ORLEANS MARINE WATER QUALITY TASK FORCE

NUTRIENT PROJECT

1997 REPORT

TOWN OF ORLEANS
19 SCHOOL ROAD
ORLEANS, MASSACHUSETTS
02653-3699

WRITTEN BY:
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SUMMARY OF NUTRIENT PROJECT 1997 REPORT

In 1987 the Town of Orleans formed a Marine Water Quality Task Force to protect its natural marine resources. The Task Force focused on road run-off problems, developing a water quality monitoring program, based on bacteriological indicators, now called the "Remediation Program". In 1997 with Coastal Zone Management grant support, the Task Force began a study of nutrients in the upper reaches of Pleasant Bay, now called the "Nutrient Project".

The primary goal of the project was to build a data base to determine the general "health" of estuary waters. In May of 1997, volunteers began the study of nutrient loading in five Orleans salt water ponds of Pleasant Bay: Meeting House, Kescayo Gansett, Areys, Paw Wah, and Quanset. Key variables assessed were: Secchi depth, dissolved oxygen, nitrate (including nitrite) nitrogen, ammonia nitrogen, particulate organic nitrogen, chlorophyll-a, phaeopigments, and fecal coliform count.

Sampling and analyses for the Project began May 18th. and continued through November 16th. Five teams of two or more persons completed 40 sampling rounds on the five ponds, at two sites per pond, and at two depths per site during the six month time frame. The study data are presented in three ways in this report: (1) In tabular form, pond by pond in Appendix 1. (2) Certain key data are displayed graphically and discussed for each pond in the "Findings" section of the report. (3) Data components which enter into the calculation of the "Buzzards Bay Eutrophication Index" are discussed in the "Findings" section under that title. Eutrophication is derived from the Greek word *eutrophos* meaning well-nourished. If a salt water body receives excess nutrients, particularly nitrogen, it may develop excess plant growth, blocking sunlight, and have reduced dissolved oxygen, threatening animal life. Such a condition may be called "eutrophic". In effect, the Eutrophication Index is a measure of the tendency for the water body to become eutrophic.

According to this index, the scale for calculating the water quality of any embayment is: good to excellent (65-100), fair (35-65), eutrophic conditions (<35). Orleans Ponds based on this study's data have the following ratings:

Pond:	Mtg.Hse.	Kes Cay	Areys	PawWah	Quanset
Points	35	24	26	62	56
Water Quality	Fair	Eutrophic	Eutrophic	Fair	Fair

Study conclusions in brief are: The Orleans Ponds of Pleasant Bay show definite signs of eutrophication, most likely due to excess nitrogen. We strongly recommend that remediation steps be planned and promptly carried out. Continued monitoring of water quality is essential to define the extent of the problems, and effectiveness of remediation.

ORLEANS MARINE WATER QUALITY TASK FORCE
NUTRIENT PROJECT

1997 REPORT

INTRODUCTION

In 1987 the Town of Orleans established a Marine Water Quality Task Force to protect its natural marine resources. The Task Force focused on issues related to contamination of marine waters by road run-off, by developing a water quality monitoring program using fecal coliform counts as its primary indicator. These efforts were applied to all three of the town's marine estuaries in what is now called the "Remediation Program". By making use of longitudinal monitoring data, other data sources and studies, the Task Force developed a priority list of problem areas. Such data has convinced town officials and voters to approve the costs of $400,000 for installation of infiltration systems at the five most critical points. The town has committed its share of funds under the 319 Storm Water Remediation program for solving problems in five additional drainage areas, four of which are to be installed in the Spring of 1999.

In 1997 with the support of a grant from the Coastal Zone management (CZM), the Orleans Water Quality Task Force initiated a study of nutrients in the upper reaches of Pleasant Bay, now called the "Nutrient Project". This study was also made possible by the helpful cooperation of John W. Portnoy, (National Park Service); and Brian L. Howes, and Dale S. Goehringer (University of Massachusetts, Dartmouth).

BACKGROUND ON ESTUARIES AND THEIR PROBLEMS

The salt water ponds of Orleans are located in the upper reaches of Pleasant Bay, which is an important estuary on the Massachusetts coast. An estuary is a semi-enclosed body of water, bounded at the mouth by the ocean and at the head by the upper limits of its tides. It drains a much larger area, and pollutants, produced near or miles away in tributaries and ground water, affect the estuary's water quality. Some of the water entering an estuary moves in from the sea. When fresh and salt water meet, the two may not readily mix. While rivers flow in a single direction, flushing out sediments and pollutants, in estuaries there is a balancing between the up-estuary salt water movement and the down-estuary freshwater flow. Often an estuary can have lengthy retention times for water borne pollutants and sediment, magnifying the adverse effect on plants and animals.

Historically, estuaries have been the receptacles for society's wastes. Over the past several decades, the signs of estuarine decline have become increasingly prevalent. Many fish and shell fish populations are near collapse, overloads of nutrients may cause chain reactions of algal bloom, threatening the balance of these systems. The sheer number of people living near the coasts continues to stress our estuaries, lagoons and other coastal waters. No

coastal areas are immune to the threat of pollution, and many estuaries share common problems, such as seasonal depletion of dissolved oxygen. The term **eutrophic**, applied to a water body, designates one in which the increase in mineral and organic nutrients has reduced the dissolved oxygen, producing an environment that favors plant over animal life. This term is derived from the Greek word *eutrophos* meaning well-nourished. Accelerated **eutrophication**, or aging of the estuary (in which there is an excess of nutrients and plant growth) often accompanies the oxygen depletion. (Ref. 20)

Estuaries are complex systems with many habitats, animal and plant species and constantly changing physical and chemical conditions. Certain properties have been shown to be suitable across-the-board indicators of environmental health such as: dissolved oxygen, nutrients (nitrogen and phosphorus, but for salt water, generally, nitrogen is the "limiting" and most important), direct or indirect measures of phytoplankton (such as chlorophyll-a and phaeo pigments), submerged aquatic vegetation, macroalgae and bacteria.

When a salt water body has water quality indicative of eutrophic conditions, the water quality has generally been degraded because of excess nutrients (usually nitrogen), or the flushing rate with high quality water has been reduced. High nutrients can result in algal blooms, reduced sunlight penetration, loss of beneficial eel grass, reductions in available oxygen, loss of fish and shellfish, and other undesirable consequences.

The following are brief summaries of a few of the previous studies of shallow, temperate, coastal lagoons, similar to the embayments of Orleans, which have led to recommendations on or actual use of remediation efforts:

(1) Lee and Olsen's study of Rhode Island Coastal Lagoons (Ref.24). The authors found that increased nitrogen loading into the ponds, primarily nitrate, resulted in markedly increased macroalgal biomass, with nutrient uptake so efficient that the nutrient concentrations in the water column were not measurably increased. In the region ground water nitrate concentrations were elevated and in some cases exceeded the 10 mg/l safety standard for potable water. Recommendations to area towns and the state focused on two issues: reducing the nutrient loadings from the already densely developed areas, and reducing the potential for future development in the, as yet, sparsely developed areas of the watershed.

(2) In Buzzards Bay (Ref.17), the Buzzards Bay Project, initiated in 1985, completed a comprehensive Conservation and Management Plan for the Bay in 1991. This plan is a blueprint for the protection and restoration of water quality and living resources in the bay and watershed. A water quality monitoring program was started in 1992. The Buzzards Bay Project originated the Eutrophication Index, integrating measurements of oxygen saturation, water transparency, chlorophyll pigments, and three forms of nitrogen. Water

quality values and nitrogen management needs are given for 27 different sub-embayments of Buzzards Bay.
(3) The Center for Marine Science and Technology (CMAST) (Ref.16) studied Popponesset Bay, two sub-embayments and the Mashpee River, focusing on whether there is currently nutrient related water quality degradation of any of the Bay's sub-embayments. The results indicate such degradation in each of the upper sub-embayments. The authors state that it is absolutely clear that in some areas the assimilative capacity of the system has already been exceeded. In the Mashpee River oxygen was depleted to only 7% saturation, and some of the highest chlorophyll-a levels for any Cape Cod embayment were reported. The authors concluded that, while the data indicated poor water quality, existing data did not provide sufficient information to develop nutrient and wastewater management plans.
(4) The Waquoit Bay Publication has the broadest discussion of management of the nitrogen loading issue (Ref. 21). The author states that the most pressing issue for Waquoit Bay is coastal eutrophication caused by nitrogen loading from various human activities in the watershed. In Waquoit Bay increased nutrients (primarily nitrate nitrogen) have lead to increased growth of algae, decreased concentrations of dissolved oxygen, habitat degradation, and changes in the community structure. Some technological approaches are: dredging, harvesting seaweed, alternative on-site waste-water treatment systems, and control or treatment of stormwater runoff.

EARLIER STUDIES OF WATER QUALITY OF PLEASANT BAY

Previous studies of water quality of Orleans waters in Pleasant Bay have been conducted by the Massachusetts Division of Marine Fisheries (DMF), Department of Natural Resources; the Massachusetts Department of Public Health (DPH); The Massachusetts Division of Water Pollution Control, Massachusetts Department of Environmental Quality Engineering (DEQE); the Town of Orleans; and two studies sponsored by the Friends of Pleasant Bay, one conducted in 1987-1988 by Robert Wilhelm of Boston College, and the other by I.E.P. Inc in 1991. (See references, items 1-4)

A few summary statements from the previous studies, which are applicable to the areas of the present study, are given in the following table:

TABLE 1: EARLIER STUDIES OF PLEASANT BAY

STUDY	YEAR	COMMENT
DPH	1965	High levels of coliform bacteria at 8 of 45 stations sampled
DMF	1965-7	Acceptable water quality
DEQE	1976	Water quality generally classified as SA, the highest water quality for estuarine waters
ORLEANS	1982	Meeting House and Paw Wah ponds shell fish bed closures due to elevated fecal coliform counts
DMF	1988	Meeting House and Paw Wah ponds shell fish bed closures among about 50 acres of shell fish bed closures in Pleasant Bay

The Wilhelm study was carried out in 1987 and the spring of 1988. Ten field samplings and sets of observations were made at up to 18 stations, ranging from Meeting House Pond to the Chatham Fish Pier. Two of the samplings were made after wet weather conditions. Sampling was conducted under ebb tide conditions. The measures made during the Wilhelm study, which are common to this study include: ammonia nitrogen, temperature, pH, salinity, dissolved oxygen and at selected stations, fecal coliform. Unlike our study Wilhelm used total coliform as the usual measure of bacterial contamination. Orthophosphate was measured by Wilhelm, rather than phosphate phosphorus, but these are inter-convertible. Finally, Wilhelm did not measure nitrate nitrogen, nor particulate organic nitrogen, so no comparisons can be made with our data on these factors.

STUDY GOALS

Prior to 1997, the Orleans Marine Water Quality Task Force monitored the quality of the water in town's three estuaries by measuring fecal coliform counts at various stations. When the present "Nutrient Project" began, the task force directed its efforts to measure a select number of additional parameters to assess the water quality of the town's largest estuary.

The primary goal of the Nutrient Project was to build a data base, using variables generally recognized to play a significant role in characterizing the general "health" of estuary waters. Field measures of water temperature, Secchi depth, salinity, dissolved oxygen, and nitrate

nitrogen were made. Samples were collected for determination of pH and fecal coliform counts in the Orleans Laboratory. Field samples also were collected for key analyses at cooperating laboratories. These were: samples for analyses for nitrate nitrogen, ammonia nitrogen and phosphate phosphorus by the National Park Service, Truro, Massachusetts; and samples, processed at the Orleans laboratory, for later analyses for particulate organic nitrogen and carbon, chlorophyll-a, and phaeopigments, by the Coastal Systems Group, Center for Marine Science and Technology, University of Massachusetts, Dartmouth.

An accompanying goal was to develop suitable data summaries and graphic portrayals so that other professionals, volunteer participants, and town citizens can understand the significance of the more important, relative measures of the "health" of water in the town's five salt water ponds of Pleasant Bay.

We were fortunate in having one of our volunteers, Paul Niles, a full time science teacher at the Lighthouse Charter School in Orleans, bring to each sampling or laboratory session a few of his students. All students were members of a school science club. The project was explained to the students and they were taught some of the specific procedures.
Thus, a better understanding of the importance of good marine water quality was spread to an important segment of the community.

STUDY DESIGN AND RATIONALE

Throughout the study we have utilized a Technical Advisory Committee for periodic reviews of procedures, and proposed changes in procedures, evaluation of results, and interpretation of data. Members include, from the town of Orleans staff, Jennifer Wood (Conservation Agent); former Town Conservation Agent, Sandra Macfarlane, and scientists who are volunteers on the Nutrition Project: Paul Marinaccio (retired Director of Research), Ken McKusick (retired Physician), Peg Wineman (retired Chemist), and Bob Wineman (retired Chemist). At our most recent meeting, outside advisors included Joe Costa (Buzzards Bay Project), John Portnoy (National Park Service), Ed Eichner (Cape Cod Commission), and Tara Nye (Waquoit Bay National Estuarine Research Reserve [WBNERR]). Kristin Gribble, formerly of WBNERR also served on the Committee in 1997.

TABLE 2: VARIABLES MONITORED AND PURPOSE

Measure	Purpose	Target Value for Good Water Quality	Footnote
Temperature of water	describe conditions		
Water depth	characterize site		
Secchi depth	transparency of water, indicator of algal growth	>3 m	(1)
Salinity	fresh water input, with possible pollution		
Dissolved oxygen	indicator of "health" for fish/shellfish	>5 mg/l	(1)
"Total nitrogen"	measure of nutrients	<0.1mg/l	(5,1,6)
pH	salt/fresh water ratio	6.5-8.5	(2)
Fecal coliform count	pollution indicator/shellfish limit	<14 col./dl	(2)
Nitrate nitrogen (NPS)	measure of nutrients	< 0.1mg/l	see Total N
Ammonia nitrogen (NPS)	measure of nutrients	<0.1 mg/l	see Total N
Phosphate phosphorus (NPS)	measure of nutrients	<.01mg/l	(1)
PON (particulate org. N);(UM)	measure of nutrients	<0.1mg/l	(6)
POC (particulate org. C);(UM)	indicator of organic carbon food source	?	?
Chl-a (Chlorophyll-a);(UM)	phytoplankton growth	<5ug/l	(3)
Chl-a +Phaeo-a; (UM)	phytoplankton growth	<3ug/l	(4)

Footnotes for Table 2:

(1) NOAA'S Estuarine Eutrophocation Survey: Vol 2 - Mid Atlantic (ref. 14)

(2) Mass Guidelines for Use Support Determinations, Coastal & Marine Waters (ref. 15)

(3) Brian Howes, Personal Communication, 11/18/97 (ref. 18)

(4) Brian Howes & D. Schlezinger, Environment Cape Cod, V.1, No.4, 1 (1998) (ref. 16)

(5) Total nitrogen is the sum of Nitrate N, (which for the NPS laboratory includes nitrite N), Ammonia N, and Particulate organic N. In this study, we do not have a measure of Dissolved Organic Nitrogen, so this is not included in our "total nitrogen".

(6) Joe Costa, Personal Communication, 10/26/98 (ref. 19)

QUALITY CONTROL

The discussion of quality control primarily concerns fecal coliform monitoring, and thus relates both to the "Remediation" Project and the "Nutrient Project". It is discussed here as part of the report on the Nutrient Project for 1997.

