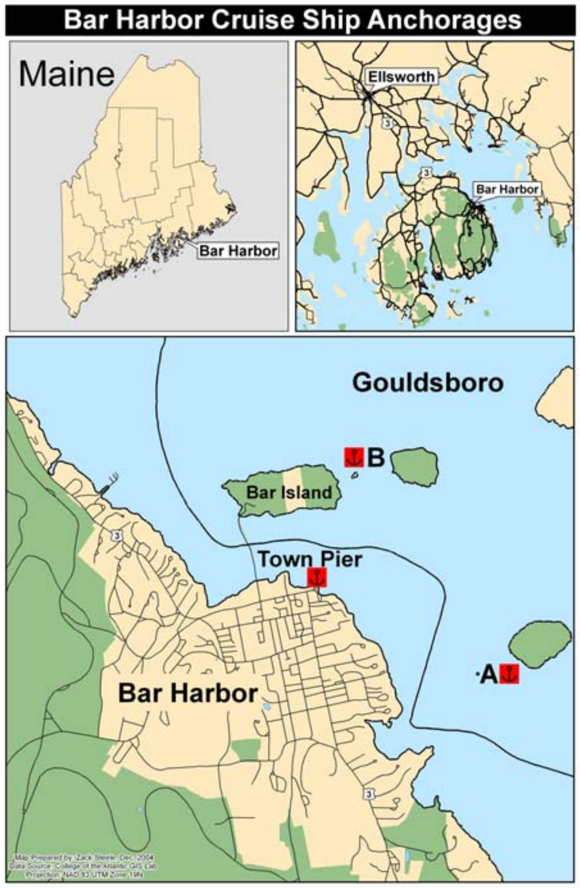
Cruise Ship Water Quality Report

Bar Harbor, Maine
May - November 2004

Prepared by:
The MDI Water Quality Coalition



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The MDI Water Quality Coalition engages citizens of all ages in preserving and improving the water quality of MDI through meaningful environmental research and community education.

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Executive Summary

The MDI Water Quality Coalition initiated a citizen-based cruise ship monitoring project in May 2004 after community members expressed concern about cruise ship industry compliance with new state legislation. The new legislation, *An Act to Protect Maine's Coastal Waters*, enacted in April 2004, prohibits discharge of graywater or a mixture of graywater and blackwater and requires licensing by 2006 of advanced wastewater treatment systems for ships discharging in the coastal waters of Maine. The Bar Harbor harbormaster's boat was used to transport monitors alongside ships in order to sample water for phytoplankton, temperature, salinity, dissolved oxygen, biological oxygen demand, *Enterococcus* bacteria, transparency, chlorine, and nitrogen. Water was sampled near 31 cruise ships between May and November 2004. Water samples were analyzed at the Community Environmental Health Laboratory, a research and education collaborative with the MDI Biological Laboratory. No significant long-term effects on the water quality in Bar Harbor were detected. Two ships discharged contaminated water; these were not members of the International Council of Cruise Lines (ICCL). ICCL ships (10 in all) appeared to be in full compliance with new state legislation. As a result of this project, the MDI Water Quality Coalition is making recommendations to the cruise ship industry, the town of Bar Harbor, the Maine State DEP, and community members. These include increased awareness of state and local regulations, support and funding for local citizen groups to ensure monitoring during future cruise ship seasons, clearly stated local harbor regulations, clearly interpreted and stringently enforced state legislation, and increased community involvement.

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Introduction

The Cruise Ship Water Quality Report for Bar Harbor, Maine was prepared by student interns, research fellows and staff of the Mount Desert Island Water Quality Coalition, a non-profit organization dedicated to engaging citizens of all ages in preserving and improving the water quality of Mount Desert Island through meaningful environmental research and community education. We hope this report and its associated appendices may serve as a model for other communities that are evaluating cruise ship impacts on water quality in their harbors.

How did we get involved with cruise ship monitoring?

The MDI Water Quality Coalition began monitoring around cruise ships in response to citizen interest in cruise ship issues. Student interns working with the MDI Water Quality Coalition attended a Natural Resources Committee hearing on proposed cruise ship legislation in Augusta on February 10, 2004. Upon their return, they felt it was important to communicate changes in proposed legislation to the citizens of Bar Harbor, and so they planned a series of *Community Conversations about Cruise Ships* to which they invited the public. These meetings were held in Bar Harbor between February and May 2004. A diversity of people from a cross section of the community (college and high school students, businesses owners, boat owners, and teachers) attended the meetings. Most citizens wondered whether cruise ships discharged wastewater into Frenchman Bay. Citizens felt that monitoring of water quality around visiting cruise ships was important for determining the impact of cruise ships on the environment. After four meetings, ideas for a monitoring program were brought to the Bar Harbor Marine Resources Committee as well as to the Bar Harbor Town Council. It was agreed that monitoring water quality around cruise ships was important. The MDI Water Quality Coalition student interns, together with the Bar Harbor harbormaster, developed a data collection protocol. Water quality monitoring around cruise ships began in May 2004.

What was the purpose of monitoring cruise ships?

This monitoring project was undertaken in order to engage local citizens in stewardship of Frenchman Bay in light of the increase in visitation by cruise ships. The variables that were used as indicators of water quality were chosen to reveal effects of discharges from these types of ships. As an outcome of this environmental stewardship project, citizens would see for themselves if cruise ships were actually having an effect on the water quality in Bar Harbor.

What is the history of wastewater discharge from cruise ships in Bar Harbor and elsewhere?

In the past, there have been questionable discharges from visiting cruise ships in Bar Harbor (J. Litteral, former assistant harbormaster, personal communication). Because there was no monitoring program established by the town or state, there are no data regarding discharges from cruise ships visiting Bar Harbor before 2004.

Between 1999 and 2001, there were 39 confirmed illegal discharges into Alaska's state waters. There was only one such violation between 2002 and 2003, following the passage of the *Alaska Cruise Ship Initiative* in 2001. The Alaska legislation requires: 1) a verified program of sampling, testing, and reporting of wastewater and air discharges from cruise ships, 2) an enforceable standard for what cruise ships may discharge into Alaska waters, 3) a method of payment for the program (http://www.serconline.org/cruiseShipPollution.html).

