the vessel and the natural circulation of the water in PS. Shellfish exposed to the water, containing the discharged viruses, bioaccumulate the viruses (Oysters were chosen as the particular shellfish for this study based on the frequency of raw consumption). The oysters are consumed, and a portion of individuals receiving an adequate dose become ill.

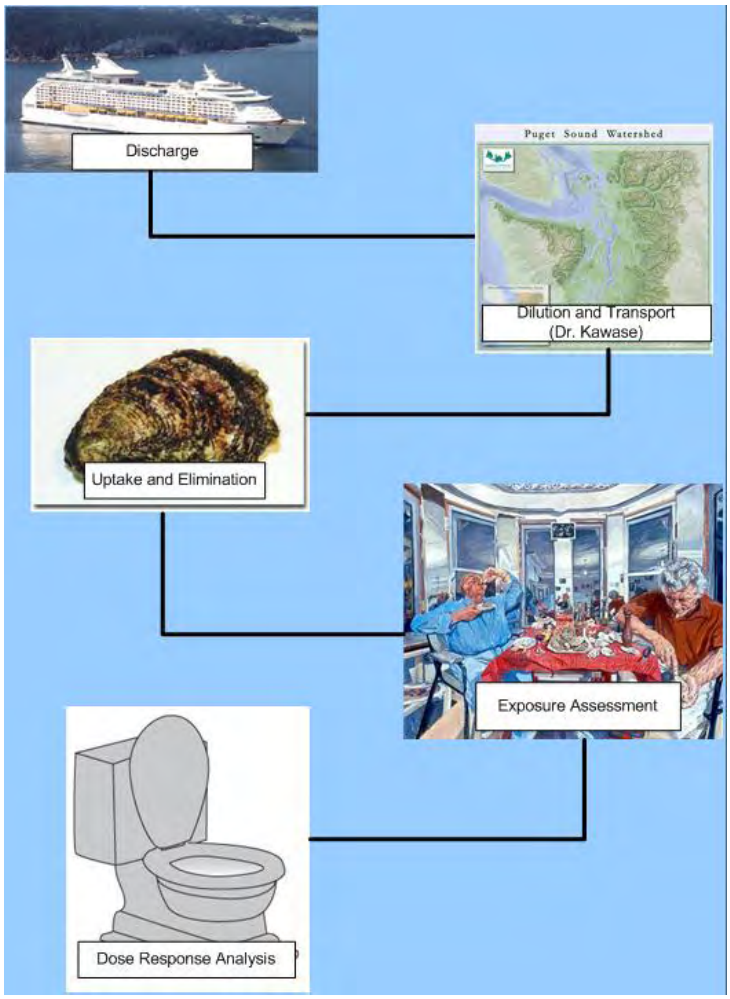


Figure 1. Problem Formulation/Hazard Identification

Risk is determined as a function of both the dose and the dose response. The purpose of the exposure assessment component is to determine the dose, while the purpose of the health effects assessment component is in part to determine the dose response.

In this case, dose may be considered to be a function of water quality (C_w), bioaccumulation of viruses in the shellfish (BAF), and the shellfish consumption rate (CR) (**Equation [1]**). For initial estimates of magnitude of risk, water quality was assumed. In subsequent models water quality was modeled based on source loadings, dilution, and virus survival. Bioaccumulation of viruses in shellfish from growing waters was based on previously published studies examining uptake and elimination of viruses from shellfish tissue. Shellfish consumption rates were derived from previously reported studies on Northwest Tribes, Asian and Pacific Islanders, and other population groups.

Equation [1] : dose

$$\text{dose}\left[\frac{\#\text{ of viruses}}{\text{event}}\right] = C_w \cdot \text{BAF} \cdot \text{CR}$$

Unfortunately very little actual dose response data are available for Noroviruses, and the data that are available were obtained at administered doses that are considerably larger than would be expected for environmental exposures. However, the minimum infectious dose for noroviruses has been estimated previously as 10-100 viruses, and this information has been used to fit an exponential model describing dose response (Masago et al., 2006). A similar approach is used in this study to characterize the risk of infection from shellfish related doses (**Equation [2]**). The equation may be rearranged to solve for the constant, λ, based on estimates of the dose for which 50% of the individuals exposed will be infected (ID_{50}).

Equation [2] : dose response (exponential model)

$$\frac{\text{risk}}{\text{event}} = P_j = 1 - \exp\left(-\lambda \times Dose\right)$$

The risk characterization component involves the integration of the dose and dose response models to obtain a quantifiable estimate of risk. The event risk can then be extrapolated to an annual risk (**Equation [3]**).

Equation [3] : annual risk

$$\frac{\text{risk}}{\text{year}} = 1 - \prod_{j=1}^{i}\left(1 - P_j\right)$$

Note on Selection Of Data For Models

Data were collected from numerous studies representing each aspect of the constructed models. Norovirus-specific data were selected whenever possible. However, Norovirus remains non-culturable in both animal and tissue culture models. As a result there is a limited amount of environmental data that is "norovirus-specific". In effort to fill gaps, data from common norovirus surrogates were used as necessary and applicable. The common norovirus surrogates include vesiviruses (e.g. San Miguel Sea Lion virus (SMSV), feline calicivirus (FCV), canine calicivirus (CaCV)), enteroviruses (poliovirus (PV), coxsackie (CV), and echoviruses (EV)), and coliphage (F+RNA coliphage (e.g. MS2)). All of these surrogates are similar to norovirus in size, shape, and type of nucleic acid. All are non-enveloped. The vesiviruses and F+ RNA coliphage are similar in that like norovirus, they have only a single major capsid protein. The Enteroviruses are more complex with four capsid proteins. The reason for inclusion of the Enteroviruses, especially poliovirus, is that they are among the best studied viruses in terms of environmental fate transport and occurrence, and have been shown to be similar in terms of survival and persistence to noroviruses. The vesiviruses would seem to make the most logical choice as surrogates for the noroviruses, since they are similarly members of the Caliciviridae. However the vesiviruses are a diverse genera of viruses that may be responsible for a variety of illness types in a wide range of hosts. SMSV is a virus of California Sea Lions causing vesicular lesions. FCV is a respiratory virus of felids and CaCV is an enteric virus of canids. The F+ RNA coliphage (leviviridae) are viruses that are commonly found in the feces of many animal species and infect the coliform bacteria. They are similar in size and shape to many human enteric viruses and have been proposed as both process and general indicators for human enteric viruses.

Risk Model

In order to estimate the magnitude of risk from consumption of oysters harvested from water of a particular quality, a 1-D stochastic risk model (in which uncertainty due to ignorance and natural population variability were lumped for consideration) was developed based on Equations 1-3 for assumed water qualities (1 virus in 100L to 1 virus in 100,000 L).

Bioaccumulation in Shellfish.

Numerous studies were reviewed in effort to perform a meta-analysis to derive distributional data for rates of virus uptake and elimination. Unfortunately, the methods used in the studies evaluated were inconsistent and the results were similarly presented inconsistently between studies, thus preventing meta-analysis. In the end a determination to use bioaccumulation factors rather than uptake rates was made. Bioaccumulation factors were determined for five studies, with shellfish tissue concentrations ranging from 3 to 1000 times the viral concentration in the water (Canzonier et al., 1971; Bedford et al., 1978; Metcalf et al., 1979; Enriquez et al., 1992; and Burkhardt et al., 2000). Other studies were excluded from consideration based on methodological considerations, or incompatibility in the presentation of the data. Studies considered reflect multiple shellfish (oysters, clams, mussels) and virus (F+ RNA coliphage, poliovirus, reovirus, and hepatitis A virus) types, however no norovirus (or other calicivirus) data was available.

Shellfish Consumption Rate.

Shellfish consumption data were considered from several sources (Degner et al., 1994; Toy et al., 1996; Sechena et al., 1999; Duncan et al., 2000) representing consumption in the general population as well as specific population groups (e.g. Native American and Asian-Pacific Islander)

that have previously been identified as having consumption rates disparate from the general population. Frequency and total annual consumption of shellfish among the Suquamish Tribe of the Port Madison Reservation was considerably greater that that of other populations considered (Duncan et al., 2000). Consumption of shellfish among the Vietnamese population was also considerably greater than the general population (Sechena et al., 1999). For this study's model, data from the Suquamish Tribe was determined to represent the most relevant data, due to magnitude and frequency of consumption, and the presence of tribal harvesting areas in the region potentially impacted by large cruise vessels. For the purposes of the model, the oyster consumption rate (g/event) was described as a log normal distribution with a 50th percentile value of 180g and a 90th percentile value of 477g. The number of consumption events per year were described as a log normal distribution with a 50th percentile value of 8 events/year and a 90th percentile value of 46 events/year.

Dose Response.

Very little human dose response data is available for noroviruses. Early Norwalk virus dose response studies were performed using poorly quantified high doses, and when plotted the dose response curves appear as a near horizontal line (Dolin et al., 1971; Wyatt et al., 1974; Graham et al., 1994). Extrapolation to lower, more realistic environmental doses was difficult. More recently human infectivity trials have been performed by Dr. Christine Moe (Emory University) using RT-PCR quantified doses of Norwalk virus and Snow Mountain virus. These studies examined a better range of doses. However, these data have not yet been published in the peer reviewed literature. The minimum infectious does for noroviruses is expected to be very low, and has been estimated at 10-100 virus particles (Moe, et al., 1998; Masago et al., 2006). In a previous risk assessment, Masago et al., used an estimate of 10-100 virus particles as a conservative estimate for the ID_{50} for noroviruses. This estimate is likely overly conservative, as acknowledged by the authors, and a more realistic ID_{50} probably falls within the range of 100 to 10,000 virus particles. The dose response model utilized in this study is based on the approach of Masago et al., which used a simple exponential model, modified for a more realistic ID_{50} of 100 to 10,000 viruses (see **Equation [2]**), where λ =0.0069 or 0.000069. Further health effects assessment is not possible at this time.

Water Quality Models

While the initial intent was to linearly couple the water quality model to the risk characterization model, it was found that decoupling the water quality model from the bioaccumulation, consumption rate, and dose response was a more appropriate approach. This was due in part to limitations in the available temporal and geospatial resolution of the harvest and consumption data, and incompatibility of the hydrodynamic flow model, used to calculate far field dilution, with the calculation of annual risk.

Two distinct models were developed in this study to characterize water quality resulting from norovirus discharges from large passenger vessels. The models used similar assumptions in terms of virus loadings associated with individuals (e.g. stool concentration, stool volume, etc). However, the models diverged in the primarily manner in which dilution was handled. One model determines dilution based on a generalized box circulation model of PS developed by Dr. Kawase's group at UW Department of Oceanography (Babson et al., 2006). This model, hereafter referred to as the box model, stochastically considered non-outbreak virus loadings (including shipboard and land-based) and deterministically evaluated dilution and die-off. The benefit of this

model was that it allowed the impacts of large cruise vessel discharges to be expressed in terms relative to discharges of land based systems. The shortcoming of this method is that since it is based on seasonal average conditions that it likely underestimates the potential impact of large cruise vessels under transient conditions. Further, it does not allow direct estimate of the annual risk associated with large cruise vessel discharges.

As a result of this shortcoming, a second model was also developed using dilution factors obtained from a more sophisticated "ship-to-shore" transport model also developed by Dr. Kawase's research group to determine far-field dilution (Sarason, 2006). This model, hereafter referred to as the transient model, stochastically estimated virus concentration at in PS resulting from large cruise vessel discharges, independently considering near-field and far-field dilution. This model was run for both outbreak and non-outbreak conditions. The application of this model was limited by temporal and spatial resolution (e.g only three shore regions representing the most adverse case locations identified were modeled in detail). In addition, complementary data on shellfish harvesting and consumption rates that would be necessary to predict event risk are not available.

Virus Loading.
The virus loading is a function of the number of symptomatic and asymptomatic individuals shedding virus, the concentration of virus in stool, the volume and frequency of stools per individual.

Number of Individuals Shedding.
Incubation Period**.** The typical incubation period for development of clinical symptoms during a norovirus outbreak is between 24 to 72 hours with a central tendency of ~30 hours (Linco and Grohman, 1980; Kapikian and Chanock, 1985; Morse et al, 1986; Leers et al.,1987; Hirakata et al., 2005) . However, incubation times of as little at 4 hours and as great as 7 days have been reported. Clinical illness from norovirus infection is an acute gastroenteritis that results in a high level of diarrhea and vomiting. Several studies have indicated that peak shedding typically occurs within 72 hours of the onset of symptoms (Thornhill et al., 1975; Graham et al., 1994). Prolonged shedding has been demonstrated in numerous cases, with shedding up to 3 weeks being common, and a majority of individuals shedding at 8 days post onset (Okhuysen et al., 1995; Marshall et al., 2001; Moe et al., 2001; Rockx et al., 2002; Goller et al., 2004). Okhuysen et al. found 70-80% of volunteers that developed symptoms were shedding virus after one week (Okhuysen et al., 1995). Extreme shedding to >100 days has also been reported (Gallimore et al., 2004; Kaufman et al., 2005; Simon et al., 2006). Studies have also indicated that noroviral shedding may precede onset of symptoms, and may extend well beyond cessation of symptoms (White et al., 1986; Lo et al., 1994; Okhuysen et al., 1995; Marshall et al., 2001). Based on the incubation period and duration of shedding common of norovirus, it is very likely that anyone infected during a typical 7 day sailing from Seattle will shed virus for the duration of the trip. Further those infected after the third day of the trip are likely to be shedding at their peak rates.

Symptomatic. According to CDC reports since 2000, eighteen norovirus (or presumptively norovirus) outbreaks have occurred on large cruise vessels (≥500 passengers) in the Pacific Northwest. The mean number of ill individuals on an outbreak cruise was 140 (±122). Thirteen of the eighteen outbreaks occurred on vessels that do or have made port in Seattle in the last three years. For four of the outbreaks the port of arrival was reported as Seattle. The average number of ill individuals on those cruises was 115 (std. dev. 25; range of 77-131). This was represented in

the box model by assuming an average symptomatic illness rate of 5% and assuming a custom distribution for the number of individuals based on the capacity of vessels sailing from the Port of Seattle. For the box model, the number of symptomatic shedders on a non-outbreak cruise was estimated based on the daily per capita incidence rate according to estimates of the annual number of norovirus cases (Mead et al., 1999) adjusted for typical duration of illness. However, this was determined to likely underestimate the number of cases on cruises that do not meet the CDC investigation threshold (3%) for cruise vessel outbreaks. As a result, for the transient model the number of symptomatic shedders on non-outbreak cruises was assumed to be 0 to 2.9% of passengers and crew on board.

Asymptomatic. Studies have demonstrated a high level of asymptomatic shedding in norovirus outbreaks. Reports of 25-35% of shedding individuals have been reported to be asymptomatic (Graham et al., 1994; Gallimore et al., 2004). In a volunteer study, shedding was observed after 7 days for 40% of asymptomatic shedders (Okhuysen et al). However the level of virus excreted by asymptomatic individuals may be assumed to be significantly less than that observed for symptomatic individuals. For the purposes of the transient model, asymptomatic shedding was ignored, on the assumption that it was insignificant relevant to symptomatic shedding in terms of total virus excretion. For the estimation of background levels using the box model, the number of individuals asymptomatically shedding was assumed to be consistent with the community prevalence. Community prevalence of asymptomatic norovirus infection has been estimated to be around 1% (0.3 to 1.1%) (Radford et al). This will be represented in the loadings portion of the box model using a triangular distribution from 0.3 to 1.1% with a mode of 0.8%.

Shedding Level.