For monitoring of fecal coliform, we use the mFC method (see reference10). This document includes discussions of the method, equipment, solutions, media preparation, safety procedures, calibration and standardization, quality control and reporting of results.

Several questions have arisen concerning the suitability of the mFC method in marine waters (see references 10-13). The mFC method is found to stress the organisms more than other methods and results in counts that on average seem to be about 15 % less than other methods. Our method of choice is the mFC procedure because of its convenience, and ease of counting compared to the mTECH method.

Split Samples: For our "Remediation Project" results are routinely checked against the Barnstable County Laboratory, because about 20% of our samples are split and are analyzed by both laboratories. Barnstable uses the mTECH procedure. A comparison of 57 split samples, collected from March through October, 1997, showed that the average count for Barnstable to be 148 compared to 178 for Orleans laboratory. The difference of 30, is about 17% based on the larger Orleans average, and 20% based on the smaller Barnstable average. These data show that the agreement between the laboratories is quite good, but in contrast to the references cited, the Orleans Laboratory mFC procedure seems to result in counts that are about 20 % greater than those of the Barnstable Laboratory, using the mTECH procedure.

A further analysis of the data was conducted by John Portnoy of the NPS (Ref. 9) From the total number of pairs, those with values below 10, and above 900 were eliminated, leaving 20 pairs for comparison. A regression analysis showed that with an R squared of 0.44, the relationship is the following:

The estimated Barnstable count = (.79) (Orleans count) + 17.3

Using an example from the regression line, for an Orleans count of 200, the corresponding Barnstable count is 175.

We will continue to check our procedures against the Barnstable County Laboratory, using split samples taken on a regular basis. These will insure that our results are meaningful, and since these samples are tested at various times using different volunteers they will also be a test of agreement between our groups.

Replicate Samples: In 1997 we took a series of 10 samples in Areys Pond. These samples were all taken at the same time and in the same location. Our intent was to determine the duplication of counts in the ten samples. Unfortunately these samples were taken late in the year and the counts were very low because of a significant drop in water temperature.

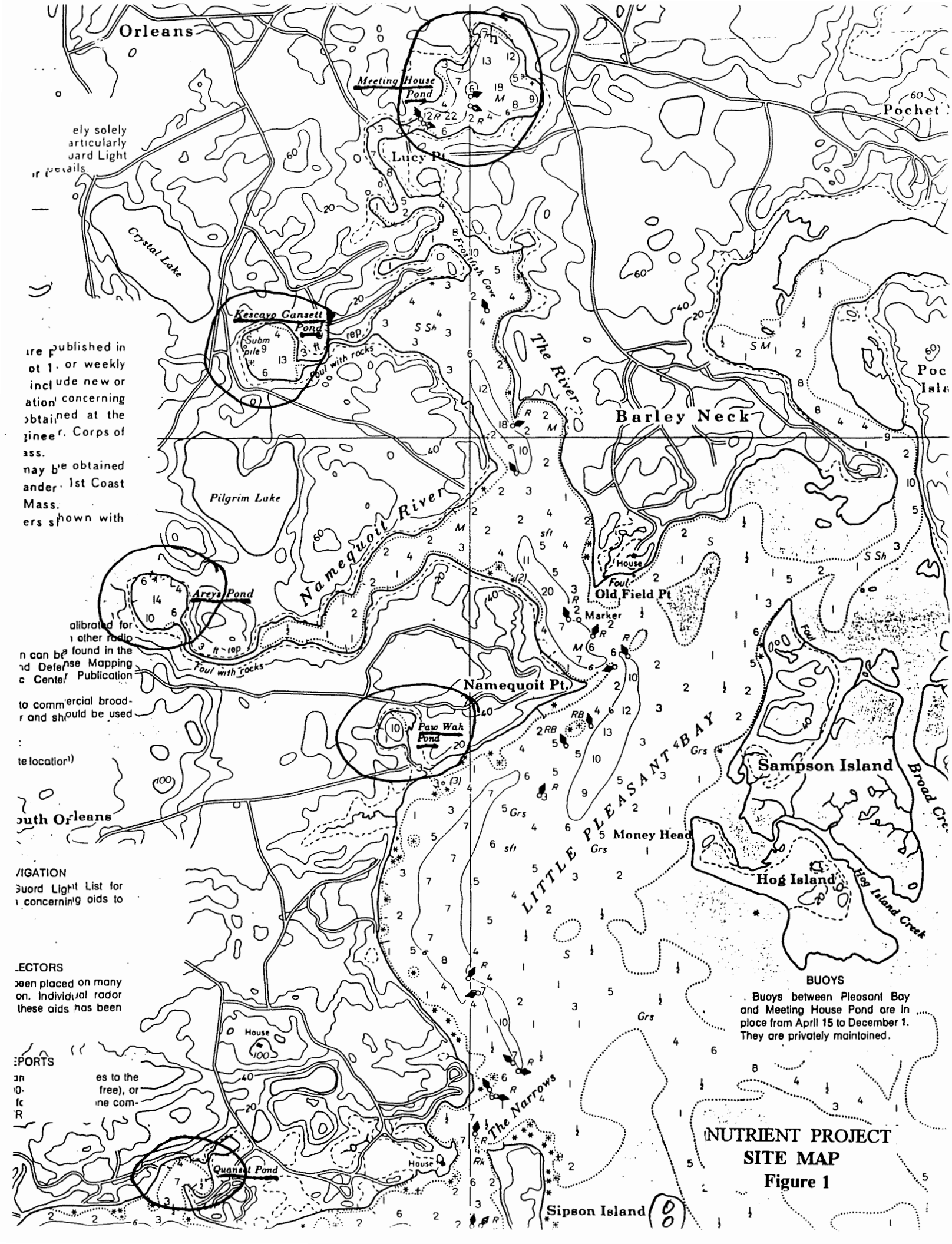
About half of these samples showed low counts and the other half showed none. The procedure will be adopted in 1998, but modified. At two different times during the year we will take ten duplicate samples in one location, which is expected to have high counts, (a total of twenty samples). The results of these samples should give us reasonable data on the reproducibility of our results. This data, plus the split data between Orleans and Barnstable Laboratories, should be sufficient for our purposes.

Blank samples: All of our laboratory tests utilize blank samples with every test session. Through 1997, we have never experienced any counts in our blank samples in five years of testing.. This procedure will continue.

CONDUCT OF THE STUDY

In May of 1997 volunteers of the Orleans Marine Water Quality Task Force began the study of nutrient loading in five salt water ponds of the upper reaches of Pleasant Bay, with support from CZM (Coastal Zone Management, part of the Massachusetts Executive Office of Environmental Affairs). The study sites were in two locations of each pond: Meeting House, Kescayo Gansett, Areys, Paw Wah, and Quanset.(See site map, figure 1) Our objectives were to measure fifteen variables, such as dissolved oxygen, coliform count, nitrate nitrogen, etc. which will reflect the relative "health" of the pond waters. Because these ponds are at the upper reaches of tidal exchange, their water quality is likely to serve as an early indicator of any general problem for the entire estuary. Generally measurements and samples were take at two depths at each site: a shallow (0.5 meters below the surface), and a deep depth (0.5 meters above the bottom). In each pond, site "one" was located in the deepest part of the pond, while site "two" was in a relatively shallow location, and, when possible, in a position to intercept a feeder stream, or incoming flow from an adjacent marsh. Samplings were scheduled to occur at half-tide of the ebb, in order to test water, characteristic of the mixed salt and ground water, typical of the pond.

The issue of whether to measure samples of bulk water, as we chose to do, or to sample many locations of ground water flowing into a given pond, and integrate these through flow measurements and tidal exchange measures to determine overall nutrient loading is a difficult problem. An important consideration for us was that we needed to have relatively simple procedures so that our sampling could be done by citizen volunteers. The consequence of bulk water sampling is the need for very sensitive test methods, especially for nitrogen. Unfortunately, the test kit we used in the field for nitrate nitrogen (the LaMotte Kit Code 3354) was not useful below 1 mg/L. In the 1998 continuation of the study we planned to use a test kit, stated to be a more sensitive nitrate nitrogen test kit, the



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NUTRIENT PROJECT SITE MAP

Figure 1

LaMotte Model NCL, Code 3615. The instructions state that it is useful in the range of 0 to 1 mg/L. After a brief trial of this kit, use was discontinued due to the potential health hazard (and practical problems) of handling toxic cadmium powder is humid conditions in an open boat. Also we tested a low range (0-1 mg/L) kit from the Hach company but found that it would not provide correct readings in salt water; therefore the Hach kit could not be used. Fortunately, in our study parallel samples for nitrate and ammonia nitrogen were analyzed by the NPS laboratory with the very low limits of detection needed.

In 1997, sampling and analyses for the Nutrient Project began on May 18th, and continued through November 16th. Five teams of two or more persons completed 40 sampling rounds on the five ponds, at two stations per pond, and at two depths per station during the six month time frame. The number of samplings per pond varied: Meeting House (10) and Kescayo Gansett (9) were sampled earliest and most often as planned. The other three ponds were sampled as follows: Areys Pond (8), Paw Wah (7), and Quanset (6). The frequencies below 8 were due to scheduling problems and individual time conflicts.

We had planned to start sampling in April but were delayed by a number of factors. Some of these were a delay in the availability of money to order equipment and supplies, our need to develop a full set of instructions for sampling and laboratory procedures, and time for training of volunteers. Four teams of volunteers conducted the laboratory work for the project. Another team was responsible for recording and entering data in the computer system. A continuing task was the cleaning and restocking of our field test kits, scheduling them with the volunteer teams, and retrieval and delivery of the kits. For the five teams, we shared the use of two full kits. Through the cooperation of the Orleans Harbormaster, Dawson Farber, marker buoys for each site were prepared for the project, and placed in position by volunteers. See the accompaning site chart (Figure 1), and the location-of-sites code sheet giving the latitude/ longitude coordinates (appendix 4), measured by a marine global position system (GPS) receiver. Boats to access the study stations were supplied by the individual team members.

A copy of all the procedures, used in both the laboratory and the field, is attached as Appendix 2.

FINDINGS OF THE STUDY

For the purpose of this report, the study data are presented in three ways: (1) All of the 1997 study data are given in tabular form in Appendix 1. The data are arranged by Pond name, for a given pond; chronologically in order of sampling date; then by site (or station) location; and finally by the depth at which the sample was taken, "surface" or "deep".

See the section on Study Goals, page 5. (2) Certain key data are displayed graphically. For the most part, the same variables are plotted for each of the five ponds, individually. (3) Data components which enter into the calculation of the Buzzards Bay Eutrophication Index (see reference 17), are given in Table 3, below. This index was developed over a period of time by Dr. Joseph Costa and colleagues in their extensive study of the multiple embayments within Buzzards Bay. Use of the Eutrophication Index provides a single number to characterize the health of a given water body, at the time of the study. Such an index enables comparisons to be made between water bodies, and also, for any given embayment, the index provides a tool to measure the "health" of the embayment over time to see whether it is improving or failing.

THE BUZZARDS BAY EUTROPHICATION INDEX

The Buzzards Bay Project developed a single index which incorporates measures of oxygen saturation, water transparency, phytoplankton pigments, dissolved inorganic nitrogen, and total organic nitrogen. The summertime mean of each indicator is used to calculate the Eutrophication Index. From the entire data record only observations between June 1st. and September 30th (the most significant) are used. In the case of oxygen saturation, the calculation uses the mean of the lowest 33% of the oxygen saturation values. Points are assigned to each of the five parameters contributing to the index. Good water quality receives higher scores, for each parameter a maximum of 100 points is possible. Poor water quality receives lower scores, down to 0 points. The following table defines the scoring system to assign points to each parameter:

Table 3: POINT VALUES FOR EUTROPHICATION INDEX PARAMETERS

PARAMETER	0 POINT VALUE	100 POINT VALUE
Oxygen saturation (lowest 1/3 of obser.)	40%	90%
Transparency (Secchi disk depth)	0.6 m	3 m
Phytoplankton pigments (chlorophyll+phaeopigments)	10 ppb	3 ppb
Dissolved Inorganic Nitrogen (DIN)	10 micromolar (=0.14 ppm)	1 micromolar (= 0.014 ppm)
Total Organic Nitrogen (dissolved+particulate)	0.60 ppm	0.28 ppm

(Reference 17)

The Buzzards Bay study uses the following point ranges of the Eutrophication Index to characterize water quality: Good to Excellent (65-100)
Fair (35-65)
Eutrophic conditions (< 35)

Table 4: EUTROPHICATION INDEX FOR ORLEANS SALT WATER PONDS
1997 (Nutrient Project Data for June 1 - September 30) POINT VALUES

POND: FACTOR	Mtg. Hse.	Kescay	Areys	Paw Wah	Quanset
Oxy. Sat'n.	12	18	0	26	52
Transparency	50	36	33	56	45
Phytopigments	38	42	36	95	61
Diss. Inorg. N	17	5	37	53	54
Partic. Org. N	59	18	25	79	69
Average	35	24	26	62	56
Water Quality	Fair	Eutrophic Conditions	Eutrophic Conditions	Fair	Fair
1997 AVERAGE VALUE OF EACH FACTOR FOR EACH POND					
Oxygen Sat'n (%)	46	49	31	53	66
Transparency (m)	1.8	1.8	1.4	1.9	1.7
Phytoplankton (ug/L)	7.3	7.1	7.5	3.4	5.7
DIN (mg/L)	0.12	0.135	0.095	0.074	0.072
PON (mg/L)	0.141	0.182	0.175	0.121	0.131

In 1997, the Orleans Nutrient Study did not make any measurements of the dissolved organic nitrogen. In consultation with Dr. Costa, he developed another scale for only particulate organic nitrogen (PON) based on the distribution of all PON data collected in 1992-1995 for the Buzzards Bay study. This PON scale specifies 0 points for 0.2 ppm (14.29um) PON and above, and 100 points for 0.1 ppm (7.14um) PON and below. The Buzzards Bay Eutrophic Index calculations on the 1997 Orleans data, used the latter factor on PON only instead of the fifth factor in table 3 (Reference 19).

It sould be noted that the Eutrophication Index does not include bacteria monitoring and is not an index of swimability or shellfish bed status. The Orleans Nutrient Project does include fecal coliform measures, but these are reported and evaluated separately from the Eutrophication Index.

OTHER DISCUSSION OF THE DATA

Following are comments to supplement the graphical data, which are discussed for each pond starting with Meeting House Pond, the uppermost, toward Quanset Pond, the Orleans Pond nearest to main Pleasant Bay and the Atlantic Ocean. Please refer to Table 2, page 6, for a listing of the purposes of each measurement and an indication of the target level of certain variables for "good" water quality.

Meeting House Pond

Chlorophyll-a is an indirect measure of phytoplankton growth, which is an indicator of availability of nutrients, particularly nitrogen, in the water. As one can observe in figure 2* chlorophyll-a concentrations ranged from 6 - 16 ug/ L during most of the summer, higher than the criteria value of <5 ug/L for "good" water quality. In the fall, concentrations declined to levels below 1 ug/L.