In California, a Crystal Cruise Line ship discharged approximately 36,000 gallons of sewage and wastewater into Monterey Bay National Marine Sanctuary in October 2002. In 2003, California prohibited the discharge of sewage sludge, oily bilge water, hazardous wastes, and other wastes such as dry cleaning chemicals and medical wastes from visiting cruise ships. Recent bills protect the ocean from graywater discharges and burning of garbage (http://www.bluewaternetwork.org). According to the National Sea Grant Law Center (http://www.olemiss.edu/orgs/SGLC), California's laws are now the most stringent in the nation.

What cruise ship legislation exists in Maine?

An Act to Protect Maine's Coastal Waters, enacted in April 2004, prohibits the operator of a large commercial vessel from discharging untreated gray water or a mixture of gray water and black water to coastal waters (coastal waters being within three miles of the shore). Operators of large commercial passenger vessels may discharge gray water or a mixture of gray water and black water into coastal waters if the effluent has been treated with a wastewater treatment system that meets state effluent standards. Beginning in January 2006, vessels with advanced wastewater treatment systems must be licensed in Maine according to the same standards as those described for ships visiting Alaska (http://janus.state.me.us/legis/).

What was the scope of our study?

In the 2004 season there were 87 cruise ship visits to Bar Harbor. These visits were made by a total of 20 ships. On many days, there were two ships in the harbor; we could only sample around one ship on these days. Altogether, we monitored the water around 16 different ships; we monitored most of them on more than one occasion (Figure 1). As a control, we took samples when ships were not in port at each of the three cruise ship anchorages (Figure 2). Most cruise ships anchored at Anchorage A or B. Ships with 250 passengers or fewer anchored at the town pier.

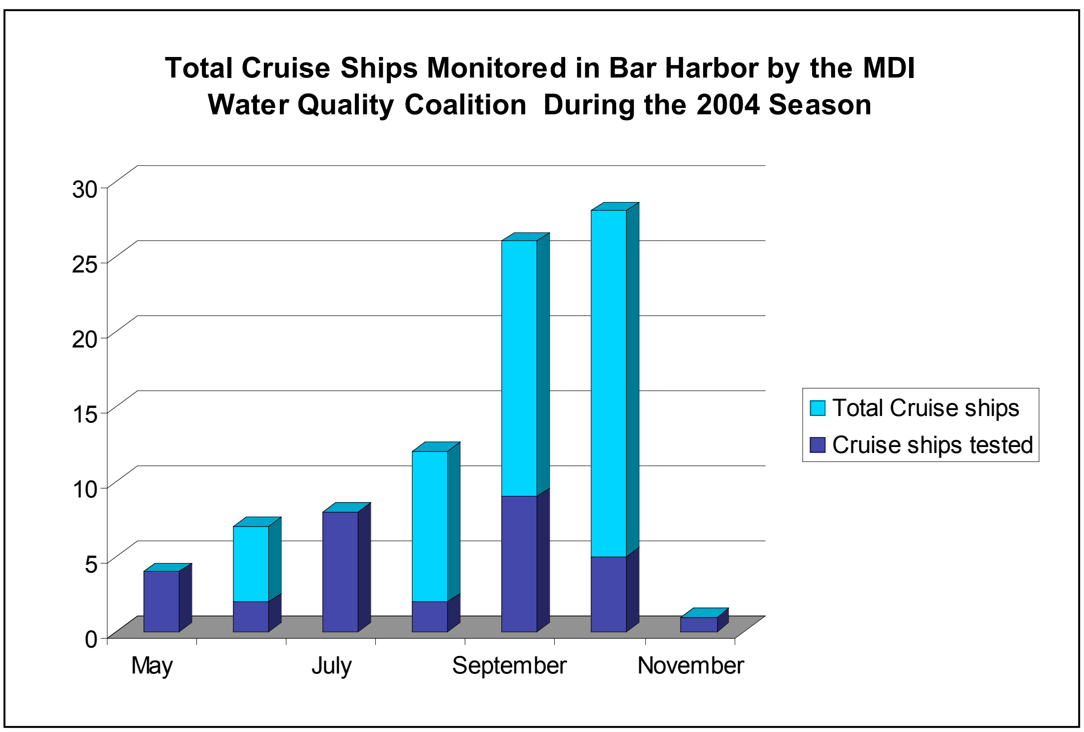

Figure 1: Cruise ships were monitored throughout the 2004 season. In all, water was monitored around 31 visiting cruise ships. There were many more ships than could be studied in the fall due to time constraints, weather conditions, and numbers of volunteers.