Symptomatic. Historically, the typical level of shedding observed in norovirus infections was on the order of 10^6 virions per milliliter (ml) (or gram) of stool (Kapikian and Chanock, 1985). However the shedding level was determined based on electron microscopy, which may not provide accurate quantitation. This level was also supported by early PCR methods on stools from patients infected with the same strain (generally Norwalk virus, the genogroup 1 type strain). More recently using PCR-based methods, Hohne and Schrier (2004) reported on the shedding levels in two groups of samples: 1) German outbreak samples, and 2) European samples. The range of shedding observed in the German outbreak samples (n=66) ranged from 10^2 to 10^{10} with a median of 1.14×10^7 genomic equivalents per ml of stool suspension. Further the distribution appeared to be nearly uniform. Similarly the range of shedding in the European stool panel (n=31) was 10^1 to 3.32×10^{10}, with a mean of 7.14×10^7 genomic equivalents per ml of stool suspension. In contrast to the German outbreak panel, the distribution of the shedding in the European panel appeared to be bimodal, with peaks between 10^5-10^6 and 10^8-10^9. This is consistent with another recent study by Chan et al. (2006) examining the norovirus shedding levels in patient stools in Hong Kong within 48 hours of development of symptoms over a one year period. Although the range of shedding was similar between viruses from genogroup 1 and genogroup2 (10^4 to high 10^{10} virus copies per ml), the median shedding level for genogroup I noroviruses (n=14) was 8.4×10^5 virus copies per ml, while the median shedding level for genogroup II noroviruses (n=46) was 3×10^8 virus copies per ml. For this study, we will use a custom distribution based on the scatterplots of detected shedding levels from Chan et al. (2006) to represent virus shedding level. This choice is consistent with recent studies and more likely to reflect the shedding rates associated with currently circulating strains compared to older shedding estimates.

Asymptomatic. Although asymptomatic shedding of norovirus is well-documented, the level of asymptomatic shedding has not been adequately characterized. A few case reports suggest that asymptomatic shedding may be as high as 10^5 to 10^6 viruses per ml of stool (Marshall et al., 2001; Hohne and Schrier, 2004). However, these case reports have involved the elderly or very young and have followed several days after cessation of clinical symptoms. In contrast another study suggested that the level of asymptomatic shedding was very low, as demonstrated by nested Reverse Transcription Polymerase Chain Reaction (RT-PCR) detection only (Gallimore et al., 2004). Considering the pathogenesis of norovirus, we can assume that asymptomatic shedding is considerably lower than symptomatic shedding. For the purposes of the models, a uniform distribution ranging from 10^0 to 10^6 virions per ml will be used to represent asymptomatic shedding for norovirus in an adult population.

Stool Frequency and Volume.

Symptomatic. In a volunteer study, the mean number of stools of symptomatic individuals varied over the course of infection (Graham et al., 1994). The mean number of bowel movements was 0.4, 1.8, 3.9, and 1.6 for 0, 1, 2, and 3 days post infection, respectively. Asssuming a 24-48 hour incubation period, this is consistent with another study examining the stool frequency in symptomatic elderly (Goller et al., 2004). This study found that in the first day of symptoms the mean number of bowel movements was 5.9, with a range of 3-10. By the 3-4 day of illness the number of stools had dropped to a mean of 0.75 with a range of 0-2. For the remainder of the period of observation (26 days), the mean number of stools ranged from 1-2, with a range of 0-4. The typical volume of stool for an individual with symptomatic norovirus has not been characterized. Acute diarrhea, as would be consistent with symptomatic norovirus infection, is characterized by >200g/day of stool (Gillies et al., 2000). With other enteric pathogens infecting the same region of intestinal tract, high volume diarrhea (up to several liters/day for non-cholera organisms) is possible. In case studies on transplant patients, noroviruses have been linked to high-volume diarrhea (4-6 liters per day) (Kaufman et al., 2003; Morotti et al 2004). However, transplant patients are immunosuppressed and perhaps represent an unrealistic extreme. For the model, daily stool volume for symptomatic individuals will be represented by a triangular distribution ranging from 0 to 5000 ml/day, with a mode of 1500 ml/day.

Asymptomatic. In contrast to the symptomatic individuals in the volunteer study, the mean number of stools for asymptomatic individuals ranged from 0.5 to 1.3 bowel movements per day (Graham et al., 1994). Typical volume of a normal stool is ~100ml (~200g/day) (Gillies et al., 2000). For the current model, daily stool volume in asymptomatic individuals will be distributed triangularly from 0 to 600 ml/day, with a mode of 200 ml/day.

Land-based Virus Inputs and Treatment.

For the purposes of the box model, loadings from land based imputs were considered. The majority of the inputs from land-based wastewater treatment systems discharging to Puget Sound come from secondary treatment systems (Vasconcelos, 2002). Most of these systems employ an activated sludge or comparable biological process prior to secondary clarification and disinfection. The land-based WWTP input component for a particular box was determined by multiplying the volumetric discharge from the WWTPs by an estimated virus concentration in the WWTP effluents. The virus concentration in WWTP effluents was estimated stochastically based on the annual estimated case of norovirus infection (Mead et al., 1999) adjusted for symptomatic and asymptomatic virus loading, as reduced based on typical virus removals for secondary treatment

and chlorine disinfection. Volumetric discharge to each box was estimated based on reported discharge rates for WWTPs discharging into Main Basin or South Sound. Estimates of volumetric discharge are 150, 30 and 200 million of gallons per day (MGD) for the surfaces box of the South Sound, surface box of the Main Basin, and the deep box of the Main Basin, respectively. Predicted levels of virus in the Main Basin were not sensitive to assumed virus inputs from AI below a level of 1 virus in 1000L, when land based inputs were considered. Land-based WWTP viral reductions were assumed to be 1.5 $\log_{10}$ by activated sludge (or similar secondary treatment) and 2 $\log_{10}$ for chlorine disinfection (Maier et al., 2000; Metcalf and Eddy, 2003).

Large Cruise Vessel Virus Inputs.

The cruise vessel virus inputs were estimated for both background and outbreak scenarios. Estimates of vessel based inputs are based on the annual number of port calls in Seattle, duration in the shipping corridor, virus loading to the ship's WWTP and efficacy of shipboard treatment. The inputs from large cruise vessels may vary significantly between outbreak and non-outbreak (background) scenarios. Therefore a relative input for each scenario is calculated for the box model.

Port Calls. The average duration of the cruise season for the Port of Seattle is ~180 days. The season begins at the end of April and extends in to the end of October or early November. In 2006, 196 calls to the Port of Seattle were made by large cruise vessels. For the 2007 season, a total of 191 calls on port are scheduled. The typical weekly pattern of port calls during the cruise season is three consecutive days of three cruise vessel port calls each followed by 4 days of no traffic. For the purposes of the model 200 non-outbreak port calls were assumed. A total of 4 outbreak port calls were assumed, based on the greatest annual number of outbreaks reported for the Port of Seattle.

Duration in Shipping Corridor. Cruise vessels typically travel through the PS shipping corridor as rapidly as vessel traffic and safety will allow. This translates to an average of ~15 hours in PS per port call with much of this time at berth. For the purposes of the box model, a triangular distribution was assumed to represent the time an individual vessel was present in the PS shipping corridor (minimum of 12 hours; likeliest of 15 hours; and maximum of 18 hours).

Shipboard Treatment. Virus loading to the shipboard WWTP is a function of the number of persons onboard, the % of individuals shedding, the level at which individuals are shedding, and the volume and frequency of bowel movements.

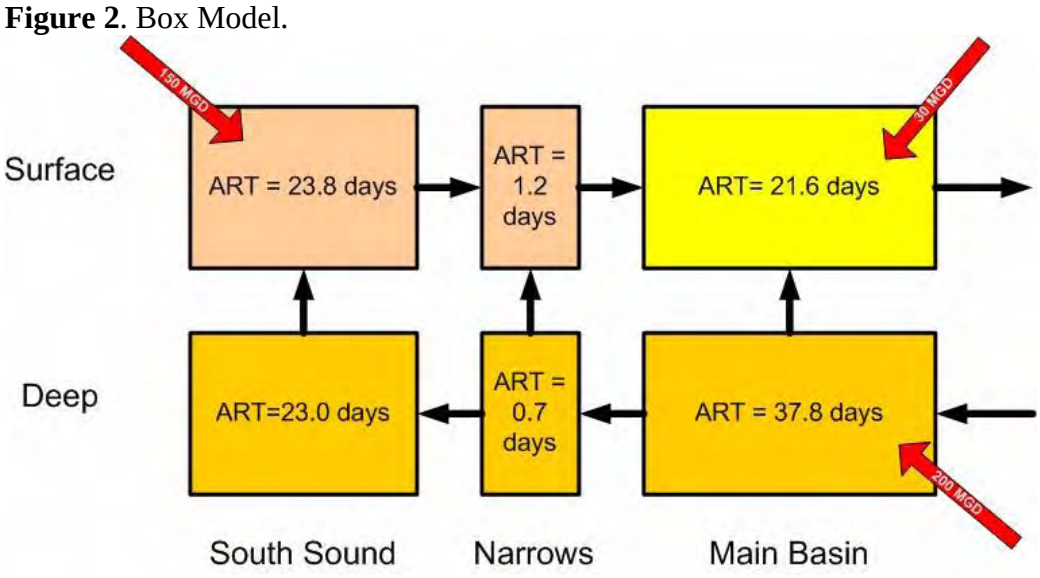
There are four main types of AWTS presently used on the NWCA cruise ships, including systems by Zenon, Rochem, Scanship, and Hamworthy. The Zenon AWTS is a membrane bioreactor system, which incorporates a biological stabilization process followed by ultrafiltration and UV disinfection. Like the Zenon system, the Hamworthy system is a membrane bioreactor system. The system includes screening via a filter press, biological stabilization in bioreactors with interstage filters, an ultrafiltration membrane, and ultraviolet disinfection. The Rochem system is a bioreactor system coupled with a reverse osmosis or ultrafiltration membrane. The system includes prefiltration, biological stabilization via bioreactors, ultrafiltration, and UV disinfection. The Scanship system also incorporates a biological reactor, but rather than ultrafiltration it incorporates flocculation/dissolved air flotation. The system includes prefiltration, biological

treatment via fixed film biofilm, chemical flocculation, dissolved air flotation, polishing filtration, and UV disinfection.

For this study, a uniform distribution of 0.5 to 2.0 $\log_{10}$ reduction was assumed for the biological treatment provided by each system (Maier et al., 2000; Metcalf and Eddy, 2003). Additional reduction (1-2 $\log_{10}$, uniform distribution) was assumed for ultrafiltration (Metcalf and Eddy, 2003). No reduction was assumed for prefiltration or microfiltration. A reduction of 0.5 to 1.5 log10 (uniform distribution) was assumed for flocculation/dissolved air flotation (NRC, 1997). A reduction of 4 $\log_{10}$ was assumed for UV disinfection under treatment conditions common for ship-based treatment systems (Thurston-Enriquez et al., 2003). No reduction from disinfection was credited for an "upset" condition. These reductions were derived from previously published studies.

Box Model.
In this adapted model, PS is divided into three regions, representing the Main Basin, Narrows, and South Sound (**Figure 2.**). Each of these regions is then divided into surface and deep boxes between which circulation can be modeled. According to the circulation model, 100% of flow into PS through AI enters the deep box of the main basin; from the deep box of the main basin most of the flow is into the surface box of the main basin (73%) with the remainder being drawn into the deep box of the Narrows; 82%of the flow from the deep box of the Narrows enters the deep box of the South Sound with the remainder entering the surface box of the Narrows; and the flow from the deep box of the South Sound enters the surface box of the South Sound then entirely flows through the surface box of the Narrows to the surface box of the Main Basin and then out through the surface of AI. The model does account for additional freshwater inputs to the surface boxes. Residence times estimated for the deep boxes are 37.8, 0.7, 23.0 days for the Main Basin, Narrows, and South Basin, respectively. For the surface boxes, residence times were modeled at 21.6, 1.2, 23.8 for the Main Basin, Narrows, and South Basin, respectively. Red arrows in Figure 2 reflect wastewater inputs into the respective boxes.

Figure 2. Box Model.



Equation 4. represents a generalized estimate of the steady state concentration of viruses in any box.

A steady state mass balance of the general form:

$$Q \cdot (C_{influent} - C_{effluent}) - k \cdot C_{effluent} \cdot V + E = 0$$

in which Q [L/day] is volumetric flow through the compartment, $C_{influent}$ [#/L] is virus concentration in entering water flow, $C_{effluent}$ [#/L] is the viral concentration in the compartment, k is decay rate [day^{-1}], V is compartment volume [L], and E is viral input from land-based wastewater treatment plants [#/day], is solved for each compartment.

Survival.
In general very little information regarding virus survival in shellfish tissues has been reported. One study examined the survival of Feline Calicivirus in seawater, and found the virus to be very stable (≤1 to 2 $\log_{10}$ reduction at 10ºC; and 2-3 $\log_{10}$ reduction at 20ºC over a period of 20 days). However, the study was performed in filter sterilized seawater and thus severely overestimates the stability of virus (Kadoi and Kadoi, 2001). Another study examining the survival of poliovirus in Olympia Oysters determined that at 5ºC 40% of seeded virus persisted in oyster tissues after 15 days (DiGirolamo et al., 1970). The study found near 2 $\log_{10}$ reduction of virus after 30 days. Perhaps the most relevant study examined, the survival of Poliovirus in sediments and seawater found reductions of 1 $\log_{10}$ reduction after 4 days in seawater and 7 days in sediments (Landry et al., 1983). By 15 days, poliovirus reductions had reached 3 $\log_{10}$ and 1.7 $\log_{10}$ in seawater and sediment, respectively. Fitting a curve to the plot of these reduction values allows a rate constant of 0.5 day^{-1} was determined for inactivation of Poliovirus in Seawater of comparable temperature to PS summer temperatures. A rate constant of 0.3 day^{-1} was determined for sediment. For the purposes of the models, a rate constant of 0.5 day^{-1} was assumed for norovirus in the surface boxes of the box model, and a rate constant of 0.3 day^{-1} was used for the deep boxes, based on a assumption that the cooler temperatures of the deep boxes would result in a slower inactivation rate, but that the rate was unlikely to be slower than observed for sediment in the Landry (1983) study. Virus survival was not considered for the transient model based on the assumption that the viruses were stable over the short time-frame considered in the model.

Transient Model.
The transient model uses stochastic estimates of virus loading coupled with either static or stochastic estimates of the near-field and far-field dilution. This model allows us to consider water quality on a more transient time scale.

Near Field Dilution.
Loehr et al. (2006) derived estimates of the near-field dilution of passenger vessel wastewater effluent in the open waters off Alaska by considering the vessel's width, draft, speed, and the volume discharge rate. This study derived near-field dilution estimates that ranged from 100000X to 270,000X at 6 knots, and 200,000X to 530,000X at 12 knots. This dilution derivation for near-field dispersion assumes a complete mixing of the wastewater effluent into the cross-sectional area of the passenger ship, accomplished by mixing provided through the large twin propellers of the ship.

In a Florida study examining the near-field dilution of wastewater effluent for passenger vessels using tracing dyes and fluorimety, EPA reported near-field dilution factors ranging from 195,000X

to 643,000X. However, the results of the EPA study were reported as averaged dilutions, whereas the NSSP standards for establishing closure zone boundaries applies to minimum (rather than averaged) plume dilutions. Reanalyzing the data from the EPA study to determine highest dye concentration (thus minimum dilution), results in near-field dilution estimates of 27,700X for the three propeller vessel and 104,300X for the two propeller vessels included in the study (Merriwether, 2007).

Questions were raised over the lower numbers for the three propeller vessel and suggestions were made that it should be eliminated it if it was an unusual propulsion system for vessels entering PS. However at the 2006 MOU meeting at the Port of Seattle, when preliminary results of this assessment were presented, it was revealed by members of the NWCA during the question and answer session that some vessels that enter PS do use a three propeller propulsion system. As a result, a near-field dilution factor of 30,000X was used for initial static runs of the transient model. While for the initial stochastic runs, the near-field dilution was represented by a uniform distribution from 30,000X to 200,000X (a value twice that of the minimal dilution observed for the two propeller vessels in order to represent the range of minimal dilutions for individual vessels).

