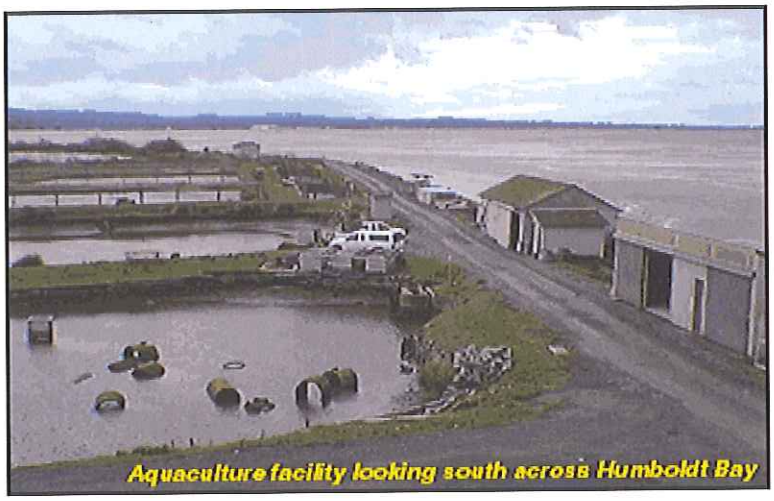

The Arcata Aquaculture Project: An Initial Study

Influents & Effluents

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Abstract

Preliminary water quality monitoring of influents and effluents was conducted at the Arcata Aquaculture Project (AAP) during February and March, 2007. The AAP consists of a series of interconnected unlined ponds, subject to tidal influx from an adjacent slough, Butcher Slough, and is fed with a mixture of treated effluent from the adjacent Arcata Wastewater Treatment Plant (AWTP) and bay water from Butcher Slough. Dissolved Oxygen (DO), pH, temperature, salinity, conductivity, ammonia, nitrate, suspended solids, biochemical oxygen demand (BOD), total coliforms, and fecal coliforms were measured each week during the two-month period, excepting the second week of March. Three sites were selected for monitoring: the treated wastewater influent source, a treated wastewater/bay water influent mixing site, and the aquaculture system effluent outfall. The results of this initial site assessment indicate that the aquaculture system, as currently operated, generally serves to beneficially enhance the water quality of both the feed wastewater effluent and feed bay water at the mixing site by lowering nutrient loads, oxygenating the water, lowering coliform counts, and removing suspended solids.

Introduction

Historical

In 1963, Dr. George Allen, a professor of fisheries at Humboldt State University, began experimenting with rearing fish in reclaimed wastewater. Though using human wastes to fertilize crops and fish ponds has been practiced for centuries in other countries, it is a relatively young practice in the United States. Dr. Allen saw Arcata's wastewater as an untapped resource. His first experiments on salmon smolts demonstrated that a wastewater aquaculture project at the Arcata Wastewater Treatment Plant (AWTP) had the potential for success. He was granted permission to build two fish rearing ponds adjacent to the oxidation ponds. A pilot project with salmon was initiated in 1971. The first adult returns were captured in 1977; the project has since expanded into what it is today. Dr. Kristine Brenneman continues to oversee the operation of the aquaculture facility. It is currently the only facility in the world to raise salmonids successfully in reclaimed wastewater.

Dr. Allen's aquaculture project played a crucial role in the creation of the Arcata Marsh and Wildlife Sanctuary (AMWS). Before getting permission to build the Sanctuary, the city of Arcata had to prove to the Regional Water Quality Control Board that the water discharged from the treatment plant would provide "enhancement" of beneficial uses of Humboldt Bay. One way they were able to successfully prove "enhancement" to the Regional Board was through the aquaculture facility. The city argued that the utilization of wastewater in an aquaculture project provided limitless educational opportunities for students ranging from grade school to graduate levels. The Regional Board agreed that this constituted "enhancement" and construction of the Sanctuary was allowed to proceed. The aquaculture project also helped to foster support from the community for the construction of a wetland wastewater treatment system. Because wastewater was already being used to rear fish, it wasn't much more of a mental leap to think about using wastewater to create freshwater wetlands. Humboldt State University students helped to design and build the facility using donated and recycled materials.

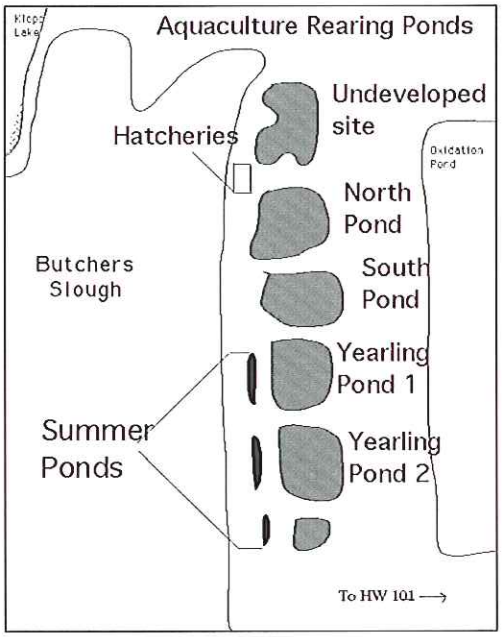

Figure 1. General map of the Arcata Aquaculture Project, showing relative pond size and locations. Image source: http://www.humboldt.edu/~ere_dept/marsh/aquawq.html

Current Arcata Aquaculture System: Site Description

Today, due to recent regulatory changes to protect wild migratory salmon species, the Arcata Aquaculture Project is no longer allowed to rear the Coho and Chinook it historically cultivated with great success and released into the region's natural waters. However, the project site is still active with the continuing production of Cutthroat Trout

to support regional recreational fisheries and the local scientific research of Humboldt State University graduate students. At the present, only the summer rearing ponds are in use for active aquaculture, though some fish are known to still be present in small numbers in the other ponds.

The Arcata Aquaculture Project consists of several interconnected ponds as displayed below in Figure 2. Circulation occurs within the system as a result of several contributing

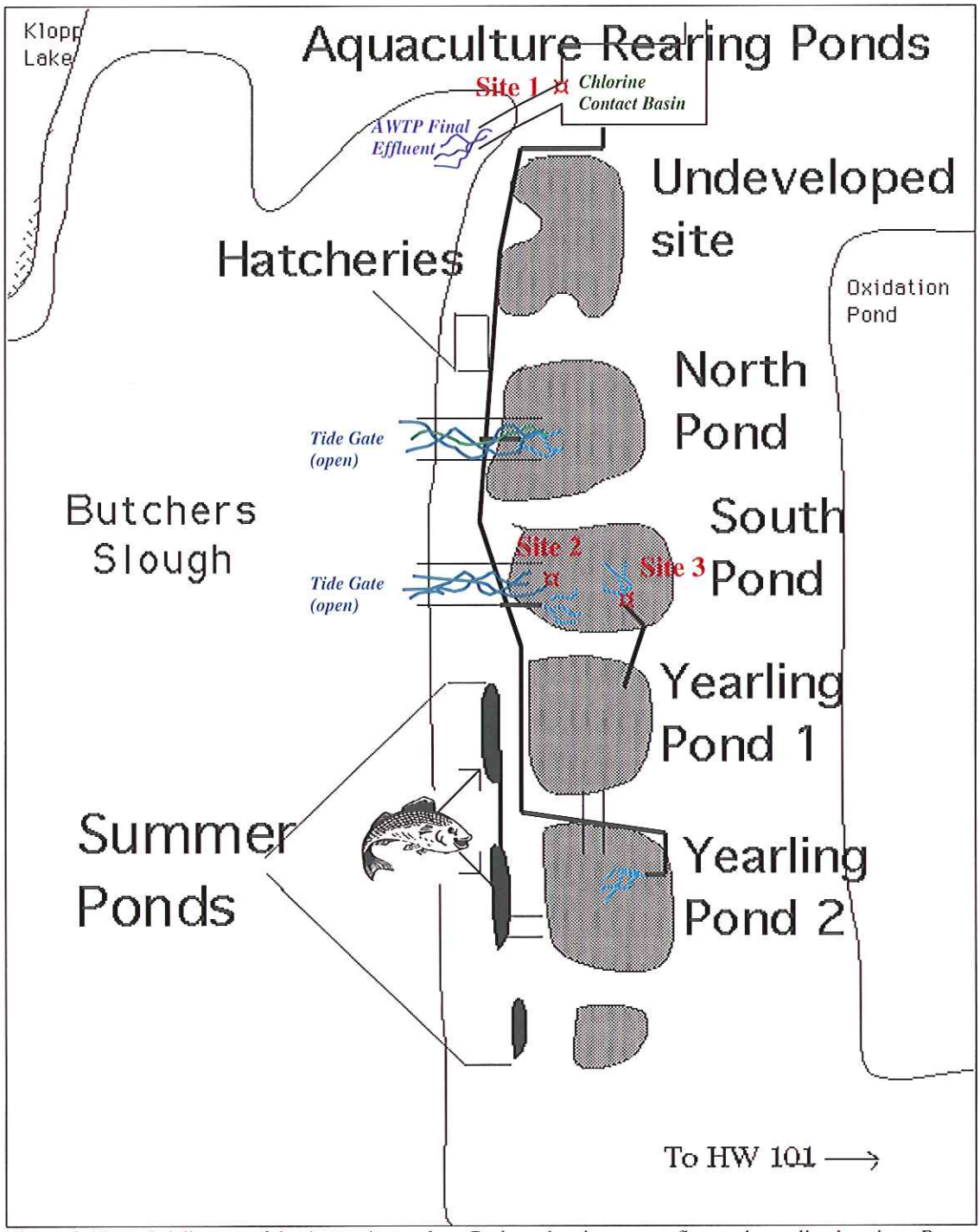

Figure 2. Expanded diagram of the Arcata Aquaculture Project, showing system flow and sampling locations. Base image source: http://www.humboldt.edu/~ere_dept/marsh/aquawq.html

factors. The relatively open system is fed by both treated waste water effluent from the AWTP and tidal influx from Butcher Slough. Influent mixing occurs at two open "T's" in the system feed pipe transporting treated wastewater effluent from the chlorine contact basin to Yearling Pond 2. The "T's" are each located next to the tide gates in North Pond and South Pond, respectively, and allow mixed waters from these two ponds to enter the Aquaculture Project influent stream pumped to Yearling Pond 2.

Once the influent is pumped into Yearling Pond 2, tidal flood and ebb allows mixing of the effluent with the waters of the summer rearing ponds, and gravity further assists mixing into Yearling Pond 1. Gravity also results in Aquaculture Project effluent from the yearling and summer rearing ponds into South Pond, where it then mixes with tidal influx, AWTP effluent, and potentially re-enters the fish pond influent stream through the "T." The entire Aquaculture Project is unlined, and thus subject to substantial tidal influx, affording a high degree of mixing throughout the system. Active (mechanical) aeration is only currently being performed in the two occupied (northernmost) summer yearling ponds, though aeration also results at the gravity-driven fish pond outfall into South Pond.

The influent into the Aquaculture Project contains treated wastewater effluent from the AWTP. This AWTP effluent is itself also a mixture of effluents of varying qualities from the treatment system. Processes involved in wastewater treatment fall into general categories of treatment, designated by virtue of their contribution to the treatment of effluent constituents. These general categories are described below:

- Primary Treatment: the initial screening and settling of large solid waste using gratings, screens, and settling basins.
- Secondary Treatment: the biological removal of "oxygen consuming wastes" (organic matter that tends to deplete oxygen when metabolized by organisms in the natural environment) through aerobic microbial activity.
- Tertiary Treatment: additional organic and inorganic nutrient removal or other treatment to enhance the effluent quality to a desired higher standard beyond that achieved by secondary treatment.

The effluent from the AWTP has been subjected to either secondary or tertiary levels of treatment. The relative amounts of each quality of effluent in the mixture vary, depending upon seasonal flow rates through the AWTP; the level of secondary treatment itself also depends on seasonal ATWP inflows. As indicated above, all utilized effluent has been chlorinated for pathogen removal, the typical final treatment prior to wastewater effluent discharge in most systems.

The AWTP utilizes oxidation ponds and constructed "treatment wetlands" for secondary treatment. The use of such a more ecologically oriented system constitutes an alternative to the more common practice of using more highly mechanical and energy intensive means of secondary treatment. Following secondary treatment, much of the effluent is diverted following chlorination to a series of constructed enhancement wetlands for tertiary treatment of nutrients, eventually to return for chlorination again before further diversion or final discharge into Butcher's Slough. During peak seasonal flow periods, the residence time during secondary treatment can be diminished by high system flow-through rates. Thus, the effluent finally discharged from the chlorine contact basin is comprised of a mixture of system effluents having various levels of treatment.

Environmental Setting

Arcata's Wastewater Aquaculture Project is located at the Arcata Marsh and Wildlife Sanctuary (AMWS), adjacent to the northeast corner of Humboldt Bay in northern California. The aquaculture ponds are situated next to the oxidation ponds within the wastewater treatment plant facility grounds, just across Butcher Slough from the enhancement wetlands and associated highly used park.

The natural setting of the Aquaculture Project and Arcata Marsh and Wildlife Sanctuary is inland tidal wetland. The both influent and effluent are constantly mixed with tidal flows in the mixing pond of the Project, and the system is essentially an open one, inseparable from its natural surroundings due to it not being lined. The adjacent wetland habitat is highly sensitive to environmental changes, and, as such, is highly regulated. For this reason tertiary treatment in the enhancement wetlands is performed at the Arcata Wastewater Treatment Plant.

Figure 3. Local of Arcata Marsh & Wildlife Sanctuary (AMWS) in relationship to Humboldt Bay. Image is from the Humboldt Bay Harbor District's Humboldt Bay Management Plan Draft Environmental Impact Report, 2006.

The wetlands and benthic tidal habitats in the area are host to a myriad of sensitive fish (e.g. salmonid), marine mammals, invertebrates, migratory bird and foul. The adjacent wetland and nearby aquatic eelgrass habitats serve significant ecological roles as both natural water treatment for Humboldt Bay and as nursing grounds for a host of species, including many with special regulatory status. The wetland and marsh habitats are highly fertile sources of food upon which many species of fish, bird, marine mammal, and migratory terrestrial mammals depend.

Of primary concern relative to the adjacent natural aquatic environment is the discharge of excessive nutrients and wastes into an already nutrient-rich environment, disrupting delicate natural balances and the aquatic food web. Also of great concern is the contribution of the Project to turbidity in the bay waters. Suspended solids decrease light

transmission and thus the productivity of benthic aquatic macrophytes. This, combined with excessive nutrient loading can result in toxic algal blooms that also adversely effect aquatic animal species in addition to harming aquatic macrophytes. Further suspended solids harbor pathogens. Decreased light penetration due to turbidity can negatively impact the sensitive eelgrass beds adjacent to the Project, which in turn affects the entire food chain all the way up to local fisheries, especially a thriving local shellfish farming industry in the adjacent waters of Humboldt Bay.

Regulatory Setting

In 2004 the EPA set national effluent guidelines and standards for aquaculture facilities. These guidelines and standards were set for facilities generating 100,000 lbs or more per year of fish stock. No numerical values were associated with the effluent, instead used the best management practice (BMP). BMP was to incorporate feed management, waste collection, material storage, and carcass removal. The aquaculture facilities are described as four types: ponds, flow through, recalculating, and net pens.