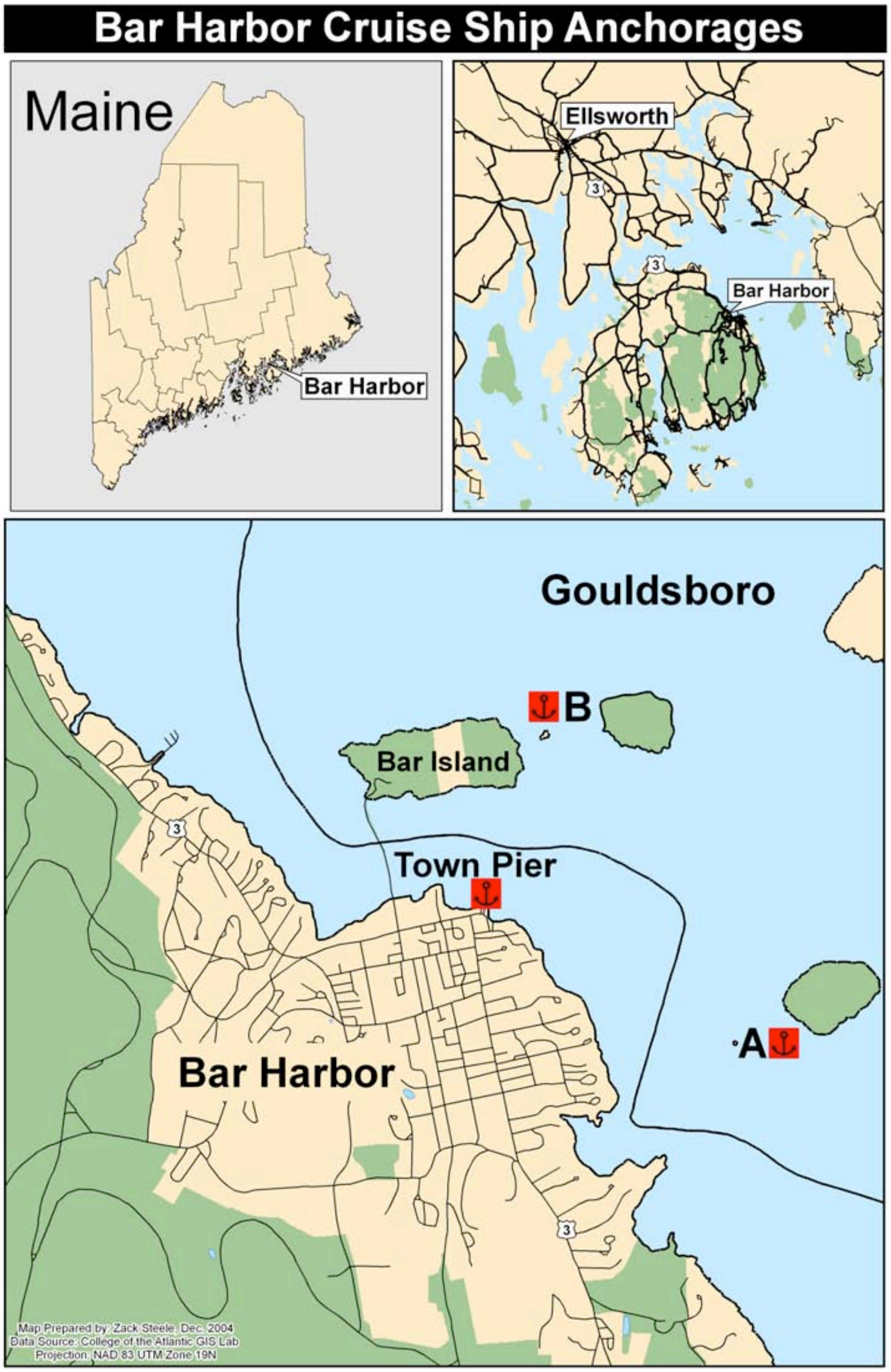

Figure 2: Cruise Ship Anchorages are in Frenchman Bay. Anchorage A is about a half-mile offshore. Anchorage B is between offshore islands and visible from Hulls Cove. The Town Pier anchorage accommodates ships with fewer than 250 passengers.

Procedures

We found no model for citizen-based monitoring around cruise ships from other states or countries. We established our own protocol for monitoring around cruise ships with input from the Bar Harbor harbormaster, the Maine State Department of Environmental Protection, and the Maine Healthy Beaches Program. We followed an EPA-approved Quality Assurance Project Plan and other established guidelines for monitoring (see references *1,2,3*).

How did we monitor around cruise ships?

The harbormaster transported water quality monitors to offshore cruise ship anchorages when ships were in the harbor and when they were not. Before approaching a cruise ship in the harbormaster boat and penetrating the "100 yard security zone" (in which no boats were allowed), the harbormaster contacted the ship. Captains and crew were informed of intentions to monitor and were asked to reveal points of discharge. Sampling was conducted as close to the point of discharge as possible. Water around ships at the town pier was monitored from a floating dock.

What were our field and lab methods?

At the sample site*:* A data sheet was completed that included Names of Monitors, Berth, Cruise Ship Name, Date, Time (24hr), Weather, Wind speed, Boat Traffic, Rainfall in the last 24 hours, Tide Stage, Water Surface, and Current (Appendix A1.1). Field comments were recorded throughout the sampling process. Water samples were taken for bacterial analysis in sterile Whirl-pak® bags and stored in a cooler at 10°C until analysis in the lab, which always took place within 6 hours (Appendix A1.2). Thermometers were placed eight inches below the surface of the water for 5 minutes to determine water temperature and in the shade for 5 minutes to determine air temperature (Appendix A1.3). Water was sampled in 60 ML bottles for determining dissolved oxygen (DO) levels. These water samples were fixed at the time of sampling using a LaMotte® DO kit. Duplicate samples were taken to determine biological oxygen demand (BOD) five days later (Appendix A1.4). Additional samples were taken to determine nitrogen and chlorine levels. The transparency of the water was found using an Oceanographic Secchi Disk and an AquaScope™ (see Appendix A1.5). Phytoplankton were collected with a 20-micron net during a 5-minute tow and the tow depth was recorded. The phytoplankton sample was stored in a jar in a cooler at 10°C or less until analysis in the lab (Appendix A1.6).

To better access the discharge pipe of the *Maasdam*, which was often located underwater, water samples for bacteria, DO and BOD were taken from 14 feet below the water surface as well as at the surface. A LaMotte® Dissolved Oxygen Sampler was used to lower sample bottles to the desired depth.

***At the Community Environmental Health Laboratory**:** The water sample that was taken for bacterial analysis was tested for *Enterococcus*, a type of bacteria found in the gut of warm-blooded animals that is used as an indicator of potential recreational water illness in marine waters in Maine (Appendix A2.1). After running the bacterial test, a sample of water from the bag was used to determine salinity (Appendix A2.2). The fixed DO samples were titrated and oxygen levels were determined (Appendix A2.3). Five days later, the water samples taken for BOD were fixed and oxygen levels were determined (Appendix A2.4). A color comparison test to measure the parts per million of nitrogen in the water was conducted on one of the additional samples of water taken near the ship (Appendix A2.5). A LaMotte® Chlorine Colorimeter was used to establish chlorine levels in samples of water taken near the ship (Appendix A2.6). Microscopic analysis of phytoplankton species was conducted by identifying and counting all species of phytoplankton observed in six fields of view on two slides. The two most dominant species of phytoplankton and the total number of organisms in six fields of view were recorded on the field sheet (Appendix A2.7).

**The Community Environmental Health Laboratory is a research and education collaborative of the MDI Water Quality Coalition and the MDI Biological Laboratory located in Salisbury Cove, ME.*

Why are these variables important?

Phytoplankton were collected by towing to determine if there were any types that could impact the results of water quality tests or if non-native species of phytoplankton had been transported in ballast water of cruise ships. Phytoplankton as small as *Alexandrium*, which are 30 microns in size, were observed. This organism tends to bloom offshore, often from natural causes, and was responsible for closures of shellfish beds in Bar Harbor in the summer of 2004.

Towing for phytoplankton

Alexandrium

Dissolved Oxygen (DO) is an indicator of water quality that is usually correlated with water temperature. Dissolved oxygen levels below 6 parts per million (ppm) cannot support most marine life. It is necessary to know the initial oxygen level to determine the biological oxygen demand.