Phaeopigments are another indirect measure of phytoplankton growth. Often phaeopigments are considered to be a measure of decaying plant material, which is another way nutrients such as nitrogen enter the water, to become available for plant growth. Figure 3 demonstrates that, by and large, concentrations of phaeopigments in Meeting House Pond were low, in the range indicative of "good" water quality.

Dissolved oxygen is another measure of water quality, with values greater than 5mg/L indicating "good" water quality. Figure 4 shows that during the summer, values for dissolved oxygen for Meeting House Pond were predominantly below 5 mg/L. The plots are for the "deep" samples from each of the two sites. Water transparency is another measure of water quality. This study uses "Secchi" depth as a measure of water transparency, with target values of >3 meters as indicative of "good" water quality. Secchi depths at site 1 in Meeting House Pond are also plotted on figure 4, which demonstrates that throughout the summer, Secchi depths ranged from 1 to 2.9 meters, with the exception of one value of 3 meters. The implication is that, based on Secchi depth, water quality was "poor" to marginal.

"Total" Nitrogen ranged from 0.21 to 0.50 mg/L. The plots of the values from the "shallow" samples at the two sites for Meeting House Pond are given in figure 5. It should

*In graphs the words "site" and "station" are used interchangeably

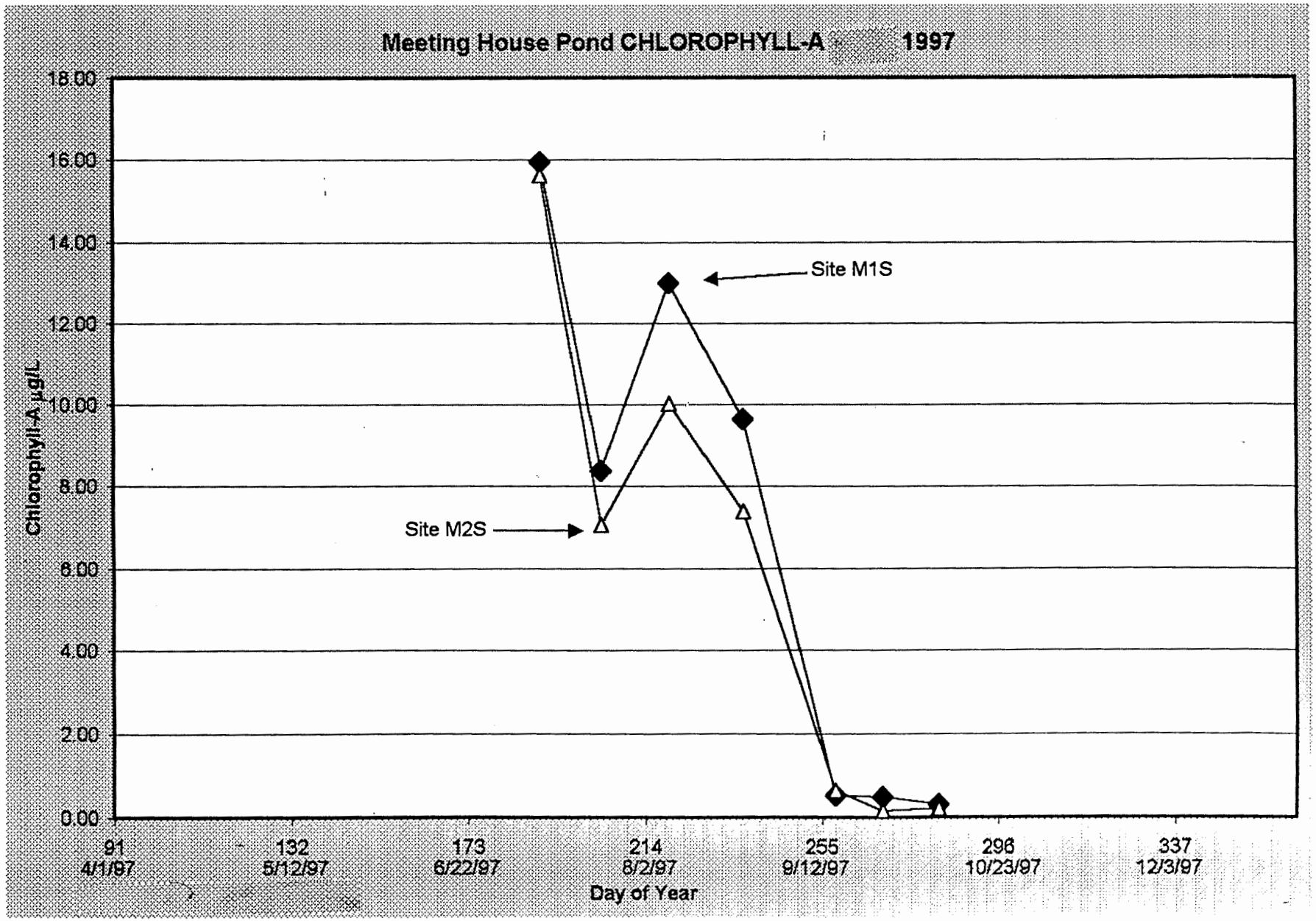
Meeting House Pond CHLOROPHYLL-A 1997
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Chlorophyll-A ug/L
Site M1S
Site M2S
91
4/1/97
132
5/12/97
173
6/22/97
214
8/2/97
255
9/12/97
296
10/23/97
337
12/3/97
Day of Year

Figure 2

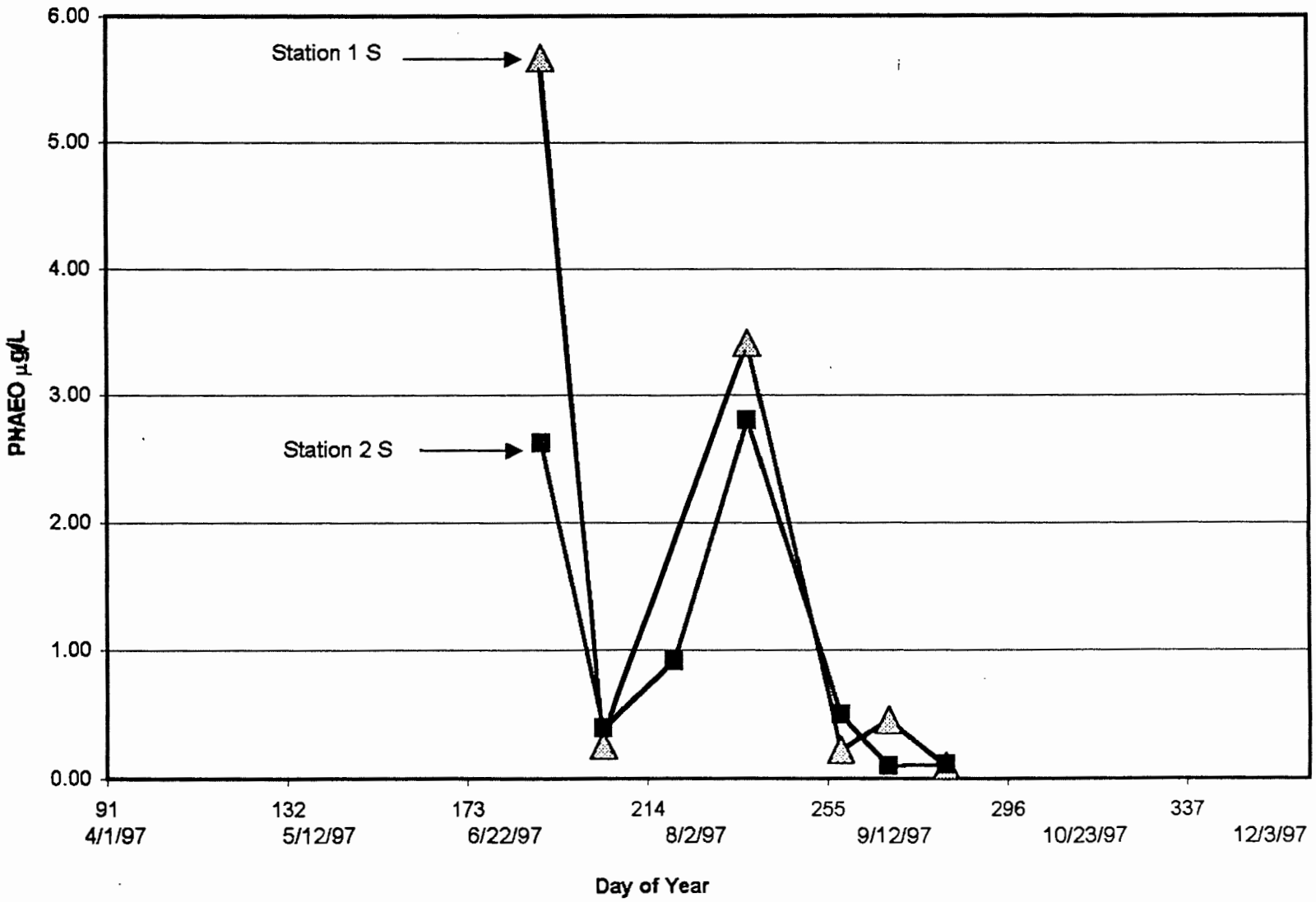
Meetinghouse Pond PHAEOPIGMENTS 1997
6.00
5.00
4.00
3.00
2.00
1.00
0.00
PHAEO µg/L
Station 1 S
Station 2 S
91
4/1/97
132
5/12/97
173
6/22/97
214
8/2/97
255
9/12/97
296
10/23/97
337
12/3/97
Day of Year

Figure 3

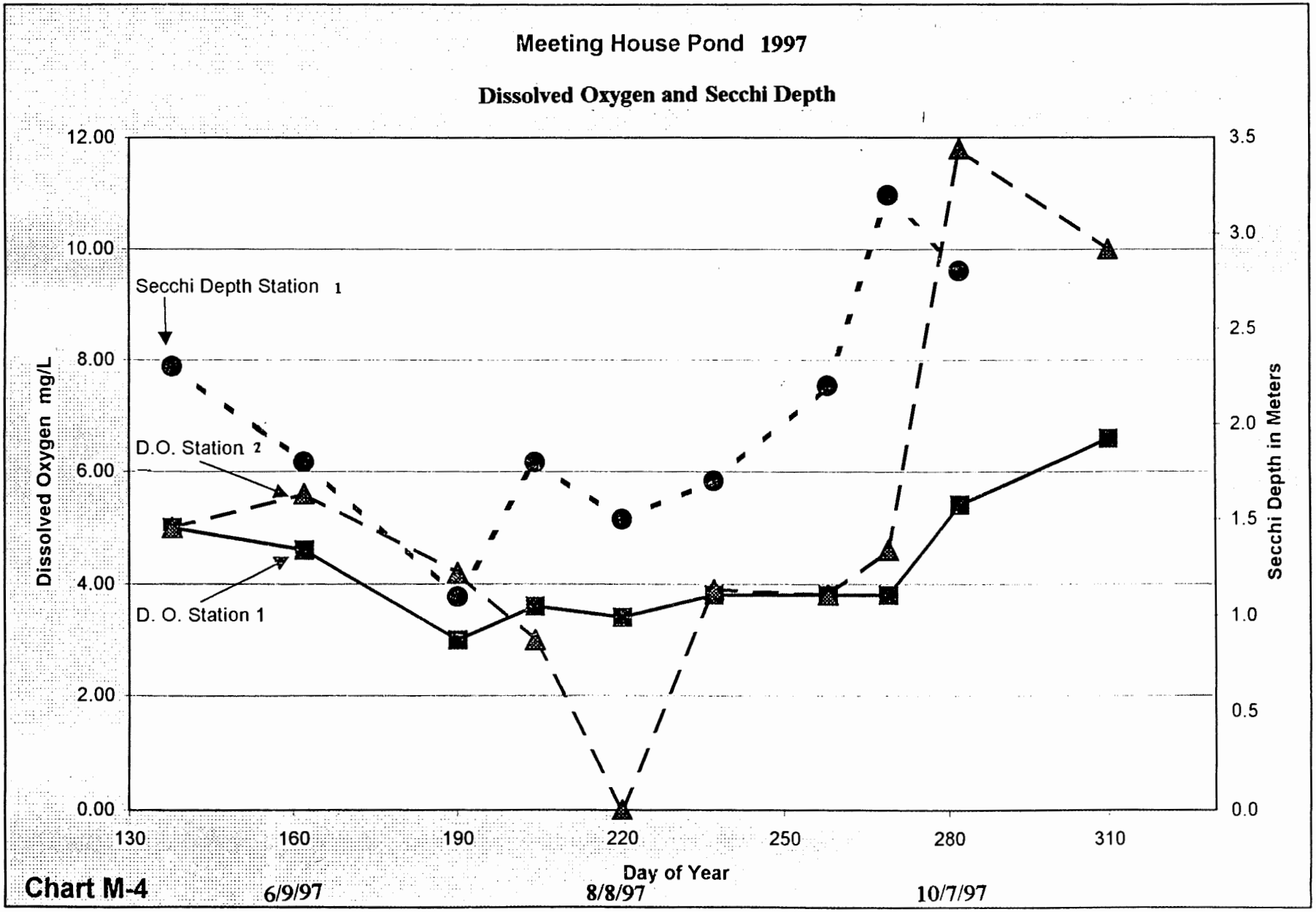
Meeting House Pond 1997
Dissolved Oxygen and Secchi Depth
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Dissolved Oxygen mg/L
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Secchi Depth in Meters
Secchi Depth Station 1
D.O. Station 2
D. O. Station 1
130
160
190
220
250
280
310
Day of Year
Chart M-4
6/9/97
8/8/97
10/7/97