In California aquaculture permitting exist with the California Coastal Commission (California Coastal Act and Coastal Zone Management Act), the Regional Water Quality Control Boards (Clean Water Act), and Department of Fish and Game (Fish and Game Code, and state lands leases and registrations). There was no specific policy with regards to "aquaculture effluent" in general, this maybe because of the different types of facilities that are onerous to this such as the net pens, though there is a policy that directly relates to net pens.

The one policy that holds true for all effluent flow is the permitting process for National Pollutant Discharge Elimination System (NPDES). So the permitting process has become the de facto policy for aquaculture facilities discharging their effluent into waterways. NPDES requirements stipulate that effluent biochemical oxygen demand (BOD) and suspended solids levels not exceed 30 mg/l each. NCRWQCB limitations on BOD also include a weekly average of $\leq$45 mg/l and daily maximum of $\leq$60 mg/l.

The North Coast Regional Water Quality Control Board's (NCRWQCB) "Water Quality Control Plan for the North Coast Region" (Basin Plan) sets policy on wastewater discharge for the North Coast region to include Humboldt Bay. Chapter 4 of this document details policies on the regulation of fish hatcheries, fish rearing facilities, and aquaculture operations. The following criteria apply to the discharge from fish hatcheries, rearing facilities, and aquaculture operations:

1. The discharge shall not adversely impact the recognized existing and potential beneficial uses of the receiving waters.
2. The discharge of waste resulting from cleaning activities shall be prohibited.
3. The discharge of detectable levels of chemicals used for the treatment and control of disease other than salt (NaCl) shall be prohibited.
4. The discharge will be subject to review by the Regional Water Board for possible issuance of Waste Discharge Requirements/NPDES permit.
5. The Regional Water Board may waive Waste Discharge Requirements for fish hatcheries, fish rearing, and aquaculture facilities, provided that the discharge

complies with applicable sections of the Water Quality Control Plan for the North Coast Region and satisfies the conditions for waiver which are described in Regional Board Resolution No. 87-113 and/or replaced or modified by Resolution R1-2002-0080, "Policy for Waiving Waste Discharge Requirements for Specific Types of Waste."

NCRWQCB Objectives for Inland Surface Waters, Enclosed Bays, and Estuaries: Humboldt Bay			
Dissolved Oxygen, mg/l	Minimum	90% Lower Limit	50% Lower Limit
	6.0	6.2	7.0
pH	Maximum		Minimum
	8.5		7.0
Suspended Materials	Not to adversely effect local "beneficial use"		
Turbidity	Not increase natural background by >20%		
Bacteria, General	"The bacteriological quality of waters… shall not be degraded beyond natural background levels…"		
Fecal Coliforms, Waters, Recreational Use	• Median from ≥ 5 samples not to exceed 50CFU/100ml per 30 days • ≤10% of samples from 30 day period exceed 400CFU/100ml		
Fecal Coliforms, Shellfish Harvesting	≤43CFU/100ml for 5-tube MPN		

Table 1. Select North Coast Regional Water Quality Board general regulatory standards for effluents into Humboldt Bay (from Chapter 4 of the current "Basin Plan."

Study Background & Design Principles

Though the Arcata Marsh and Wildlife Sanctuary Aquaculture Project, established in 1971, has been operational for over thirty years, no formal monitoring of its effluents has ever been performed. As the Project has only been using treated effluent from the Arcata Wastewater Treatment Plant, it has been considered only an enhancement over the years. However, in the recent climate of growing public concern of the environmental impacts of fish hatcheries and aquaculture, and the associated regulatory climate, it has become important that this information gap be bridged.

The need has been determined to exist for a clear understanding of the benefits and impacts associated with the Arcata Aquaculture Project in the face of growing scrutiny and emerging regulation of aquaculture facilities. To address this need, an initial study has been designed to monitor the Arcata Aquaculture Project's influents and effluents over a two month period and then conduct a comparative analysis of the findings to assess environmental benefits associated with the Project.

The parameters and methods of their measurement chosen for this study were selected based on access to laboratory equipment, research team size, and time availability of team members. It was decided in consultation with Dr. Kristine Brenneman that monitoring would include dissolved oxygen (DO), pH, temperature, salinity, conductivity, nitrogen content (ammonia, $N\text{-}NH_3$, and nitrate, $N\text{-}NO_3^-$), biochemical oxygen demand (BOD), suspended solid, and coliform (both total and fecal) measurements.

DO is a very important indicator of an aquatic system's ability to support aquatic life, as most aquatic organisms are aerobic, requiring sufficient oxygen for respiration. Oxygen enters natural waters through diffusion from the surrounding air, aeration resulting from surface turbulence, and aquatic macrophyte and algal photosynthesis, and is removed by respiration and decomposition of organic matter. Dissolved oxygen analysis measures the amount of gaseous oxygen (O_2) dissolved in an aqueous solution.

Total dissolved aquatic oxygen concentrations in water should not be over 110 percent. Concentrations above this level can be harmful to aquatic life. Fish in waters containing excessive dissolved gases may suffer from "gas bubble disease," though a very rare occurrence. Oxygen deficiency, however, is a more common problem. As dissolved oxygen levels in water drop below 5.0 mg/l, aquatic life is put under stress. As the DO diminishes further, the stress elevates. Oxygen levels that remain below 1-2 mg/l for a few hours can result in substantial fish kills.

Dissolved oxygen concentration in a pond fluctuates in a diurnal cycle. DO increases during daylight hours when photosynthesis is occurring and then decreases at night when respiration continues in the absence of photosynthetic activity. Under conditions of extremely high productivity, or eutrophication, in some aquatic systems, this can result in anoxic conditions during the night.

Most aquatic organisms are extremely sensitive to pH, temperature, and salinity levels, and thus these parameters serve well in determining overall environmental health. pH is the measure of aquatic hydrogen ion concentration, or the acidity of the water. pH is measured on a logarithmic scale of 1 to 14, with 7 being "neutral" (or the pH of pure water), values below 7 indicating acidity, and values above 7 indicating basic conditions. Most aquatic organisms can only live within relatively narrow windows of pH, usually falling between 6 and 8, and thus its measure serves as an important indicator of environmental health. Just as aquatic species are sensitive to pH, they are also quite sensitive to temperature, and have specific ranges in which they can survive. Dramatic fluctuations in temperature over short periods can stress or even kill an aquatic organism depending on time and intensity. Aquatic organisms also have very specific salinities at which they thrive. Changes in salinity can also result in the shock or death of aquatic organisms.

Monitoring DO levels reveals relative respiration and primary productivity levels in the waters being analyzed, and DO content serves as a strong indicator of overall environmental health. DO levels are also very closely related to the temperature and salinity of the water being sampled, and also typically reflect relative nutrient abundance.

Specific Conductance measures how well water can conduct an electrical current for a unit length and unit cross-section at a certain temperature. Conductivity increases with increasing concentration and mobility of ionic species, which conduct electricity due to their negative or positive charge in solution. SC thus serves as an indirect measure of the presence of dissolved nutrients and salts such as calcium, sodium, chloride, magnesium, nitrate, sulfate, phosphate, and iron, and as such can be used to assess water quality.

SC is measured in µmhos or microseimens of conductance. There aren't usually regulatory standards for SC. Instead, the concentrations of solids or turbidity are often regulated. However, SC can serve as a good indicator of the amount of dissolved solids in water, and thus can be used to detect aquatic pollution. Depending on the aquatic system involved, a higher level of specifically conductivity is usually associated with poor environmental quality, often from excessive nutrient loading.

One nutrient often measured to determine water quality is nitrogen. In aquatic systems, nitrogen can exist in several forms: nitrogen gas (N_2), ammonia (NH_3), nitrite (NO_2^-) nitrate (NO_3^-). The nitrogen compounds ammonia and nitrate are both sources of bio-available nitrogen (most organisms can't "fix" nitrogen gas to use it), which is used by aquatic primary producers for protein synthesis (growth and reproduction). Though essential macronutrients for aquatic plants at the base of aquatic food chains, if present in excess these substances result in a deterioration of environmental health, their accumulation directly chemically toxic to organisms and their abundance causing blooms of algae species which also end up negatively impacting overall environmental health. Thus the levels of nitrogen species present also serve as an excellent indicator of overall environmental health and forecast excessive nutrient loading.

Bio-available phosphate species are actually more often the limiting nutrient in (particularly freshwater) aquatic systems. However, due to time and personnel constraints, the brackish nature of the Arcata Aquaculture Project, and the toxicity of phosphate testing reagents, phosphate level monitoring was not conducted in this study.

The amount of particles that suspend in a sample of water is called total suspended solids (TSS). To remain permanently suspended in water (or suspended for a long period of time), particles have to be light in weight (they must have a relatively low density or specific gravity), be relatively small in size, and/or have a surface area that is large in relation to their weight. Suspended solids generally consist of an inorganic fraction (silts, clays, etc.) and an organic fraction (algae, zooplankton, bacteria, and detritus) that are carried along by water as it runs off the land, however in the case of the Arcata Aquaculture Project it is the influence of remaining TSS from the AWTP and the influence of tidal waters from Humboldt Bay. The inorganic portion is usually considerably higher than the organic. Both contribute to turbidity, or cloudiness of the water. Excessive suspended materials can inhibit photosynthesis in both aquatic macrophyte and phytoplankton species, harbor pathogens, and interfere with fish respiration. Thus, suspended solids also serve as a very useful measure in assessing aquatic environmental health.

BOD is an indirect measure of DO content of a water sample. This test ultimately measures how much dissolved oxygen is required for the organic matter in a sample to decompose. BOD measurement also serves to indicate overall environmental health by revealing the loading of biologically available organic matter and inorganic measure. Thus BOD is an indirect measure of not only DO, but also of nutrient levels in an aquatic system. It is a very commonly used test due it being such an inclusive measure, and being a safe, non-toxic test with very simple procedure. For these reason BOD is frequently used in the regulating and monitoring wastewater effluent quality.

Coliform bacteria exist throughout the aquatic and terrestrial environment, adapted to most natural conditions on earth, though preferring moist soils, and the external surfaces and interiors of larger organisms as a habitat. A coliform is officially defined as a gram–negative, rod-shaped bacterium which is capable of fermenting lactose. Coliforms are nearly always present in healthy natural waters, though usually in relatively small numbers. Their presence in large numbers, however, is usually an indicator of poor environmental health, and often of contamination from excessive run-off or anthropogenic inputs.

Fecal coliform is a particular classification within the general category of coliforms, referring to species which have adapted specifically to the conditions in the digestive systems of larger animals. Thus, when present in aquatic systems, they usually indicate an excessive contamination of feces. They also indicate a recent fecal contamination, as they are typically short-lived under the harsh conditions in the natural aquatic environment, to which they are not well adapted, preferring the conditions found in larger organism digestive systems. As a result, the presence of fecal coliforms in substantial numbers serves a very good indicator of environmental health, as the presence of excessive fecal contamination does not typically constitute and favorable environment for most aquatic organisms. Further there is a correlation between the presence of coliforms and pathogens, which thrive in similar conditions and usually have common sources.

Though potentially an indicator of human fecal contamination, it must also be considered that fecal coliforms exist in the feces of all mammals and most higher aquatic organisms. Thus, any fecal coliform results must be interpreted in the context of the natural environment in which they have been detected with full consideration of all probable sources. There are, however, advanced and more specific means of isolating or directly identifying fecal coliform species to determine their origin, such as DNA testing.

Materials and Methods

Sample Collection and Storage

The collection and storage of samples was conducted in accordance with *Standard Methods* (SM) sections 1060 and 9060. Quality assurance and control measures were instituted in accordance with SM 1020 and SM 9020, by following previously existing laboratory guidelines and procedures for the Humboldt State Wastewater Quality Laboratory, established already in accordance with SM 1020 and 9020.

Physical Parameters

All tests (Dissolve Oxygen, Salinity, Conductivity, Temperature, and pH) were completed immediately on site using the following three instruments: YSI DO Meter Model # 55/ 12ft, SN: 04C4609 Al. Salinity, Conductivity, and Temperature were taken using a YSI Model # 30M/100ft, SN: 05D1524 AA. pH measurements were taken using a Hanna Model #HI98127 pH meter, SN: H198127.

Nitrogen

The methods used for testing ammonia and nitrates were in accordance with colorimetry instructions delineated on the testing apparatus: HACH DR/890 Colorimeter S/N 020690021005; associated HACH standard pre-measured reagents were also used. The collection of samples was conducted in accordance with SM as described above. During the first NH3 tests for Site 1 and Site 2, it was found that dilutions of 1/100 and 1/10, respectively, were required to obtain readings from the HACH meter. This dilution rate was maintained throughout the study and factored into the results obtained.

Total Suspended Solids (TSS)

The TSS testing procedure followed SM section #2540 D (APHA 1997) with no changes made. The following formula was used to calculate TSS:

$$\text{mg total solids/L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$

Where:
A = weight of filter + dried residue, mg, and
B = weight of filter, mg.

Biochemical Oxygen Demand (BOD)

The BOD dilution procedure used for this experiment was referenced from SM section #5210 (APHA 1997) with no changes made. The DO meter used was a YSI model 58 DO meter, item #062078. The dilution water was prepared using HACH BOD nutrient buffer pillows, cat. 14861-66 PK/50. The following formula was used to calculate 5-day BOD:

$$BOD_5(mg/L) = \frac{(DO_i - DO_5)^{mg/L}}{P}$$

Total and Fecal Coliforms

Total and fecal coliform levels were determined in accordance with SM 9020 through 9060, using techniques and procedures outlined in SM 9221 and 9222 simultaneous. As treated wastewater effluent was involved, we utilized the dechlorination procedure specified in SM 9060 A, involving the addition of sodium thiosulfate to our sample bottles. For weeks 1 through 6 of the study, SM 9222 was directly utilized for both total and fecal membrane filtration. For most probable number (MPN) determination, SM 9221 A was followed as procedurally specified through to first completion, using dual EC-broth and brilliant green lactose bole broth for completion following presumptive and confirmatory runs. 5-tubes were utilized for all MPN determination runs throughout the study.

MPN determination was discontinued for weeks 2 and 3 of the study due to time limitations, though membrane filtration procedures were continued. It was then decided in week four to resume MPN using a modification of SM 9221, in which EC-broth was used to move directly to fecal testing in the confirmatory incubation. Then, following EC-broth confirmation for fecal MPN, eosin-methylene blue (EMB) agar plates were utilized for completion, to select and differentiate for strains of *Escherichia coli*, with 24-hour incubation periods at 44.5°C.

MPN determination was performed using Table 9221.IV, from SM 9221. Membrane filtration results were assessed in coliform forming units (CFU), using the following equation prescribed in SM 9222:

$$CFU/100mL = \frac{(Fecal\ Coliform\ Colonies\ Counted)(100)}{mL\ Sample\ Filtered}$$

The following key materials were utilized:

- Market Forge "Sterilmatic" autoclave, at 250°C for 15min.
- Fischer Scientific "Isotemp 128" water bath incubator, at 44.5°C
- Blue M "Stabli-Therm" dry incubator, at 37°C
- Millipore filters, 0.45µm, Lot #F5SN30872Q.
- Matheson, Coleman, and Bell sodium thiosulfate.
- DIFCO "Bacto" EC-broth for fecal MPN determination, control # 659601.
- DIFCO "Bacto" EMB agar for *E. coli* MPN completions, lot # 103474JC.