Preparing dissolved oxygen samples

Biological Oxygen Demand (BOD) is the measure of the amount of oxygen consumed by microorganisms in the water over a 5-day period. It is an indicator of organic matter in the water that could be a result of gray water or black water discharges. BOD levels above 2 ppm indicate that water may be polluted with organic matter.

Setting up BOD bottles

Enterococci**,** bacteria that come from the gut of warm-blooded animals, may indicate that untreated or inadequately treated sewage discharges have occurred. There is an increased risk of contracting a recreational water illness when bacterial levels rise above 100 colonies per 100 ml. of water. Water samples were taken in sterile plastic bags. The test for *Enterococcus* was run at the Community Environmental Healthy Laboratory.

Running an Enterococcus test

Taking a water sample for bacterial analysis

Nitrogen is a nutrient that, when introduced into the ocean in large amounts, can lead to an unhealthy bloom of plant life, ultimately resulting in a depletion of oxygen. Human waste has high nitrogen levels, and if a cruise ship were dumping raw sewage, then high levels of nitrogen may be present in the water. Ships with advanced wastewater treatment systems are permitted to discharge treated wastewater. Nitrogen is one of the few contaminants that cruise ships with these advanced systems cannot eliminate from their effluent (4).

Chlorine is used to treat wastewater in some ships using Marine Sanitation Devices. Chlorine can be damaging to the environment when discharged even at low levels. The recommended maximum for all fish and aquatic life is 0.01 ppm. Most marine plankton are killed when levels reach 0.1 ppm (*5*).

The *Maasdam*, equipped with an operational advanced wastewater treatment system, visited Bar Harbor 22 times in 2004 accounting for 25% of all cruise ship visits.

Upwelling is evident near *Maasdam* discharge pipe on May 16, 2004.

A LaMotte® nitrogen test kit was used to determine nitrogen levels in water samples taken near the discharge pipe. Samples with high nitrogen levels turn pink using this colorimetric test. Here, results of a positive control are shown.

Sampling for chlorine and nitrogen near the *Maasdam* discharge pipe October 10, 2004. Significant levels of nitrogen and chlorine were *not* detected in samples taken near *Maasdam.*

Results

We learned more than we anticipated on our trips out to visiting cruise ships. In addition to gathering observational data and water samples for lab analysis, we were able to communicate with ship captains about their wastewater treatment systems. We discovered that not all captains and crews were knowledgeable about their own systems, Maine Legislation, or the “code of behavior” expected by the Bar Harbor harbormaster.

What did we find out about water quality?

When ships that belong to the International Council of Cruise Lines (ICCL) were in the harbor, test results indicated that water quality was good: bacterial levels were low, transparency was high, and oxygen levels were above 6 ppm, the level necessary to sustain most marine life (see Table 1). When looking at a number of other parameters such as temperature, salinity, chlorine, and nitrogen levels, we found results similar to control samples (taken when cruise ships were not in the harbor, see full data set with control samples and phytoplankton data, Appendix B).

There was a natural bloom of an unusual phytoplankton species called *Phaeocystis* just prior to the arrival of the first cruise ship in May. This event correlated with a rise in the BOD level in water samples; this result was consistent whether cruise ships were in the harbor or not (Appendix B).

Some water quality problems arose when sampling around ships that were not members of the ICCL. We discovered evidence of discharges from two non-ICCL ships (Figure 3). These were the *American Glory* (Figure 4) and the *Deutschland*.

The *American Glory*, a passenger vessel that docked at the town pier on ten different visits in 2004, was draining seawater from tanks used to hold water for flushing toilets (Figure 5 and Table 2). They were unaware that the water was potentially contaminated. Water quality monitors detected bacteria above background levels when sampling around the cruise ship on three occasions.

The Deutschland was intermittently discharging wastewater that had been treated with an AquaMar MSD Type II system when it visited Bar Harbor on September 30, 2004. Monitors detected 41 colonies of *Enterococcus* per 100 ml. of water in the vicinity of the ship (Table 2).

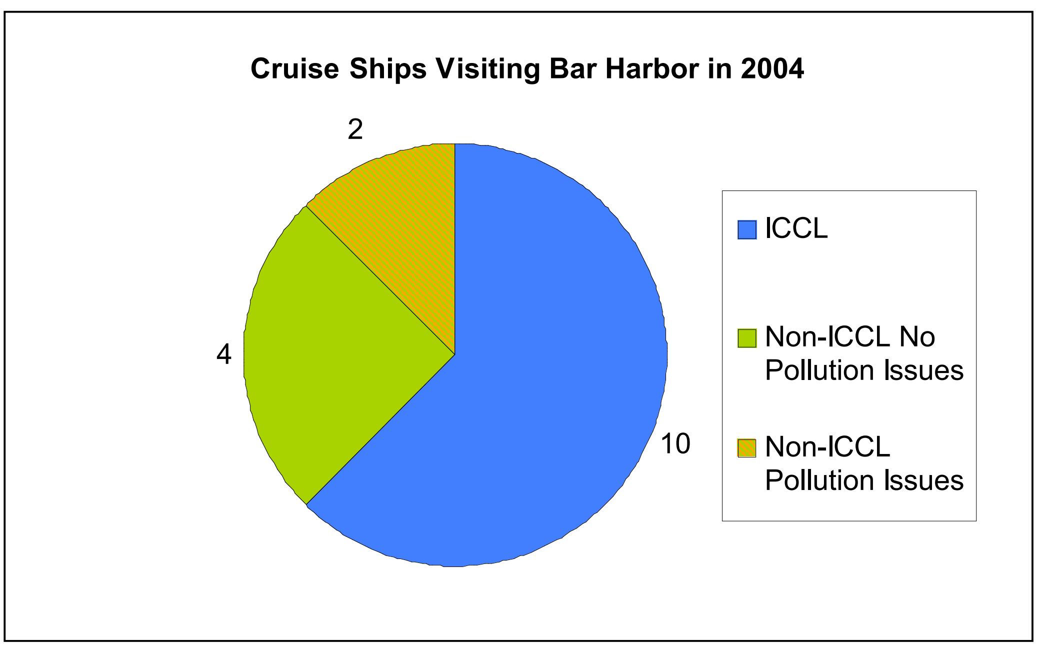

Figure 3: Water quality was assessed around 16 of the 20 ships that visited Bar Harbor in the 2004 cruise ship season. Of those, two discharged contaminated water into Frenchman Bay.