Figure 4

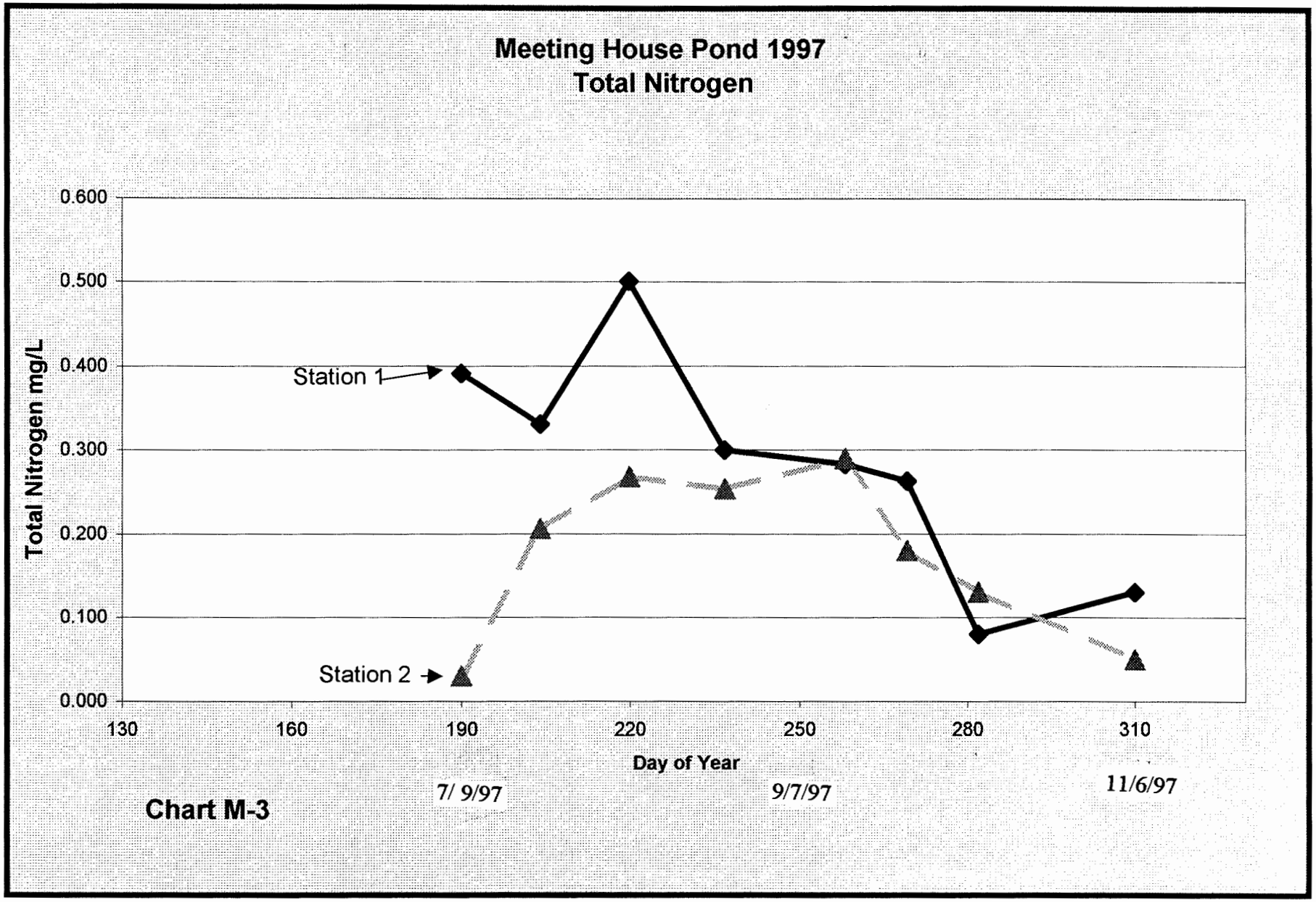
Meeting House Pond 1997
Total Nitrogen
Total Nitrogen mg/L
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Station 1
Station 2
130
160
190
220
250
280
310
Day of Year
7/ 9/97
9/7/97
11/6/97
Chart M-3

Figure 5

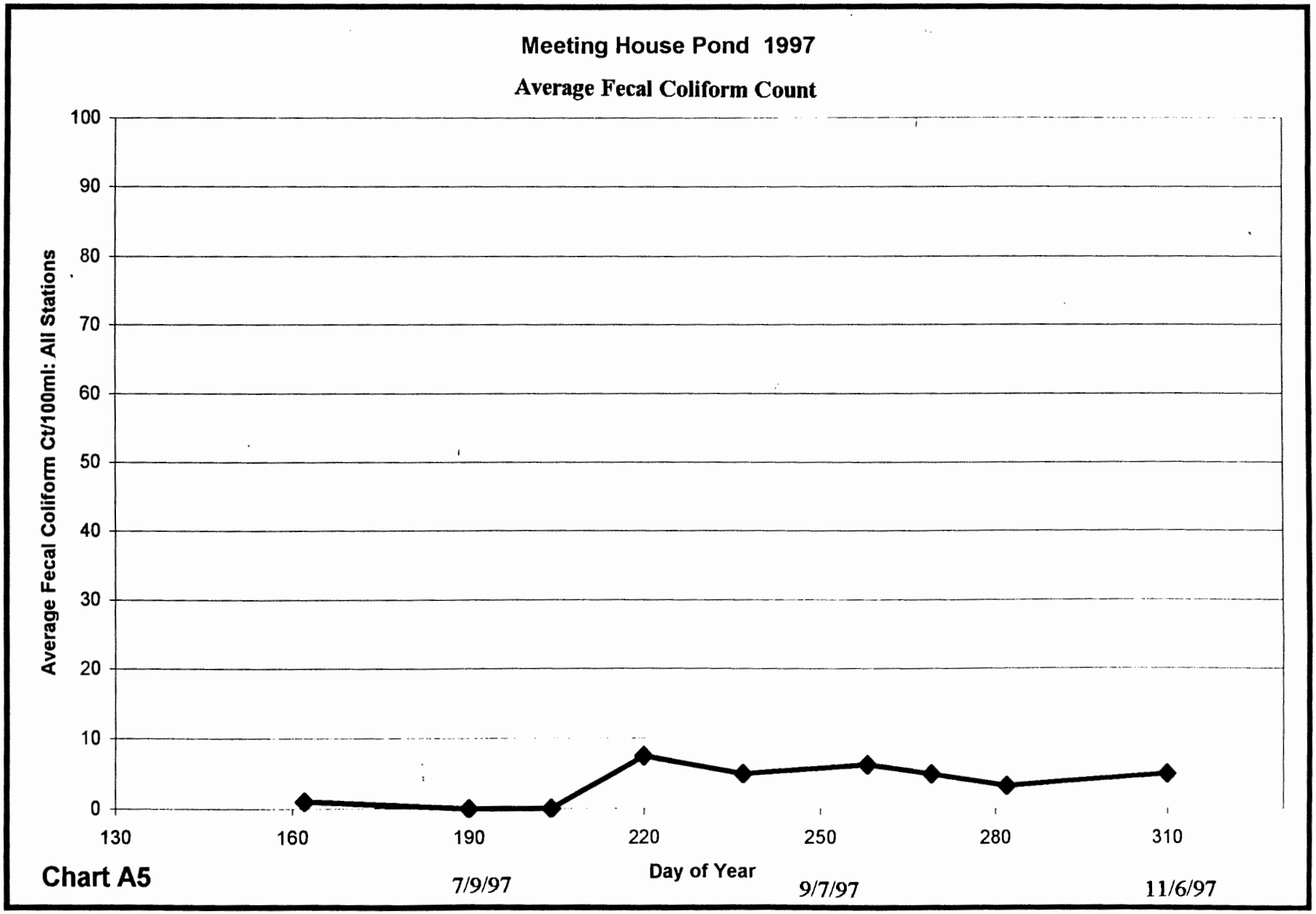
Meeting House Pond 1997
Average Fecal Coliform Count
Average Fecal Coliform Ct/100ml: All Stations
100
90
80
70
60
50
40
30
20
10
0
130
160
190
220
250
280
310
Day of Year
7/9/97
9/7/97
11/6/97
Chart A5

Figure 6

be noted that our data do not have any value for dissolved organic nitrogen. If we were to have that value our numbers would be somewhat higher. Our interpretation is that the values for nitrogen are indicative of poor water quality, especially in the early part of the season. In the fall values are in the range for "good" water quality.

On each sampling date four samples were taken for measurement of fecal coliform count. While not a measure of "nutrients" in the pond, fecal coliform count is a useful indicator of potentially pathogenic bacteria, and thus safety of the pond waters for fish, shellfish, and human use, such as swimming. For the taking of shellfish, fecal coliform counts should be below 14 colony counts per 100 ml. For swimming up to 200 counts are considered safe. In 1997, fecal coliform counts/100ml on all samples from Meeting House Pond ranged from zero up to fifteen. Average fecal coliform counts from all four samples taken on each sampling date are plotted on Figure 6, showing that Meeting House Pond waters were safe for shell fishing and swimming. Note that fecal coliform counts of this study are not the "official" governing count for shellfishing. The latter are made by the State's Division of Marine Fisheries. Similarly local Boards of Health officially measure bacterial counts for monitoring swimming areas.

Kescayo Gansett Pond

Chlorophyll-a concentrations for Kescayo Gansett Pond ranged from a low of 0.4 ug/L to a high of 14.6 ug/L. The target value for "good" quality water for chlorophyll-a concentration, (< 5ug/L), was exceeded 54% ot the time. Thus the data show that during the summer season, phytoplankton activity was strong. Chlorophyll-a concentrations are plotted in figure 7, showing that this pond's concentrations ranged from 8-14+ ug/L in the summer, dropping to about 2 ug/L or below in the fall.

Phaeopigments for Kescayo Gansett Pond ranged from a low of 0.2 ug/L to a high of about 2 ug/L. Figure 8 is a plot of these values, which are generally in the range for "good" water quality.

Dissolved oxygen ranged from a minimum of 2.3 mg/L to a maximum of 7.2mg/L, which occurred in the fall. Figure 9 shows the dissolved oxygen values for the deep samples of sites 1 and 2 for Kescayo Gansett Pond. With three exceptions, during the summer values for dissolved oxygen were below the target value of 5mg/L for "good" water quality. Secchi depths at site 1 are also plotted on Figure 9 (right hand scale) and show that 67% of the values are greater than 3 meters, indicative of "good" water quality. In this instance the two quality indicators are conflicting.

"Total" nitrogen for this study is the sum of nitrate nitrogen, nitrite nitrogen (which is

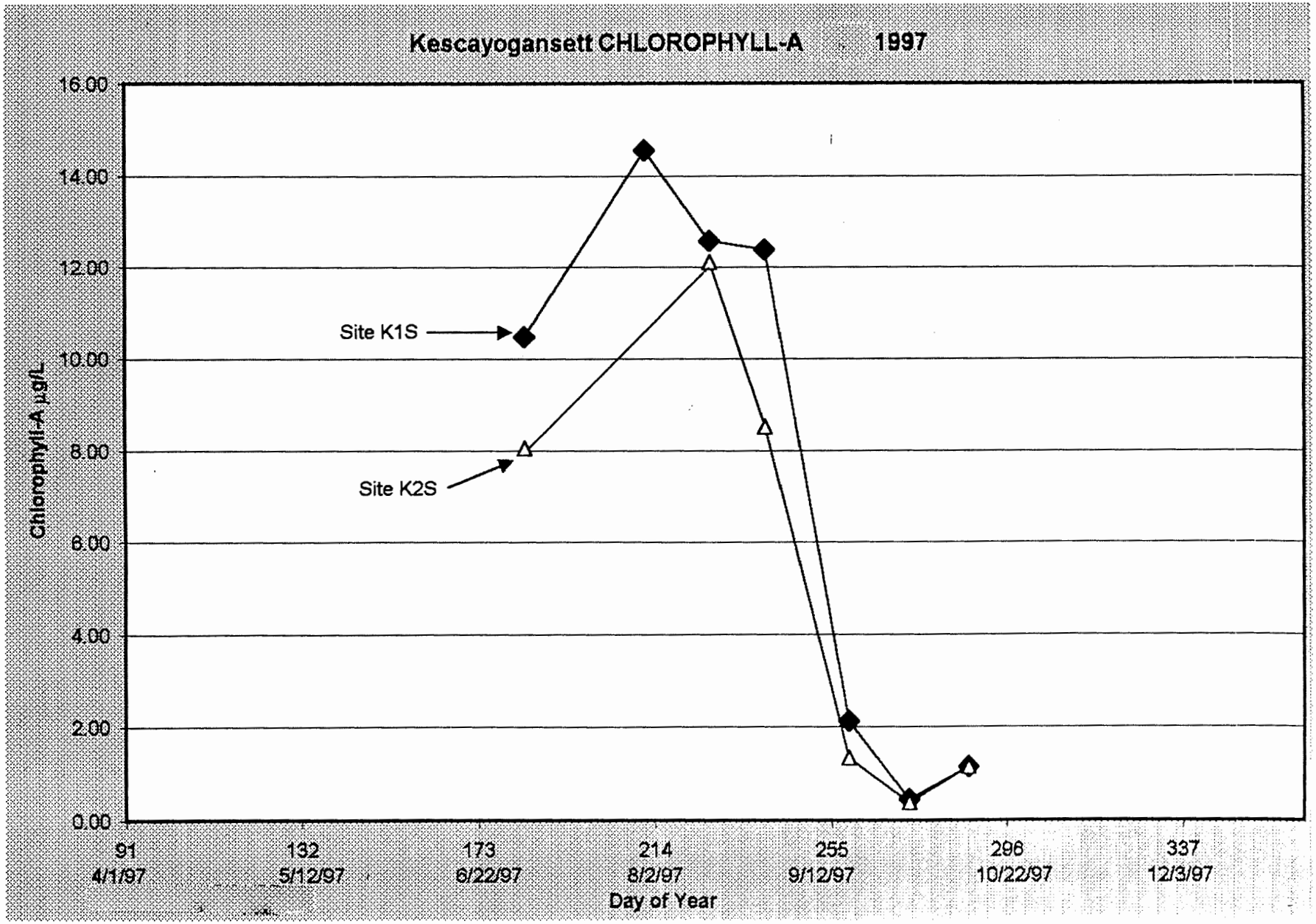
Kescayogansett CHLOROPHYLL-A 1997
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Chlorophyll-A µg/L
Site K1S
Site K2S
91
4/1/97
132
5/12/97
173
6/22/97
214
8/2/97
255
9/12/97
296
10/22/97
337
12/3/97
Day of Year

Figure 7

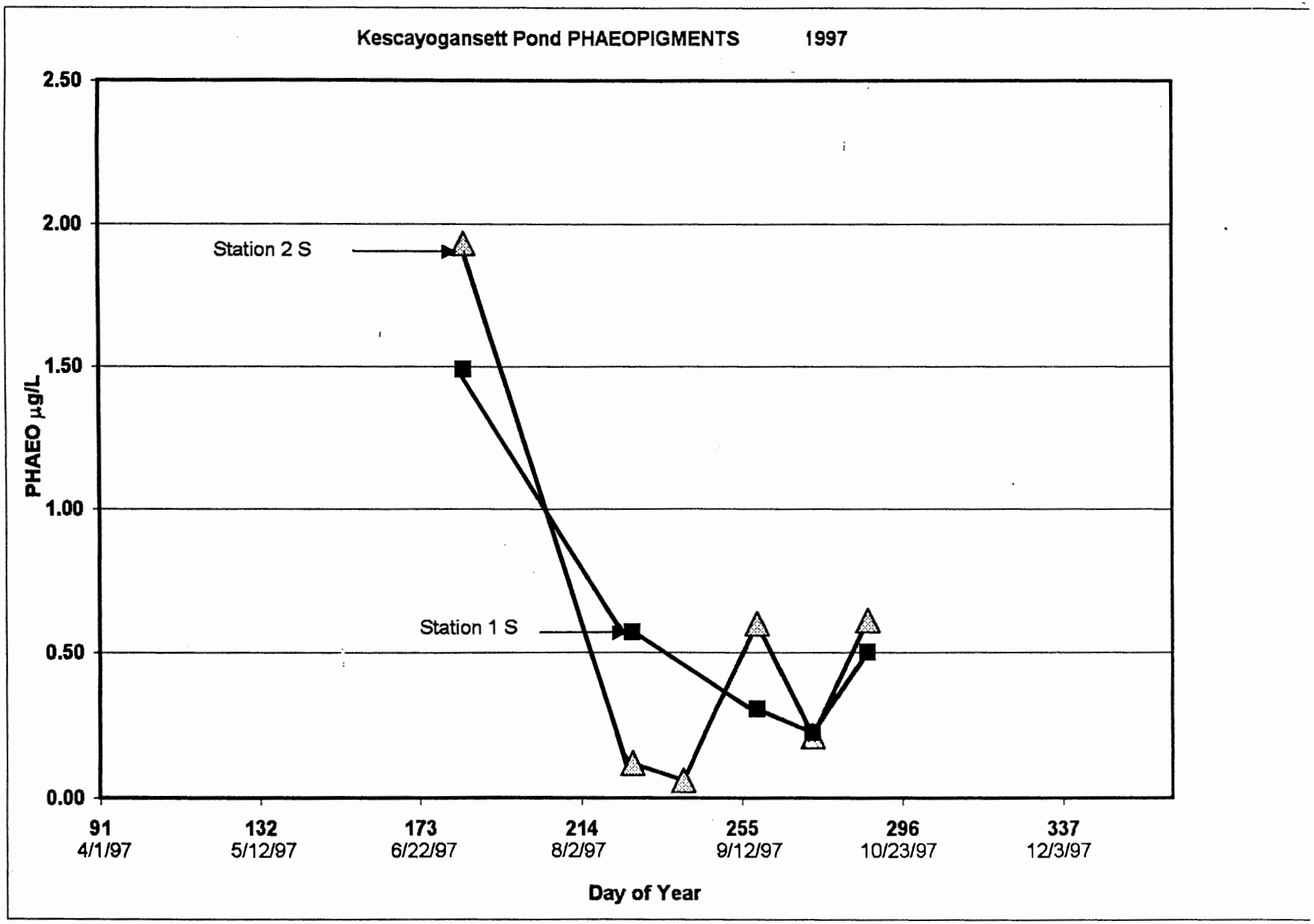
Kescayogansett Pond PHAEOPIGMENTS 1997
2.50
2.00
1.50
1.00
0.50
0.00
PHAEO µg/L
Station 2 S
Station 1 S
91
4/1/97
132
5/12/97
173
6/22/97
214
8/2/97
255
9/12/97
296
10/23/97
337
12/3/97
Day of Year