Additional arguments were raised for the rejection of the three propeller vessel data based on an incomplete mixing of the dye in the tank prior to discharge during the EPA plume tracking study (EPA, 2002). This may have been appropriate for estimation of average near field dilution. However there is no guarantee that waste with in the tanks is homogenous with regard to viral concentration prior to discharge. In fact significant aggregation of the viruses would be expected in the waste stream (though the level of aggregation would be limited by the pore size of the membranes). However for the sake of comparison, the static runs of the model were repeated with a minimal near field dilution of 105,000X, and the stochastic model runs were repeated using a range of dilution from 105,000X to a dilution factor of 1,000,000X suggested by Lincoln Loehr as a more appropriate representation of near field dilution (personal communication).

Two additional concerns regarding the near field dilution data should be noted: first, the equations were derived from a relative small data set; and secondly the EPA data was generated at much higher discharge rates than the vessels would typically be discharging in PS (in fact the rates look higher than what seems to be the maximum discharge possible according to the department of ecology vessel reports; are these bilge discharges in addition to WW discharges), as a result there is uncertainty as to whether the dilution numbers are scalable to typical discharge levels. It is our general view that the precautionary principle suggest that the uncertainty associated with near field dilution be conservatively considered.

Far Field Dilution.

Far field dilution was modeled based on dilution factors derived from a complex “ship to shore” hydrodynamic model (Saranson et al., 2006; Appendix B). The “ship to shore” hydrodynamic model predicted dilution occurring at three locations in PS over a time course. Dilution factors ranged from ~50X to >1700X. The model found that peak concentrations of discharges from a vessel reached the shore within 4-8 hrs of release (~50X at Point Jefferson). The model also found that following the peak ebb tide that concentrations fall to ~1000X. Previous studies on virus bioaccumulation have typically examined constant or steady state levels of virus challenge, where as the “Ship to Shore” dilution model (from which the far-field dilution factor was derived) predicts an ephemeral exposure of 4 to 8 hours at elevated concentrations of virus before the tidal

cycle and circulation patterns significantly reduce the concentrations. For the purposes of the transient model, 50X is used as the dilution factor for the static runs (which was intended to model the dilution during peak shoreline concentration at the most impacted location), and a triangular distribution ranging from 50-2000 (mode of 750) is used for the stochastic runs (which were intended to represent the range of far field dilution at peak shoreline concentrations in the impacted corridor).

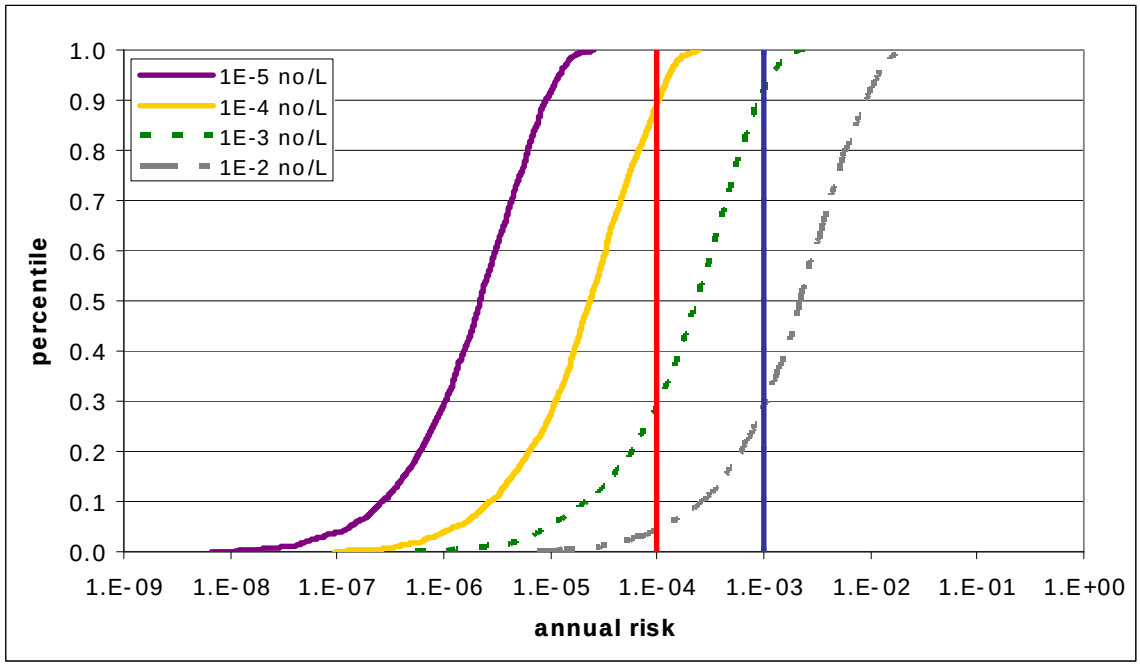
RESULTS

Risk Model

Results from 2000 trials of the risk model run are shown in Figure 3. The results of the risk model indicate predicted annual risk of norovirus illness for shellfish consumers whose consumption pattern matches that reported for members of the Suquamish Tribe, and who only consume shellfish grown in water of a specified quality. Oyster consumption rates for the general population are reported to be more than an order of magnitude less than that of the Suquamish Tribe. This translates to roughly an order of magnitude less annual risk.

There are no formally established accepted risks for norovirus infection as a consequence of shellfish consumption. In fact very few acceptable risks for any type of microbial exposure have been well defined. However, a 1 in 10,000 annual risk, below which EPA has defined as acceptable for microbial exposures in drinking water, is represented by a red vertical line (NPDWR, 1989). It is suspected that the level of risk that shellfish consumers are willing to accept is considerably higher than a 1 in 10,000 annual risk. Based on the current estimated number of cases of norovirus arising from raw oyster consumption annually divided by the US population, the annual risk of norovirus illness for oyster consumers may be currently estimated at 1 in 1000 (represented as a blue vertical line in Figure 3.). Since only a fraction of the population actually consumes shellfish, the actual annual risk of norovirus illness is greater than 1 in 1000 for raw oyster consumers.

Figure 3. Predicted annual population risk for a native American population (Suquamish tribe) using an exponential dose-response curves based on a ID50 of 100 to 10000 viruses (uncertainty bounds are not shown because they would overlap adjacent risk curves).



Box Model

Assuming no upset condition (i.e. treatment, including disinfection is working properly) and typical community levels of non-outbreak symptomatic and asymptomatic norovirus shedding, the results of the box model suggest that the steady state concentration of viruses in the surface box of the main basin would be 2.2 x 10^{-4} viruses per ml considering land-based and large cruise vessel discharges. Oysters grown in water of this quality would be expected to carry a median annual risk of approximately 1 in 50000 for NV and a 90th percentile risk of approximately 1 in 2000 for NV for consumers with consumption patterns similar to that reported for the Suquamish Tribe (provided that oysters grown in this water quality represented all the oysters they consumed over a year). Relative to the land-based discharges the overall magnitude of cruise vessel discharges was small. Based on the box model, only the surface box of the main basin is impacted by discharges from large cruise vessels. Considering only the inputs from large cruise vessels with properly functioning systems, the median predicted water concentration in the surface box of the main basin is 3 x 10^{-7} to 8 x 10^{-7} per liter for systems employing ultrafiltration and dissolved air flotation prior to UV disinfection, respectively. Oysters grown in water of this quality would be expected to carry a median annual risk of much less than 1 in ten million for consumers with consumption patterns similar to that reported for the Suquamish Tribe (provided that oysters grown in this water quality represented all the oysters they consumed over a year and that all oysters were consumed raw). This model calculates a steady state concentration based on inputs averaged over the entire season and does not consider geospatial variation within a box. As a result the concentrations predicted by this model likely over predict the concentration in some less impacted areas, while under predicting the virus concentrations at highly impacted areas. Still these results might be considered as a "baseline" by which to evaluate the impacts of specific scenarios predicted by the transient model.

On a side note the model predicts the virus concentration in the surface boxes of the Narrows and South Basin and the deep box of the Main Basin as an order of magnitude higher due to the large land-based inputs in these boxes.

Table 1. Steady state virus concentrations in Puget Sound boxes

Babson Model Boxes	Steady State Virus Concentration
Surface	**(#/L)**
Main	2.2E-04
Narrows	1.4E-03
S Sound	2.3E-03
Deep	**(#/L)**
Main	1.0E-03
Narrows	8.3E-04
S Sound	8.4E-05

Main Basin (only) Box Model

The adaptation of the PS box model was further simplified to consider only the main basin and inputs from the large cruise vessels alone. This was done in effort to estimate a maximal (steady state) dilution factor. The steady state dilution factor calculated was dependent on the number of boats and concurrent discharge rates the Main Basin at a given time and ranged from 4×10^6 for a single boat discharging at 18 m^3/hr to 9×10^5 for three boats discharging at 30 m^3/hr (Jankowiak et al., 2004; Amy Jankowiak personal communication). For all large cruise vessel discharges averaged over the entire cruise season the dilution factor was estimated at 3×10^6. Based on the 2006 cruise season, the temporal pattern of port calls for the bulk of the season is three consecutive days of three port calls, followed by four days of cruise traffic. Based on this temporal pattern (and the assumption that the box is well mixed) , the best maximal dilution factor is estimated to be ~1×10^6.

The model considers each box to be a continuously stirred reactor. Based on the model, the flushing rate of the surface box of the Main Basin is 0.046 day^{-1}. This translates to a $\log_{10}$ reduction time due to flushing alone of ~45 days (3 half-lives). The more important $\log_{10}$ reduction factor is virus die-off, with a log10 reduction time of ~4 days (3 half-lives). This means about one $\log_{10}$ reduction in viruses discharged by the third day of three port calls may be expected prior to the next loading occurs. Stated another way; assuming average discharge conditions for cruise vessels during their time in the PS, the concentration of viruses in the water attributable to the cruise ships would vary by ~1 $\log_{10}$ during a week of the cruise season.

Transient Model

The transient model was run for two conditions: an outbreak scenario and a non-outbreak scenario. The outbreak scenario assumed at least 3% of individuals onboard the cruise vessel presenting with symptomatic norovirus illness (the actual number of symptomatic individuals in each run of the trial was determined stochastically based on a distribution of the number of individuals

involved in NW cruise vessel outbreaks of norovirus). The results for 10,000 trials of the transient model under the outbreak scenario are presented in Table 2. The non-outbreak scenario assumes that less than 3% of individuals on board a vessel present with symptomatic illness from norovirus (the actual percentage of symptomatic individuals for each trial chosen randomly from a uniform distribution ranging from 0 to 2.9%). The results for 10,000 trials of the transient model run under the non-outbreak scenario are presented in Table 3. Two dilution conditions, static and stochastic, were evaluated for both the outbreak and non-outbreak scenario. For the static dilution conditions, the near field dilution factor was based on the minimal dilution observed in the EPA plume tracking study as interpreted in an OSWP memorandum (EPA, 2002; Merriwether, 2007). The far field dilution was based on the minimal dilution identified in the "Ship to Shore" model developed by Mitsuhiro Kawase's research group (Sarason et al., 2006). For the stochastic conditions, near field dilution values were selected from a uniform distribution representing minimal to typical dilution as indicated in the EPA plume tracking study, and far field dilution values were selected from a triangular distribution representing the range of dilution values identified in the ship to shore model, with a mode of 750X. For comparison sake, additional runs of the model were performed with static near-field dilution factor of 105,000X, and stochastic near field dilutions ranging from 105,000X to 1,000,000X, due to concerns previously discussed. Far field dilution factors were not changed. These results are represented in Tables 4 and 5, respectively. The increased dilution resulted in predicted virus concentrations at the beds being a factor of 3 to 5 lower than when the more conservative dilution factors were used.

Table 2. Summary of water concentration at beds (#/L) (runs of 10000 trials of the transient model under outbreak scenario [>3% onboard population ill])

	water concentration at beds (#/L)		
	50th %ile	90th %ile	95th %ile
static dilution factors			
water conc at beds (mbr/uf + uv)	5.8.E-06	5.9.E-04	1.7.E-03
water conc at beds (mbr/uf)	6.1.E-02	5.7.E+00	1.7.E+01
water conc at beds (mbr/daf + uv)	1.9.E-05	2.0.E-03	5.5.E-03
water conc at beds (mbr/daf)	1.9.E-01	1.9.E+01	5.4.E+01
stochastic dilution factors			
water conc at beds (mbr/uf + uv)	9.4.E-08	1.1.E-05	3.2.E-05
water conc at beds (mbr/uf)	1.0.E-03	1.1.E-01	3.3.E-01
water conc at beds (mbr/daf + uv)	3.0.E-07	3.6.E-05	1.0.E-04
water conc at beds (mbr/daf)	3.1.E-03	3.7.E-01	1.1.E+00

mbr/uf = membrane bioreactor/ ultrafiltration; mbr/daf = membrane bioreactor/dissolved air flotation; uv = uv disinfection

results are from runs of Norovirus 7.24.07.xls

static dilution factors : near field/prop wash (30000); far field (50); stochastic dilution factors : near field/prop wash (uniform : min = 30000, max =200000); far field (triangular : min = 50, mode = 750, max = 2000)

Table 3. Summary of water concentration at beds (#/L) (runs of 10000 trials of transient model under non-outbreak scenario [<3% onboard population ill])

	water concentration at beds (#/L)		
	50th %ile	90th %ile	95th %ile
static dilution factors			
water conc at beds (mbr/uf + uv)	5.9.E-06	5.5.E-04	1.6.E-03
water conc at beds (mbr/uf)	6.4.E-02	5.2.E+00	1.5.E+01
water conc at beds (mbr/daf + uv)	1.8.E-05	1.8.E-03	5.2.E-03
water conc at beds (mbr/daf)	1.9.E-01	1.7.E+01	5.1.E+01
stochastic dilution factors			
water conc at beds (mbr/uf + uv)	1.0.E-07	1.0.E-05	3.1.E-05
water conc at beds (mbr/uf)	1.1.E-03	9.8.E-02	3.0.E-01
water conc at beds (mbr/daf + uv)	3.2.E-07	3.4.E-05	1.1.E-04
water conc at beds (mbr/daf)	3.2.E-03	3.2.E-01	1.0.E+00

mbr/uf = membrane bioreactor/ ultrafiltration; mbr/daf = membrane bioreactor/dissolved air flotation; uv = uv disinfection

results are from runs of Norovirus 7.24.07.xls

static dilution factors : near field/prop wash (30000); far field (50); stochastic dilution factors : near field/prop wash (uniform : min = 30000, max =200000); far field (triangular : min = 50, mode = 750, max = 2000)

Table 4. Summary of water concentration at beds (#/L) (runs of 10000 trials of the transient model under outbreak scenario with elevated dilution [>3% onboard population ill])

	water concentration at beds (#/L)		
	50th %ile	90th %ile	95th %ile
static dilution factors			
water conc at beds (mbr/uf + uv)	1.9E-06	1.8E-04	5.0E-04
water conc at beds (mbr/uf)	1.8E-02	1.7E+00	4.8E+00
water conc at beds (mbr/daf + uv)	5.7E-06	5.6E-04	1.5E-03
water conc at beds (mbr/daf)	5.6E-02	5.8E+00	1.5E+01
stochastic dilution factors			
water conc at beds (mbr/uf + uv)	2.3E-08	2.7E-06	7.7E-06
water conc at beds (mbr/uf)	2.2E-04	2.6E-02	7.3E-02
water conc at beds (mbr/daf + uv)	7.2E-08	8.3E-06	2.5E-05
water conc at beds (mbr/daf)	6.9E-04	8.4E-02	2.5E-01

mbr/uf = membrane bioreactor/ ultrafiltration; mbr/daf = membrane bioreactor/dissolved air flotation; uv = uv disinfection

results are from runs of Norovirus 11.12.07.xls

static dilution factors : near field/prop wash (105000); far field (50); stochastic dilution factors : near field/prop wash (uniform : min = 105000, max =1000000); far field (triangular : min = 50, mode = 750, max = 2000)

Table 5. Summary of water concentration at beds (#/L) (runs of 10000 trials of transient model under non-outbreak scenario with elevated dilution [<3% onboard population ill])

	water concentration at beds (#/L)		
	50th %ile	90th %ile	95th %ile
static dilution factors			
water conc at beds (mbr/uf + uv)	1.8E-06	1.5E-04	4.7E-04
water conc at beds (mbr/uf)	1.7E-02	1.7E+00	4.8E+00
water conc at beds (mbr/daf + uv)	5.4E-06	4.9E-04	1.4E-03
water conc at beds (mbr/daf)	5.5E-02	5.1E+00	1.5E+01
stochastic dilution factors			
water conc at beds (mbr/uf + uv)	2.2E-08	2.4E-06	7.2E-06
water conc at beds (mbr/uf)	2.2E-04	2.5E-02	7.5E-02
water conc at beds (mbr/daf + uv)	6.5E-08	7.2E-06	2.3E-05
water conc at beds (mbr/daf)	6.7E-04	8.1E-02	2.2E-01

mbr/uf = membrane bioreactor/ ultrafiltration; mbr/daf = membrane bioreactor/dissolved air flotation; uv = uv disinfection

results are from runs of Norovirus 11.12.07.xls

static dilution factors : near field/prop wash (105000); far field (50); stochastic dilution factors : near field/prop wash (uniform : min = 105000, max =1000000); far field (triangular : min = 50, mode = 750, max = 2000)

DISCUSSION AND CONCLUSIONS

This study applied a risk assessment framework to evaluate the risks posed by consumption of raw oysters harvested from areas impacted by wastewater discharges from large cruise vessels. A series of models were developed in accordance with this framework to deterministically and stochastically predict the risk associated with consumption of raw oysters grown in a particular water quality and to derive estimates of water quality in PS resulting from wastewater discharges from large cruise vessels.