Figure 4: *American Glory* docked at town pier in Bar Harbor. *American Glory*, a 165 ft. boat with 49 passengers and 18 crew members, visited on ten occasions in 2004, all of which were overnight stays.

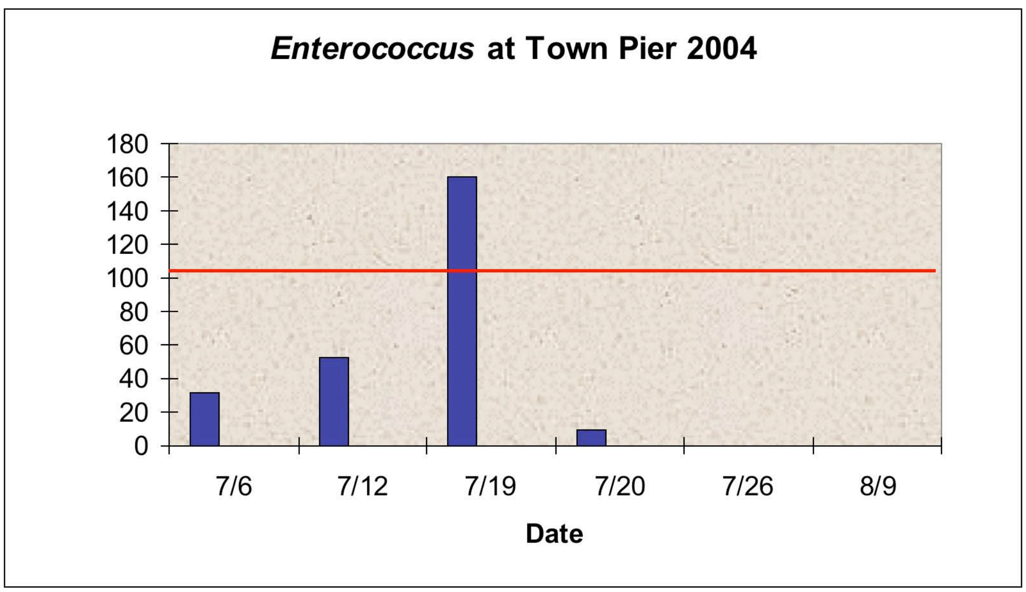

Figure 5: *Enterococcus* levels at the Town Pier were higher than background levels when *American Glory* was docked (7/6-7/19). The ship had left port when the 7/20 water sample was taken. The ship was in port on 7/26 and 8/9. The red line indicates the EPA-established level above which there is increased risk of contracting swimming illness. The harbormaster asked the ship captain to refrain from draining holding tanks after the 7/19 visit. Over the next couple of weeks, the bacterial counts went down to zero.

Water Quality Around ICCL Ships in Bar Harbor 2004

Cruise Ship Name	Date	Transparency (meters)	*Enterococcus* (colonies /100 ml)	DO (ppm)	BOD (ppm)
Crystal Symphony	9/23/2004	4.225	0	8.2	1.4
Grand Princess	9/16/2004	5.1	0	8.1	2.05
Grandeur of the Seas	10/6/2004	4	0	8.8	2
Jewel of the Seas	9/12/2004	4.5	0	7.6	1.15
Maasdam	5/16/2004	9.1	0	10.1	5
Maasdam	5/28/2004	7.4	0	9.7	2.9
Maasdam	7/2/2004	6.5	0	8.95	0.6
Maasdam	7/16/2004	8.15	0	8.55	0.55
Maasdam	7/23/2004	9.35	0	9	1.35
Maasdam	9/10/2004	6.5	0	7.5	0
Maasdam	9/24/2004	2.98	0	8.85	1.75
Maasdam	10/10/2004	4.8	0	9	2.2
Norwegian Crown	9/15/2004	5.9	0	7.92	0.65
Queen Mary 2	9/27/2004	3.15	0	8.75	1.95
Regal Princess	9/14/2004	5.6	0	8.05	0.65
Regal Princess	11/4/2004	5.14	0	8.35	1.35
Rotterdam	10/26/2004	4.875	0	7.3	0.4
Seven Seas Navigator	6/11/2004	4.2	0	7.95	0.35
Seven Seas Navigator	8/6/2004	7.1	0	8.55	0.95

Table 1: All of the ships listed in this table are members of the International Council of Cruise Lines (ICCL). It is the policy of the ICCL that all ships hold all waste until 4 miles out to sea. This data set indicates that these ships did not discharge any wastewater while anchored in Bar Harbor. With a discharge of improperly treated graywater or a mixture of graywater and blackwater, transparency may have been lower (due to increased turbidity of the water), bacterial counts may have been high (due to septic waste), and Biological Oxygen Demand (BOD) may have been higher (due to an increase in organic matter in the water). These data are consistent with data collected when cruise ships were not in the harbor (see Controls in Appendix B).

Water Quality Around Non-ICCL Ships in Bar Harbor 2004

Cruise Ship Name	Date	Transparency (meters)	*Enterococcus* (colonies /100 ml)	DO (ppm)	BOD (ppm)
American Glory	7/6/2004	--	31	9	3.5
American Glory	7/12/2004	--	52	8.3	4.5
American Glory	7/19/2004	--	160	8.55	0.15
American Glory	7/26/2004	2.9	0	9	1.3
American Glory	8/9/2004	3.4	0	8.2	1.15
Deutschland	9/30/2004	5.7	41	7.95	1.925
Deutschland	10/18/2004	4.975	0	7.7	0.7
Grande Caribe	7/23/2004	8.63	0	9.6	1.125
Le Levant	5/20/2004	8.1	0	8.95	0.45
Nantucket Clipper	6/10/2004	--	0	9.4	7
Orion	5/31/2004	6.9	0	9.4	3
Orion	10/10/2004	--	0		

Table 2: The ships listed in this table are not members of the International Council of Cruise Lines (ICCL). The *Enterococcus* levels were higher than levels obtained on days that cruise ships were not in port (see Controls in Appendix B). The BOD levels were not significantly higher than in control samples.

What actions were taken?