- DIFCO lauryl tryptose broth, for presumptive MPN, lot #111500JB.
- DIFCO "Bacto" brilliant green bile broth, for confirm/completion MPN, control # 552716
- DIFCO mENDO agar LES, for total coliform membrane filtration, lot # 113042JA.
- DIFCO "Bacto" mFC agar, for fecal coliform membrane filtration, lot # 122485JD

Results

Physical Environmental Parameters

The results from our Dissolved Oxygen (D.O.), Salinity (ppt), Conductivity (mS), Temperature (°C), and pH are displayed by date and sampling site as well as the average means over a six-week period for each tests by site.

Site	Description
1	"Chlorine contact basin"- treated wastewater effluent, pre-aquaculture system
2	Mixing site, bay water/wastewater effluent, pre-aquaculture system
3	Aquaculture terminal discharge outfall

Table 2.

Date	Description of Day and Tide Fluctuation
16-Feb-07	Rainy Day-Low tide approx. 4:20a.m. Ht. 2 ft. / High tide approx. 10:19 a.m. Ht. 7.6 ft.
21-Feb-07	Rainy Day-Low Tide Approx. 8:39am Ht. 0.4 ft.
28-Feb-07	Rainy Day-High Tide Approx. 9:04am ht. 6.9 ft.
7-Mar-07	Rainy Day-Low Tide approx. 7:53am Ht. 1.0 ft.
21-Mar-07	Sunny Day-Extremely Low Tide Approx. 8:28am Ht. -0.7 ft.
28-Mar-07	Sunny Day-High Tide Approx. 8:50am Ht. 6.2 ft.

Table3.

Site#1

Date	D.O. (mg/L)	Salinity (ppt)	Conductivity (mS)	Temperature (°C)	pH
16-Feb-07	2.38	0.20	0.40	12.00	7.1,7.1,7.0 **Mean**:7.10
21-Feb-07	1.94	0.20	0.40	11.10	6.9,6.7,6.6 **Mean**:7.10
28-Feb-07	3.50	0.10	0.30	7.20	6.7,6.7,6.7 **Mean**: 6.7
7-Mar-07	4.00	0.10	0.30	12.50	6.6,6.6,6.5 **Mean**:6.57
21-Mar-07	2.50	0.20	0.30	13.00	6.6,6.7,6.6 **Mean**:6.64
28-Mar-07	2.43	0.20	0.40	10.70	6.7,6.7,6.7 **Mean**:6.70

Table 4. On site physical measurements at Site 1 for each week of the study period.

Site#2

Date	D.O. (mg/L)	Salinity (ppt)	Conductivity (mS)	Temperature (°C)	pH
16-Feb-07	5.19	18.30	22.06	11.50	7.3,7.3,7.3 **Mean**:7.30
21-Feb-07	7.17	10.90	18.40	11.80	7.0,7.1,7.0 **Mean**:7.04
28-Feb-07	5.55	11.50	18.05	6.90	7.1,7.1,7.1 **Mean**:7.10
7-Mar-07	5.34	15.00	20.53	11.60	7.4,7.4,7.4 **Mean**:7.40
21-Mar-07	7.00	8.60	10.60	9.80	7.9,7.8,7.8 **Mean**:7.84
28-Mar-07	6.67	16.30	18.81	9.80	7.8,7.8,7.8 **Mean**:7.80

Table 5. On site physical measurements at Site 2 for each week of the study period.

Site#3

Date	D.O. (mg/L)	Salinity (ppt)	Conductivity (mS)	Temperature (°C)	pH
16-Feb-07	9.07	7.20	15.06	10.00	8.0,8.0,8.0 **Mean**:8.17
21-Feb-07	9.07	7.20	15.06	10.00	8.0,8.0,8.0 **Mean:**8.0
28-Feb-07	8.45	4.80	8.35	6.70	8.4,8.4,8.4 **Mean:**8.40
7-Mar-07	6.24	12.40	17.60	13.10	8.0,7.9,8.0 **Mean**:7.97
21-Mar-07	6.70	6.30	8.09	13.50	8.0,7.9,8.0 **Mean**:7.97
28-Mar-07	8.70	4.70	6.30	11.90	8.0,7.9,8.0 **Mean**:7.97

Table 6. On site physical measurements at Site 3 for each week of the study period.

Dissolved Oxygen, mg/l

Week	Sample Date	Site 1	Site 2	Site 3
1	16-Feb-07	2.38	5.19	7.10
2	21-Feb-07	1.94	7.17	9.07
3	28-Feb-07	3.50	5.55	8.45
4	7-Mar-07	4.00	5.34	6.24
5	21-Mar-07	2.50	7.00	6.70
6	28-Mar-07	2.43	6.67	8.70
	Mean	2.79	6.15	7.71
	Median	2.47	6.11	7.78

Table 7. Dissolved Oxygen for each site, by week.

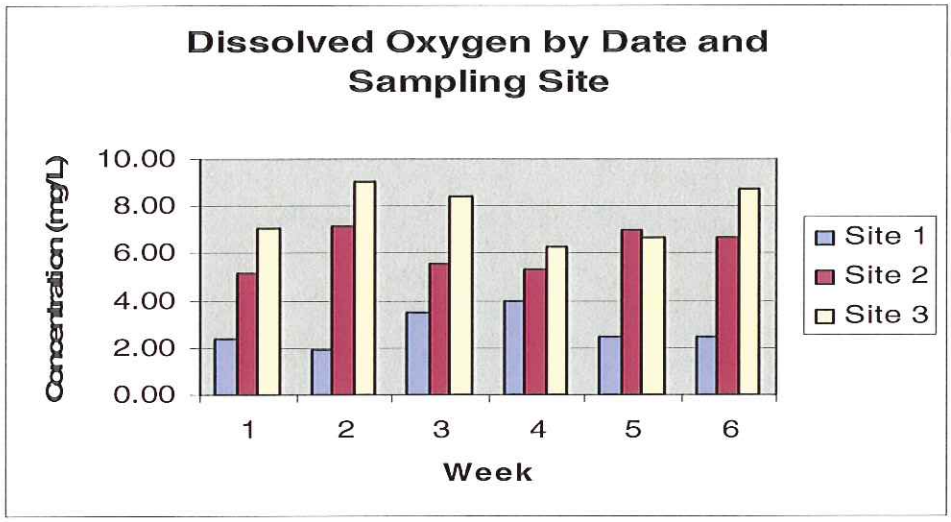

Figure 4. Dissolved Oxygen for each site, by week.

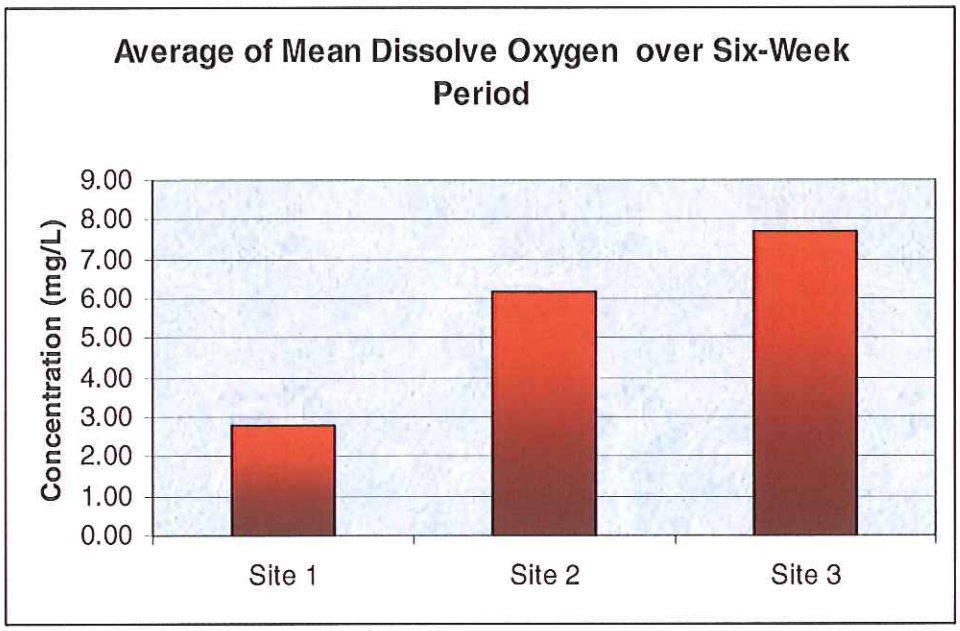

Figure 5. Mean dissolved oxygen levels across the entire study period for each site.

Salinity (ppt)

Week	Sample Date	Site 1	Site 2	Site 3
1	16-Feb-07	0.20	18.30	15.00
2	21-Feb-07	0.20	10.90	7.20
3	28-Feb-07	0.10	11.50	4.80
4	7-Mar-07	0.10	15.00	12.40
5	21-Mar-07	0.20	8.60	6.30
6	28-Mar-07	0.20	16.30	4.70
	Mean	0.17	13.43	8.40
	Median	0.20	13.25	6.75

Table 8 Salinity for each site, by week.

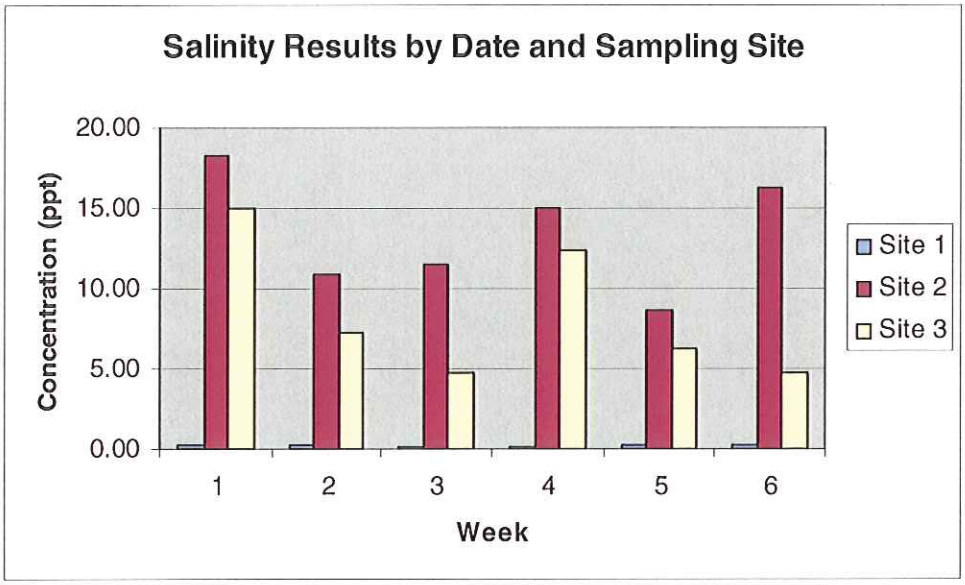

Figure 6. Salinity for each site, by week.

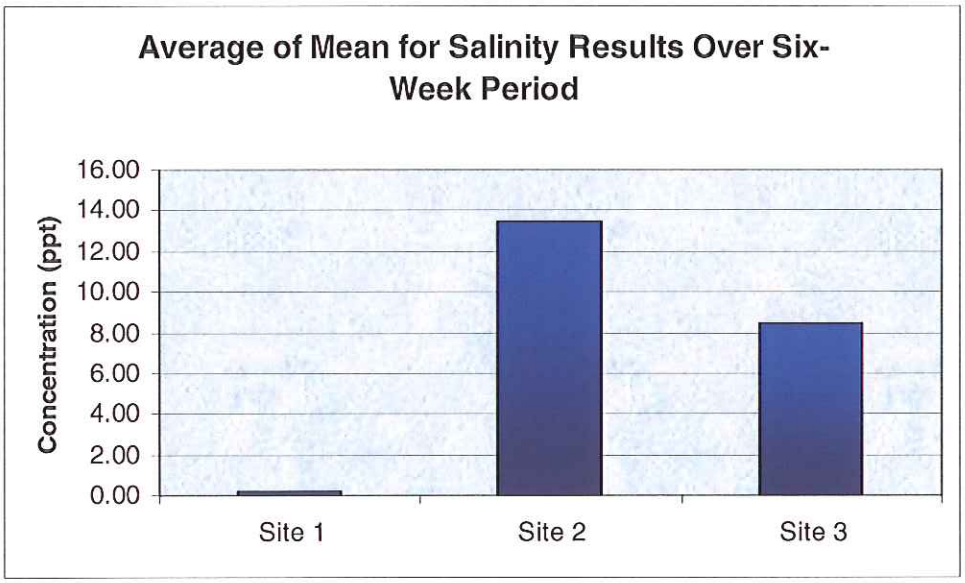

Figure 7. Mean salinity for each site, across the entire study period.

Conductivity, mS

Week	Sample Date	Site 1	Site 2	Site 3
1	16-Feb-07	0.40	22.06	18.27
2	21-Feb-07	0.40	18.40	15.06
3	28-Feb-07	0.30	18.05	8.35
4	7-Mar-07	0.30	20.53	17.60
5	21-Mar-07	0.30	10.60	8.09
6	28-Mar-07	0.40	18.81	6.30
	Mean	0.35	18.08	12.28
	Median	0.35	18.61	11.71

Table 9. Conductivity for each site, by week.

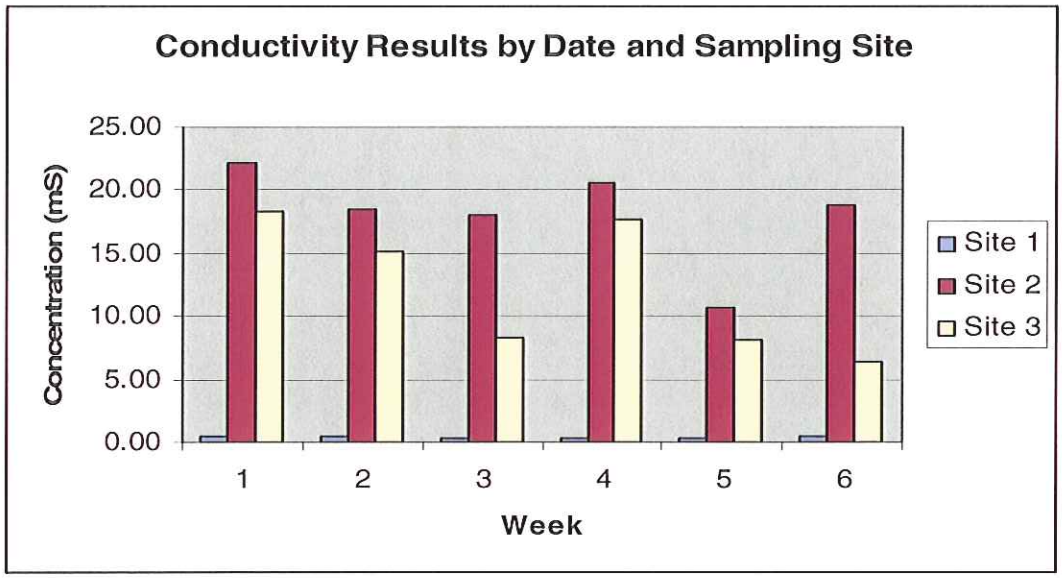

Figure 8. Conductivity for each site, by week.