The organization of this report progresses from consideration of risk given an assumed water quality to consideration of possible water quality outcomes. This organization was chosen deliberately to avoid overstatement of quantitative results. Many of the model inputs are highly uncertain, but nevertheless grounded in measurements reported in the literature. For some other aspects of this problem, it was felt that relevant parameters were so poorly grounded that quantitative estimates could not be justified. For example, no information is available on the spatial and temporal characteristics of shellfish consumption by individuals (i.e., the extent to which persons repeatedly consume shellfish from beds most vulnerable to cruise ship impacts is unknown). Rather than speculate on these points, or conceal this lack of information in a large model, we have separated prediction of risk from prediction of water quality. The risk curves presented in Figure 3 represent median estimates of annual risk based on consumption, at tribal rates, of oysters grown to maturity in water of a specified quality. With respect to water quality, we have utilized two approaches. A steady-state box model was employed to provide an estimate of long term seasonal average virus concentration in the shellfish growing areas of the Sound. This effort is intended to provide some perspective on the curves in Figure 3 (and sampling to confirm/refute this modeling result is recommended). A stochastic, transient water quality model that utilizes information from an EPA field test of dispersion from cruise ships and hydrodynamic

modeling of Puget Sound by Kawase and co-workers was also created. The results of the second model provide additional insight into the short-term effect of cruise ships on water quality. However, the complexity and spatial and temporal resolution of the Kawase model does not permit either direct integration with the risk model developed here or prediction of water quality at specific shellfish beds over durations relevant to shellfish growth and harvest.

Like any risk assessment the results of the study were limited by the quality of the data used to develop and run the models. Uncertainty and variability were handled through stochastic means (1-D analysis), where possible. For example, the transient water quality model (outbreak or non-outbreak) is most sensitive to the assumed concentration of viruses in shedders' stools; while the annual risk model is most sensitive to the assumed number of consumption events per year and the dose response model chosen. However it is recognized that many of the values used in the models contained considerable uncertainty in addition to natural population variability, which would warrant a 2-D analysis if the quality of data were better.

The results of the risk model predict the annual risk associated with consumption of raw oysters grown in a particular quality of water for consumers that eat raw oysters at a rate comparable to those reported for the Suquamish Tribe (assuming all oysters consumed were grown in that quality of water). The results suggest that oysters grown in water with a concentration of ~1 norovirus per 10000 liters, a level comparable to the background concentration level determined by the box model, would present a median annual risk of less than the suggested 1 in 10,000 risk benchmark that has been established for drinking water. At virus concentrations higher than that the model predicts the risk of infection would be greater. For the general population which consumes oysters at a much lower intake rate, the annual risk would be expected to be considerably less. The ability of the risk model to accurately assess the annual risk of infection is limited by the resolution of the available oyster harvest and consumption data. While several studies have reported annual ingestion rates for specific populations, this information is not coupled with an indication of where, or from what quality of water (with respect to viruses), the oysters consumed were harvested. Another issue is that large cruise vessel discharges would only be expected to impact the water quality during the cruise season, and for a limited period thereafter, during which virus die-off and flushing of the surface box of the Main Basin would return virus concentrations to background levels. Unfortunately, there is currently no available data, let alone seasonal data, for the virus concentration in waters of PS. Therefore the model was limited to assessing risks from oysters grown in a particular quality of water, assuming that only raw oysters grown in that water quality were consumed.

Another limitation of the risk model was the quality of the data on rates by which oysters uptake and eliminate norovirus from their growing waters. While several studies have reported uptake and elimination rates for enteric viruses by various shellfish (including oysters), few have adequately examined noroviruses. The results in these various studies are frequently difficult to interpret between studies, due to significant differences between the methods employed. These include differences in: the concentration of viruses with which shellfish are challenged, the type of viruses used, the circulation scheme, and assay methods. Few of these studies performed an adequate mass balance for the viruses introduced into the system. Additionally, most of these studies have been performed at unnaturally high challenge concentrations of viruses and few, if any, standardize the observations in terms of the activity of the shellfish (i.e. filtration rate). In contrast, there is a study in which low levels of poliovirus were used to challenge oysters and hard

clams (Landry et al., 1982). This study found little if any viral accumulation below virus levels of 0.01 plaque-forming units (PFU)/ml, a concentration several orders of magnitude above the levels assumed in the current model. However the study apparently did not account for shellfish activity during the experiments. Recent studies suggesting significant norovirus occurrence in market shellfish (Cheng et al., 2005; Constantini et al., 2006) suggest that oysters do accumulate noroviruses from water of a low virus concentration. Recent studies have shown a specific binding of the virus to oyster tissues which may partially explain the bioaccumulation. The general conclusions of virus uptake and elimination studies tend to be that viruses are rapidly accumulated by shellfish and are slowly eliminated. The model in this study tried to encompass the uncertainty and variability of the virus uptake and elimination represented in the published literature by using a uniform distribution from 3X to 1000X to represent the bioaccumulation factor.

The available dose response data for norovirus is also a major limiting factor for the risk model. Norovirus is not readily culturable in tissue or animal models. Any reports of dose response information have resulted from human volunteer studies. The early studies were performed with high, poorly quantified doses of Norwalk virus. As a result, they were difficult to extrapolate to low doses that might be expected from environmental exposures. More recently, human volunteer studies have been performed using a more reflective range of better quantified doses. Unfortunately this data is not yet available in the peer reviewed literature. The current dose response model has relied on a simple exponential model and previous assumptions regarding minimal infectious dose. Until better dose response data is peer reviewed and available, the current dose response model will be a major source of uncertainty. Additionally, all of the dose response data available is for single lineages of a very limited number of strains (i.e. Norwalk virus and Snow Mountain virus). There are numerous strains of norovirus, and the circulating strains are constantly shifting. As a result even when the existing dose response data is fully available, considerable uncertainty will exist.

Results of the box model suggest that virus discharges from the large cruise vessels represent on a fraction of overall viral inputs to PS. This is a logical conclusion considering that cruise traffic represents only about 500,000 person-days per year in terms of wastewater discharges, as compared to more than 3 million permanent residents on the shores of the Main Basin contributing daily discharges to PS. However, the box model does not offer adequate resolution of local impacts of wastewater discharges. Impacts of Land-based treatment outfalls are expected to be localized. As a result outfalls are individually modeled and closure zones tailored to specific conditions. The box model calculates steady state conditions within the individual boxes based on seasonal average loading. It does not consider localized variability in water quality, and as a result under estimates the water quality in some areas, while over estimating in others. Still the results of the model might be used to estimate the relevant magnitude of land-based and large cruise vessel loadings to PS in similar terms.

The transient model was developed to examine specific large cruise vessel discharge scenarios. The model was run for a matrix of scenarios representing loading conditions (outbreak and non-outbreak), treatment conditions (type and upset condition), event frequency and dilution. Non-outbreak loading conditions were stochastically modeled based on a number of symptomatic individuals representing 0 to 2.9% of individuals onboard the vessel. Outbreak conditions were stochastically modeled based on the distribution of symptomatic individuals reported for previously recorded cruise outbreaks. The results of the model suggest that no clear difference can

be drawn between outbreak and non-outbreak scenarios, in terms of the virus loading. This is likely largely due to the several order of magnitude variability in virus shedding reported among symptomatic individuals.

The transient model was initially developed to examine the rare scenario, i.e. a vessel with an outbreak discharging to PS under upset conditions. However, cruise vessel traffic into PS is not a rare occurrence. Since based on the model no significant difference may be assumed between outbreak and non-outbreak scenarios, the level of loading due to cruise ship discharges can be adjusted based on the weekly traffic pattern. During the season, vessels make port calls at very predictable frequency. Typically during the peak cruise season, there are three consecutive days of three vessels making port followed by 4 days of lesser to no traffic. Based on the flushing and virus die-off rates used in the models, virus concentration in the water would expected to vary by only on order of magnitude during a typical week during the season (assuming average loadings).

There are two basic treatment configurations employed in large cruise vessel wastewater treatment systems (among vessels party to the MOU): a membrane bioreactor coupled with integrated ultrafiltration followed by UV disinfection; and a membrane bioreactor couple with dissolved air flotation followed by UV disinfection. The model results show very little difference (~0.5 $\log_{10}$) between the two types of systems. DOH employs an upset condition when establishing closure zones around wastewater treatment outfalls. This upset condition is typically defined as a failure in disinfection. While the AWTS onboard the large cruise vessels are very efficient systems when properly functioning, the assumption of an upset condition (no UV disinfection) reduce their effectiveness by several orders of magnitude ($4\log_{10}$).

The transient model was constructed to evaluate near-field and far-field dilution separately. Near field dilution was based on the immediate mixing as treatment effluent was discharged through the propwash of the vessel. Far-field dilution was based on a hydrodynamic model of the tidal and circulation patterns in Puget Sound. The model was run with dilution considered both deterministically (using static dilution factors representing perceived worst case dilution) and stochastically (using dilution factors based on distributions based on published values and the results of the hydrodynamic model). The static near-field dilution factor (30,000X) was determined from an interpretation of the EPA plume tracking regarding the maximum dye concentration observed in the wake of the vessels examined (Merriwether, 2007). The static far-field dilution factor (50X) was assumed to be the minimum dilution calculated at Point Jefferson (minimum overall dilution reported) by the “Ship to Shore” hydrodynamic dilution model. Together the static dilution factors contribute an overall dilution of 1.5×10^6. This was anticipated to represent the minimum dilution that could be represented. For comparison, the box model can also be used to estimate dilution of discharges to the Main Basin. The box model considers each box in the model to be well mixed; as such it estimates the best case (maximal) dilution. Considering only the surface box of the Main Basin (into which all large cruise vessel discharges are input), a maximum dilution of around 10^6. Dilutions calculated by the transient model are based on hydrodynamic models that suggest that the main basin is in fact not well mixed and that apparent local dilution may be significantly greater than predicted by the box model.

If the upset condition assumed for the purposes of establishing a closure zone around the cruise vessels is to assume no disinfection, the transient model predicts resulting water quality (at least ephemerally) during the cruise season that would be likely be unacceptable for harvest and

consumption of oysters in much of the Main Basin. Unfortunately the lack of resolution of the oyster harvest, acquisition, and consumption data from a temporal and geospatial aspect, prevent a coupling of the risk model and either the box or transient models to estimate an event or annual risk of norovirus infection from consumption of raw oysters grown in waters impacted by large cruise vessels.

It is important to consider that the large cruise vessels have very good treatment systems relevant to other types of vessels, and even most land-based systems. Although the large cruise vessels may represent the greatest number of vessel passengers impacting PS, other vessels with smaller number of passengers make many more Port Calls (>1000) annually (or may be resident in the case of small vessels), and have less effective treatment systems. The potential for discharges by vessels, other than large cruise vessels, to impact shellfish beds may be significant.

Based on the results of this study, several areas warranting additional investigation have been identified. Additional analysis is needed on the dose response relationship for noroviruses, once better data from human feeding studies are published in a peer-reviewed journal. Investigation into shellfish harvest and consumption patterns at a more compatible temporal and geospatial resolution would improve the precision of modeling efforts and the ability to predict seasonal or event risks. Similarly, investigation into the actual efficacy of ship-based treatment systems for virus removal under typical operating conditions would improve the ability to assess impacts of vessel discharges. Further information is also needed on the bioaccumulation rates for viruses by shellfish during exposure to low virus concentrations. Assessment of the virus concentration in shellfish tissues at impacted beaches would allow a better evaluation of the model. Finally, virus discharges by other vessels (e.g. day cruise vessels, private boats, military vessels, etc.) should be evaluated, and land-based WWTPs should be re-evaluated for virus discharges.

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APPENDIX Parameters used in Models

Table A1. *Selected parameters and assumptions used in the risk and dose-response models*

model parameter	variable	units	value / distribution type
water concentration	C_w	# viruses / L	assumed
bioaccumulation factor	BAF	L / kg oyster	log uniform (0.5 - 3.0)
oyster consumption rate	CR	g / event	logN (50th %ile = 180; 90th %ile = 477)
number of events per yr	--	events /yr	logN (50th %ile = 8; 90th %ile = 46)
alpha / beta values (for the approximate Beta-Poisson dose-response model)			
Snow Mountain virus	SMV	--	0.33 / 230
Norwalk virus	NV	--	0.096 / 14

Table A2. *Stochastic parameters and assumptions used in the loadings model*

model parameter	units	value / distribution type
wastewater treatment plant – main deep, main surface, south sound surface (symptomatic individuals)		
log stool concentration	--	custom (combined genotypes 1 & 2 data)
daily stool volume	ml / day	triangular (min = 0; mode = 1500; max = 5000)
wastewater treatment plant – main deep, main surface, south sound surface (asymptomatic individuals)		
log stool concentration	--	uniform (0 - 6)
daily stool volume	ml / day	triangular (min = 0; mode = 200; max = 600)
wastewater treatment plant – main deep, main surface, south sound surface (symptomatic & asymptomatic individuals)		
log base treatment reduction	--	uniform (0.5 - 2)
shipboard background – symptomatic individuals		
persons / ship	persons / ship	custom
log stool concentration	--	custom (combined genotypes 1 & 2 data)
daily stool volume	ml / day	triangular (min = 0; mode = 1500; max = 5000)
shipboard background – asymptomatic individuals		
percentage of asymptomatic shedders	%	triangular (min = 0.3; mode = 0.8; max = 1.1)
log stool concentration	--	uniform (0 – 6)
daily stool volume	ml / day	triangular (min = 0; mode = 200; max = 600)
shipboard background – symptomatic & asymptomatic individuals		
log activated sludge filtration	--	uniform (0.5 – 2)
log membrane / dissolved air flotation filtration	--	uniform (0.5 – 2)

Table A3. *Stochastic parameters and assumptions used in the transient model*

model parameter	units	value / distribution type
symptomatic shedders		normal (mean = 115; std dev = 25)
log stool concentration	--	custom (combined genotypes 1 & 2 data)
daily stool volume	ml / day	triangular (min = 0; mode = 1500; max = 5000)
log activated sludge reduction	--	uniform (0.5 - 2)
log membrane filtration reduction	--	uniform (1 - 2)
log dissolved air flotation reduction	--	uniform (0.5 – 1.5)
in corridor time	hrs	triangular (min = 12; mode = 15; max = 18)
shipboard wastewater treatment plant flow	m^3 / hr	custom
near field/propeller wash dilution factor	--	uniform (30000 - 200000)
far field dilution factor	--	triangular (min = 50; mode = 750; max = 2000)

Appendix B.

Saranson C.P., M. Kawase, and L.B. Curry. 2006. Dilution of Norovirus from "Ship to Shore" in Puget Sound, Wa. Report to Washington Department of Health. *Submitted June 2006*.