On the occasions when discharges were detected from ships without advanced wastewater treatment systems, Charlie Phippen, the Bar Harbor harbormaster, communicated with the ship's agent. In the case of *American Glory*, a simple change in practice, refraining from draining holding tanks while in the harbor, correlated with a decrease in bacterial levels at the town pier (Figure 5). In the case of *Deutschland*, the harbormaster communicated by e-mail with ship's agent:

> The presence of any detectable amount of *Enterococcus* or any other waste being discharged into the waters of anchorage A or for that matter anywhere inside the three mile limit of the state of Maine is of great concern. All cruise ships calling on Bar Harbor, with the exception of the *Maasdam*, hold all wastewaters whether black, gray or otherwise … until at a minimum of four miles off shore. This is a policy of the ICCL and was stressed by their President at a Cruise Symposium held in Bar Harbor in the spring of 2004. The *Deutschland* is scheduled for a second port visit to Bar Harbor on October18, 2004 from 12 noon until 6:00 pm. The ship will not be allowed into anchorage B, north of Bar Island, unless the ship is capable of holding all on board wastewater, with no discharge into the waters of Frenchman Bay. (October 5, 2004)

When the *Deutschland* returned for a second port visit, the MSD Type II system was not discharging and monitors detected no bacteria or other pollutants in the harbor (Table 2).

What do we recommend for the future?

After completing a season of monitoring around visiting cruise ships, we have specific recommendations for the cruise ship industry, the town of Bar Harbor, community members and the Maine State Department of Environmental Protection. These recommendations include:

Cruise Ship Industry:

- Increase understanding of state and local regulations concerning wastewater treatment and discharge.
- Make information available to the harbormaster about wastewater treatment systems and holding capacity of each visiting ship.
- Refrain from discharging in port unless the cruise ship has an advanced wastewater treatment system.
- Support local citizen groups working to preserve and improve water quality in Frenchman Bay by opening lines of communication and providing funds for monitoring initiatives.

Town of Bar Harbor:

- Direct the Harbor Committee to develop local harbor regulations clearly stating Bar Harbor's policy for wastewater discharge.
- Ask cruise line agents to instruct the captain and crew of the ship about harbor regulations and no-discharge policies before ship arrival.
- Give the harbormaster the authority to make specific requests of cruise ship captains and crew.
- Support local citizen groups working to preserve and improve water quality in Frenchman Bay by continuing to provide transport to cruise ship anchorage sites and providing funds for monitoring initiatives.

Community Members:

- Become involved in the cruise ship monitoring project by volunteering time or making financial donations.
- Ask questions.

Department of Environmental Protection:

- Make the *Act to Protect Maine Waters* clear and easily accessible so that all citizens understand the legislation concerning the fast growing cruise ship industry in Maine.
- Earmark funds for local organizations to test the water quality around cruise ships.
- Help to refine the protocol described in this report so that it is an exemplary model for other Gulf of Maine organizations planning to test around cruise ships.

More volunteers and additional funding are necessary to continue the cruise ship monitoring program in 2005. We believe that a second season of monitoring is important for several reasons: (1) Nitrogen and chlorine testing did not commence until fall 2004. A comprehensive look at nitrogen is imperative since this is one of the pollutants that advanced wastewater treatment systems cannot eliminate from effluent. (2) In 2004, few samples were taken at Anchorage B and in the cruise ship lane due to time and funding constraints. These areas warrant further study. (3) Some provisions of *An Act to Protect Maine's Coastal Waters* will not be in effect and others will not be enforced until 2006. Citizens need to get involved now.

Appendix A: Cruise Ship Monitoring Procedures:
A How-To-Guide

Appendix A1: At the sample site

Appendix A1.1: Field Sheet

Cruise Ship Monitoring Data Sheet

Monitor Name: ___________________
Cruise Ship Name: ________________
Berth: ___________________________
Cruise Ship Name: ________________
Date: ____________________________
Time (24hr): _____________________

Inches of Rain Past 24 hrs (www.rainwise.com)	Tide Stage 1-12 in/out		Time of Low Tide/High Tide (www.maineharbors.com)	

Cooler Temp (in °C)	Water Temp (in °C)	Air Temp (in °C)	Wind Speed (in knots)	Tow Depth (in meters)	Transparency Depth (in meters) Fall/Rise		Salinity (in ppt)	Dissolved Oxygen (ppm) Test 1/Test 2/ Avg		

Enterococcus /100ml	Nitrogen (ppm)	BOD (ppm) Test 1	Test 2	Avg.	BOD	Chlorine Free	Total	Assoc.	Phytoplankton Dominant 1	Dominant 2	Total

Current	Weather	Wind on Water	Traffic	Observations
Calm	Clear	Calm	None	Oil on Surface
Low Current	Partly Cloudy	Ripple	Other Cruise Ship	Abnormal Color
Medium Current	Overcast	Waves	Small Boats	Fish Kills
Strong Current	Rain	Whitecap	Other: ____________	Foam
	Snow			Marine Mammals
				Water Fowl
				Other

Field Comments:

Appendix A1.1: Student interns, using the data sheet from the Maine Healthy Beaches Program and the DMR Volunteer Phytoplankton Monitoring Program as models, developed this field sheet.

Appendix A1.2: Collecting Water Sample

1) Write the date, site, and time sampled on sterile Whirl-pak® sample bag.
2) Attach clips of sampling tongs to white tabs on the side of the bag, keeping tongs closed.
3) Submerge the closed bag underwater, open it, and swirl bag until it is 2/3 full.
4) Close bag underwater and bring out of water.
5) Keeping tongs closed, undo clips.
6) Grasping yellow tabs, whirl bag away from body until airtight.
7) Twist the yellow tabs.
8) Place sample in a cooler at 10^{o}C.

Appendix A1.3: Taking Water and Air Temperature

Water Temperature:

1) Place armored thermometer at least 8 inches underwater for 5 minutes.
2) Read temperature underwater (in degrees Celsius).

Air Temperature:

3) Place armored thermometer in the shade for 5 minutes.
4) Read air temperature in shade (in degrees Celsius).
5) Record water and air temperature on data sheet.

Appendix A1.4: Collecting Water Samples for Measuring Dissolved Oxygen or Biological Oxygen Demand (BOD) using a LaMotte® DO Test Kit

1) Label site, date, time collected on four sample bottles, two of which are covered with aluminum foil to prevent exposure to light.
2) Rinse out bottles 3 times in ocean.
3) Fill bottles underwater and cap, making sure no air bubbles remain in bottles.
4) Set the two bottles with aluminum foil aside for BOD analysis 5 days later.
5) To each of the other two bottles, add 8 drops of #1 solution (manganese sulfate).
6) To these same bottles, add 8 drops of #2 solution (alkaline azide).
7) Place caps on bottles and invert gently 8 times.
8) Let samples sit while precipitate forms and settles below the neck of each bottle.
9) Add 8 drops of #3 solution (sulfuric acid) to each bottle.
10) Cap and shake samples vigorously until samples are clear and no particles are seen.
11) Place fixed DO samples and BOD samples in cooler until returned to the lab.