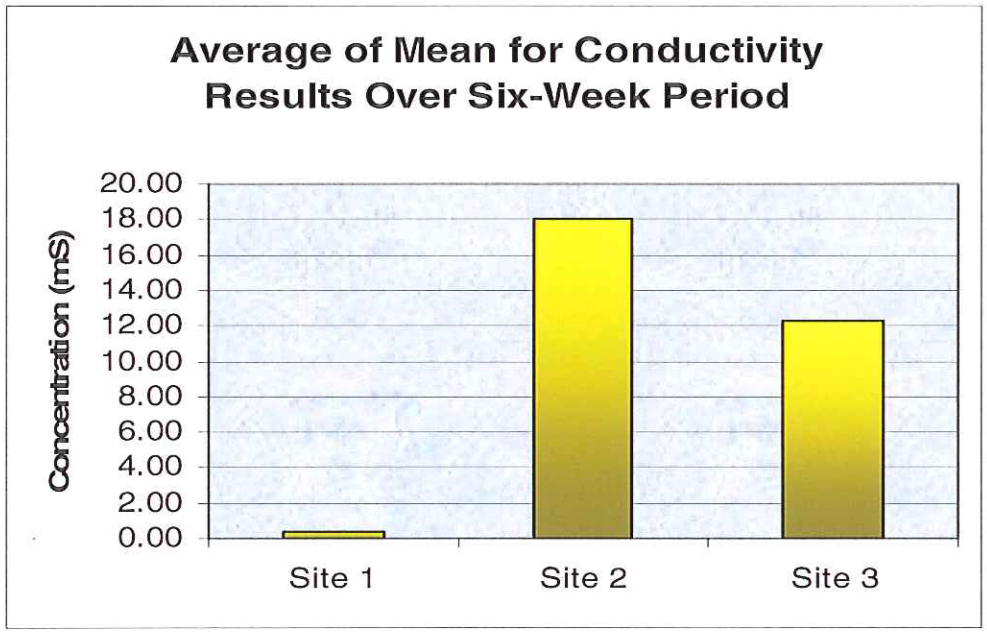

Figure 9. Mean conductivity for each site, across the entire study period.

Temperature, °C

Week	Sample Date	Site 1	Site 2	Site 3
1	16-Feb-07	12.00	11.50	11.80
2	21-Feb-07	11.10	11.80	10.00
3	28-Feb-07	7.20	6.90	6.70
4	7-Mar-07	12.50	11.60	13.10
5	21-Mar-07	13.00	9.80	13.50
6	28-Mar-07	10.70	9.80	11.90
	Mean	11.08	10.23	11.17
	Median	11.55	10.65	11.85

Table 10. Temperature for each site, by week.

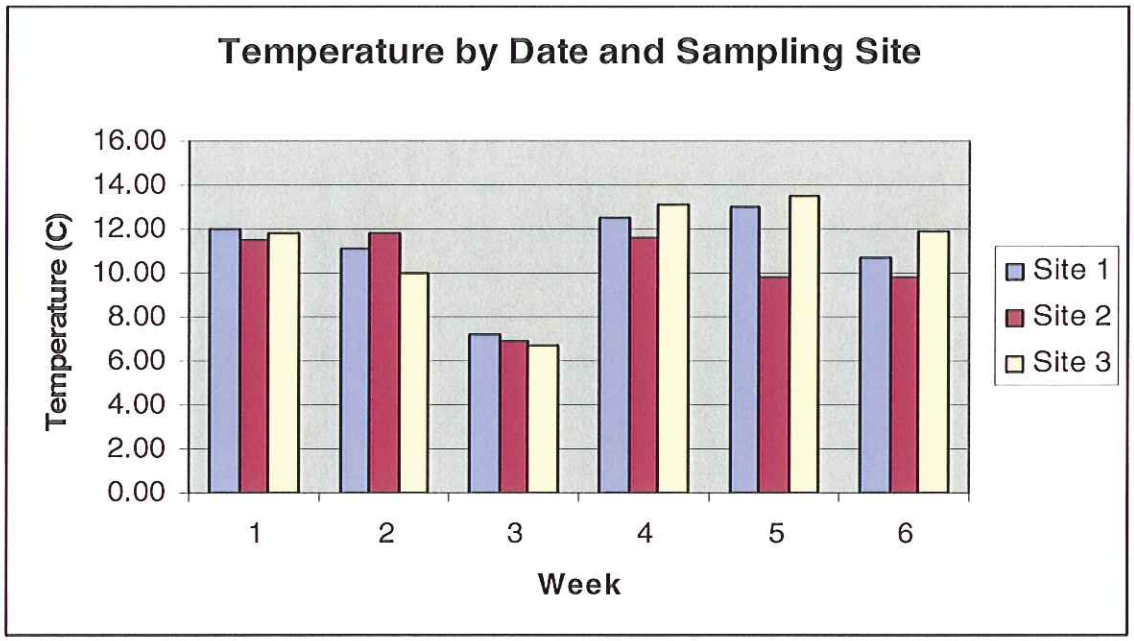

Figure 10. Temperature for each site, by week.

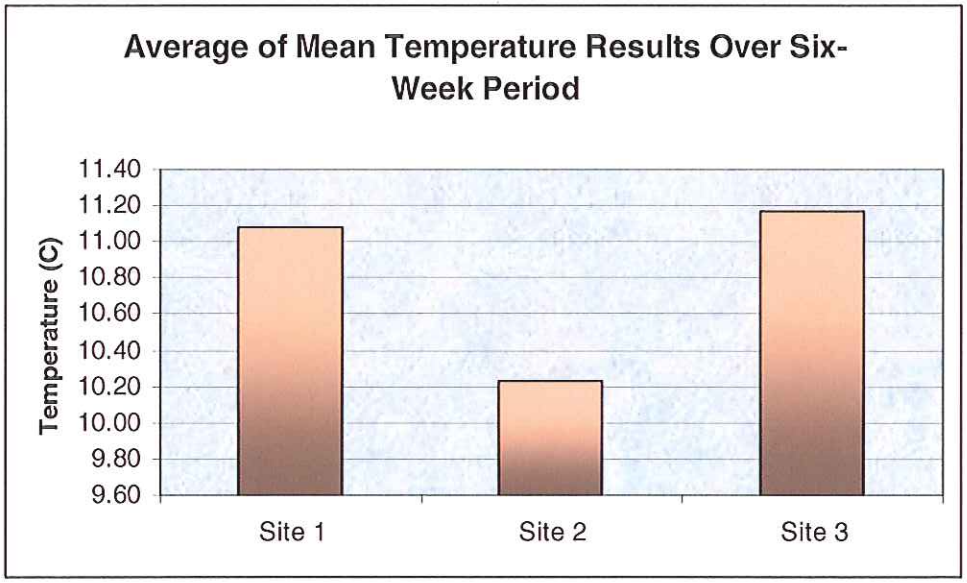

Figure 11. Mean temperature measurements for each site across the entire study period.

pH

Week	Sample Date	Site 1	Site 2	Site 3
1	16-Feb-07	7.10	7.30	8.17
2	21-Feb-07	6.75	7.04	8.00
3	28-Feb-07	6.70	7.10	8.40
4	7-Mar-07	6.57	7.40	7.97
5	21-Mar-07	6.64	7.84	7.97
6	28-Mar-07	6.70	7.80	7.97
	Mean	6.74	7.41	8.08
	Median	6.70	7.35	7.99

Table 11. pH (means) for each site, by week.

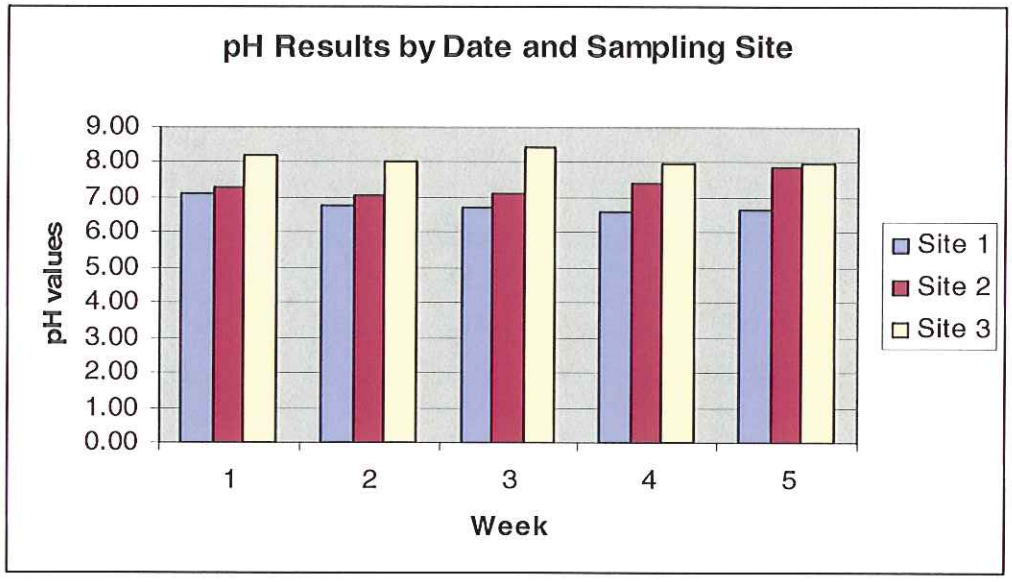

Figure 12. pH (means) for each site, by week.

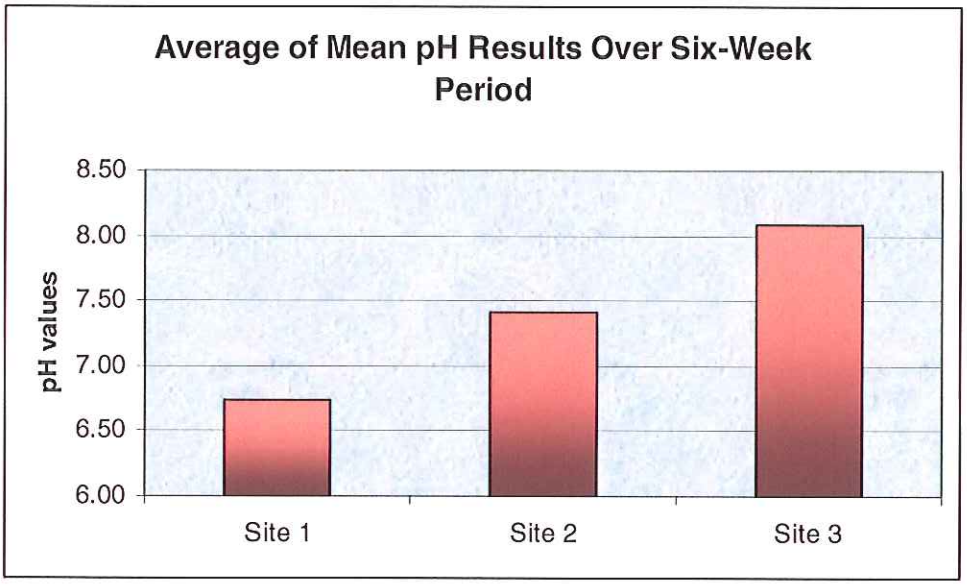

Figure 13. Mean of means for pH for each site across the entire study period.

Total Suspended Solids TSS

The TSS levels measured during this study ranged from 1.3 to 18.6mg/l throughout the study period. The following hierarchy was consistently maintained throughout the study in terms of TSS levels:

Mixing Site (Site 2) > Aquaculture Discharge (Site 3) > Chlorine Contact Basin (Site1)

Total Suspended Solids, mg/l				
Week	Sample Date	Chlorine Contact Basin	Mixing Site	Aquaculture Discharge
1	16-Feb-07	3.1	9.9	2.9
2	21-Feb-07	1.3	8.2	6.2
3	28-Feb-07	2.0	9.5	3.3
4	07-Mar-07	3.2	18.6	12.5
5	21-Mar-07	2.2	7.9	3.7
6	28-Mar-07	1.6	11.3	3.3
	Mean	2.2	10.9	5.3
	Median	2.1	9.7	3.5

Table 12. TSS levels for each site, for each week of the study period.

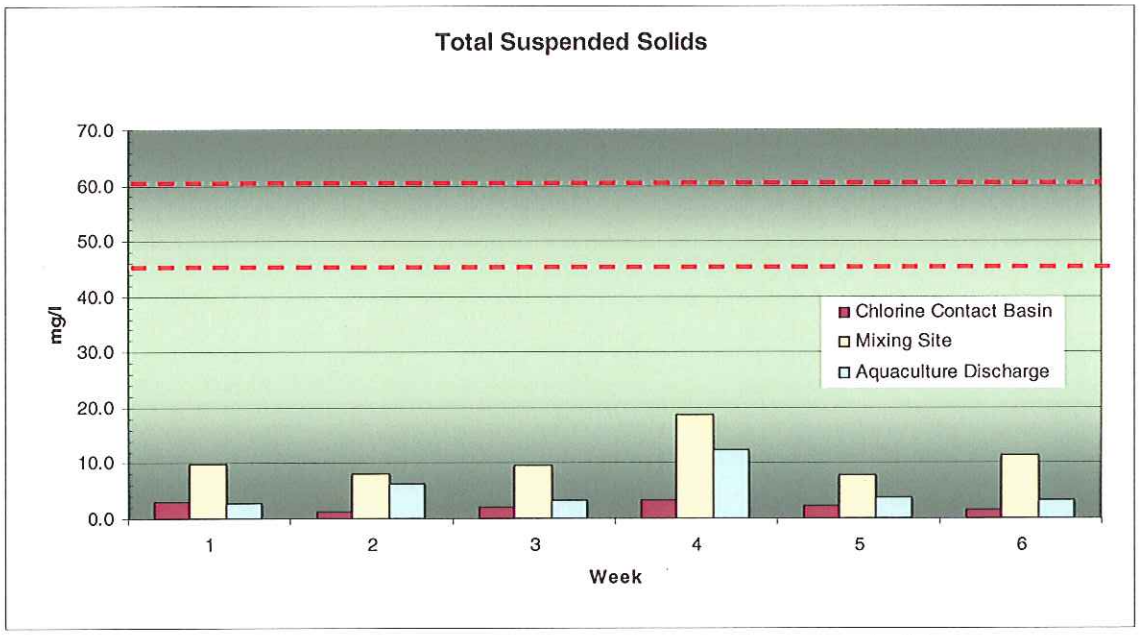

Figure 14. Total Suspended Solids in the effluent is a measure of the amount of solids that remain suspended after treatment. During the period Feb – April 07 both the weekly and monthly concentrations were below AWTP permit limits.

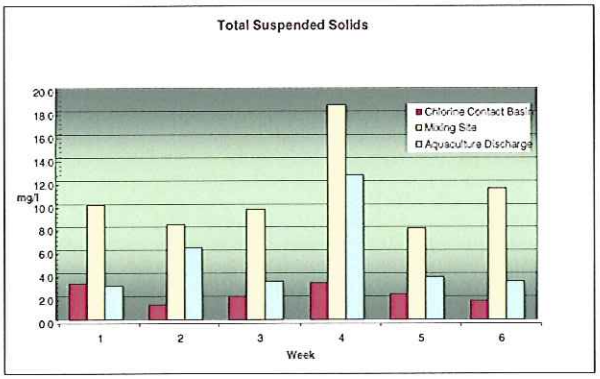

Figure 15: Mean TSS for the Arcata aquaculture facility ranged from 18.6mg/l at the mixing site to 2.9 mg/l at the discharge point.

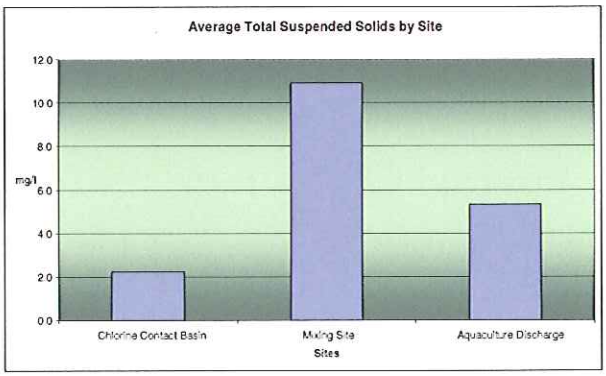

Figure 16: Average of the Mean comparing the three sites in total.