Figure 8

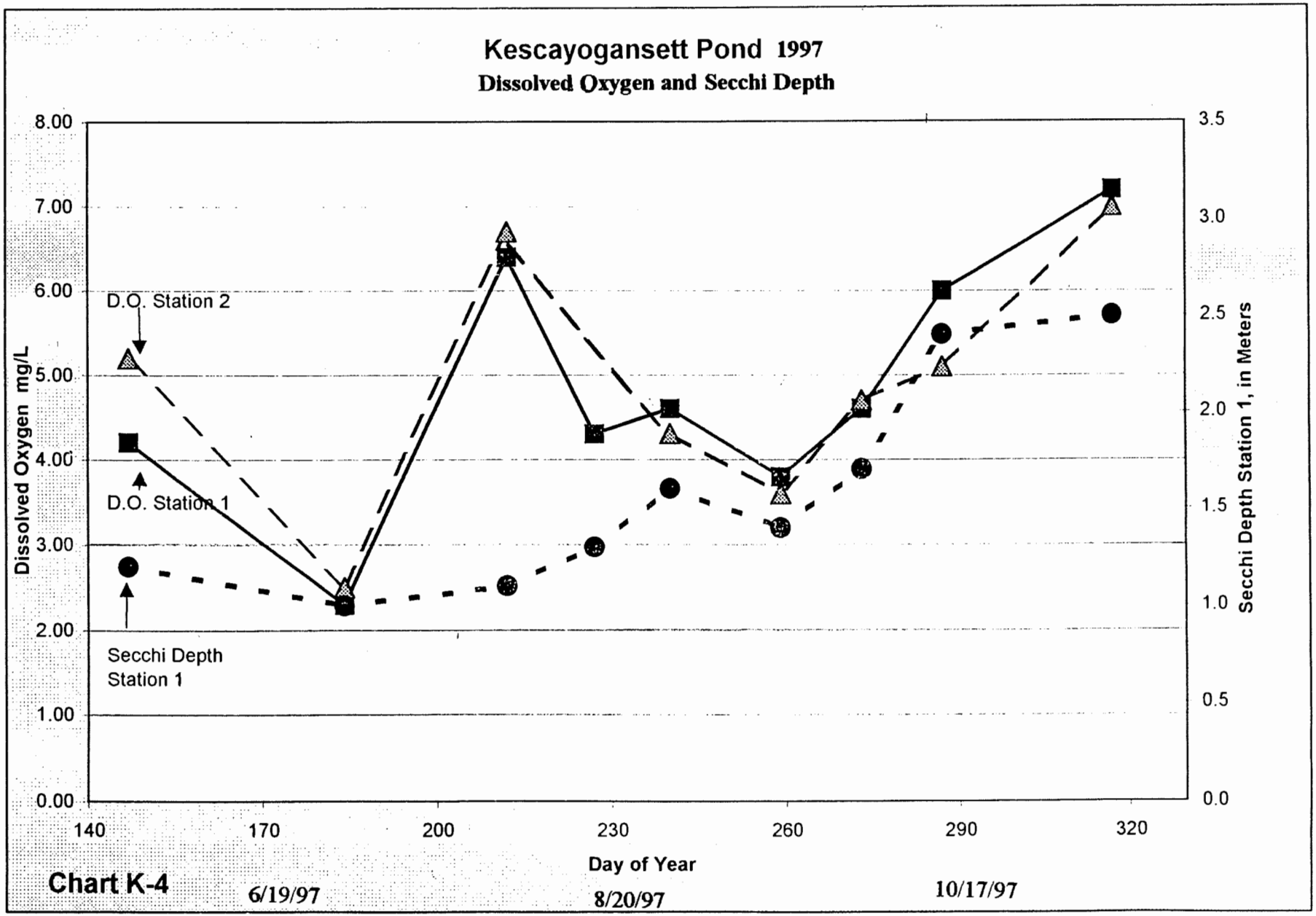
Kescayogansett Pond 1997
Dissolved Oxygen and Secchi Depth
Dissolved Oxygen mg/L
Secchi Depth Station 1, in Meters
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
D.O. Station 2
D.O. Station 1
Secchi Depth
Station 1
140
170
200
230
260
290
320
Day of Year
Chart K-4
6/19/97
8/20/97
10/17/97

Figure 9

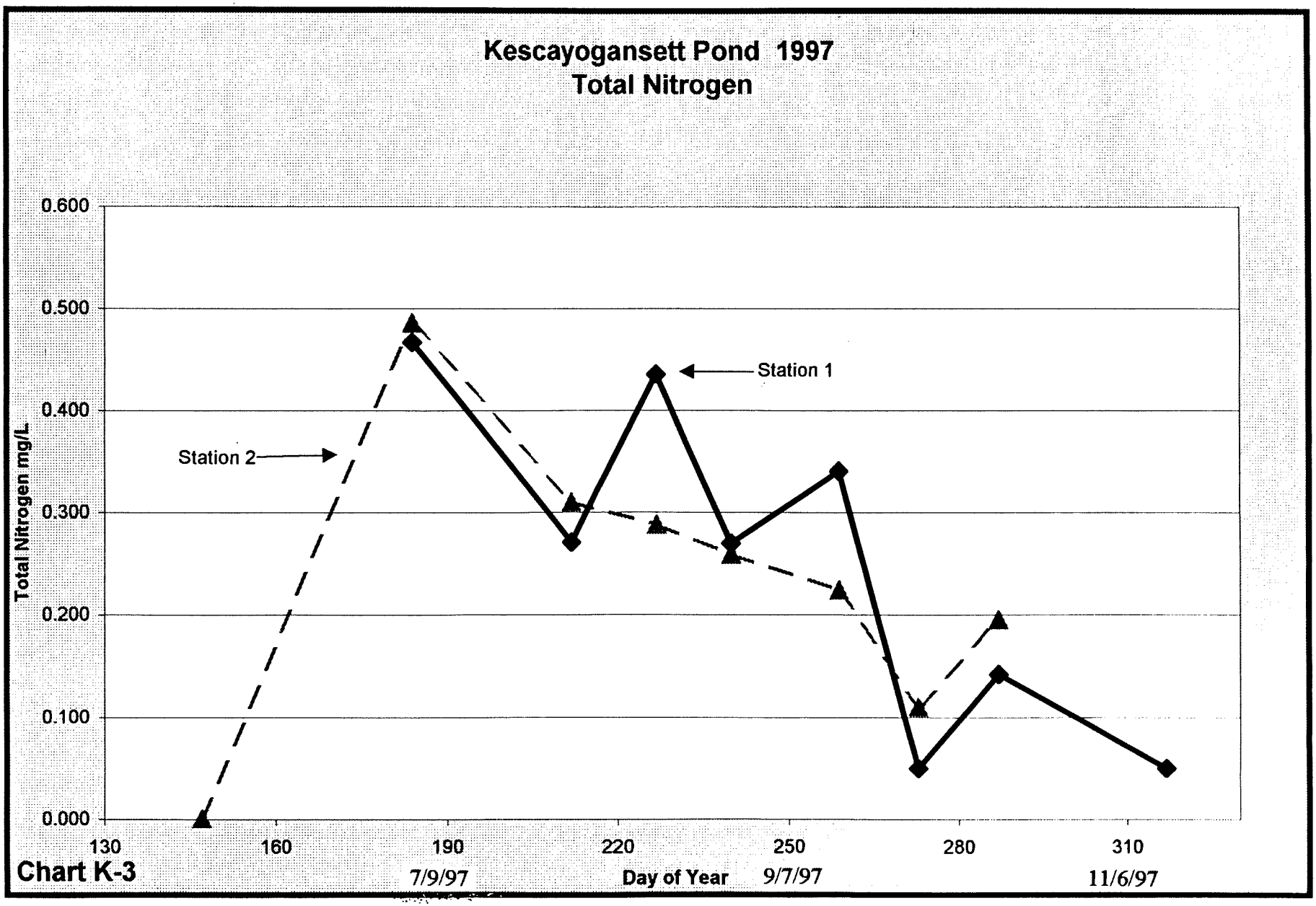
Kescayogansett Pond 1997
Total Nitrogen
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Total Nitrogen mg/L
Station 1
Station 2
130
160
190
220
250
280
310
7/9/97
Day of Year
9/7/97
11/6/97
Chart K-3

Figure 10

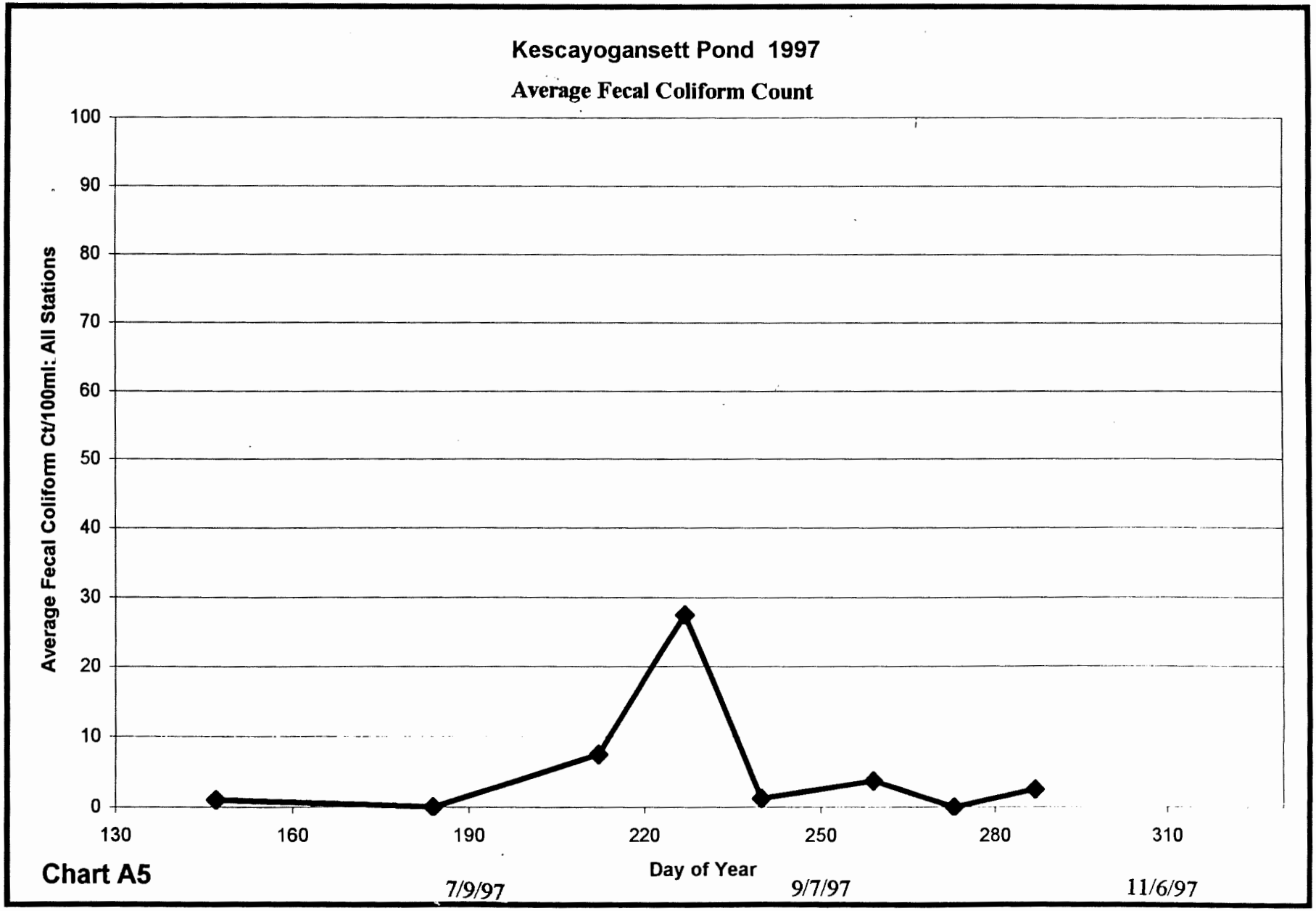
Kescayogansett Pond 1997
Average Fecal Coliform Count
Average Fecal Coliform Ct/100ml: All Stations
100
90
80
70
60
50
40
30
20
10
0
130
160
190
220
250
280
310
Day of Year
7/9/97
9/7/97
11/6/97
Chart A5

Figure 11

included in the nitrate nitrogen) determined by the National Park Service Laboratory, ammonia nitrogen, and particulate organic nitrogen (determined by CMAST). We do not have a measure of dissolved organic nitrogen in our data set. Figure 10 gives plots of the "total" nitrogen in mg/L, from the "shallow" samples taken at each of the two sites in the pond. "Total" nitrogen remained in the "poor" water quality range through the middle of September.

Figure 11 shows the average fecal coliform counts from all four samples taken on a given sampling day. With one exception, average counts are within the <14 shellfish limit, indicating overall very good bacteriological quality.

Areys Pond

Chlorophyll-a concentrations for Areys Pond ranged from a low of 0.19 ug/L to a high of 100.99 ug/L, which was the highest value recorded in this study. Plots of chlorophyll-a concentrations are given in figure 12 for both sites. Based on the chlorophyll-a data as well as other observations, such as Secchi depths, it is clear that Areys Pond experienced some algal blooms during the summer. About half of the time, values for chlorophyll-a for Areys Pond were in the range for "good" water quality based on this parameter.