Appendix A1.5: Transparency

1) While having a partner look through the AquaScope™, lower an oceanographic Secchi disk on a heavy line until it disappears from sight.
2) Have a partner clip the line at the water surface.
3) Lower Secchi disk approximately one additional meter.
4) Raise the Secchi disk until it comes into view and again, have a partner clip the line at water surface.
5) Measure from top of Secchi disk to each clip. The average of both measurements is the transparency depth.
6) Record measurements on field sheet.

Appendix A1.6: Phytoplankton Collection

1) Submerge the net and let the air bubbles out.
2) Snap the clip on the net's catch basin closed.
3) Tow vertically from just below the surface to the approximate transparency depth and back up. Repeat this action for five minutes.
4) Pull the net out of the water vertically and let the water drain out the bottom of the net.
5) Open the clip and let the water in the catch basin drain into a sample bottle.
6) Tightly cap the bottle and place it in the cooler.

Appendix A2: In the Lab

Appendix A2.1: Idexx Enterolert™ Protocol for Testing *Enterococcus*

1) Check that incubator is reading 41º C (+/- 0.5º C).
2) Turn on sealing machine.
3) Measure 90 mL sterile water into a sterile 100-mL graduated cylinder, then pour into a sterile plastic jar.
4) Shake water sample in the plastic bag vigorously at least 100 times.
5) Open sterile pipette and attach bulb, aspirate 10 ml. of water sample into pipette.
6) Add 10 mL of water sample to jar.
7) Add *Enterococcus* media to jar.
8) Replace lid and invert jar until media is completely dissolved (minimizing bubbles).
9) Label multi-well plate with site, date and time.
10) Separate plate backing from the wells.
11) Pour contents of jar into plate making sure to empty every last drop.
12) Vigorously tap plate until all of the air bubbles are out of the wells.
13) Place plate face down on orange sealer pad.
14) Run plate and pad through sealer.
15) Place plate in incubator immediately.
16) For a positive control: Bring pellet of *Enterococcus faecium* from MicroBioLogics, Inc. to room temperature for 30 min, place in sterile plastic jar, dissolve in 90 mls of sterile water for 30 min. by shaking periodically. Repeat steps 7-15.
17) For a negative control: Repeat steps 3-15 substituting sample water with sterile water or using non-reactive bacteria (*Serratia marcescens* and/or *Aerococcus viridans* from MicroBioLogics, Inc) using the method described in step 16 for a positive control.
18) Incubate multi-well plates for 24 hours +/- 1 hour.
19) Read plate in black box with UV light.
20) Use Most Probable Number (MPN) chart to determine number of organisms per 100 ml.
21) Record findings in lab log and on data sheet.

Appendix A2.2: Performing Refractometry on a Sample (Determining Salinity)

1) Clean off prism with distilled water.
2) Put 1-2 drops of distilled water on the prism, close the daylight plate and use a small screwdriver to set at zero parts per thousand (ppt).
3) Clean out pipette with distilled water.
4) Collect sample in pipette.
5) Place 1-2 drops of sample on prism; close the daylight plate.
6) Read salinity through eyepiece in ppt.
7) Record salinity reading on data sheet.
8) Clean off prism and clean out pipette with distilled water.

Appendix A2.3: Measuring (Titrating) Dissolved Oxygen Samples

1) Measure 20 mL of sample with graduated cylinder.
2) Pour the 20 mL in titration tube and cap.
3) Fill titrator with 10 units of sodium thiosulfate until base of plunger is at zero with no air bubbles present in the titrator.
4) Put titrator in the hole of the titration tube cap; add drops of sodium thiosulfate (swirling the titration tube after each drop) until light yellow.
5) Add 8 drops of starch and swirl (should be dark purple).
6) Drop by drop, add the sodium thiosulfate and swirl titration tube each time a drop exits titrator. Continue this until solution is clear.
7) Read titrator (This is oxygen in parts per million).
8) Record on data sheet.
9) Repeat procedure for a second sample bottle; record average on data sheet.

Appendix A2.4: Biological Oxygen Demand

1) Store designated BOD sample bottles in a drawer or other light sealed container for 5 days.
2) Fix the BOD samples as described for DO samples in A1.4.
3) Titrate the BOD samples as described for DO samples in A2.3.
4) Subtract sample results designated BOD level for DO level to determine BOD. BOD levels above 2.0 indicate there may be organic matter in the water from a pollution source.
5) Record BOD on a data sheet.

Appendix A2.5: Nitrogen Testing Using a LaMotte® Nitrogen Test Kit

1) Collect water in sample bottle at test site, bring bottle back to lab to run test
2) Put on protective gloves, goggles and mask.
3) Fill sample tube to the 2.5 mL line with water from the sample bottle.
4) Add Mixed Acid Reagent to 5 mL line on test tube using sterile pipette. Cap tightly and invert several times to mix. Wait 2 minutes.

5) Uncap sample tube. Using 0.1g spoon add one level measure of Nitrate Reducing Reagent. Cap tightly once more and invert gently 60 times in one minute. Let sit for 10 minutes.
6) Insert sample tube into Nitrate-N Comparator to compare with measures of pink. Once similar pink is determined multiply that measure by 4.4 to record on data as ppm (parts per million) nitrate.
7) Clean up test area and dispose of sample tube contents in proper toxic waste containers.

Appendix A2.6: Chlorine Testing Using the LaMotte® Chlorine Colorimeter

1) Rinse colorimeter tubes with sample water then fill to 10 mL line. Cap and wipe dry making sure no fingerprints or water is on sample tube.
2) Insert the tube into the chamber of colorimeter. Close the lid and push the READ button to turn the machine on.
3) Press ZERO button and hold for 2 seconds until BLA appears. Release the button to take a zero reading.
4) Take tube out of chamber and empty all but a few drops from the tube.
5) Crush one Chlorine DPD #1 tablet and fill tube again to 10 mL line with sample water. Cap and mix until tablet is completely dissolved.
6) Insert tube into chamber again and push READ button. This is the Free Available Chlorine reading recorded in ppm. Record on data sheet.
7) Remove tube again and add one Chlorine DPD #3 Tablet and crush. Cap and dissolve as in Step 5. Wait 2 minutes.
8) Insert the tube once more into chamber and push READ button. This reading will be the Total Residual Chlorine measured in ppm. Record on data sheet.
9) Subtract the Free Available Chlorine reading from the Total residual Chlorine reading to determine Combined Chlorine in ppm. Record on data sheet.