Nitrogen

Ammonia level measurement results were found to consistently conform to the following hierarchy:

Chlorine Contact Basin (Site 1) >> Mixing Site (Site2) > Aquaculture Discharge (Site3)

Nitrate level measurement results were found to consistently conform to the following hierarchy:

Chlorine Contact Basin (Site 1) > Aquaculture Discharge (Site3) > Mixing Site (Site2)

The mean values of from three tests per sample per sampling day are displayed below, and further averaged across the study period for comparative analysis in Tables 13-14 and Figures 17-20.

Ammonia ($N\text{-}NH_3$), in mg/l				
Week	Sample Date	Chlorine Contact Basin	Mixing Site	Aquaculture Discharge
1	16-Feb-07	35.00	2.30	0.10
2	21-Feb-07	2.67	0.10	0.03
3	28-Feb-07	14.67	2.33	0.07
4	07-Mar-07	14.00	1.30	0.26
5	21-Mar-07	N.D.	N.D.	N.D.
6	28-Mar-07	15.00	3.90	0.60
	Mean	16.3	2.0	0.2
	Median	14.7	2.3	0.1

Table 13. Ammonia means for three tests per site per sample day, by site and date of sample collection.

Nitrate ($N\text{-}NO_3$), in mg/l				
Week	Sample Date	Chlorine Contact Basin	Mixing Site	Aquaculture Discharge
1	16-Feb-07	4.67	3.73	2.50
2	21-Feb-07	1.63	0.13	0.63
3	28-Feb-07	2.03	0.17	0.63
4	07-Mar-07	2.23	0.00	1.40
5	21-Mar-07	N.D.	N.D.	N.D.
6	28-Mar-07	1.20	0.47	0.73
	Mean	2.35	0.90	1.18
	Median	2.03	0.17	0.73

Table 14. Nitrate means for three tests per site per sample day, by site and date of sample collection.

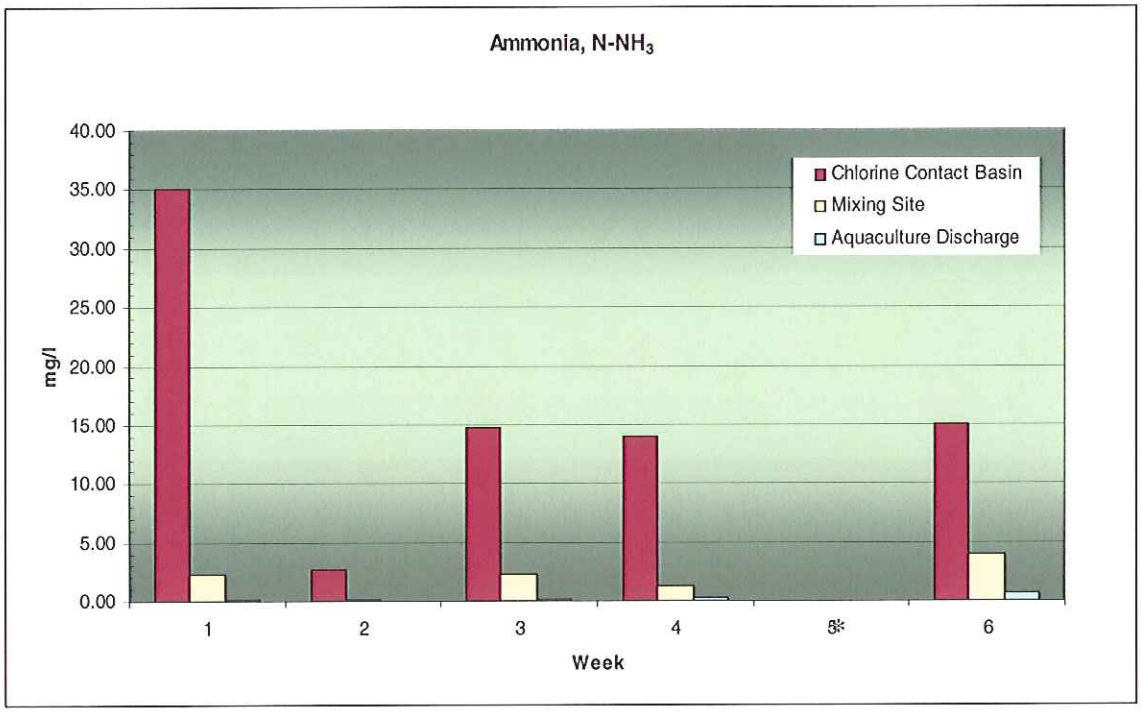

Figure 17. Relative mean ammonia levels, by week, across the study period. *Please note that in week five of the study period there was no data collected, and the blank space does not represent zero values.

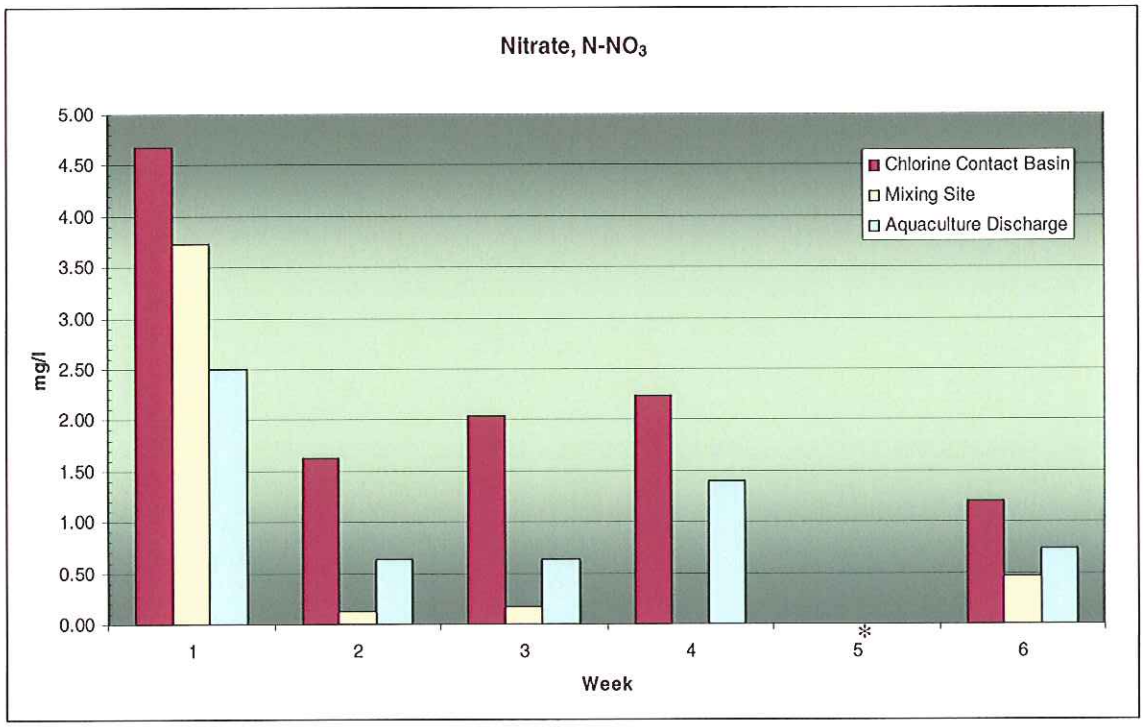

Figure 18. Relative mean nitrate levels, by week, across the study period. *Please note that in week five of the study period there was no data collected, and the blank space does not represent zero values.

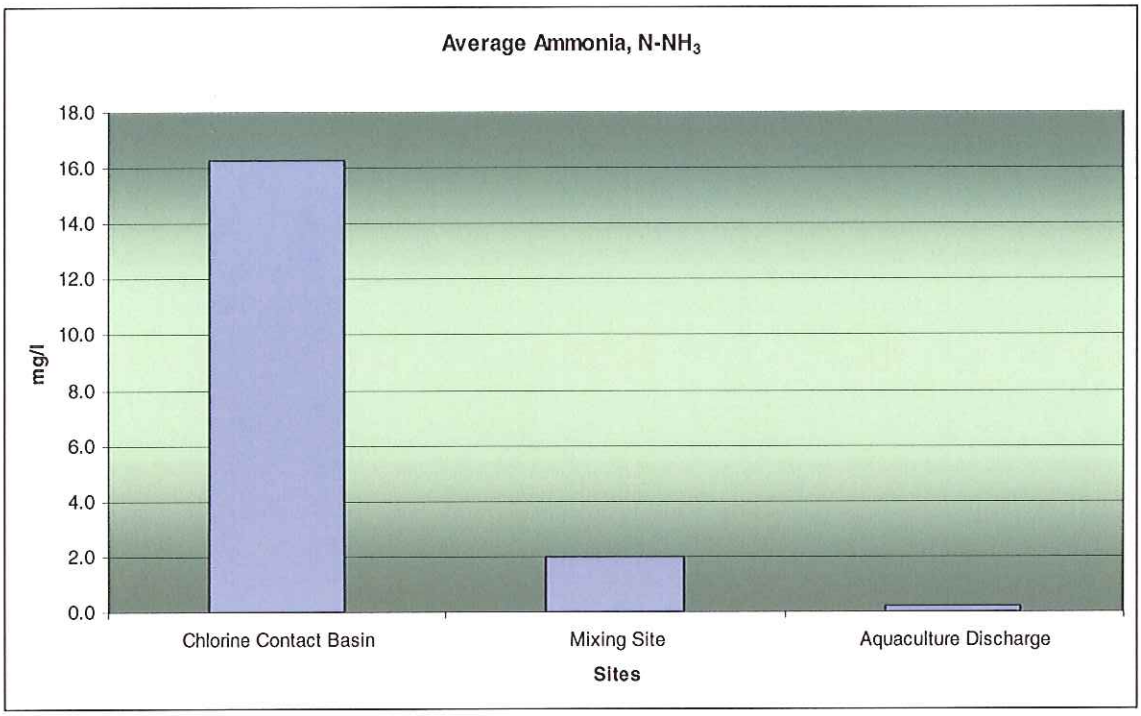

Figure 19. Relative mean ammonia levels for each site, averaged across the entire study period.

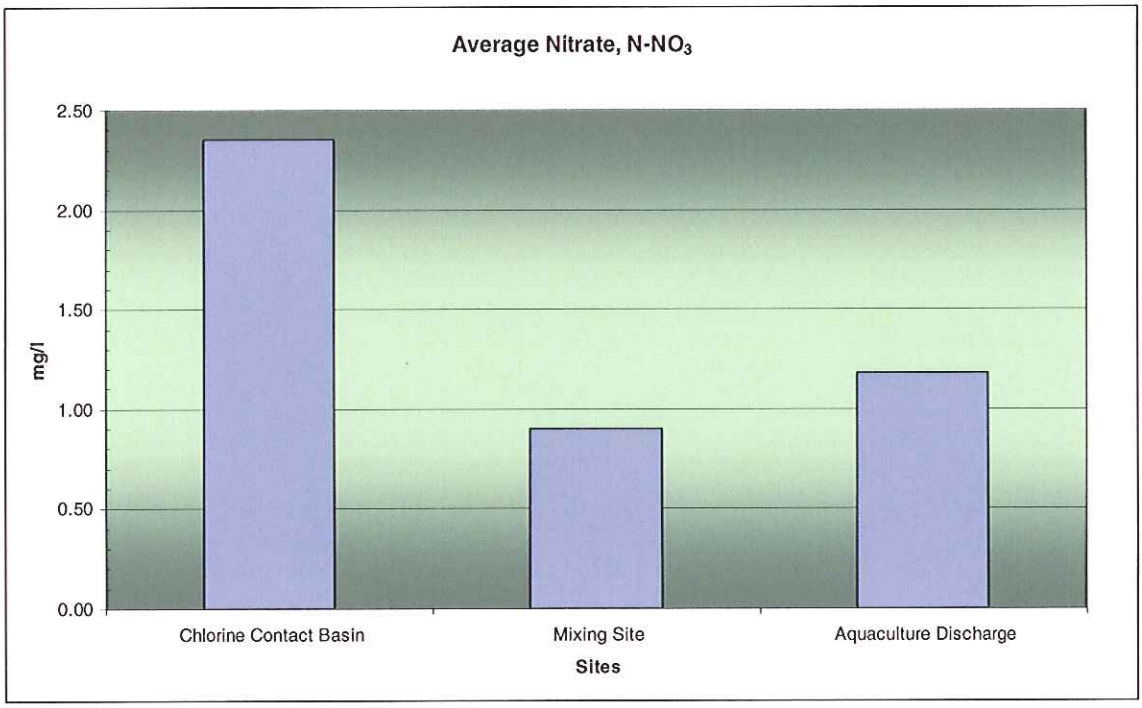

Figure 20. Relative mean nitrate levels for each site, averaged across the entire study period.

BOD

5-day blank values for each week were negative, and for purposes of this experiment, will be considered to be zero. Initial and 5-day temperatures were not recorded for weeks 1-3; for initial temperatures, on site measurements are used for these weeks, and room temperature is assumed after 5 days (approx 20 deg C). Four out of six weeks' results show that direct outfall of the AWTP (Site 1) has higher BOD values than either sites in the aquaculture facility. Weeks 1-6, however, show higher BOD values for the outfall of the aquaculture facility (Site 3), than those found at the inflow (Site 2). The highest values for each site were recorded during week 3; the lowest values were found during week 5. Average BOD values for each site through six weeks of testing were calculated:

Site	Average BOD-5, mg/l
1	8.9
2	3.1
3	7.5

Table 15. Average BOD-5 results across entire study period

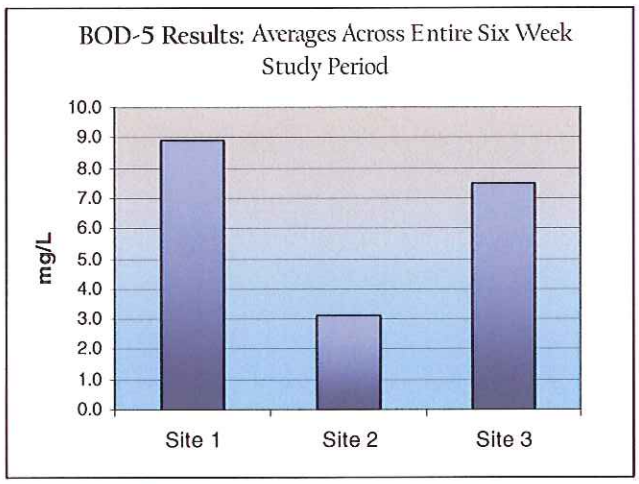

Figure 21. Histogram displaying average BOD-5 values from Table X for visual comparison.