Phaeopigments for Areys Pond ranged from a low of 0.07 ug/L to a high of 6.17 ug/L, as shown in figure 13. With the exception of one high value for site one, the remainder of the values for phaeopigments are in the range for "good" water quality.

Figure 14 shows plots of dissolved oxygen concentrations for the "deep" samples from both sites of Areys Pond. Generally values for dissolved oxygen greater than 5 mg/L are indicative of "good" water quality. As one can observe for site 1 of Areys Pond, dissolved oxygen was consistently below 2 mg/L except for one value about 3. Samples from the shallower site, exhibited considerable variation, with 3 values greater than 5 mg/L. Overall dissolved oxygen indicated poor water quality. Secchi depths from site 1 are also plotted in figure 14, using the right hand Y-axis as the scale. Only one measurement equalled a depth of 3 or more meters. The remainder varied between 0.5 and 2, indicating marginal or "poor" water quality.

"Total" nitrogen ranged from a minimum of 0.06 mg/L to 1.72 mg/L in Areys Pond. The plots of the values from the "shallow" samples from each of the two sites are given in Figure 15. The values are in the "poor" water quality range until October. The values are exceedingly high.

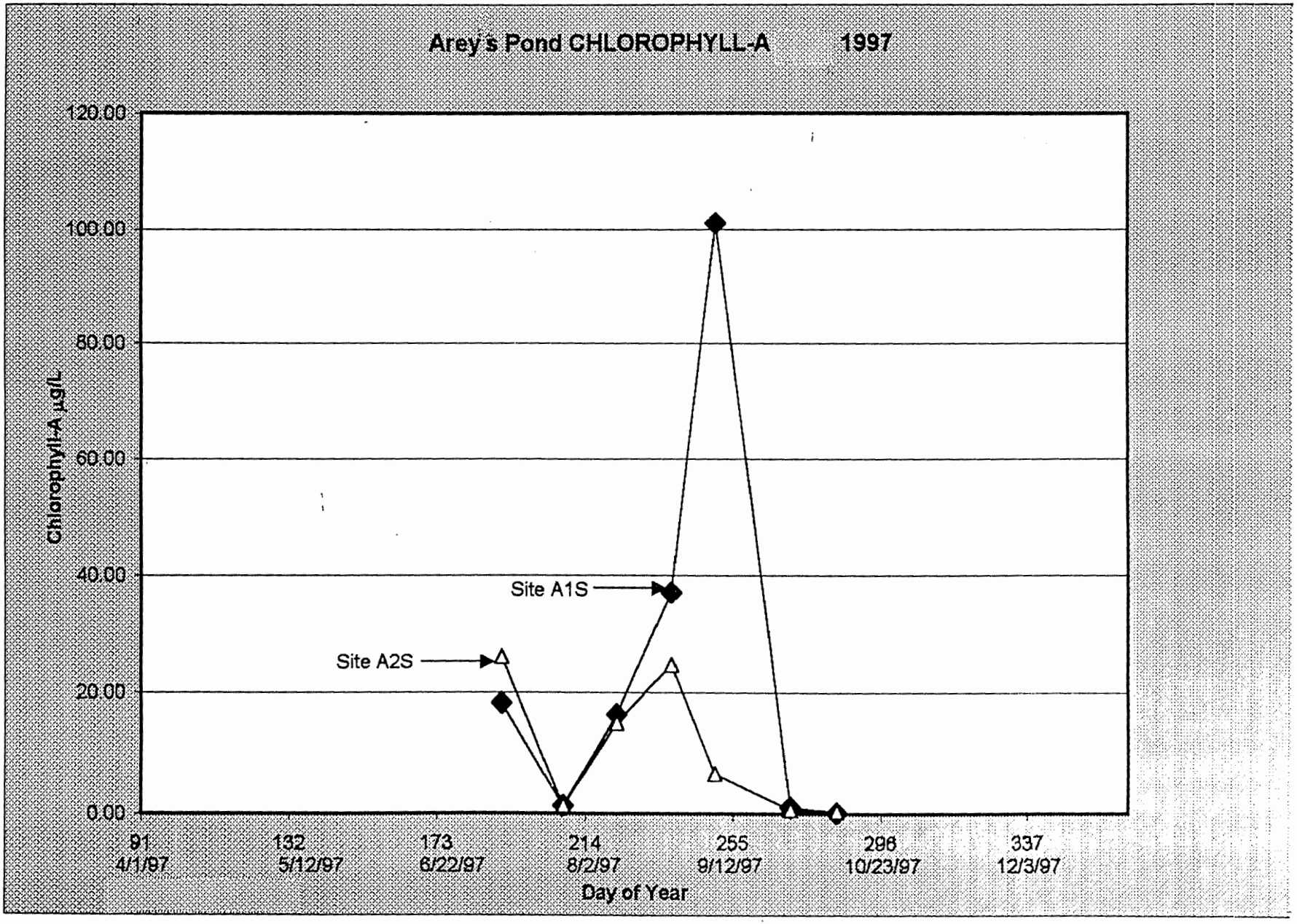
Arey's Pond CHLOROPHYLL-A 1997
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Chlorophyll-A µg/L
Site A1S
Site A2S
91
4/1/97
132
5/12/97
173
6/22/97
214
8/2/97
255
9/12/97
296
10/23/97
337
12/3/97
Day of Year

Figure 12

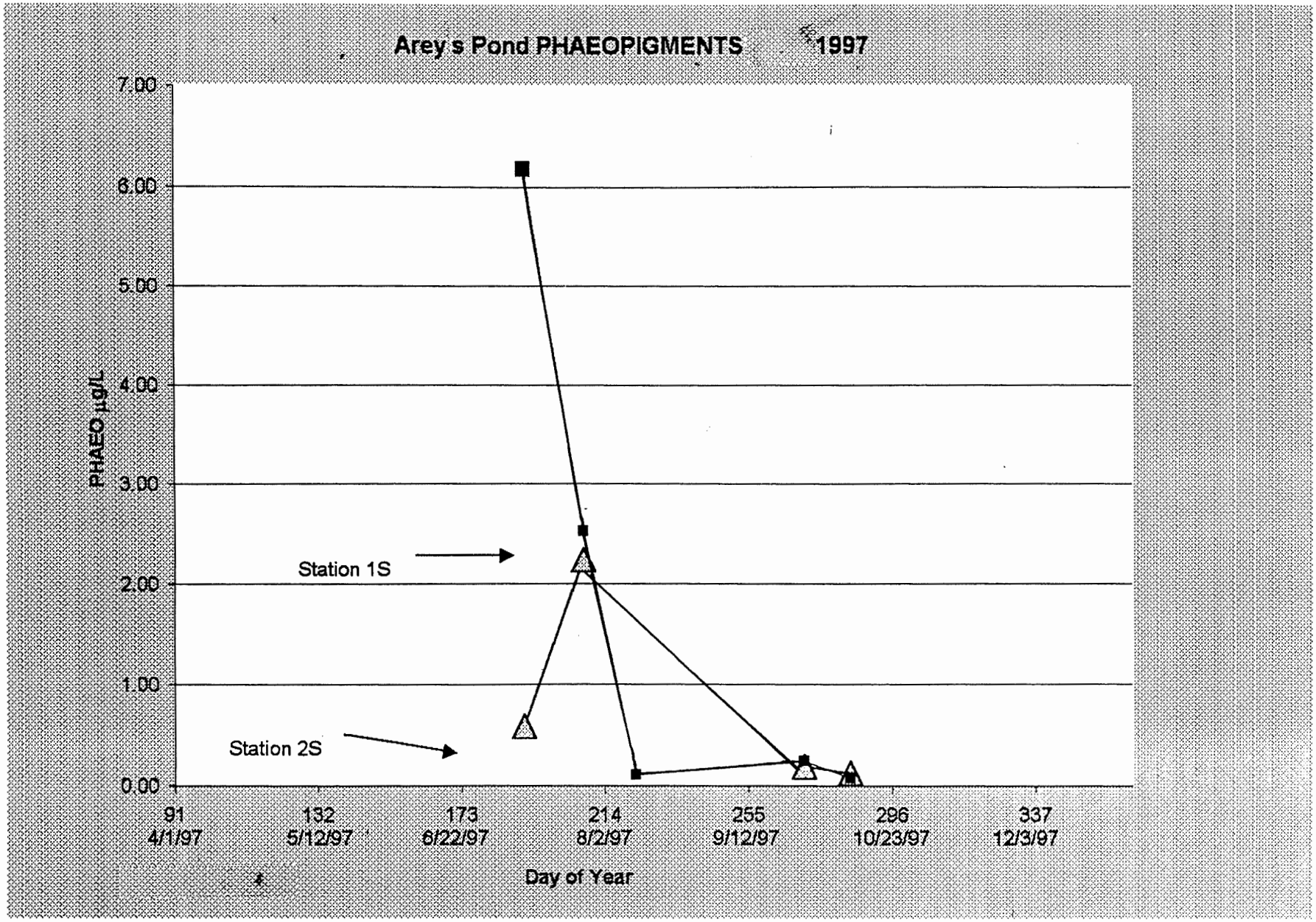
Arey's Pond PHAEOPIGMENTS 1997
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
PHAEO µg/L
Station 1S
Station 2S
91
4/1/97
132
5/12/97
173
6/22/97
214
8/2/97
255
9/12/97
296
10/23/97
337
12/3/97
Day of Year

Figure 13

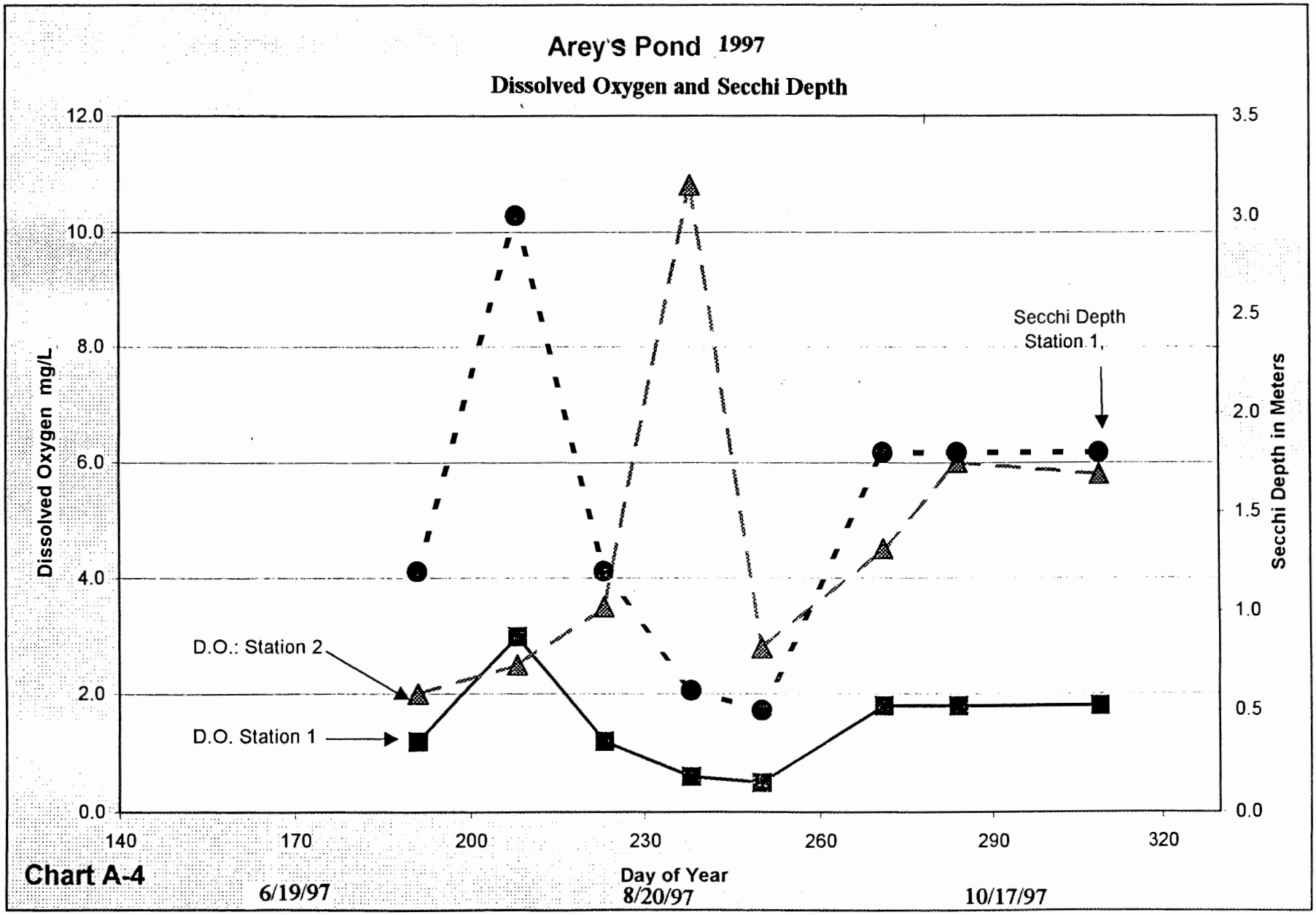
Arey's Pond 1997
Dissolved Oxygen and Secchi Depth
Dissolved Oxygen mg/L
Secchi Depth in Meters
12.0
10.0
8.0
6.0
4.0
2.0
0.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Secchi Depth
Station 1,
D.O.: Station 2
D.O. Station 1
140
170
200
230
260
290
320
Day of Year
Chart A-4
6/19/97
8/20/97
10/17/97