Appendix A2.7: Phytoplankton Analysis

1) Using a pipette put two drops of water from the phytoplankton sample bottle on a microscope slide.
2) Look at three random fields of view on the slide under the microscope and record the number and types of phytoplankton seen.
3) Repeat steps 1-2 with another slide and record the total number and types of phytoplankton from both slides on the data sheet.

Appendix B: 2004 Bar Harbor Cruise Ship Season All Data

Water Quality Data in Bar Harbor With and Without Cruise Ships in Anchorages

Anchorage and Cruise Ship	*Date*	*Water Temp degrees Celcius*	*Transparency meters*	*Salinity ppt*	*DO ppm*	*Enterococcus colonies/100ml*	*BOD ppm*	*Chlorine (total)ppm*	*Phytoplankton Most Dominant*	*Total Cells*
Alpha										
Control	5/13/2004	6.0		34	9.9	0	6.2		Phaeocystis *	35
Control	7/9/2004	10.0	7.85	34	9.2	0	0.9		Leptocylindrus	167
Crystal Symphony	9/23/2004	11.0	4.225	35	8.2	0	1.4	0.01	Chaetocerous	51
Deutschland	9/30/2004	12.0	5.7	35	7.95	41	1.925	0	Chaetocerous	31
Grand Princess	9/16/2004	11.0	5.1	35	8.1	0	2.05		Rhizosolenia	45
Grande Caribe	7/23/2004	12.0	8.63	34	9.6	0	1.125		Leptocylindrus	76
Grandeur of the Seas	10/6/2004	11.0	4	35	8.8	0	2.0	0.01	Chaetocerous	56
Jewel of the Seas	9/12/2004	12.0	4.5	35	7.6	0	1.15		Pseudonitzchia	41
Le Levant	5/20/2004	15.0	8.1	35	8.95	0	0.45		Chaetocerous	10
Maasdam	5/16/2004	6.0	9.1	35	10.1	0	5.0		Phaeocystis *	21
Maasdam	5/28/2004	9.0	7.4	35	9.7	0	2.9		Chaetocerous	31
Maasdam	7/2/2004	7.5	6.5	33	8.95	0	0.6		Thalassiosira	51
Maasdam	7/16/2004	11.0	8.15	35	8.55	0	0.55		Leptocylindrus	85
Maasdam	7/23/2004	12.0	9.35	34	9	0	1.35		Dinophysis	44
Maasdam	9/10/2004	11.0	6.5	34	7.5	0	0.0		Guinardia	60
Maasdam	9/24/2004	12.0	2.98	35	8.85	0	1.75	0.01	Chaetocerous	36
Maasdam	10/10/200	11.5	4.8	35	9	0	2.2	0	Chaetocer	18
Maasdam 14ft.	7/16/2004	11.0		35	8.7	0	0		Leptocylindrus	85
Maasdam 14ft.	7/23/2004	12.0		34	9.4	0	2.3		Dinophysis	44
Maasdam 14ft.	9/10/2004	11.0		34	7.6	0	1.46		Guinardia	60
Maasdam 14ft.	9/24/2004	12.0		34	7.8	0	0.7	0	Chaetocerous	36
Maasdam 14ft.	10/10/200			35	9.25	0	1.85	0	Chaetocerous	
Norwegian Crown	9/15/2004	11.0	5.9	35	7.92	0	0.65		Rhizosolenia	49
Orion	5/31/2004	7.0	6.9	35	9.4	0	3.0		Chaetocerous	42
Queen Mary 2	9/27/2004	12.0	3.15	35	8.75	0	1.95	0.01	Rhizosolenia	20
Regal Princess	9/14/2004	13.0	5.6	35	8.05	0	0.65		Rhizosolenia	52
Regal Princess	11/4/2004	8.0	5.14	35	8.35	0	1.35	0	Rhizosolenia	110
Rotterdam	10/26/200	9.5	4.875	35	7.3	0	0.4		Cocinodiscus	39
Seven Seas	6/11/2004	9.5	4.2	35	7.95	0	0.35		Chaetocerous	42
Seven Seas	8/6/2004	14.0	7.1	34	8.55	0	0.95		Nizschia	21
Bravo										
Control	10/15/200	10.0	6.33	35	8.2	0	0		Chaetocerous	8
Deutschland	10/18/200	10.5	4.975	35	7.7	0	0.7	0	Chaetocerous	37
Cruise ship lane										
Orion	10/10/200					0		0		
Town Pier										
American Glory	7/6/2004	10.0		36	9	31	3.5		Thalassiosira	51
American Glory	7/12/2004	11.0		34	8.3	52	4.5		Leptocylindrus	82
American Glory	7/19/2004	12.0		34	8.55	160	0.15		Chaetocerous	35
American Glory	7/26/2004	14.0	2.9	34	9	0	1.3		Chaetocerous	27
American Glory	8/9/2004	13.0	3.4	33	8.2	0	1.15		Leptocylindrus	22
Control	7/20/2004	12.0	3	34	8.85	10	0.85		Skeletonema	39
Control	8/2/2004	13.0	4.19	34	8.05	10	0.55		Chaetocerous	14
Control	9/7/2004	13.0	3.9	35	8.2	0	2.4		Chaetocerous	13
Control	9/21/2004	12.0	3.01	35	6.9	0	0.3		Rhizosolenia	25
Nantucket Clipper	6/10/2004	12.0		37	9.4	0	7.0		Chaetocerous	63

Appendix B: This data set lists ships by anchorage and includes the control samples that were taken on days when there were no cruise ships at any of the anchorages. There were no significant water quality issues on these days. *Phaeocystis* (indicated by a star in the table) is a colonial algal species that may add enough organic matter to the water column to affect the biological oxygen demand. In both cases where *Phaeocystis* is the dominant species, there is an elevated biological oxygen demand.

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(5) *Water Quality Criteria*, US Environmental Protection Agency, July 1976.

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