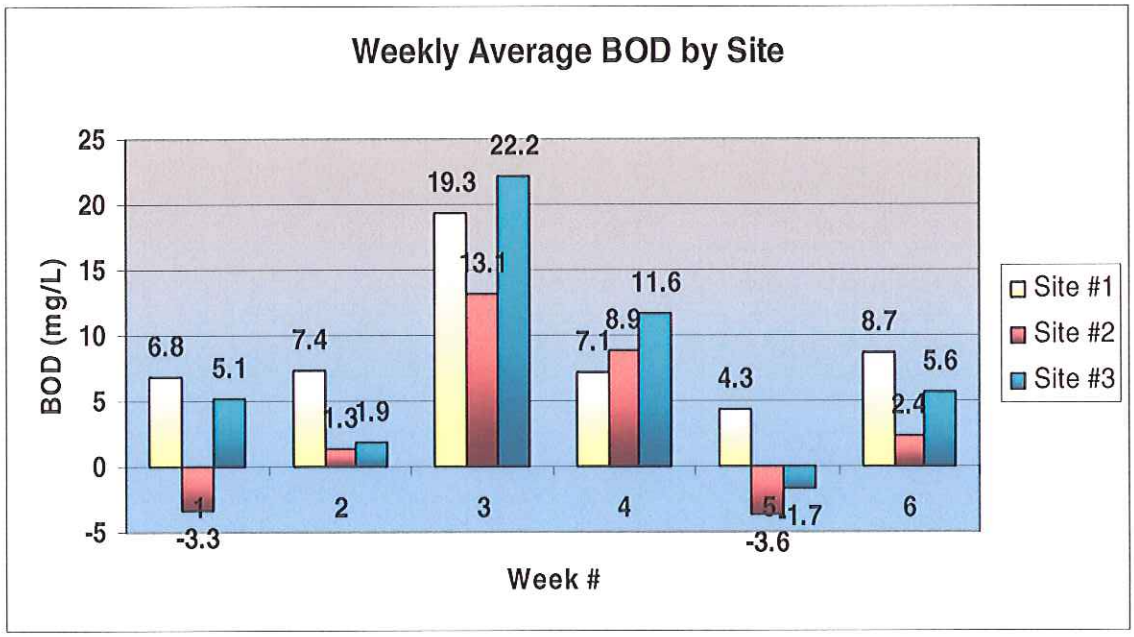

Figure 22. Weekly mean BOD-5 values for each sample site, by week, for each week of the study period.

Table 16: BOD-5 Results

WEEK #1

Site 1 Initial		5-Day (2/21)	BOD
Dilution (mL)	D.O. (mg/L)	D.O. (mg/L)	(mg/L)
	8.3	6.7	6.4
100	8.2	6.5	5.1
150	8.7	6.0	5.4
200	8.5	6.1	3.6

Salinity = 15.0 ppt

average = 5.1 mg/L

Site 2 Initial		5-Day (2/21)	BOD
Dilution (mL)	D.O. (mg/L)	D.O. (mg/L)	(mg/L)
50	6.0	7.4	-8.4
100	6.1	7.1	-3.0
150	5.9	6.6	-1.4
200	6.0	6.2	-0.3

Salinity = 18.3 ppt

average = -3.3 mg/L

Site 3 Initial		5-Day (2/21)	BOD
Dilution (mL)	D.O. (mg/L)	D.O. (mg/L)	(mg/L)
75	6.0	4.4	6.4
100	5.8	3.9	5.7
150	5.4	0.8	9.2
200	4.9	1.0	5.9

Salinity = 0.3 ppt

average = 6.8 mg/L

	Initial DO	5-Day	BOD
Blank	6.7	8.2	-1.5

WEEK #2

Site 1 Initial		5-Day (2/26)	BOD
Dilution (mL)	D.O. (mg/L)	D.O. (mg/L)	(mg/L)
100	8.9	8.5	1.2
150	9.3	8.3	2.0
200	9.9	8.2	2.6

Salinity = 10.9 ppt

average = 1.9 mg/L

Site 2 Initial		5-Day (2/26)	BOD
Dilution (mL)	D.O. (mg/L)	D.O. (mg/L)	(mg/L)
100	9.1	9.0	0.3
150	9.5	8.8	1.4
200	9.9	8.4	2.3

Salinity = 7.2 ppt

average = 1.3 mg/L

Site 3 Initial		5-Day (2/26)	BOD
Dilution (mL)	D.O. (mg/L)	D.O. (mg/L)	(mg/L)
100	9.0	6.5	7.5
150	8.9	5.4	7.0
200	8.9	3.8	7.7

Salinity = 0.2 ppt

average = 7.4 mg/L

	Initial DO	5-Day	BOD
Blank	8.9	9.45	-0.55

WEEK #3

Site 1 Initial		5-Day (3/5)	BOD
Dilution (mL)	D.O. (mg/L)	D.O. (mg/L)	(mg/L)
100	10.6	0.8	29.4
150	11.1	0.7	20.8
200	11.7	0.7	16.5

Salinity = 4.8 ppt

average = 22.2 mg/L

Site 2 Initial		5-Day (3/5)	BOD
Dilution (mL)	D.O. (mg/L)	D.O. (mg/L)	(mg/L)
100	9.6	6.3	9.9
150	9.7	2.0	15.4
200	10.0	0.6	14.1

Salinity = 10.5 ppt

average = 13.1 mg/L

Site 3 Initial		5-Day (3/5)	BOD
Dilution (mL)	D.O. (mg/L)	D.O. (mg/L)	(mg/L)
100	9.6	0.6	27.0
150	9.5	0.6	17.8
200	9.4	0.6	13.2

Salinity = 0.1 ppt

average = 19.3 mg/L

	Initial DO	5-Day	BOD
Blank	9.5	13.8	-4.3

WEEK #4

Site 1 Initial			5-Day (3/12)		BOD
Dilution (mL)	Temp (deg C)	D.O. (mg/L)	Temp (deg C)	D.O. (mg/L)	(mg/L)
100	19.1	7.3	21.4	1.7	16.8
150	17.3	7.4	21.4	2.1	10.6
200	16.0	7.5	21.3	2.5	7.5

Salinity = ~13.0 ppt

average = 11.6

Site 2 Initial			5-Day (3/12)		BOD
Dilution (mL)	Temp (deg C)	D.O. (mg/L)	Temp (deg C)	D.O. (mg/L)	(mg/L)
100	18.4	7.1	21.3	2.8	12.9
150	16.7	7.0	21.3	3.0	8.0
200	15.6	7.1	21.3	3.3	5.7

Salinity = ~14.0 ppt

average = 8.9

Site 3 Initial			5-Day (3/12)		BOD
Dilution (mL)	Temp (deg C)	D.O. (mg/L)	Temp (deg C)	D.O. (mg/L)	(mg/L)
100	18.1	7.6	21.3	4.0	10.8
150	17.0	7.4	21.3	4.2	6.4
200	15.6	7.3	21.3	4.6	4.1

Salinity = ~0.1 ppt

average = 7.1

	Initial DO	Temp	5-Day DO	5-Day Temp	BOD
Blank	8.4	20.9	9.1	21.3	-0.7

WEEK #5 Table 16 (continued) WEEK #6

Site 1 Initial / **5-Day (3/26)** / **BOD**

Dilution (mL)	Temp (deg C)	D.O. (mg/L)	Temp (deg C)	D.O. (mg/L)	BOD (mg/L)	Sal =	Not adjusted
	20.6	8.7	22.1	10.0	-3.9		
150	19.7	8.5	22.2	9.1	-1.2	avg =	-1.7
200	18.6	8.4	22.2	8.3	0.1		

Site 2 Initial / **5-Day (3/26)** / **BOD**

Dilution (mL)	Temp (deg C)	D.O. (mg/L)	Temp (deg C)	D.O. (mg/L)	BOD (mg/L)	Sal =	Not adjusted
100	19.9	8.0	22.2	10.5	-7.5		
150	19.3	7.9	22.2	9.1	-2.4	avg =	-3.6
200	18.5	7.9	22.4	8.5	-0.9		

Site 3 Initial / **5-Day (3/26)** / **BOD**

Dilution (mL)	Temp (deg C)	D.O. (mg/L)	Temp (deg C)	D.O. (mg/L)	BOD (mg/L)	Sal =	Not adjusted
100	20.1	6.6	22.1	6.1	1.5		
150	19.7	6.0	22.2	3.7	4.6	avg =	4.3
200	19.0	5.3	22.1	0.7	6.9		

	Initial Temp	Initial DO	5-day Temp	5-Day DO	BOD
Blank	21.5	8.8	22.0	11.7	-2.9

Site 1 Initial / **5-Day (4/3)** / **BOD**

Dilution (mL)	Temp (deg C)	D.O. (mg/L)	Temp (deg C)	D.O. (mg/L)	BOD (mg/L)	Sal =	4.2 ppt
100	18.3	11.1	22.1	10.1	3.0		
150	16.3	11.4	22.0	8.8	5.2	avg =	5.6
200	14.5	11.9	21.9	6.2	8.6		

Site 2 Initial / **5-Day (4/3)** / **BOD**

Dilution (mL)	Temp (deg C)	D.O. (mg/L)	Temp (deg C)	D.O. (mg/L)	BOD (mg/L)	Sal =	16.0 ppt
100	17.9	9.7	21.9	9.2	1.5		
150	16.1	9.8	21.8	8.1	3.4	avg =	2.4
200	14.5	10.1	21.8	8.6	2.3		

Site 3 Initial / **5-Day (4/3)** / **BOD**

Dilution (mL)	Temp (deg C)	D.O. (mg/L)	Temp (deg C)	D.O. (mg/L)	BOD (mg/L)	Sal =	0.4 ppt
100	17.9	10.0	21.9	6.5	10.5		
150	16.6	9.5	21.9	5.8	7.4	avg =	8.7
200	15.0	9.1	21.8	3.6	8.3		

	Initial Temp	Initial DO	5-day Temp	5-Day DO	BOD
Blank	21.3	10.6	22.3	11.4	-0.8

Membrane Filtration

The results from our membrane filtration are displayed below as averages and medians from three filtration volumes utilized for each sampling date. The filtration volumes are listed below in Table 18, for reference. The volumes were increased in the second week to adjust for low counts in the first week; the high total coliform counts in the second week then prompted us to return to the original total coliform volumes, which we maintained for consistency.

Site	Description
1	"Chlorine contact basin"—treated wastewater effluent pre-mixing/pre-aquaculture
2	Mixing site, baywater/wastewater effluent, pre-aquaculture system.
3	Aquaculture terminal discharge outfall.

Table 17.

Sample Date	Total Coliforms, Volumes, mL	Fecal Coliforms, Volumes, mL
16-Feb-07	0.1, 1, 10	0.1, 1, 10
21-Feb-07	1, 10, 50	1, 10, 50
28-Feb-07	0.1, 1, 10	1, 10, 50
7-Mar-07	0.1, 1, 10	1, 10, 50
21-Mar-07	0.1, 1, 10	1, 10, 50
28-Mar-07	0.1, 1, 10	1, 10, 50

Table 18. Filtration volumes for each sample date within the study.

M-ENDO: (Arithmetic Means CFU/100mL)

Week	Sample Date	Site 1	Site 2	Site 3
1	16-Feb-07	0	10	367
2	21-Feb-07	0	1980	920
3	28-Feb-07	0	173	53
4	7-Mar-07	73150	TNTC	TNTC
5	21-Mar-07	8150	13557	18843
6	28-Mar-07	430	3450	800
	Mean	13622	3834	4197
	Median	215	1980	800

Table 19. Total coliforms; means from three filtration volumes with mean and median values across entire study period. TNTC: too numerous to count. CFU: colony forming units (of bacteria).

M-ENDO: (Medians CFU/100mL)

Week	Sample Date	Site 1	Site 2	Site 3
1	16-Feb-07	0	15	100
2	21-Feb-07	0	1980	920
3	28-Feb-07	0	200	80
4	7-Mar-07	73150	TNTC	TNTC
5	21-Mar-07	1100	3900	2800
6	28-Mar-07	290	3450	400
	Mean	12423	1909	860
	Median	145	1980	400

Table 20. Total coliforms; medians from three filtration volumes with mean and median values across entire study period. TNTC: too numerous to count. CFU: colony forming units (of bacteria).

M-FC: (Arithmetic Means CFU/100mL)

Week	Sample Date	Site 1	Site 2	Site 3
1	16-Feb-07	0	383	3
2	21-Feb-07	0	97	171
3	28-Feb-07	0	121	14
4	7-Mar-07	TNTC	TNTC	TNTC
5	21-Mar-07	0	3	34
6	28-Mar-07	0	252	51
	Mean	0	171	55
	Median	0	121	34

Table 21. Fecal coliforms; means from three filtration volumes with mean and median values across entire study period. TNTC: too numerous to count. CFU: colony forming units (of bacteria).

M-FC: (Medians CFU/100mL)

Week	Sample Date	Site 1	Site 2	Site 3
1	16-Feb-07	0	100	5
2	21-Feb-07	0	140	200
3	28-Feb-07	0	110	12
4	7-Mar-07	TNTC	TNTC	TNTC
5	21-Mar-07	0	5	2
6	28-Mar-07	0	300	30
	Mean	0	131	50
	Median	0	110	12

Table 22. Fecal coliforms; medians from three filtration volumes with mean and median values across entire study period. TNTC: too numerous to count. CFU: colony forming units (of bacteria).

Figures 23 through 28 display our results in various manners for visual comparative analysis.

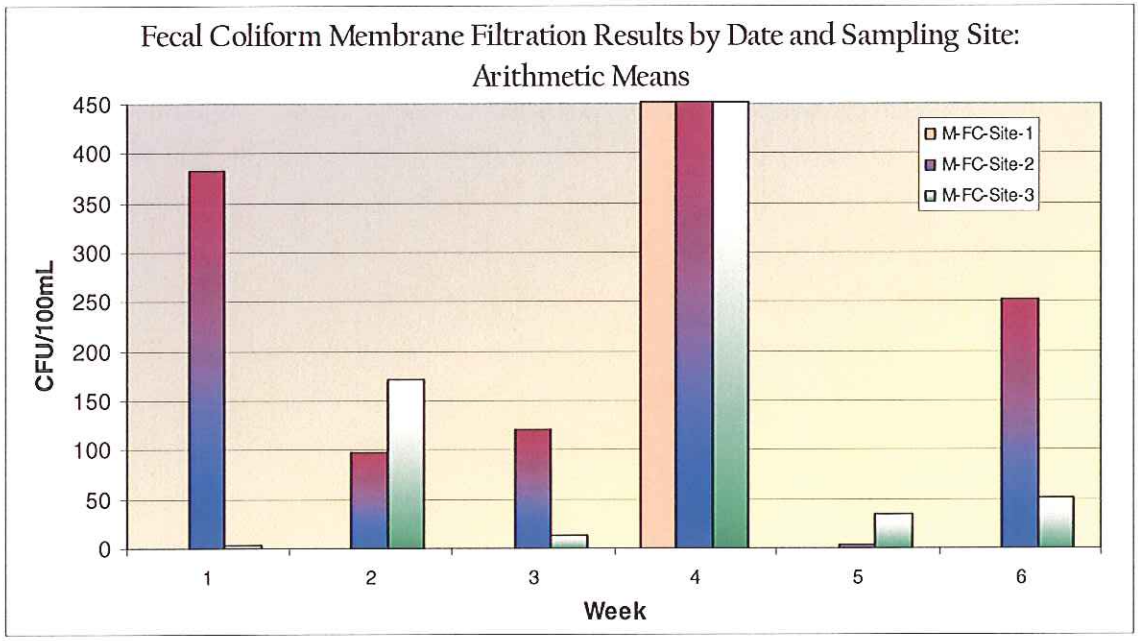

Figure 23. Histogram showing the relative fecal coliform levels at each site across the study period, based on mean calculations across filtration volumes. Please reference Tables X through X above corresponding sample dates and numerical values Please note that on week four of our study, the plates contained colonies in exceeding accurate counting (TNTC).