Figure 14

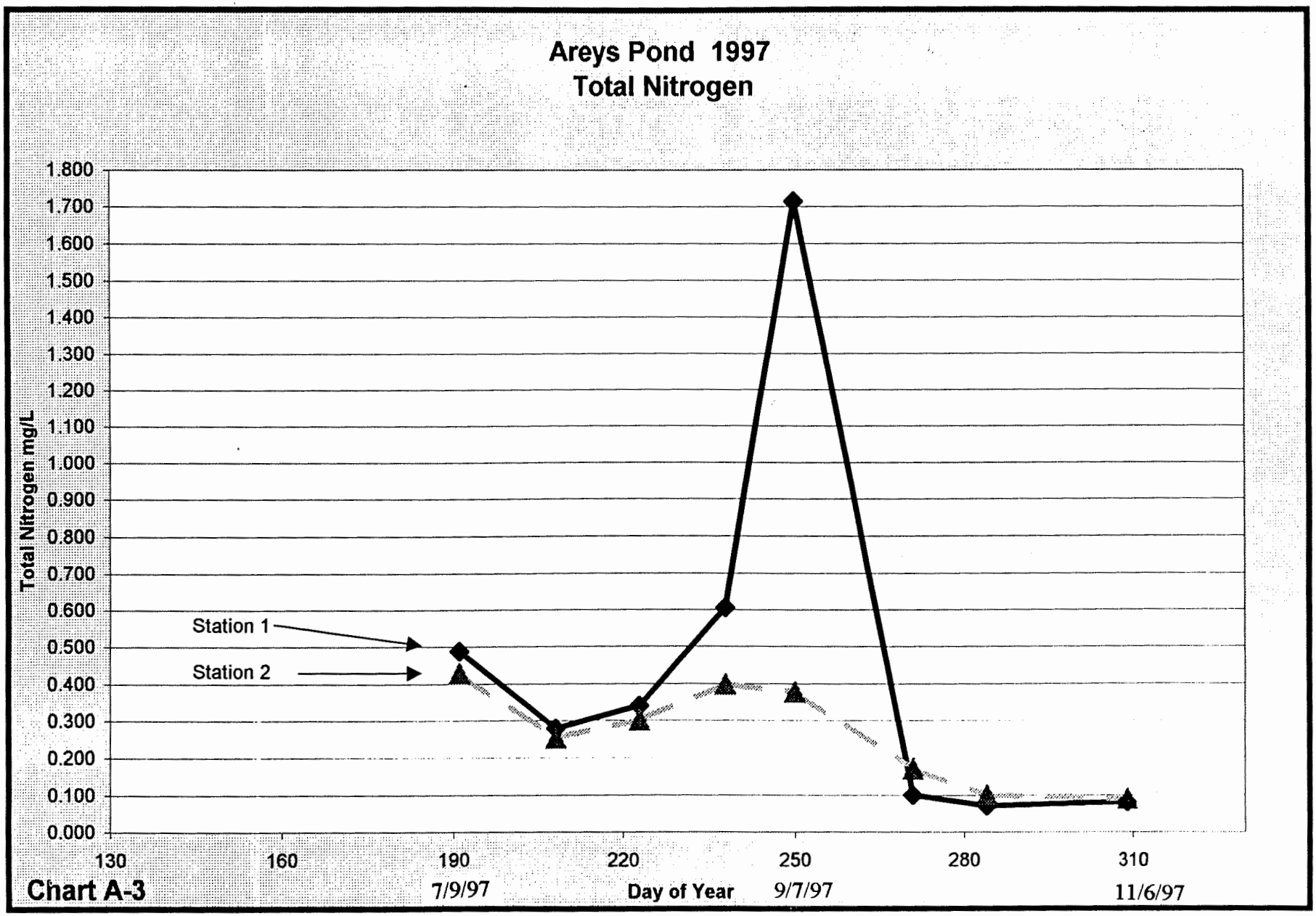
Areys Pond 1997
Total Nitrogen
Total Nitrogen mg/L
1.800
1.700
1.600
1.500
1.400
1.300
1.200
1.100
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Station 1
Station 2
130
160
190
220
250
280
310
7/9/97
Day of Year
9/7/97
11/6/97
Chart A-3

Figure 15

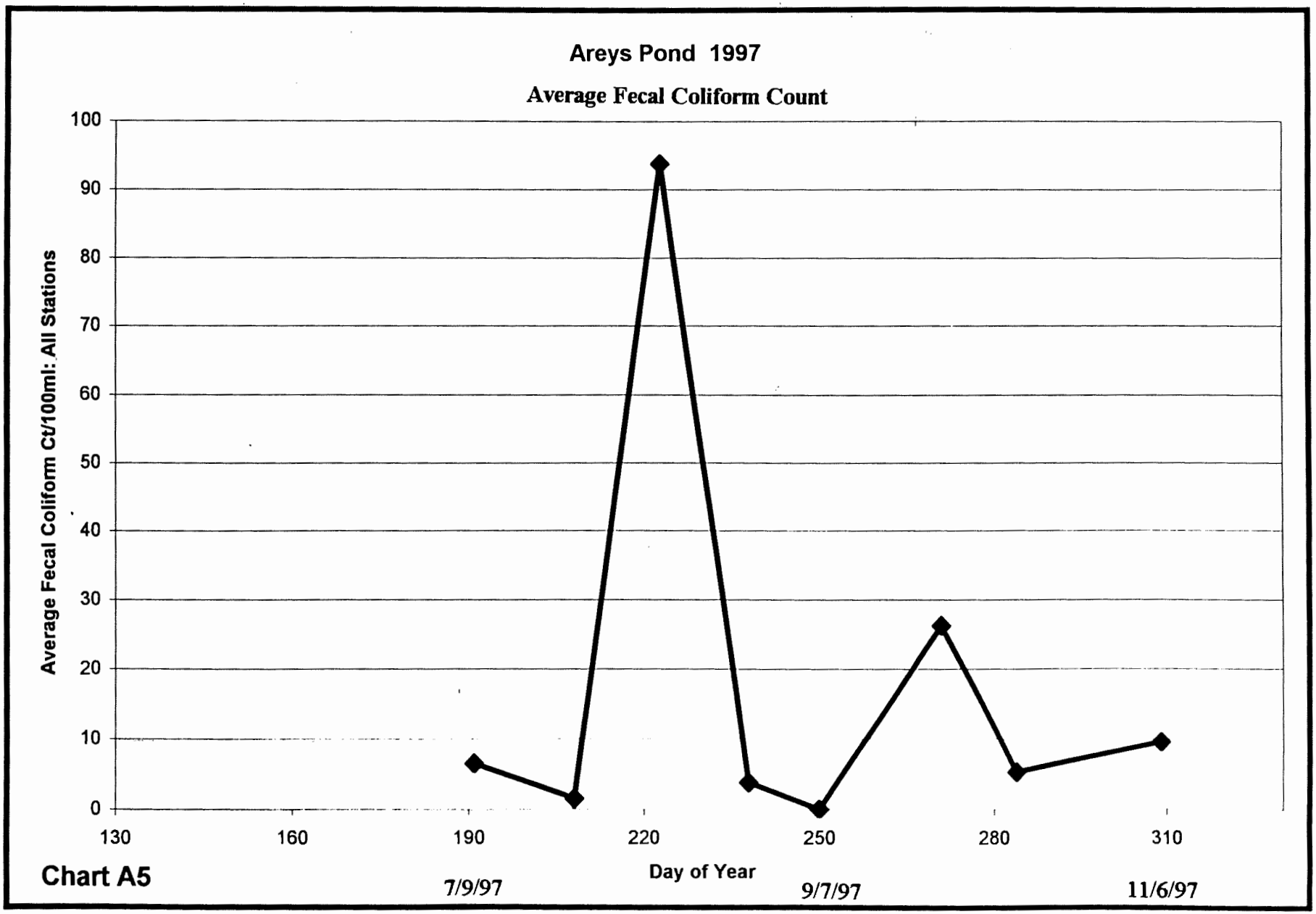
Areys Pond 1997
Average Fecal Coliform Count
Average Fecal Coliform Ct/100ml: All Stations
100
90
80
70
60
50
40
30
20
10
0
130
160
190
220
250
280
310
Day of Year
7/9/97
9/7/97
11/6/97
Chart A5

Figure 16

Figure 16 is a plot of the average fecal coliform count from the four samples (two at each site) in colonies/100 ml as measured over the 1997 season. There were nine individual values which exceeded the shellfish limit of 14, and one in which the count exceeded the recommended limit for swimming of 200. For the latter, samplers noted several Canada Geese in the vicinity of the site at that time. Overall this indicates marginal water quality from a bacteriological point of view.

Paw Wah Pond

Chlorophyl-a concentrations for PawWah Pond ranged from a low of 0.13 ug/L to a single high measurement of 59.22 ug/L. With one other exception (5.51ug/L), the other values were under 5 ug/L, as one can observe in the plots on Figure 17. Predominantly, the chlorophyll-a concentrations are in the range for "good" water quality.

As the plots of phaeopigments in Figure 18 demonstrate, all values remained below 3 ug/L, indicating "good" water quality for Paw Wah Pond according to this measure.

Figure 19 shows the dissolved oxygen concentrations for the "deep" samples from both sites of Paw Wah Pond. Values of 5 mg/L or above are indicative of "good" water quality. During the summer each site had one value of 5 or above, but most values were in the 4-5 mg/L range, indicating fairly good conditions. Secchi depth for site one, plotted on the same graph, remained at 1.7 meters or greater, but only once reached a value of 3 meters. The Secchi data indicate only "fair" water quality.

Figure 20 shows that the concentration of "total nitrogen" remained quite low, although a number of data points had the component of particulate organic nitrogen missing because of a machine malfunction at the Woods Hole Laboratory. Water quality is "fair" in the mid season. In the fall water quality is "good".

The average fecal coliform count of all four samples taken each sampling day are shown in Figure 21. Of the 28 samples included in the data only two had counts of 15, the remainder were less. As shown, the average count stayed below 10, indicating "good" water quality for shellfishing and other uses.

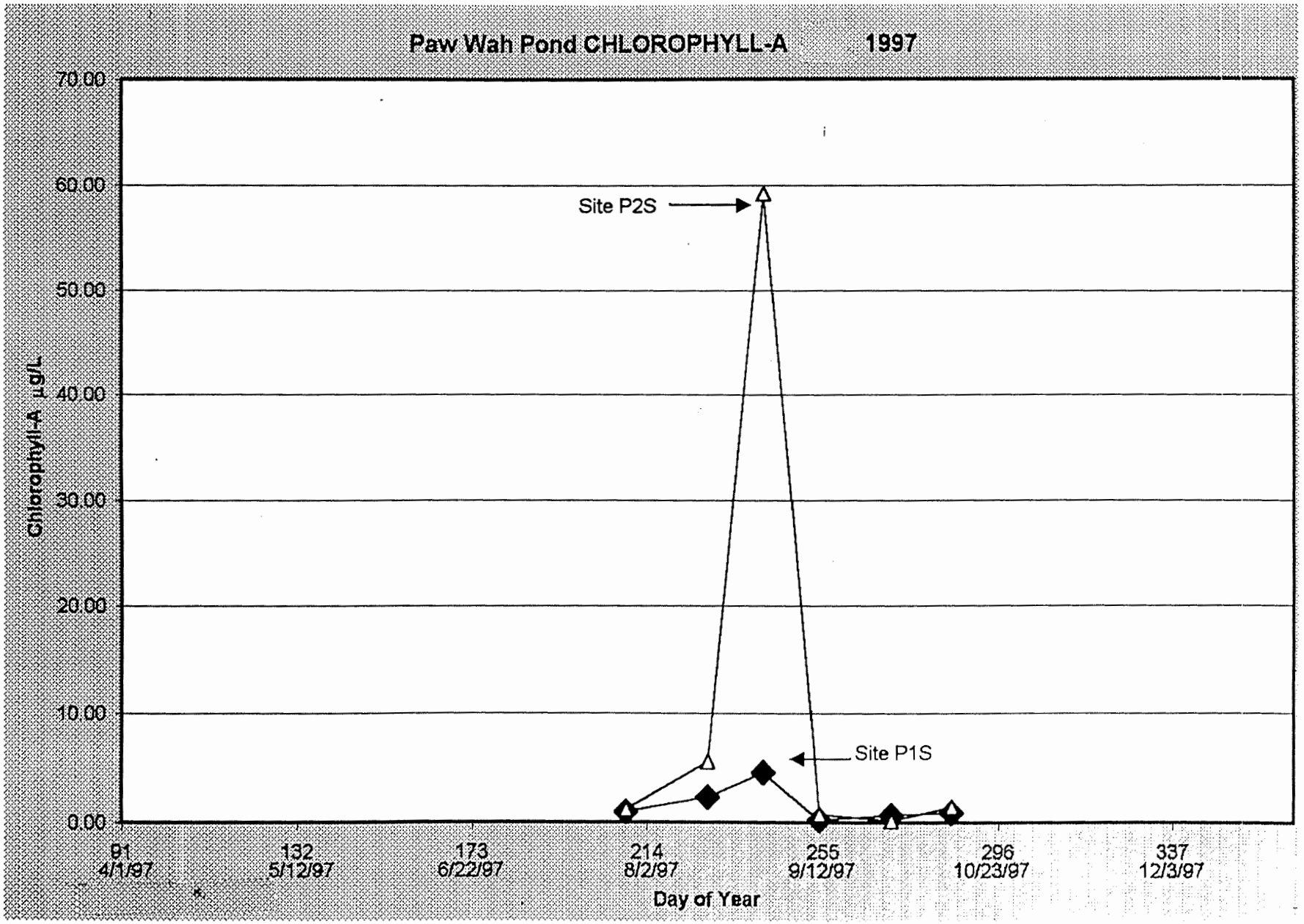
Paw Wah Pond CHLOROPHYLL-A 1997
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Chloroptyll-A µg/L
Site P2S
Site P1S
91
4/1/97
132
5/12/97
173
6/22/97
214
8/2/97
255
9/12/97
296
10/23/97
337
12/3/97
Day of Year

Figure 17

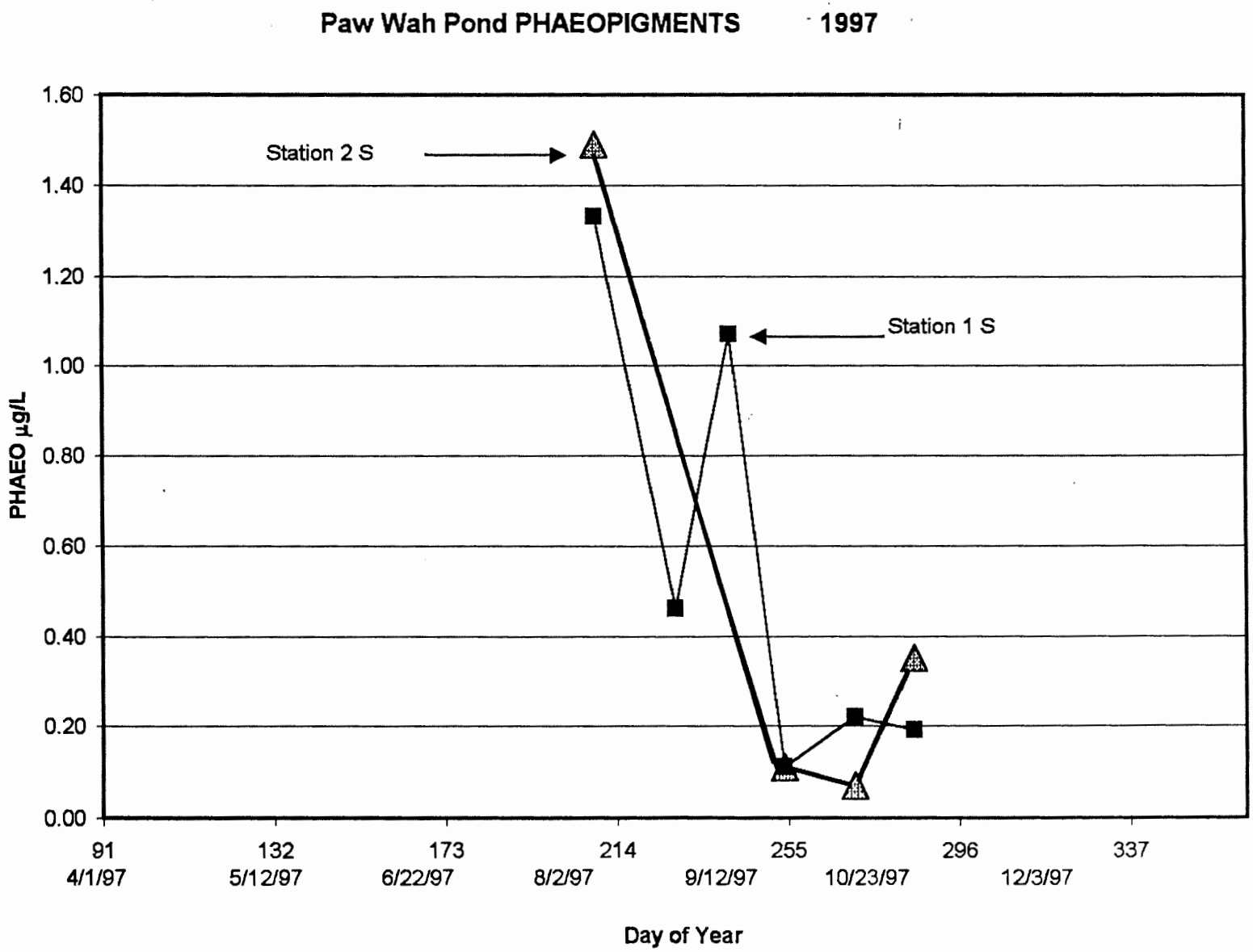
Paw Wah Pond PHAEOPIGMENTS 1997
Station 2 S
Station 1 S
PHAEO µg/L
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
91
4/1/97
132
5/12/97
173
6/22/97
214
8/2/97
255
9/12/97
296
10/23/97
337
12/3/97
Day of Year