Dilution of Norovirus from "Ship-to-Shore" in Puget Sound, WA

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Final Report submitted to Washington State Department of Health, June 2006

ABSTRACT

Puget Sound, Washington State's largest inland sea, is both the largest fjord in the lower forty-eight states and closest to the substantial urban centers of Seattle, Tacoma, Everett and surrounding communities. Since 1998, cruise ship traffic has increased significantly in Puget Sound, and has brought substantial infrastructure development for the Port of Seattle. In 2004, the industry had 150 port calls and 562,000 passengers, a 62% increase from 2003. In April of 2004, the Port of Seattle, the Washington State Department of Ecology and the Northwest Cruise Ship Association (NWCA) signed an agreement which prohibits the discharge of untreated wastewater and requires strict monitoring, although accidental discharges of wastewater are always possible. In 2005, 96% of port calls to Seattle by large passenger vessels were made by NWCA member ships.

To estimate dilution and transport of possible discharges from a cruise ship in Puget Sound, we use a three-dimensional hydrodynamic model, based upon the Princeton Ocean Model (POM) of Blumeberg and Mellor (1987). This model is maintained by the Puget Sound Marine Environmental Modeling (PSMEM) partnership (http://www.psmem.org), and runs in daily hindcast mode. We compare a 2-D Lagrangian particle trajectory model, which utilizes the output from daily hindcasts, with conservative tracers released within the 3-D POM circulation. Monte Carlo simulations with the 2-D model show particles released at the surface in Admiralty Inlet tend to leave the Sound quickly and enter the eastern Strait of Juan de Fuca. The 2-D model predicted that the highest levels of particles that hit landfall did so in the general areas of Admiralty Bay and Useless Bay. Conversely, particles released in the eastern Strait of Juan de Fuca do not tend to move into Puget Sound at the surface.

We compared these 2-D results to those predicted by releasing both particles and a conservative tracer within the 3-D POM circulation model. The resulting model runs took significantly longer, making Monte-Carlo simulations impractical, and showed our assumption that particles remain in the surface layer was a poor one. Particle trajectories identified similar locations of beached particles, but the timing of arrival was different between the 2-D and 3-D model runs. Because the goal of the study was to estimate dilution in Admiralty Inlet using the model, we released passive, conservative tracer dye into the model as a continuous stream along the shipping lanes, mimicking the locations used in the 2-D modeling. Dye released south of the sill at Admiralty Inlet showed a smaller dilution than on top of the sill (see Figure 5a-e) A time-series of dye concentration at Pt. Jefferson showed peak dye concentrations were diluted by a factor of 45X by the time the dye released from the shipping lanes reached the shore, although the average levels of dilution were more like 100X or more. Concentrations in Admiralty Bay and Useless Bay were greater that 100X. This behavior is expected, as vertical circulation over the sill at Admiralty Inlet greatly

increases mixing. The 100X dilution factor estimated by Loehr et al. in their analysis of far-field dilution is similar to the tracer dye concentrations at Point Jefferson in the 3-D model runs.

STUDY DESIGN

In general, the goal of this study is to understand the possible impacts of cruise ship discharge on shellfish beds along the shores of Puget Sound. There are 3 important processes which need to be understood to derive the appropriate risk assessment: 1) the concentration and dilution of discharge at the ship, 2) the ship-to-shore (or "far-field") dilution and 3) the uptake of any viruses by shellfish at the shore. In general, we can view this as a simple back-of-the-envelope problem:

(ship discharge concentration) X (ship dilution) X (ship-to-shore dilution)	=	estimate of concentration at shellfish bed.

This study addresses the second process, the estimate of "ship-to-shore" dilution between the shipping lanes and Puget Sound beaches.

Hydrodynamic Model

The PRISM/PSMEM Puget Sound model runs daily in "hindcast" mode. The model is an implementation of the Princeton Ocean Model (POM; Blumberg and Mellor 1987), and simulates the circulation and stratification of Puget Sound. The model equations are those of the standard primitive equation (hydrostatic) dynamics. Given initial and boundary conditions, the model predicts sea-surface elevation, three components of velocity, temperature, salinity, turbulent kinetic energy, and turbulent mixing length. The latter two quantities are used to parameterize vertical mixing by eddies in terms of the turbulence closure scheme of Mellor and Yamada (1974). Surface elevation and depth-averaged velocities are integrated separately from internal quantities in a split-explicit formulation.

The setup of the model was developed to address overall circulation in Puget Sound (Kawase 1998). The model domain covers the entire Puget Sound from Admiralty Inlet inwards, as well as a part of the Strait of Juan de Fuca, at a 360-m resolution in the east-west direction and 540-m in the north-south direction (Fig. 1). Bathymetry is gridded at 30-m horizontal resolution. The model's surface boundary conditions are the turbulent fluxes of momentum, heat, and fresh water, as well as radiative fluxes. All are provided by the UW Atmospheric Sciences forecast group[2] and are derived from the output of the Penn State University/National Center for Atmospheric Research mesoscale atmospheric model, known as MM5. The turbulent fluxes are derived from MM5 air temperature, humidity, and wind speed using a bulk flux algorithm (Appendix C, Mellor 2003), while the radiative fluxes are directly output from MM5. A no-flux boundary condition is applied at the bottom for mass, heat and salt, while bottom stress is obtained from a quadratic drag law.

The model has an open boundary in the Strait of Juan de Fuca, where tidal forcing is incorporated as a boundary condition using the scheme of Flather (1976). Seven tidal constituents (M2, K1, S2,

[2] (http://www.atmos.washington.edu/mm5rt/)

N2, O1, P1, M4) are used, in emulation of an earlier channel model of Puget Sound tides (Lavelle et al. 1988). A radiation boundary condition is applied to external and internal modes of velocity, while temperature and salinity are either advected out of the model domain or set to a prescribed value when advected in. At the northern edge of the domain, boundary conditions come from climatology of cruise data from the Joint Effort to Monitor the Straits. Within the domain, river inputs are specified as mass and freshwater sources at the grid points nearest to the mouths of major rivers, using USGS stream gauge data. Inputs from non-gauged streams were extrapolated following Lincoln and Collias (1975). Freshwater input to the water surface from precipitation is not included, but is expected to be small. A significant validation of the model's hydrodynamics was completed to help site the King County Brightwater sewage treatment facility; subsequent validation using data from the MIXED experiment (Edwards et al., in prep) shows excellent reproduction of circulation features in Carr Inlet.

Particle Tracking

Particle tracking using the POM model takes a significant amount of time to run (1 day of simulation takes 12 hours on a dual Pentium 4 with 2GB of RAM) so multiple simulations for long time periods are not practical. The model is run daily, however, and provides "nowcast" simulations, with various properties saved every ½ hour. To learn where particles on the surface (top 50 meters of water) would go, we developed a post-simulation 2D Lagrangian particle trajectory routine, and traced the particle's position by applying currents saved at the ½ hour timesteps from daily nowcasts.

To assess the trajectory of cruise ship discharge, we chose a discrete number of release locations within the shipping lanes in Puget Sound. Five hundred particles were released from each location. Locations are shown in figure 1a, and are indicated by different colors for tracking purposes. To get quick insight into the distribution of particles being released, we have visualized the particle trajectories in a number of ways.

First, (figure 2a) we keep track of whether a trajectory has passed through a grid cell or not. Cells which have every trajectory in them (such as the start point) will show "500" in on this surface; cells with no trajectories are blank. We term this type of plot a "trajectory density plot" or TDP. Secondly, (figure 2b) to determine whether particles move quickly through a given region or not, we track the total number of times any particle traverses a grid cell. Thus, for M particles that transit a cell N-times, this surface plot will show MxN hits. For the purpose of the 2D trajectories, we assume particles remain on the surface layer. This assumption is not a good one when thinking about fluid parcels (see discussion of 3D results below), although the 2D tool is useful for quick insight into surface circulation patterns. Figure 2c shows a cross-section view of a TDP showing the vertical distribution of particles. This figure clearly shows our assumption that particles stay in the surface layer may be suspect; we compare both the 2D and 3D results below.

To ease our analyses, we developed a MATLAB tool to select regions of interest and calculate a histogram plot of the number of particles expected to make landfall in that region. By color coding the histogram, we achieve an estimate of the distribution of particles from a given release point, as well as minimum time to landfall in that region (figure 3). The histogram plots show time on the x-axis and all initial particle hits within the region (shown by the blue box in the subfigure map) as bars on the y-axis. The colors of the histogram boxes correspond to release locations as shown in

figure 1. Figure 3a shows the histogram plot for all of Admiralty Inlet using 3D trajectories; figure 3b shows the histogram plot for the same region using the 2D Monte Carlo simulation. Particles were released randomly throughout the tidal cycle in both cases. These particle trajectory plots were useful to identify beach areas which may or may not be affected by cruise ship discharges. Because of the uncertainty introduced in our 2D assumption, we present the particle trajectory histogram plots from the full 3D calculation in Figure 4 (panels a-c). Each of these three panels show particle hits for individual beaches. In general, almost all of the particle landfalls were in Admiralty Inlet, along the shores of Whidbey Island, with a smaller subset in the Main Basin, and the majority of landfalls occur in the first 8 hours.

Passive, Conservative Dye Releases

To derive the minimal amount of mixing expected between ship and shore, we released a passive, conservative tracer into the 3D simulation. The tracer was initialized in a continuous line along the ship corridor. Figure 5 shows successive frames from the simulation at 1, 6, 12, 18 and 24 hours after the start of the model run. To assess the dilution between ship and shore, we focus on 3 areas (North, Middle and South release areas, corresponding to Admiralty Bay, Useless Bay and Pt. Jefferson). These release points are located near kilometer 40, 60 and 100 respectively on the Thalweg cross section (shown later in Figure 5). Because of the time required to perform simulations with dye tracers, we release dye along the expected track of a cruise ship simultaneously in the tidal cycle at the beginning of the day and watch where the dye travels through time. We record the peak concentration at the release location, based upon a ship traveling along the ship corridor, and compare to the concentration at the 3 land locations. We monitor the dye concentration through time, and use the peak dye concentration to estimate the minimal dilution expected.

RESULTS

2D vs. 3D calculation

We initially designed the 2D lagrangian particle tool to get a sense of surface circulation in Puget Sound, as well as to allow us to run large numbers of simulations to help derive statistics for particle motions. The 2D assumption was based upon the fact that we had data saved out of the model at ½ hour timesteps, and we wondered how vertical mixing on a smaller time scale would affect the particle trajectories. In figure 2a and 2b, we see horizontal mixing similar to what we would expect from a simple 2D calculation; figure 2C shows the 2D assumption may not reflect actual mixing conditions; the cross-section trajectory density plot show a significant fraction of particles move between 0 and 50 meters, with a smaller number of particles making it all the way to the bottom of the sound. Vertical motions are most significant over the sill (shallow area) of Admiralty Inlet. Accordingly, while the 2D tool is useful for deriving general patterns of surface circulation, we decided to focus on the 3D results to estimate dilutions.

Histogram Plots

Both the 3D and 2D histogram plots (Figures 3a and 3b) show landfall along Admiralty Inlet occurs mostly on the shores of Whidbey Island. The largest concentration of particles which make landfall in a short time are found in Admiralty Bay, about 2/3 of the way through Admiralty Inlet.

Although the character of the histogram plots is somewhat different between the 2D and 3D case (the 2D case in Figure 3b shows more landfalls of particles released in the Main Basin, as shown by the blue colors), both show a peak at about 4 hours after release (because the particles are released randomly throughout the tidal cycle, we do not need to worry about whether the release is on the incoming or outgoing tide.) The 3D results show fewer particles released in the Main Basin make landfall within our box, and in general fewer particles make landfall in the later hours of the simulation. We speculate this is due to vertical motions which reduce the likelihood of making landfall versus the 2D surface calculation.

Dye plots

To derive bulk estimates for "ship-to-shore" dilution, we released conservative tracers at the same locations shown in Figure 1. After releasing dye for the first hour of the simulation, we recorded the maximum concentration in a grid cell along the cruise track (termed "maximum concentration @ release" in Figure 5) and then compared this concentration to the peak concentration recorded at 3 land stations shown in Figure 5. As we expected from looking at the trajectory density plot cross-section, there is significant vertical mixing of the dye, with the most mixing occurring over the sill (shallow area in Admiralty Inlet). In general, we find the maximum concentrations in Main Basin, which is consistent with previous field work showing mixing is vigorous over the sill, and less vigorous in the Main Basin (Ebbesmeyer and Barnes, 1980).

Northern Dye Release

Dye released nearby to Admiralty Bay shows significant vertical mixing (the dye patch furthest to the left on the cross-section plots shown in Figure 5). The maximum concentration along the cruise track is 3.4×10^{-5}[3]. As this dye mixes and spreads horizontally and vertically, it eventually reaches the sampling location in Admiralty Bay. Here we find concentration has a broad peak after about 16 hours. We are most concerned with the relative dilution of the tracer, and we may normalize the concentrations shown in Figure 5 to any value. The peak concentration at Admiralty Bay is $\sim 0.2 \times 10^{-7}$; this is a dilution of more than 1000X the peak concentration at the release location.

Middle Dye Release

Dye released near to Useless Bay (the "Middle" release shown in Figure 5) shows even higher levels of dilution. Peak values at this sampling location hardly even register on the plot giving a relative dilution of greater than 2000X. The peak in concentration is long lived (e.g. it persists over many timesteps) and is delayed compared with the Admiralty Bay location.

Southern Dye Release

Dye released near to Pt. Jefferson in the Main Basin (the "Southern release shown in Figure 5) remains most concentrated. The peak concentration at the release point is 3.4×10^{-5}; and the time series plot at the sampling location shows a peak concentration at hour 4 of 8×10^{-7} at Point Jefferson, which gives a dilution of ~40X. This lower dilution can be estimated just by looking at

[3] The dye along the cruise track is initialized at this concentration based upon a discharge of 16.66 m^3 over 5 minutes; the absolute magnitude of discharge is not as important as the relative dilution from the initial concentration.

the cross-section plot of concentration, which shows the southern release gets mixed the least, retaining a concentration peak much longer than the releases closer to the sill at Admiralty Inlet. A second, somewhat broader peak shows up around hour 8, and likely reflects mixing of dye from many locations along the track of the cruise ship. After the peak ebb tide, however, concentrations fall considerably, closer to the ~1000X dilution seen in the northern sampling site.

One limitation with this type of analysis is we are only saving out parameters every ½ hour, which means transient concentrations at our sampling stations may well exceed those shown here. In addition, it is possible the release time of the dye in relation to the tidal cycle plays an important role in dilution, as well as possible concentration of discharge from along the cruise track. Because of the uncertainty involved with estimating a bulk dilution factor for many different cases, we find using a ship-to-shore dilution factor of 100X is a conservative estimate of far-field dilution.

RECOMMENDATION

In general, these results indicate it is preferable to delay discharge from cruise ships until at least being parallel with the Southern tip of Whidbey Island, where there will be more mixing due to proximity to sill. If a dilution factor of 100X results in permissible risks after assessing the dilution at the ship discharge as well as shellfish uptake rates (parts 1 and 3 of our back of the envelope model), then discharges in the Main Basin may have no potential health effects on harvestable shellfish.

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FIGURE CAPTIONS

Figure 1: Basemap of modeling domain, showing release locations superimposed upon bathymetry. Release locations for particle tracking experiments are color coded to match histograms in Figures 2 and 3.

Figure 2: a) For particle release modeling, we keep track of whether a trajectory has passed through a grid cell or not. Cells which have every trajectory in them (such as the start point) will show "500" in on this surface; cells with no trajectories are blank. We term this type of plot a "trajectory density plot" or TDP. b) To determine whether particles move quickly through a given region or not, we track the total number of times any particle traverses a grid cell. Thus, for M particles that transit a cell N-times, this surface plot will show MxN hits. Areas showing high amounts of "hot" colors are where particles spend a large amount of time. c) This cross-section TDP shows particles move a significant distance in the vertical dimension; most of the particles stay in the top 50 meters, but a significant number make it all the way to the bottom near the sill.