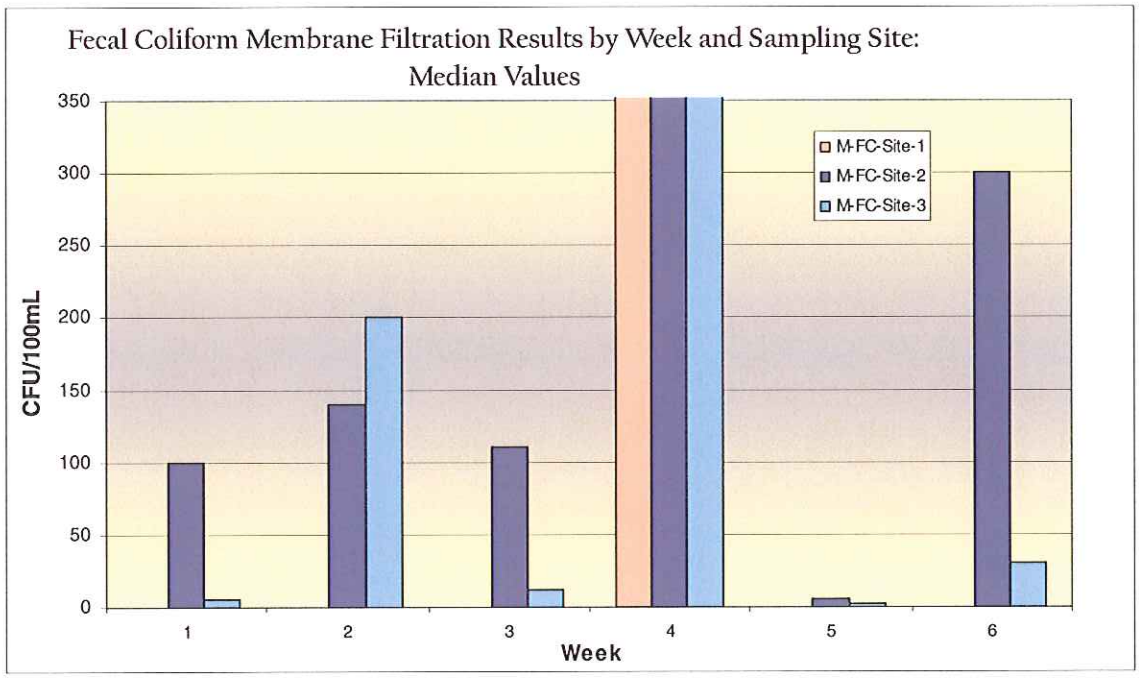

Figure 24. Histogram showing the relative fecal coliform levels at each site across the study period, based on median calculations across filtration volumes. Please reference Tables X through X above corresponding sample dates and numerical values. Please note that on week four of our study, the plates contained colonies exceeding levels permitting accurate counting (TNTC).

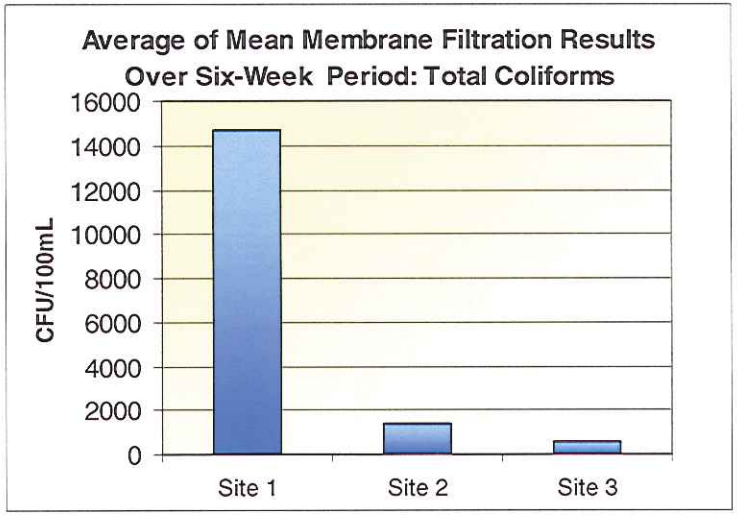

Figure 25. Total coliform membrane filtration means averaged over the entire study period. Please reference Table X for the source data.

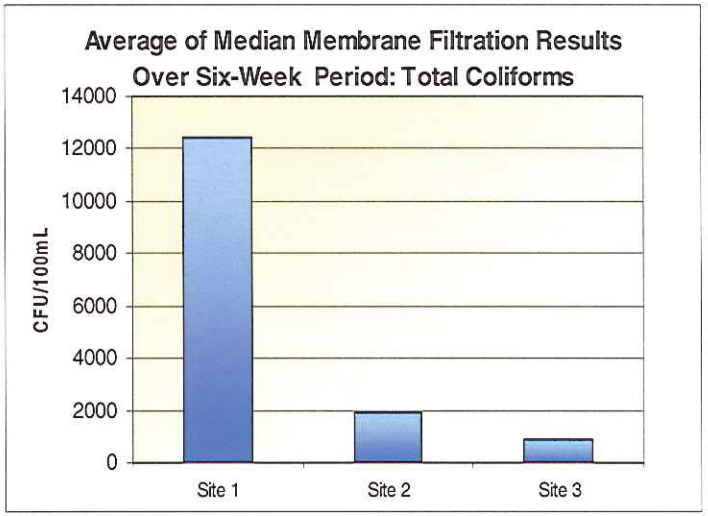

Figure 26. Total coliform membrane filtration medians averaged over the entire study period. Please refer to Table X for source data.

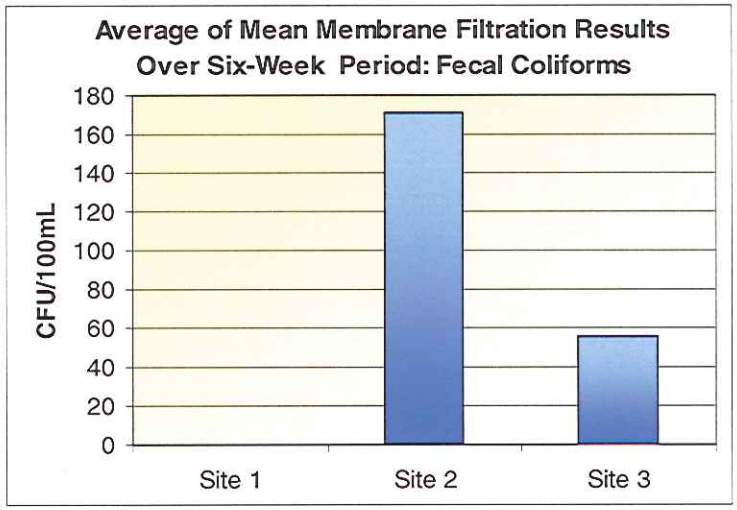

Figure 27. Total coliform membrane filtration means averaged over the entire study period. Please reference Table X for the source data. Please note zero value for Site 1.

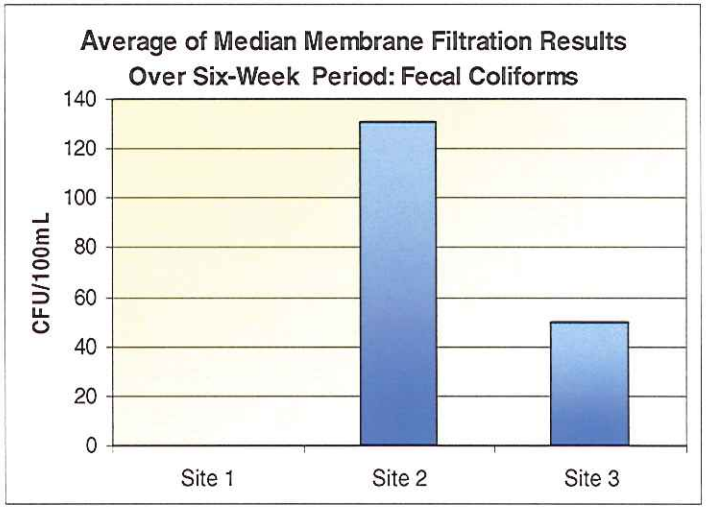

Figure 28. Total coliform membrane filtration medians averaged over the entire study period. Please refer to Table X for source data. Please note zero value for Site 1.

5-Tube Most Probable Number (MPN)

As MPN evaluation was temporarily discontinued during the second and third weeks of the study due to time constraints, and then EC-broth was used to confirm only for fecal coliforms once MPN evaluation was later resumed in week four, MPN data from this study is limited for total coliforms. However, included below in Table 23 are the data from the first week, in which the standard total coliform confirmation was conducted, to give some indication of relative total coliform levels indicated by MPN testing.

After the first week, confirmation tests were only done for fecal coliforms; the MPN's for each sample date are displayed below in Table 24. Please note that each MPN is exactly that, a probability, and that each MPN has an associated confidence range (not included, to simplify the presentation of data), as is demonstrated by the data set in Table 23, which does include the standard confidence intervals always associated with an MPN. In Figure 29, a histogram has been utilized to provide a visual representation of our MPN findings. Also below is Figure 30, which graphically compares MPN and membrane filtration results across the entire study period, also via histogram. Finally, the raw findings from eosin-methylene blue (EMB) testing for *Escherichia coli*, have been provided in Tables 25 through 27, contrasted therein with raw EC-broth confirmation results for comparative interpretation.

5-Tube MPN, Confirmed, 16-Feb-07						
	Number Positive			MPN	95% Conf. Int.	
	10mL	1.0mL	0.1mL		Lower limit	Upper Limit
Site 1	5	0	0	23	9	86
Site 2	5	1	1	50	20	150
Site 3	5	4	0	130	50	390

Table 23. Total coliform MPN data for the first week of the study.

MPN-Confirmed Fecal Coliforms

Week	Date	Site 1	Site 2	Site 3
1	16-Feb-07	8	50	130
2	21-Feb-07	N.D.	N.D.	N.D.
3	28-Feb-07	N.D.	N.D.	N.D.
4	7-Mar-07	0	13	0
5	21-Mar-07	13	4	23
6	28-Mar-07	2	500	8
	Mean	6	142	40
	Median	5	32	16

Table 24. Most probable number (MPN) determinations for each sampling site and date, with calculated means and medians for each site across the entire study period. N.D. = no data.

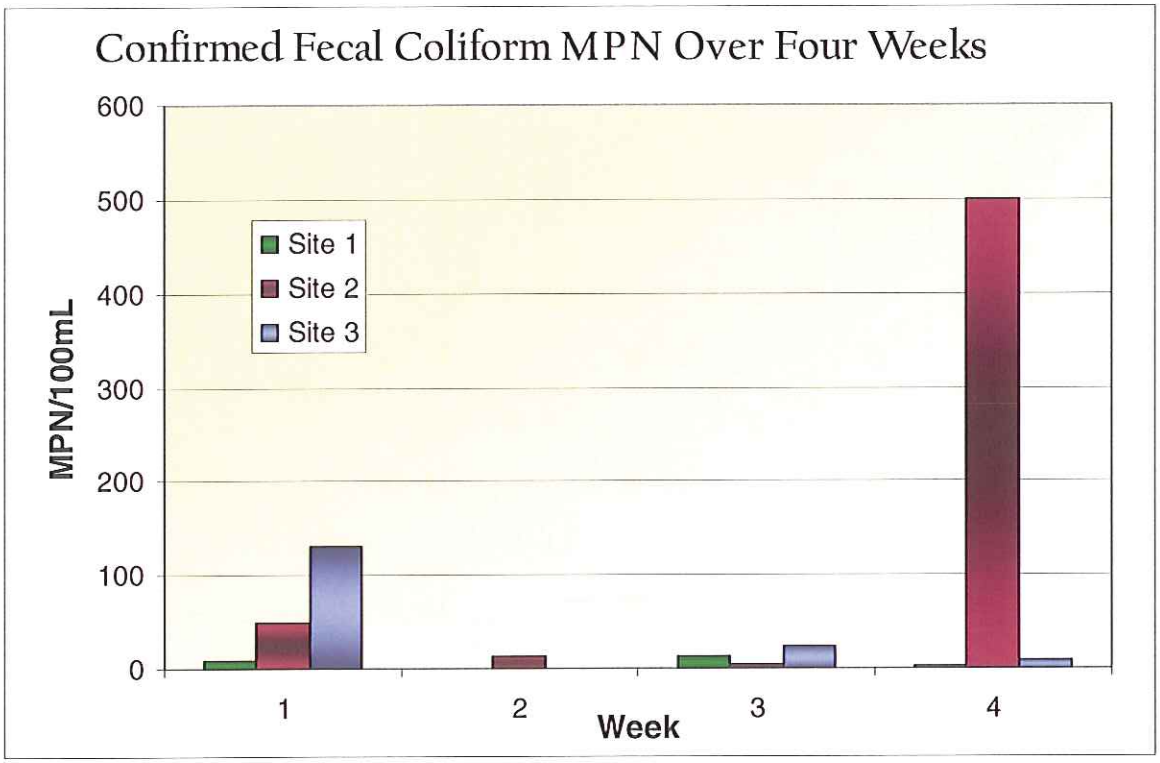

Figure 29. Comparison of determined MPN values for each site and sample date. Please note that we jump from week one of the study period to week four, as MPN testing was not conducted during weeks 2 and 3. Please reference Table 24, above, for the sample date associated with each week and exact MPN values.

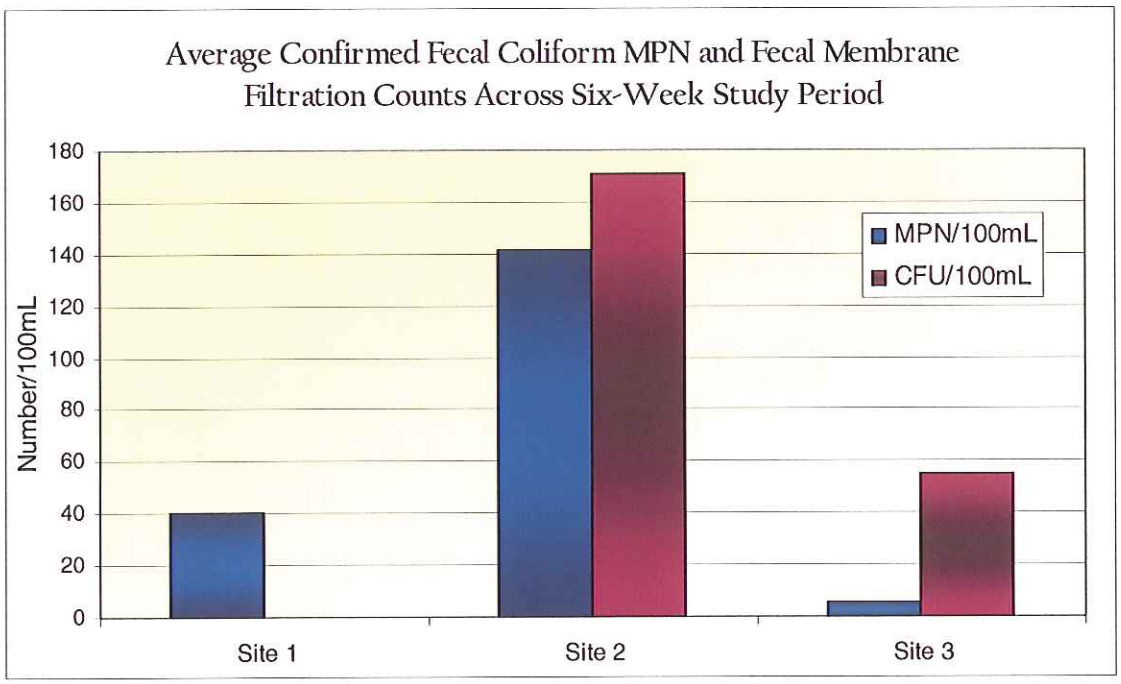

Figure 30. Comparison of averages for fecal coliform MPN and membrane filtration across the entire study period. Please refer to Tables 21 and 24 for associated data, and note that for Site 1 average CFU/100mL was determined to be zero.

EMB Plate Results 7-Mar-07 (week 4)						
	# Positive Plates, EMB			# Positive Tubes, EC-Broth		
Site	10mL	1mL	0.1mL	10mL	1.0mL	0.1mL
1	-	-	-	0	0	0
2	3	0	-	4	2	0
3	-	-	-	0	0	0

Table 25. Eosin-methylene blue (EMB) completion results compared to confirmation fecal coliform MPN raw tube counts. All positive tubes from EC-broth incubation were included in EMB testing. Positive EMB results confirm the presence of *E. coli* in the sample. "–" mark indicates no test for EMB associated with that volume.

EMB Plate Results, 21-Mar-07 (week 5)						
	# Positive Plates, EMB			# Positive Tubes, EC-Broth		
Site	10mL	1mL	0.1mL	10mL	1mL	0.1mL
1	2	-	-	4	0	0
2	1*	-	-	0	2	0
3	4	-	-	5	0	0

Table 26. Eosin-methylene blue (EMB) completion results compared to confirmation fecal coliform MPN raw tube counts. All positive tubes from EC-broth incubation were included in EMB testing. Positive EMB results confirm the presence of *E. coli* in the sample. "–" mark indicates no test for EMB associated with that volume. *Please note that for Site 2, 10mL, a positive EMB result was obtained, while no EC-broth tubes were reported to be "positive;" this has resulted from the inclusion of "weakly positive" EC tubes (no gas evolution, but some color change and precipitation) in EMB completion tests.

EMB Plate Results, 28-Mar-07 (week 6)						
	# Positive Plates, EMB			# Positive Tubes, EC-Broth		
Site	10mL	1mL	0.1mL	10mL	1mL	0.1mL
1	0	-	-	1	0	0
2	2	2	1	5	5	2
3	3	-	-	3	0	0

Table 27. Eosin-methylene blue (EMB) completion results compared to confirmation fecal coliform MPN raw tube counts. All positive tubes from EC-broth incubation were included in EMB testing. Positive EMB results confirm the presence of *E. coli* in the sample. "–" mark indicates no test for EMB associated with that volume.

Discussion

Prior to further discussion, it should be noted that with the exception of total and fecal coliforms found in the natural bay waters, all tested parameters were within local and national standards for wastewater effluent. Due to the fact that measurements were taken during both high and low tides, and during wet and dry conditions, average values were found to be more useful as they reveal general trends and provide an overview of the water quality conditions in the aquaculture facility. Many of the parameters appeared to be inconsistent from week to week, but when data results were averaged over the six week testing period, many consistent overall patterns emerged.

pH & Conductivity

It is of worthy note to mention that pH levels gradually increased as water moved from Site 1 to Site 3. Lower pH values are to be expected at the Arcata Wastewater Treatment Plant (AWTP) outfall (average pH = 6.74), due to dechlorination processes and the fact that it is freshwater. As the effluent mixes with bay water, the pH slowly raises to the average value of 7.41, which was found at Site 2. The average pH of 8.08 found at the Arcata Aquaculture Project (AAP) outfall, however, is a product of multiple factors, including infiltration from bay waters and increased CO_2 concentrations (which subsequently lead to increased HCO_3^-) from algal constituents. Although a pH above 8 is not sustainable for many freshwater organisms, salmonids are thriving in it; further, National Pollution Discharge Elimination System (NPDES) limitations for pH delineate a maximum of no greater than 9.0, which Site 3 falls well under.

On-site conductivity measurements were conducted to find dissolved solid and colloidal content at all three sites. Conductivity was consistently lowest at Site 3, which had average of 0.35 mS; this is to be expected, after ox pond/treatment wetland and dechlorination processes at the AWTP. After release into the bay, significant amounts of ions and colloidal material are picked up by the effluent, and conductivity rose to an average value of 18.08 mS at Site 2. The lower average value found at Site 3 of 12.28 mS could be due to many factors, such as: organic dissolved solids are being utilized by both macro and microorganisms in the pond system, or simply the lower value is the result of effluent and bay water mixing. Either way, the AAP showed some ability to lower natural conductivity levels that were found at Site 2.

Total Suspended Solids (TSS)

NPDES limitations for TSS are a daily maximum of 60 mg/l, and a weekly average of 45 mg/l; our results for all three sites never approached these limits. Site 2 consistently experienced the highest TSS levels (mean = 10.9 mg/L); this is largely due to the fact that of the three sites, the water at Site 2 has experienced the most turbulence (being largely comprised of bay water), and would therefore contain the most sedimentation. In contrast, the water at sites 1 and 3 has had considerable amounts of time to settle out most of their solids; particularly at site 3, where removal of solids must meet regulatory standards. The AWTP's oxidation pond/treatment wetland system is efficient at removal of solids, and TSS at the outfall never exceeded 3.2 mg/L throughout the testing period; these were the lowest observed values of all sites. The AAP showed some ability to also reduce TSS content, as levels were consistently lower at the AAP outfall (Site 3) than at Site 2 each week (mean = 5.3 mg/L). This is due largely to settling of

solids in the pond system, and removal through bio-utilization of organic particulates by aquatic species present in the system..

The highest TSS levels were experienced during week 4 for all sites. This is consistent with coliform levels for that week, which were too numerous to count; BOD values for that week were also higher relative to the other weeks. The reason for the increase in organic matter at all three sites during that week could be attributed to increasing temperatures and subsequent organism activity. Week 4 water temperature values were among the warmest of all of the tested weeks (average for all three sites = 12.40°C). Because the sample was taken during low tide conditions that week, the amount of bay water influence was minimal.

NH_3 and NO_3^-

No local regulations could be found for NH_3 in effluent, but the EPA has set a maximum contaminant limit of MCL of 45 mg/l as a daily maximum for NO_3^-; here again, our results never approached these limits. Eventually NH_3 will be oxidized to NO_3^-, so it seemed logical to also include measurements of NO_3^-. Nitrogen is a limiting nutrient, and increased levels must therefore be monitored in receiving water bodies.

For both NH_3 and NO_3^-, Site 1 consistently showed the highest levels, with an average NH_3 content of 16.3 mg/l and an average NO_3^- content of 2.35 mg/l. Both levels are to be expected at this site, due to human waste products, fertilizer runoff, and decomposition of organic matter which characterizes wastewater effluent. Week 1 results were particularly interesting as it was the first big rain of the year, and "first flush" conditions were experienced. Both NH_3 and NO_3^- levels were more than twice the amounts found during all of the subsequent weeks that week, particularly at Site 1. The AAP proved its ability to remove NH_3, as levels were significantly lower each week than both of the other sites (average NH_3 content = 0.2 mg/l). The bacteria in the pond system effectively oxidize the NH_3 in the effluent, as NO_3^- levels were generally higher than those found at Site 2 (1.18 mg/l, compared with 0.9 mg/l found at Site 2); they were, however, lower than NO_3^- levels found at Site 1 (2.35 mg/l), showing the ability of the microorganisms to uptake these much needed nutrients. While both NO_3^- and NH_3 can be detected in most natural waters (Site 2 levels best illustrates this), the abnormally low average amounts found at the AAP outfall for both forms of Nitrogen prove the effectiveness of the AAP at nutrient removal.

BOD

NPDES limitations for BOD are a daily maximum of 60 mg/l, and a weekly average of 45 mg/l. Again, our results for all three sites never approached these limits. For purposes of this experiment, all negative BOD values in the data are considered to be zero, and average values are the focus.

On average, Site 1 showed the largest average BOD value during six weeks of testing, at 8.9 mg/l, while outfall of the AAP, Site 3, showed an average BOD of 7.5 mg/L and Site 2 had an average BOD of 3.1 mg/L. The data illustrate that as the effluent moves from the WWTP outfall, it experiences conditions similar to estuarine mixing, as the organic content most likely forms

precipitates with cations in the bay water, resulting in a decreased BOD amount when it reaches Site 2. Additionally, as the water moves and mixes with the receiving water of the bay, it picks up more DO. Also, Site 1 continually exhibited the highest levels of NH_3 and NO_3^-; this should be accounted for, as there is an oxygen demand associated with both (3 moles of oxygen for every 2 moles of NH_3, 1 mole of oxygen for every 2 moles of NO2-, resulting in NO3-). After Site 2, the water receives more effluent which is pumped directly from the AWTP. As it moves through the AAP, it should pick up more salinity and organic matter from bay water infiltration and tidal movement, and from photosynthesis within the pond system.

Salinity was always highest at Site 2 (average = 13.43 ppt), slightly lower at Site 3 (average = 8.40 ppt), and lowest at Site 1 (average = 0.17 ppt), which is essentially fresh water. Decreased average BOD at the outfall of the AAP relative to the outfall of the AWTP shows an ability of the AAP system to act as tertiary treatment for further removal of organic matter. Data from four out of six weeks of testing showed Sites 2 and 3 to have lower BOD than Site 1. On site DO measurements also consistently found Site 1 to have the lowest DO (2.79 mg/l average), and Site 3 to have the highest (7.71 mg/L average). This is due not only to the aeration of the yearling ponds, but to decreased organic matter. Because measurements were taken in the morning, DO concentrations found in the pond system are also expected to be lower than those which would be found later in the day, due to increased photosynthetic activity.

There were considerable discrepancies in the BOD data including negative values, as well as values that did not match up to those reported by the AWTP. A difference in temperatures, and subsequent microbial activity, between lab and on site conditions should be remembered; there was approximately an 8-10°C difference between on-site and laboratory temperatures. Additionally, the residual Cl^- content found in Site 1 waters were of concern, as was the salinity content found at Sites 2 and 3. *Standard Methods* was referenced, however no provisions could be found for conducting the 5-day BOD test in such brackish waters; ultimately, the freshwater test was used without modification. Experimental errors also were possibly made, as DO meter calibration varied from week to week. This is most likely the main reason for the negative blank and sample values. Additionally, the prepared dilution water for weeks 1-3 was aerated all the way through until testing time, which could have resulted in super saturated conditions. Initial blank DO values exceeded 9.0 mg/L three out of six weeks.

Total and Fecal Coliforms-

NPDES limitations for fecal coliforms in effluent are a daily maximum of 43 MPN/100mL; total coliform limitations are a daily maximum of 230 MPN/100mL. It should be noted that Site 2 essentially consists of natural waters, so any coliform levels found there could be considered natural background levels.

The raw results of the microbial analysis conducted in this study seemed to be inconsistent at first glance, that is, until they were compared across the course of the study as averages to determine overall trends associated with this study period. The dramatic fluctuation and the apparent inconsistency from week to week, even between testing methods for the same sample, can, however, be readily explained by the incredible variability of microbial populations

associated with ambient environmental conditions and even within a one liter sample bottle, from which fractions of milliliters to milliliters are drawn for analysis.

Considering the nature of this aquaculture system, unlined, with large tidal and highly variable treated wastewater effluent influx, and given its natural setting in a wetland system heavily utilized by migratory birds and various terrestrial animals, great variability in the findings is not surprising. In most probable number (MPN) determination, it is well understood that the best that can be afforded is the provision of an approximate value and range of confidence based on probability. For these reasons, it was decided that both MPN and membrane filtration should be utilized in this study, and it was not a surprise to find variability in the raw data gained from each test and across sampling periods.

It is important also to note that great outliers from our total coliform membrane filtration counts been included in the presentation of our results, through their inclusion in average calculation or in producing graphic displays (ref. Tables 19 and 20, and Figures 25 and 26). Other major included outliers are "too numerous to count" TNTC and elevated results from the membrane filtration testing conducted on March 7th (ref. Tables 19-22 and Figures 23-24). For some reason they comprise a relative anomaly in the data. It must be noted that their inclusion in our figures drastically changes the portrait that might have been created by their exclusion.

This is especially true for Figures 25 and 26, where the inclusion of the unusually high levels of total coliforms experimentally observed for Site 1 on March 3rd has greatly distorted the relative proportions of the histogram, since the plates for the other two sites could not be counted and the unusually high levels have greatly exaggerated the apparent average difference between sites across the entire study period (ref. Tables 19 and 20). Even with this being said, however, it must be duly noted that an overall increase in total coliform levels was observed in the latter weeks of the study, most likely attributed to seasonal warming and increased biological activity and microbial metabolism. It must also be noted that March 7th was the only occasion on which a less experienced team member performed the membrane filtration in lieu of the usual team member responsible for conducting microbial testing; it is also possible that different results might be associated with this substitution.

Despite data anomalies not untypical for microbial testing, the overall picture provided by the data, when averaged across the six-week study period and compared between the utilized testing methods, is quite clear (ref. Figures 25 through 28, and 30). On average, Site 3, the terminal discharge pipe from the aquaculture facility, had the lowest levels of both total and fecal coliforms. Site 2, the wastewater effluent/bay water mixing site (pre-aquaculture) had the greatest level of fecal coliforms, but was moderate in total coliforms. Site 1, the effluent discharge point from the wastewater treatment system, had the lowest average fecal coliform levels, but ended up with the highest average total coliform levels due to the results from March, despite zero levels observed during the first two weeks (in February). The lower average levels of coliforms immediately post-aquaculture at Site 3 can be explained by the constant flushing with bay water tidal influx and the extra retention time under highly aerobic conditions affording greater bioremediation of both effluent and any fish detritus produced in the aquaculture system.

The higher fecal levels at Site 2 suggest the possibility that recovery of fecal coliforms from treated effluent is occurring as the effluent is mixing with bay water. It is important to note here that not only is treated effluent being pumped to Site 2, but the tidal influx occurs from a narrow slough adjacent to the aquaculture facility, Butcher Slough, into which the treated effluent from the wastewater facility is also discharged. Further, the adjacent bay consists of highly productive shallow mud flats, home to a host of wildlife; particularly migratory and other coastal bird species, which are also a highly probable source of fecal coliforms.

The extremely low levels of fecal coliforms at Site 1, immediately following final chlorine treatment at a wastewater treatment plant, is exactly as anticipated. Surprising, however, were the increased levels of total coliforms observed in March. The best possible explanation for this includes a combination of potentially contributing factors: decreased treatment and retention due to increased wastewater system influx with winter rains, increased microbial metabolic activity with warming temperatures, and recovery of total coliforms in our de-chlorinated samples and during our microbial incubations.

The general, big picture trends in the data suggest that aquaculture system enhances wastewater treatment for coliforms through its tertiary utilization of the wastewater effluent, with the increased retention time it affords of both directly utilized effluent pumped in to the system from the chlorine contact basin and effluent mixed with bay water and washed into the system upon the tides.

Conclusion

Results from this study indicate that the Arcata Aquaculture Project, as currently operated, is effective at further removal of TSS, BOD, NH_3, and NO_3^- from the AWTP effluent. Additionally, levels of dissolved solids and total and fecal coliforms were lowered, and dissolved oxygen levels were raised at the outfall of the aquaculture facility from levels measured in mixed bay water and effluent at Site 2. Although pH levels were elevated after aquaculture facility treatment, they were well below NPDES limitations. Further assessment during a greater variety of tidal conditions throughout all seasons, however, is strongly recommended due to the limited scope of this initial study.

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