Figure 3: For the region along Admiralty Inlet (shown in the inset box in upper right) we record the number and timing of landfall using a histogram plot. The colors of the boxes correspond to the release points shown in figure 1 (cool colors to the south, warm colors to the north). a) The 3D trajectory calculation shows a large number of particles (2039/5060, or ~40%) hit within 24 hours of being released, with most of them hitting withing 8 hours of release. b) The 2D calculation gives a similar number of landfalls (924/2300, or ~40%) within the first 24 hours, with less of a drop-off after 8 hours (e.g. they continue to hit land for a longer time period than with the 3D calculation).

Figure 4: Histogram plots for 3 subregions using 3-D trajectory modeling (see inset box for region used to calculate histograms). The subregions shown are a) Admiralty Bay, b) Useless Bay and c) Main Basin/Pt. Wells. In panels a) and b), we see the particles make landfall well within the first 8 hours; for the Main Basin (panel c) it takes longer for particles to make landfall. This is likely due to the difference in current regimes; Admiralty Inlet is characterized by strong flows and vigorous mixing, the Main Basin has relatively weaker flows and less vigorous mixing.

Figure 5: Successive frames from the simulation at 1, 6, 12, 18 and 24 hours after the start of the model run are presented in panels a-e. To assess the dilution between ship and shore, we focus on 3 areas (North, Middle and South release areas, corresponding to Admiralty Bay, Useless Bay and Pt. Wells). These areas are shown by green dots on the inset map of the model domain. Dye is released only in the first hour of the tidal cycle and subsequently advected for the remainder of the day. After the dye release is complete (at hour 1), we record the peak concentration at the release location, and compare to the concentration at the 3 land locations. We monitor the dye concentration through time, and use the peak dye concentration to estimate the minimal dilution expected. Also presented in each panel is a colormap of the dye concentration, both in plan view (inset map) and cross-section view. The cross-section is shown on the inset map as the green line down the center of the basin. Distance along the cross-section is measured from north to south. Note the significant dilution of the dye concentration and vertical mixing over the sills (shallower areas shown in the cross-section) when compared with areas in the Main Basin.

FIGURES

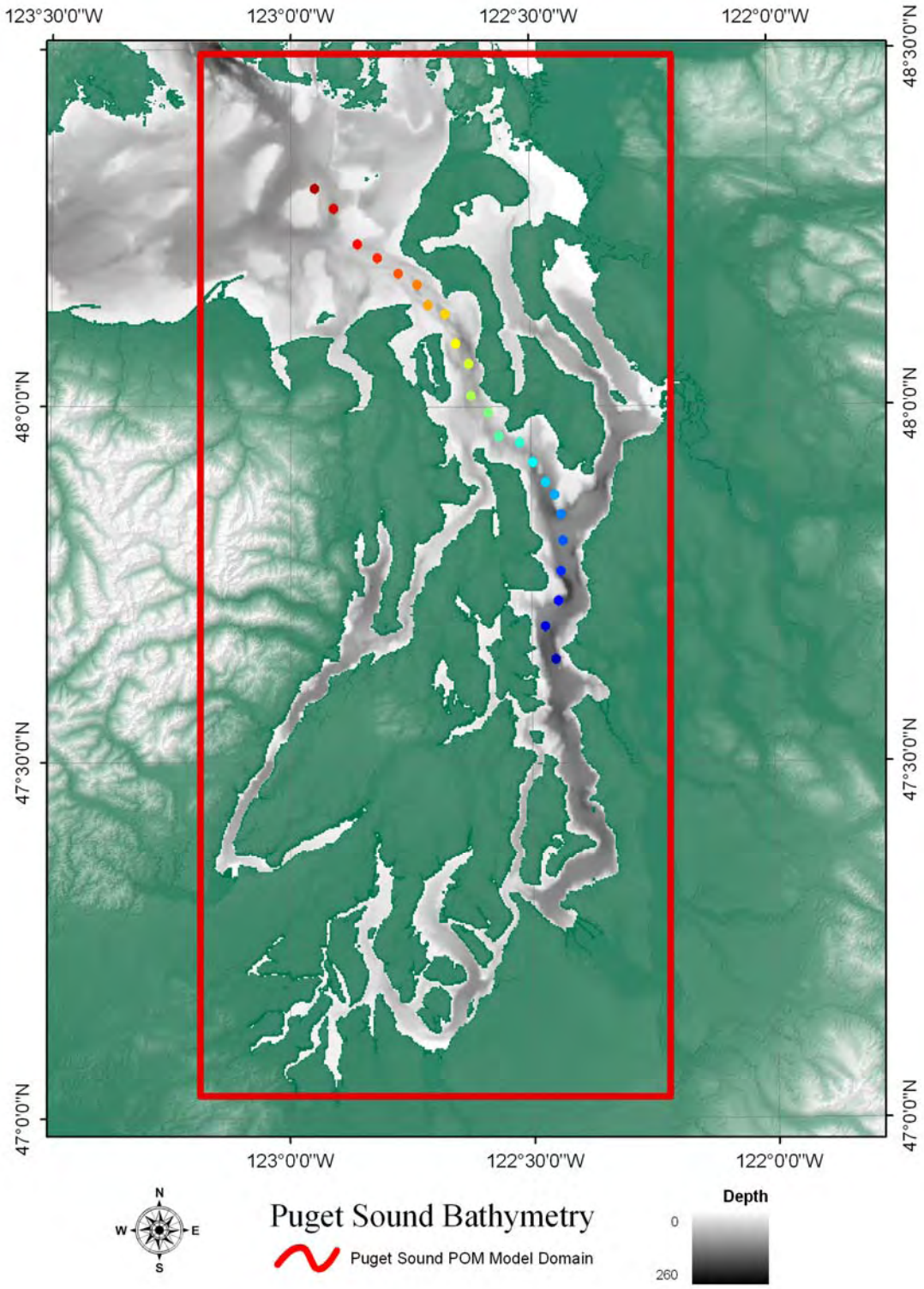


Figure 1

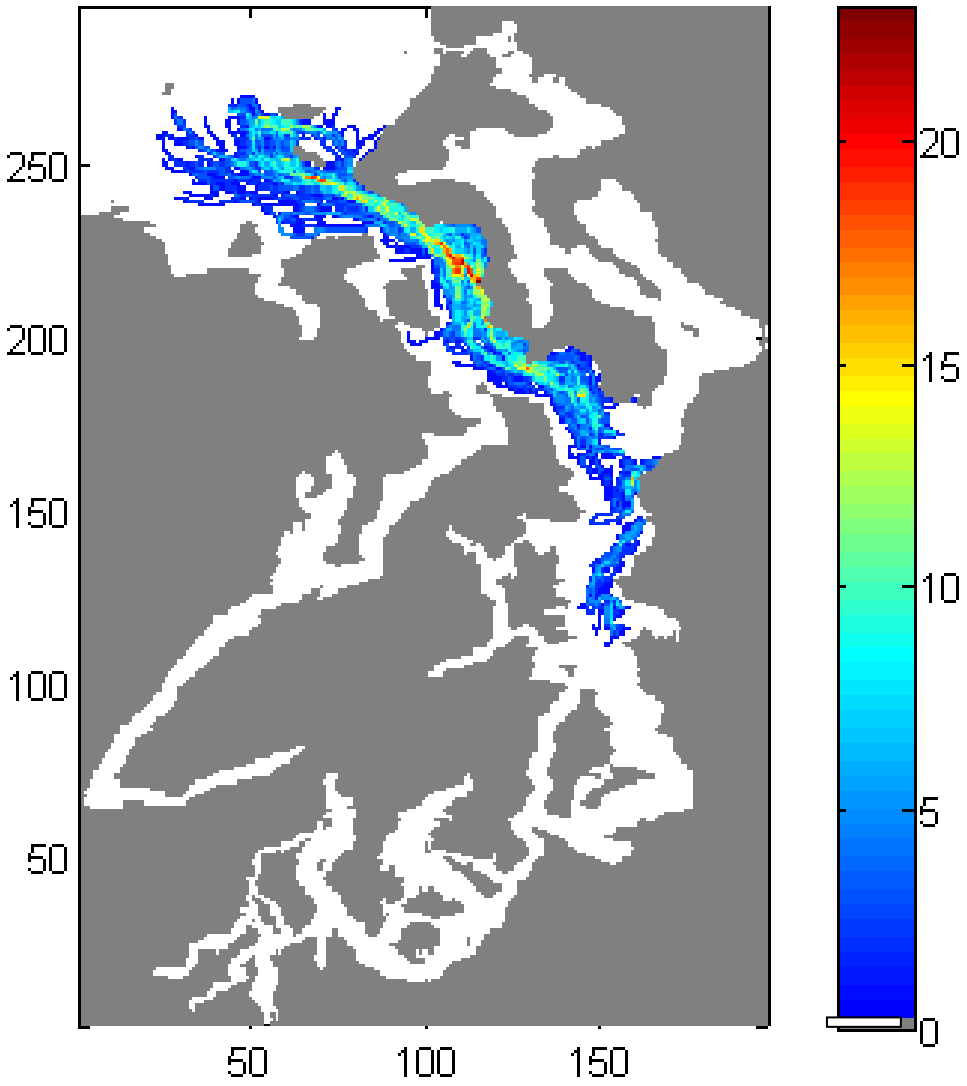


Figure 2a

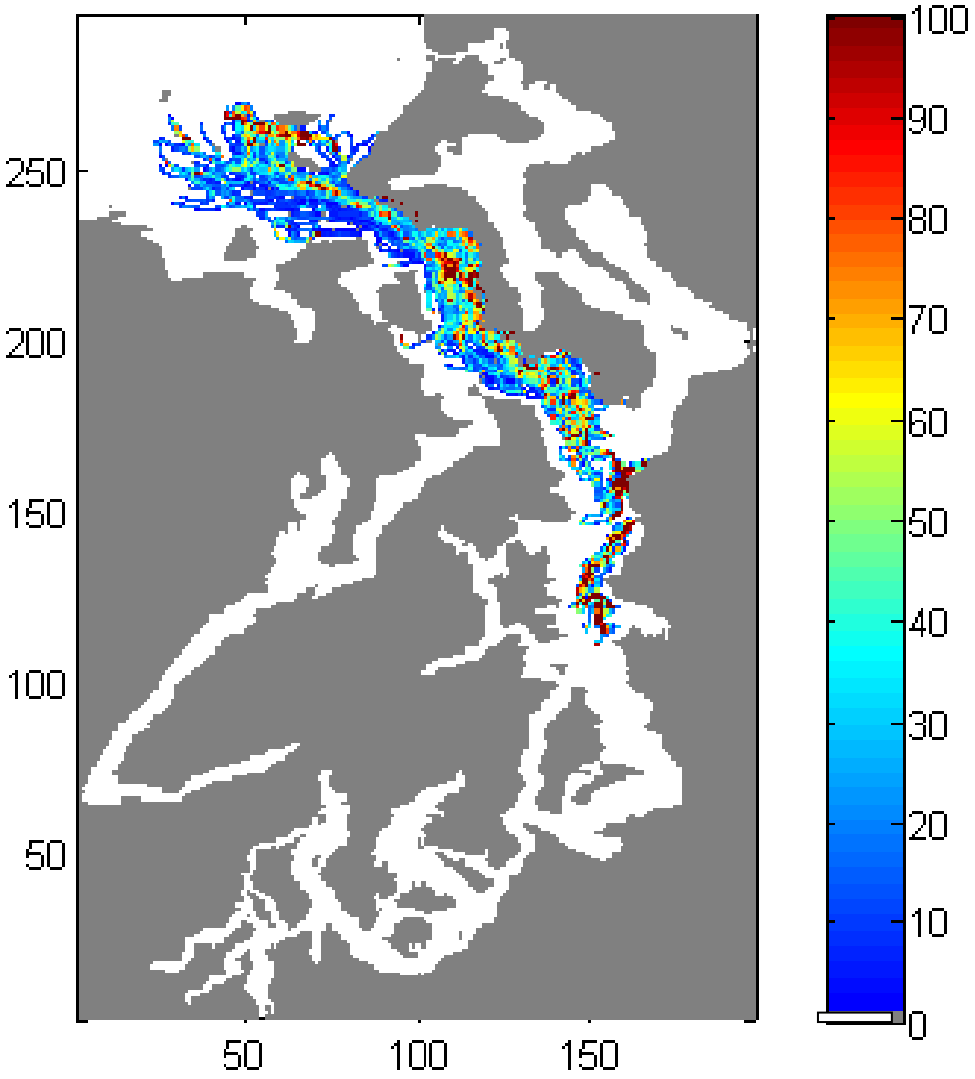


Figure 2b

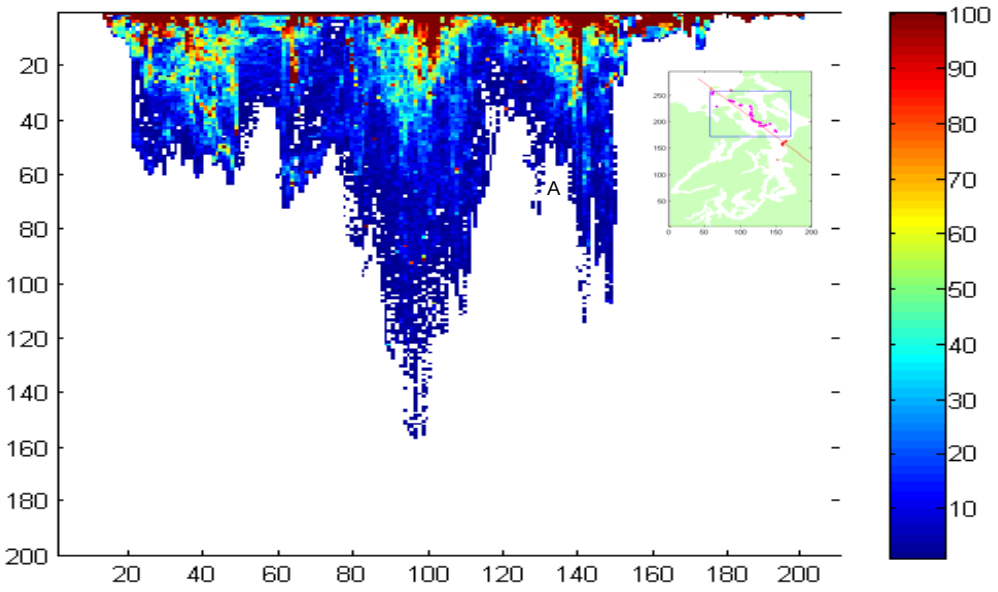

20
40
60
80
100
120
140
160
180
200
20
40
60
80
100
120
140
160
180
200
A
100
90
80
70
60
50
40
30
20
10

Figure 2c

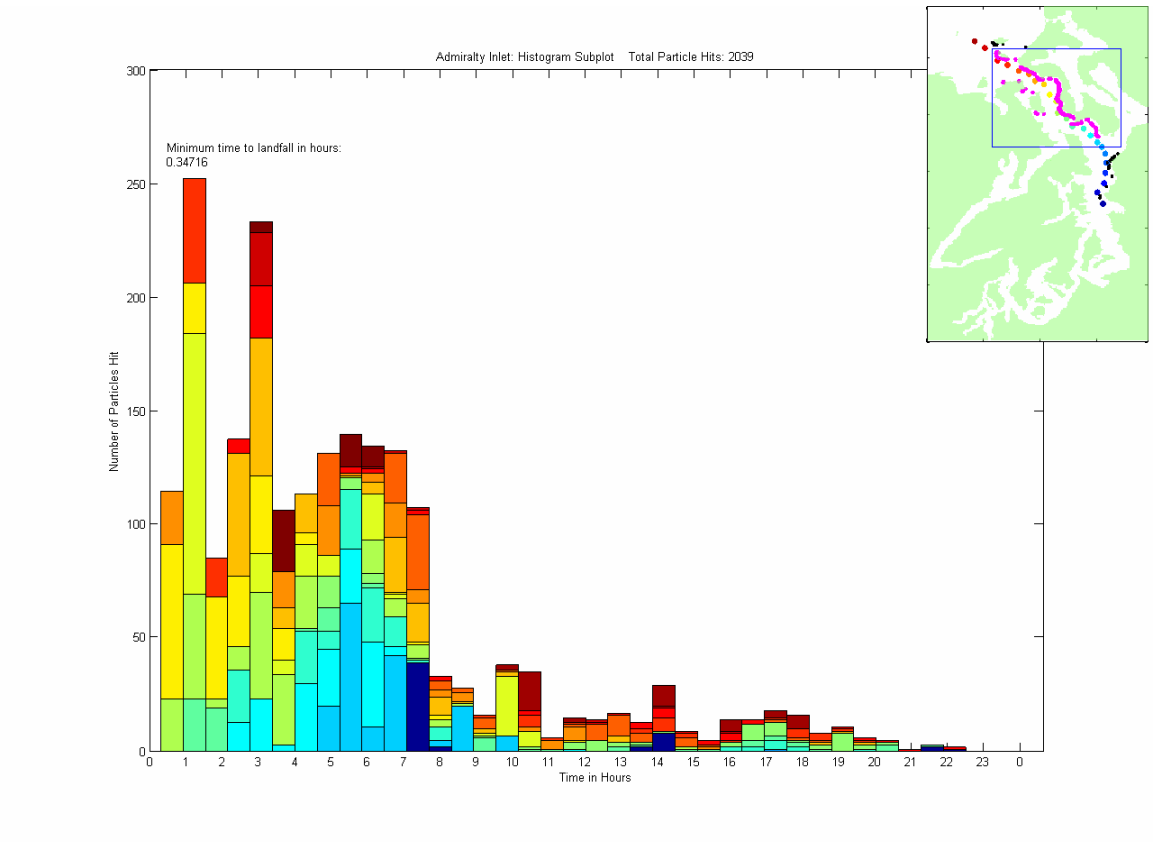

Admiralty Inlet: Histogram Subplot Total Particle Hits: 2039
Minimum time to landfall in hours:
0.34716
Number of Particles Hit
Time in Hours
0
50
100
150
200
250
300
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 0

Figure 3a

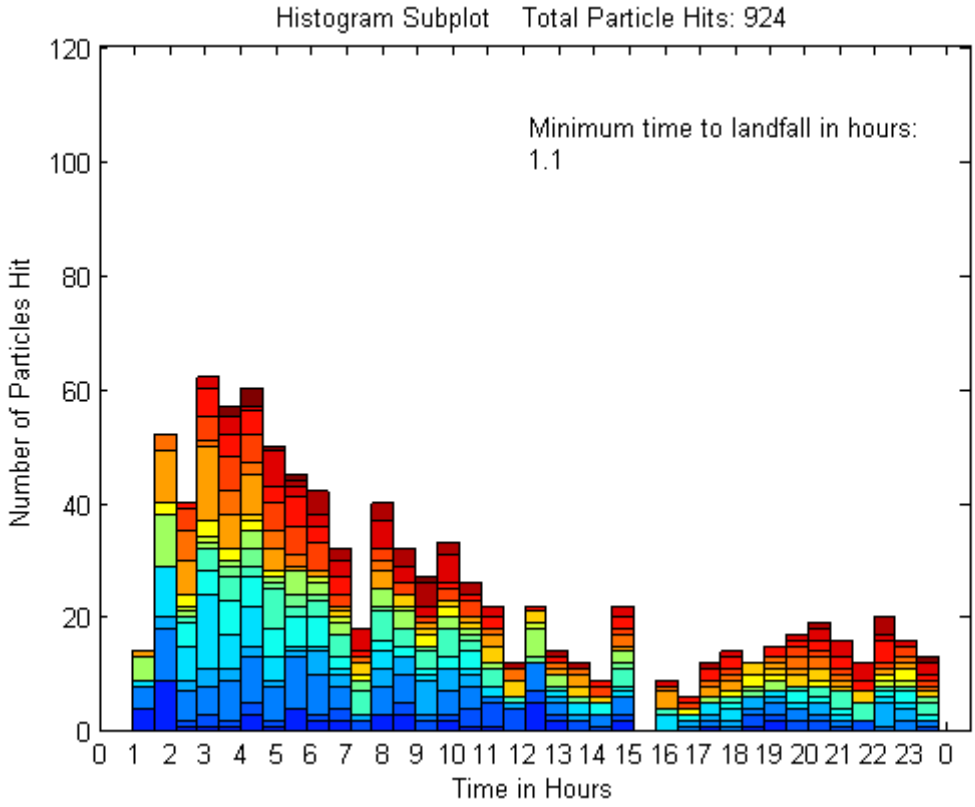

Histogram Subplot Total Particle Hits: 924
Minimum time to landfall in hours:
1.1
Number of Particles Hit
Time in Hours
0
20
40
60
80
100
120
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 0

Figure 3b

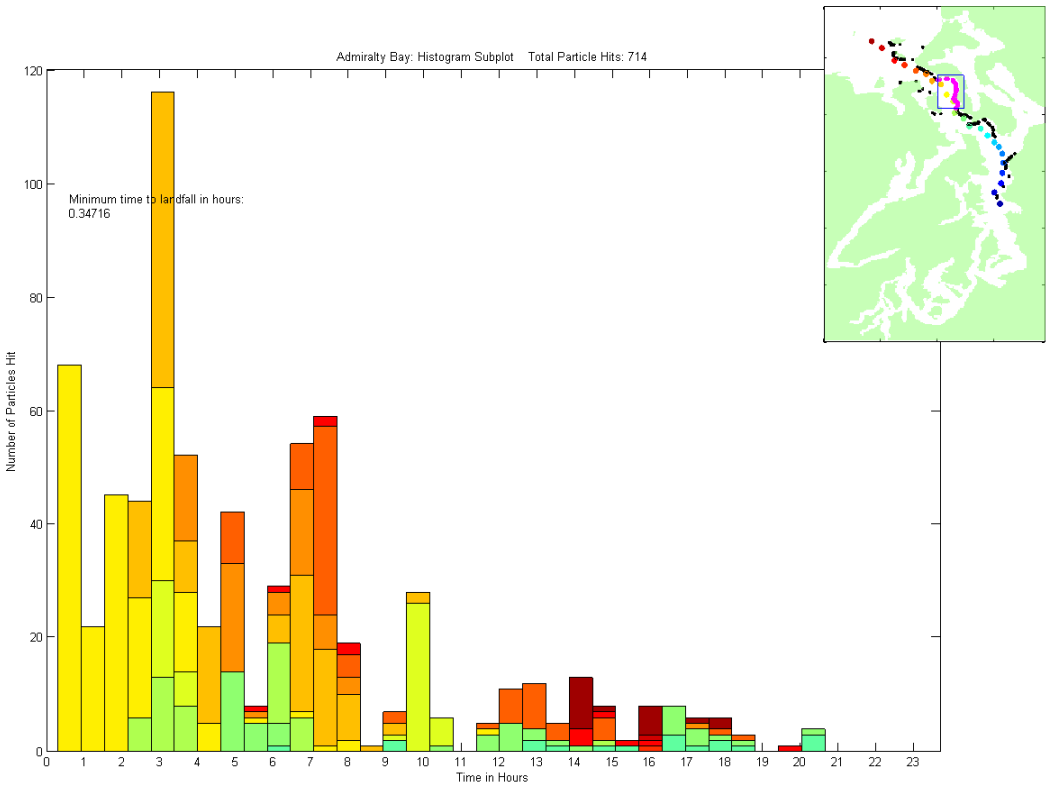

Admiralty Bay: Histogram Subplot Total Particle Hits: 714
Minimum time to landfall in hours:
0.34716
Number of Particles Hit
Time in Hours

Figure 4a

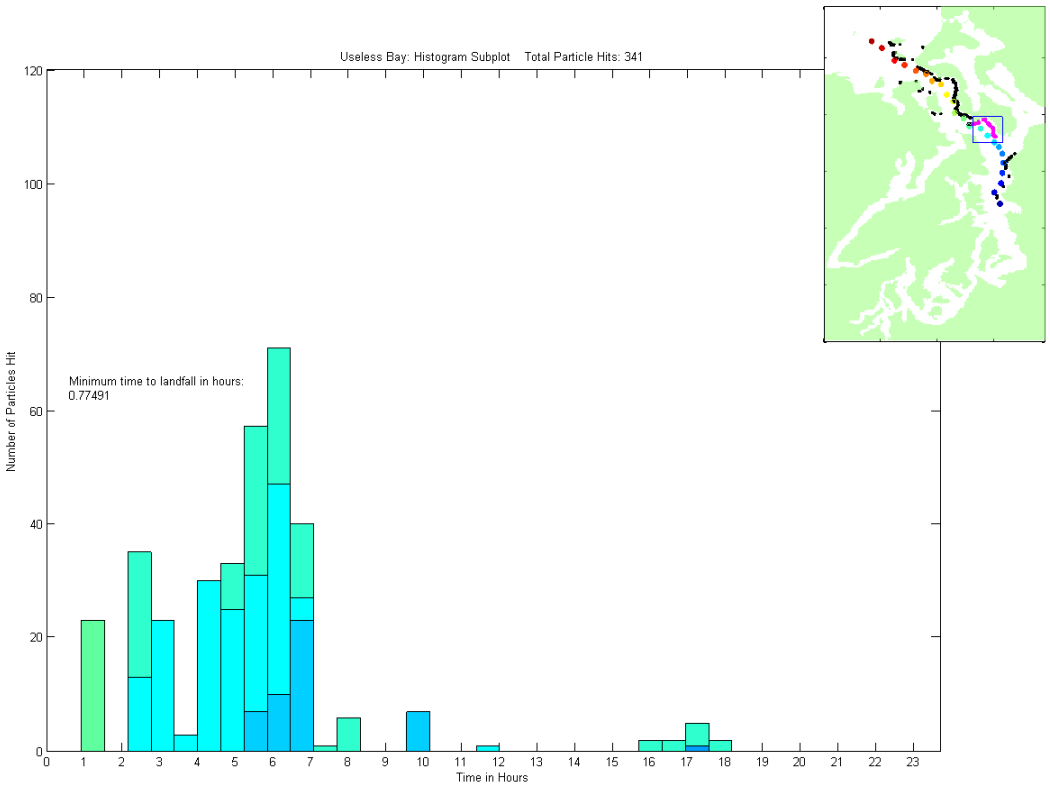

Useless Bay: Histogram Subplot Total Particle Hits: 341
Minimum time to landfall in hours:
0.77491
Number of Particles Hit
Time in Hours

Figure 4b

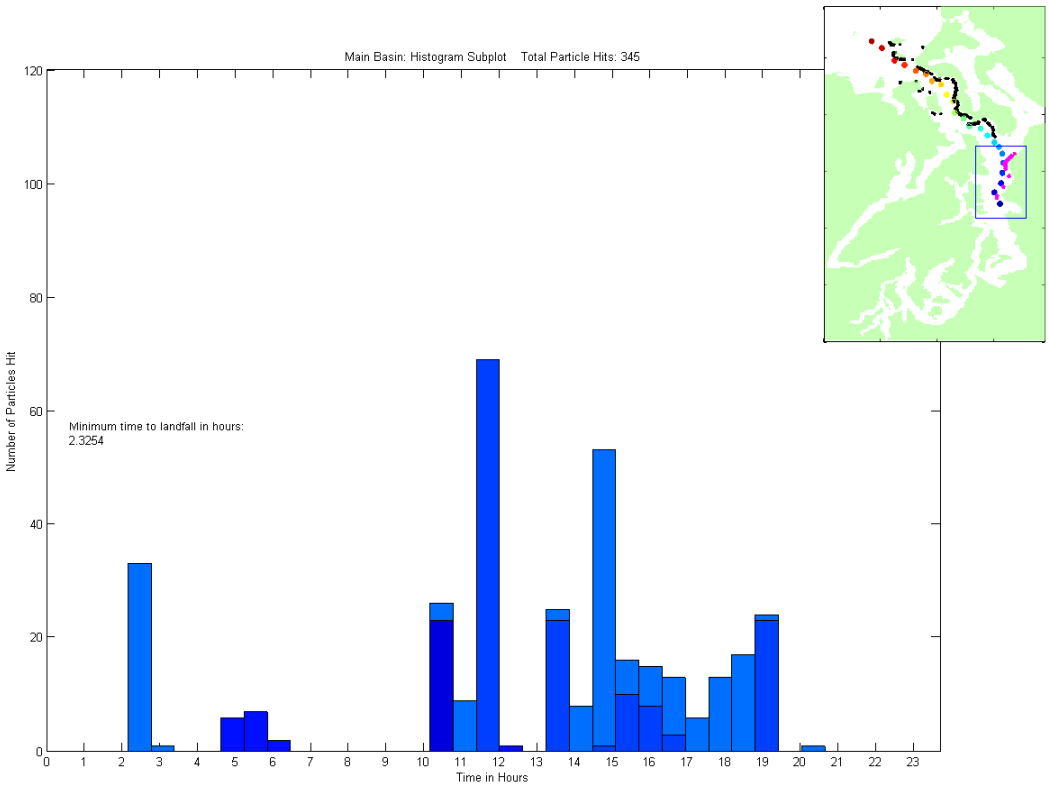

Main Basin: Histogram Subplot Total Particle Hits: 345
Number of Particles Hit
Time in Hours
Minimum time to landfall in hours:
2.3254

Figure 4c

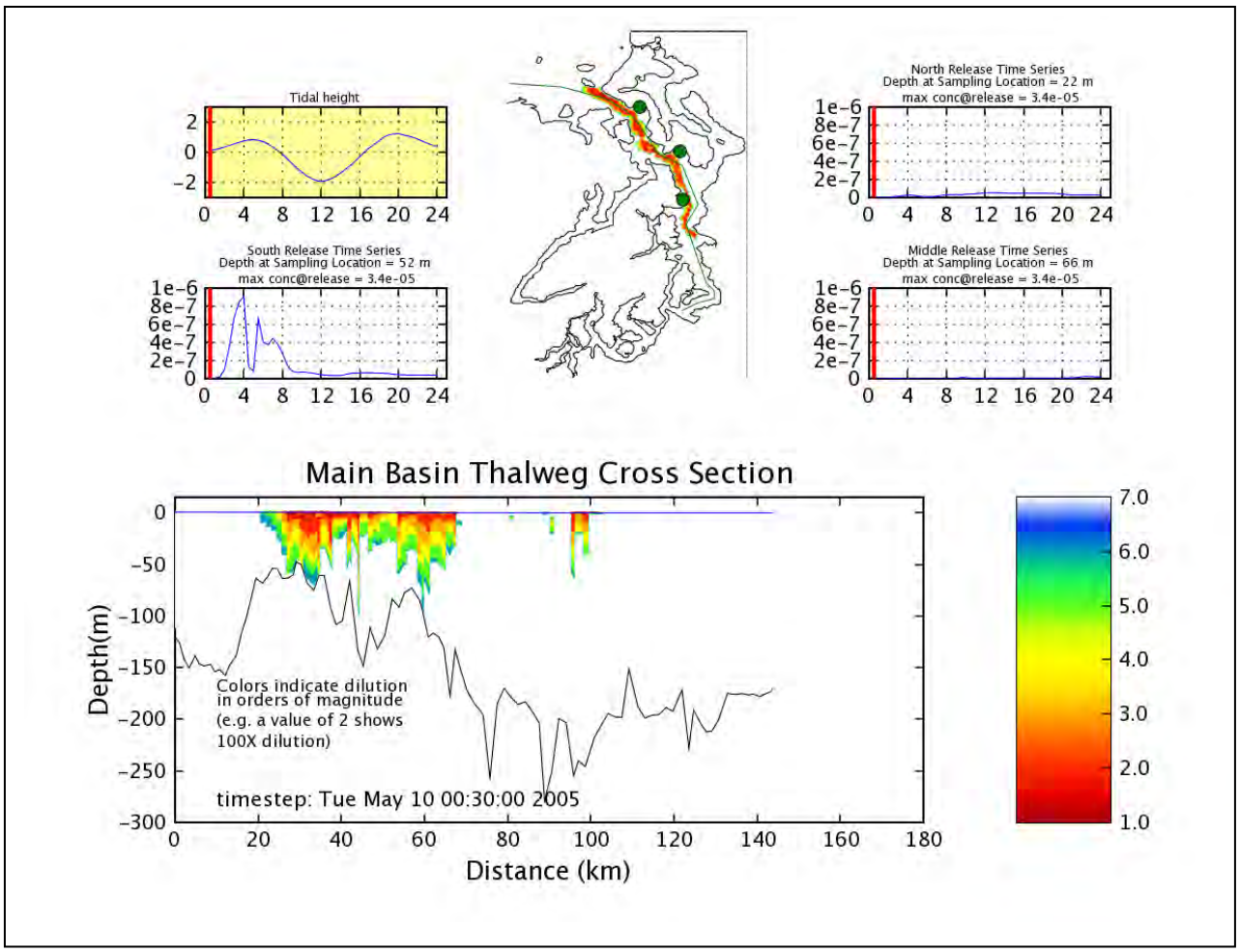
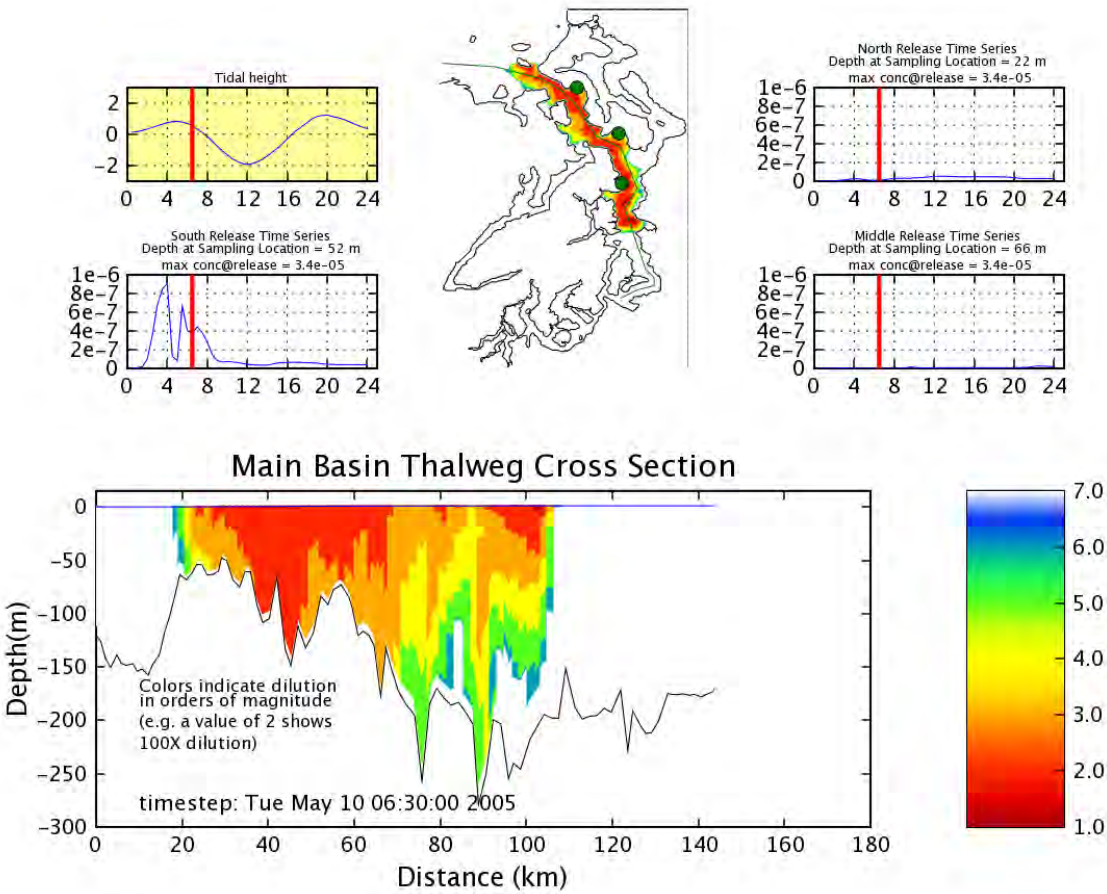

Tidal height
South Release Time Series
Depth at Sampling Location = 52 m
max conc@release = 3.4e-05
North Release Time Series
Depth at Sampling Location = 22 m
max conc@release = 3.4e-05
Middle Release Time Series
Depth at Sampling Location = 66 m
max conc@release = 3.4e-05
Main Basin Thalweg Cross Section
Depth(m)
Distance (km)
Colors indicate dilution
in orders of magnitude
(e.g. a value of 2 shows
100X dilution)
timestep: Tue May 10 00:30:00 2005

Figure 5a


Tidal height
South Release Time Series
Depth at Sampling Location = 52 m
max conc@release = 3.4e-05
North Release Time Series
Depth at Sampling Location = 22 m
max conc@release = 3.4e-05
Middle Release Time Series
Depth at Sampling Location = 66 m
max conc@release = 3.4e-05
Main Basin Thalweg Cross Section
Depth(m)
Distance (km)
Colors indicate dilution
in orders of magnitude
(e.g. a value of 2 shows
100X dilution)
timestep: Tue May 10 06:30:00 2005

F

Figure 5b

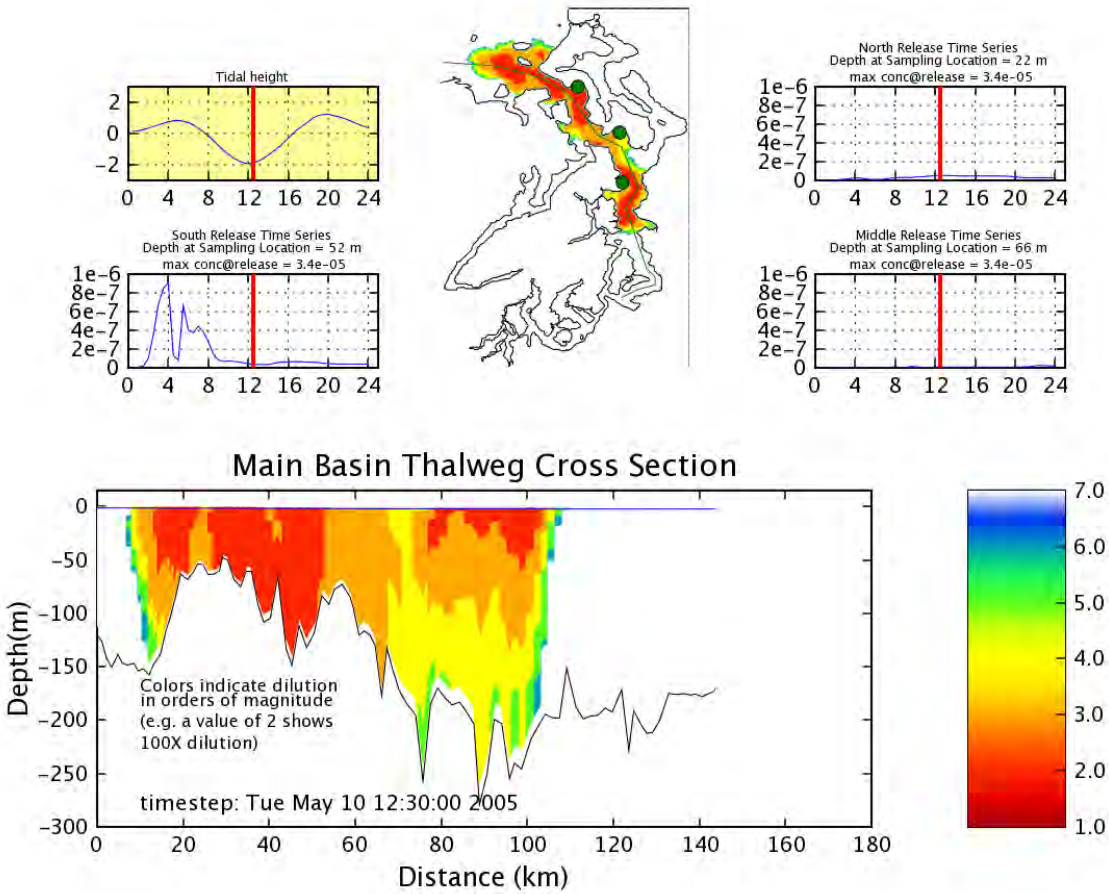

Tidal height
North Release Time Series
Depth at Sampling Location = 22 m
max conc@release = 3.4e-05
South Release Time Series
Depth at Sampling Location = 52 m
max conc@release = 3.4e-05
Middle Release Time Series
Depth at Sampling Location = 66 m
max conc@release = 3.4e-05
Main Basin Thalweg Cross Section
Depth(m)
Distance (km)
Colors indicate dilution
in orders of magnitude
(e.g. a value of 2 shows
100X dilution)
timestep: Tue May 10 12:30:00 2005

Figure 5c

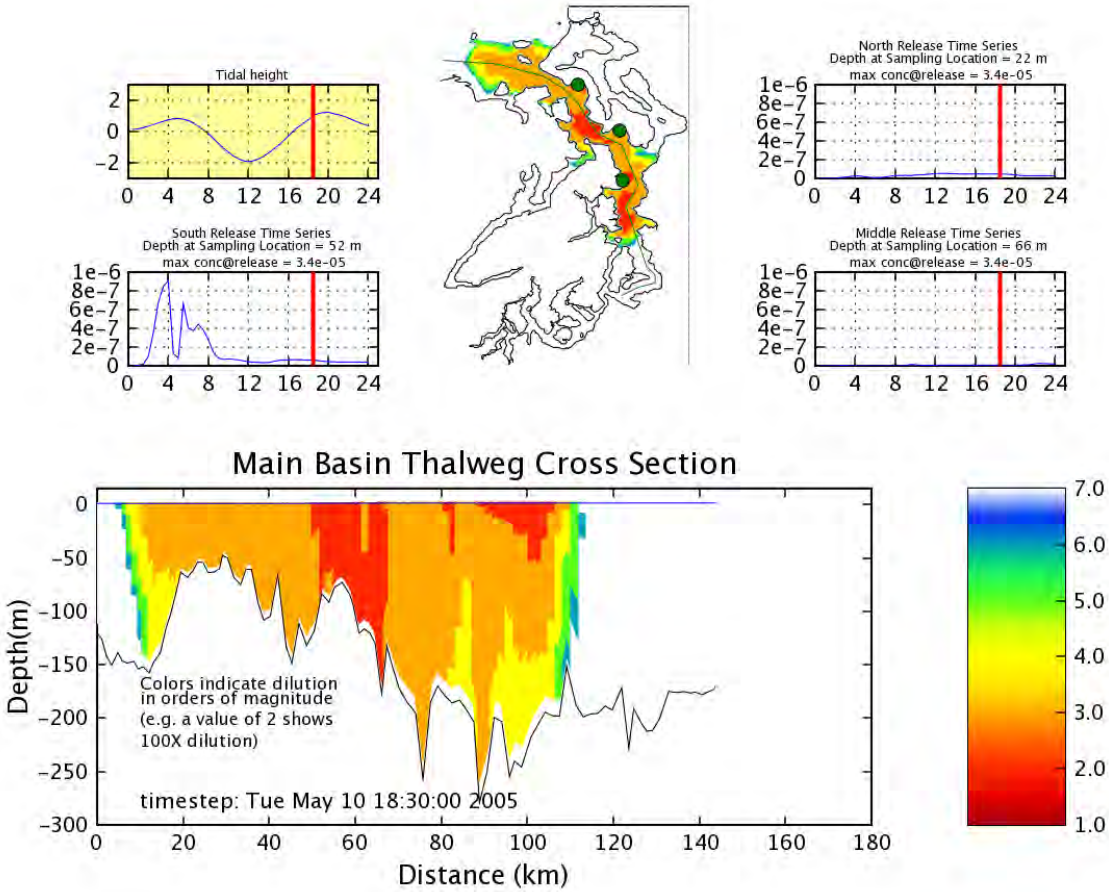

Tidal height
South Release Time Series
Depth at Sampling Location = 52 m
max conc@release = 3.4e-05
North Release Time Series
Depth at Sampling Location = 22 m
max conc@release = 3.4e-05
Middle Release Time Series
Depth at Sampling Location = 66 m
max conc@release = 3.4e-05
Main Basin Thalweg Cross Section
Depth(m)
Distance (km)
Colors indicate dilution
in orders of magnitude
(e.g. a value of 2 shows
100X dilution)
timestep: Tue May 10 18:30:00 2005

Figure 5d

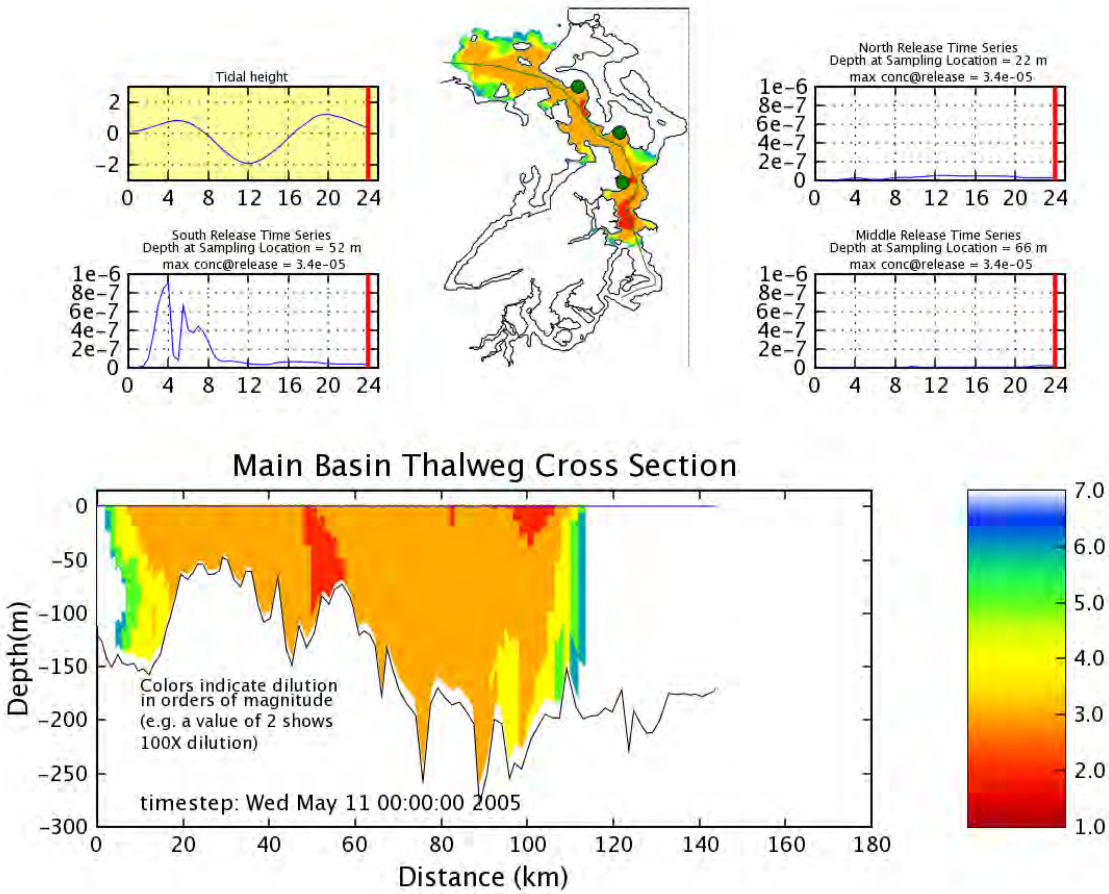

Tidal height
South Release Time Series
Depth at Sampling Location = 52 m
max conc@release = 3.4e-05
North Release Time Series
Depth at Sampling Location = 22 m
max conc@release = 3.4e-05
Middle Release Time Series
Depth at Sampling Location = 66 m
max conc@release = 3.4e-05
Main Basin Thalweg Cross Section
Depth(m)
Distance (km)
Colors indicate dilution
in orders of magnitude
(e.g. a value of 2 shows
100X dilution)
timestep: Wed May 11 00:00:00 2005

Figure 5e