Figure 18

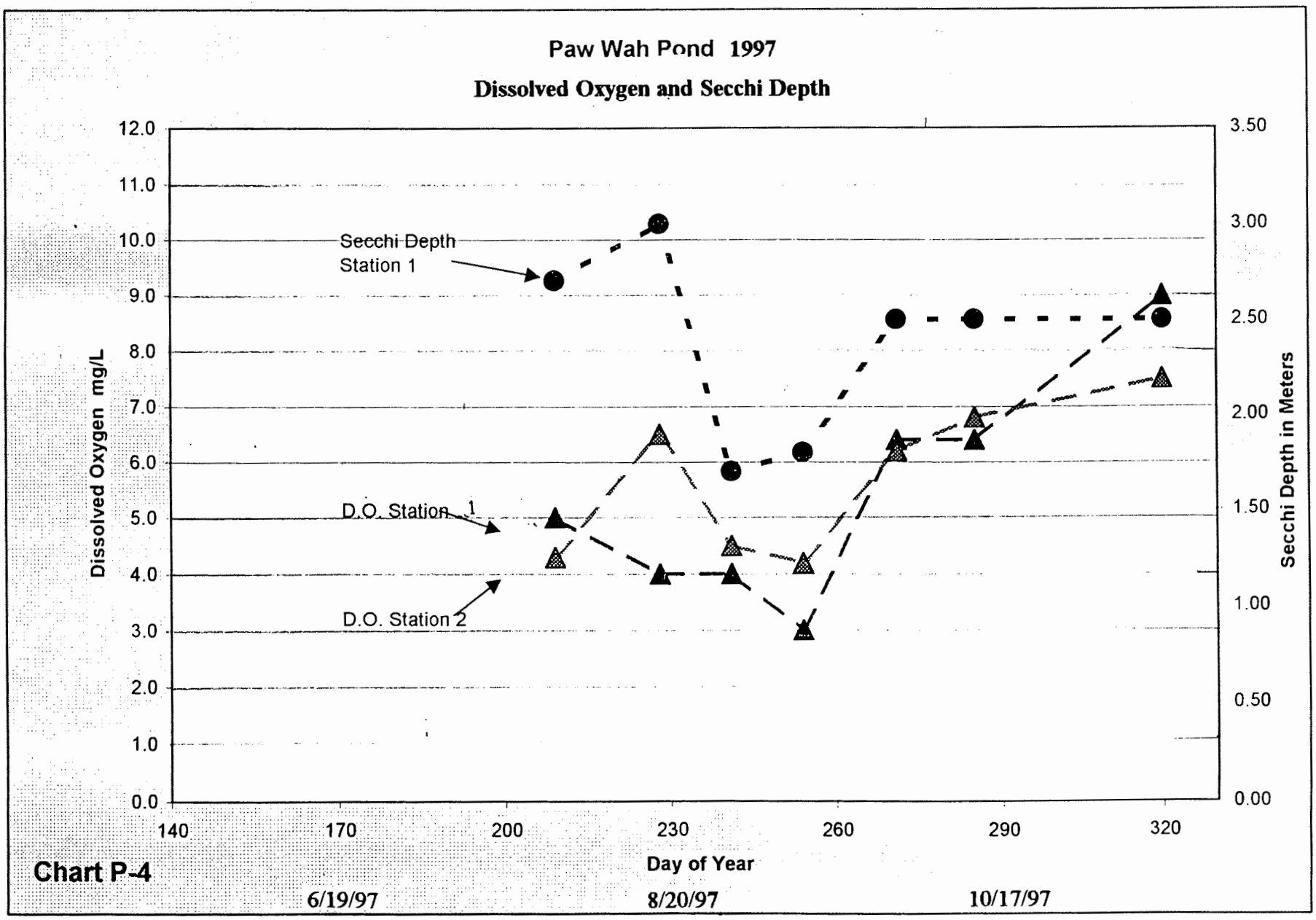
Paw Wah Pond 1997
Dissolved Oxygen and Secchi Depth
Secchi Depth
Station 1
D.O. Station 1
D.O. Station 2
Dissolved Oxygen mg/L
Secchi Depth in Meters
Day of Year
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
140
170
200
230
260
290
320
Chart P-4
6/19/97
8/20/97
10/17/97

Figure 19

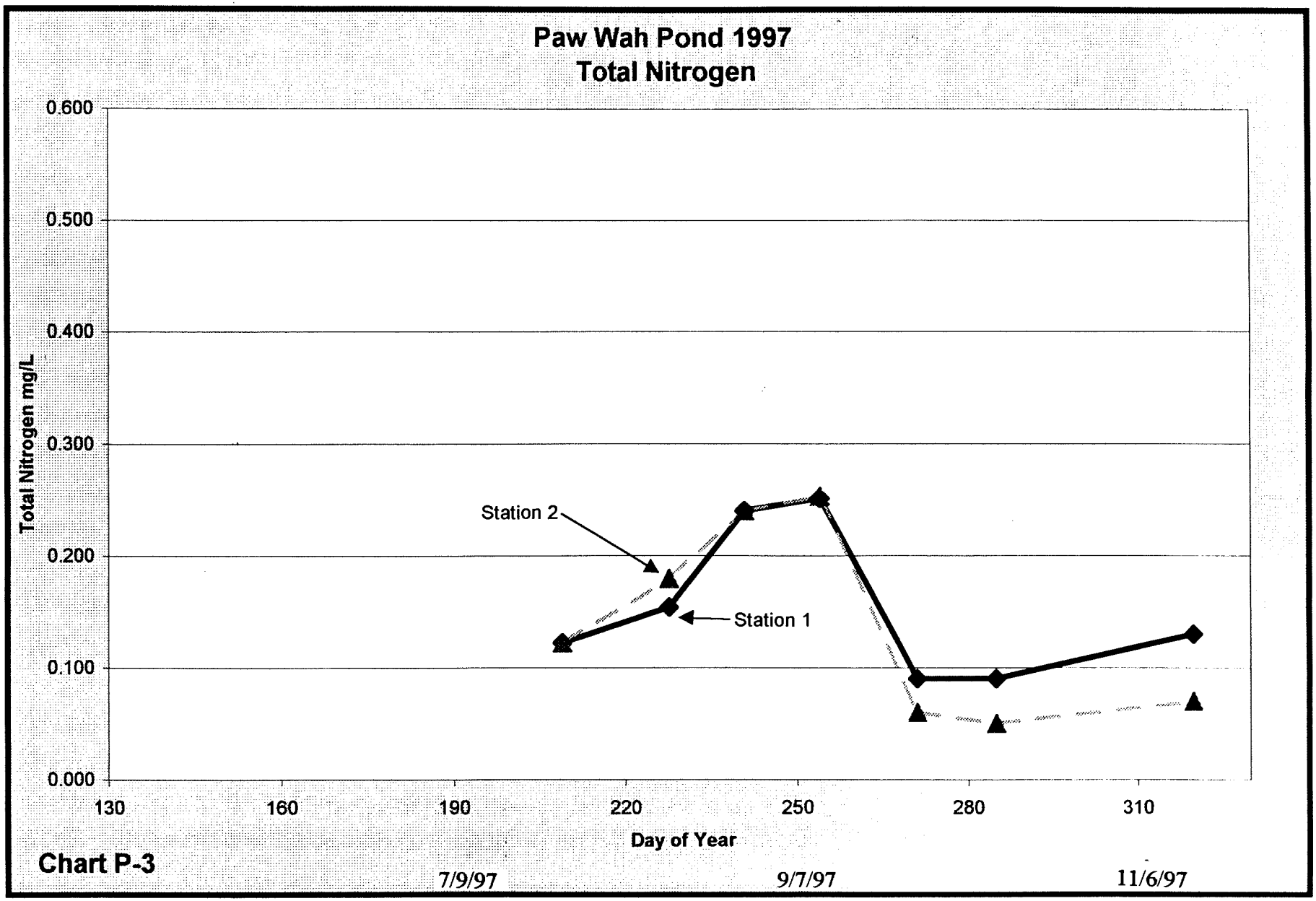
Paw Wah Pond 1997
Total Nitrogen
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Total Nitrogen mg/L
Station 2
Station 1
130
160
190
220
250
280
310
Day of Year
Chart P-3
7/9/97
9/7/97
11/6/97

Figure 20

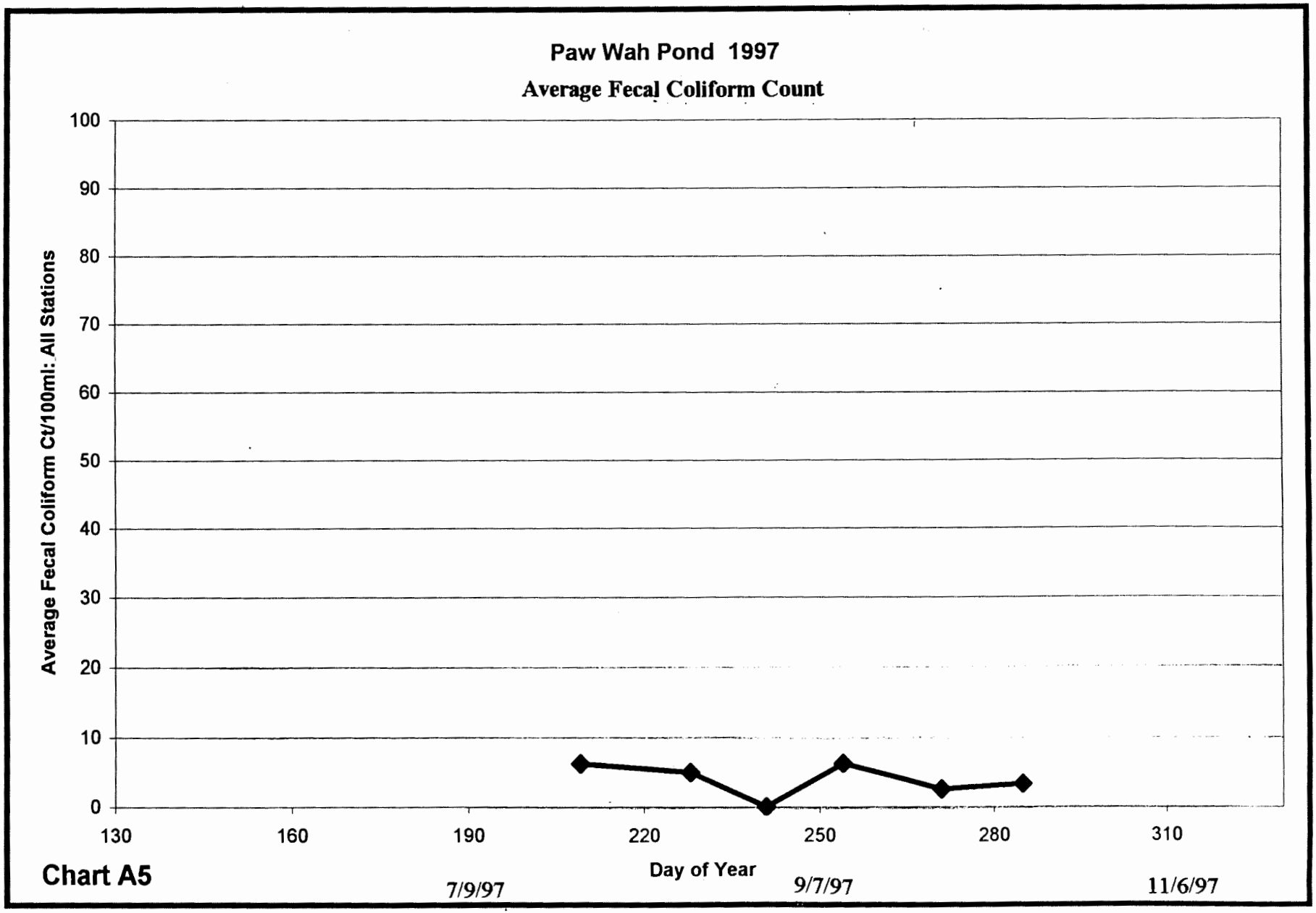
Paw Wah Pond 1997
Average Fecal Coliform Count
Average Fecal Coliform Ct/100ml: All Stations
100
90
80
70
60
50
40
30
20
10
0
130
160
190
220
250
280
310
Day of Year
7/9/97
9/7/97
11/6/97
Chart A5

Figure 21

Quanset Pond

Chlorophyll-a concentrations for Quanset Pond are shown in Figure 22. Initially high values at the first sampling of the season on July 19, fell to values below 4 ug/L on August 13, and even lower in the remainder of the season. The earliest value indicated high phytoplankton activity, the remaining values are indicative of "good" water quality.

Phaeopigment concentrations for Quanset Pond are shown in Figure 23, where they fluctuate between values of 0.35 and 2.00 ug/L. Such values are indicative of "good" water quality.

Figure 24 gives the dissolved oxygen concentrations for the "deep" samples for both sites of Quanset Pond. Values of 5 mg/L or higher are indicative of "good" water quality. While the first two points for the deeper site (#1) are at the 4.5 mg/L level, all the remaining values are at 5 mg/L or above. Overall, the dissolved oxygen data show the water quality to be "good" in Quanset Pond.

Figure 24 also provides a plot of the Secchi depth at site 1 of Quanset Pond. This plot relates to the right hand Y-axis scale. Initial measures of the water transparency, (Secchi depth), are at 1.5 meters, increasing to 3 and later, falling to 1.5 again. The Secchi depths are fairly "good" relative to our data in the other ponds; that is, very small Secchi depths are not observed in Quanset Pond.

"Total" nitrogen ranged from a minimum of 0.12 mg/L to a maximum of 0.25 mg/L for the "shallow" samples taken from sites 1 and 2 of Quanset Pond. Plots of the values for each of these sites are given in figure 25. Water quality through the mid-season is "fair", improving to "good" in the fall.

Figure 26 is a plot of the average fecal coliform count from the four samples (two at each site) in colonies/100 ml as measured over the 1997 season. Of the 23 samples taken only one exceeded the shellfish limit of 14, and that count was 30. From a bacteriological point of view, the data show that Quanset Pond water in 1997 was safe for shellfishing and swimming.

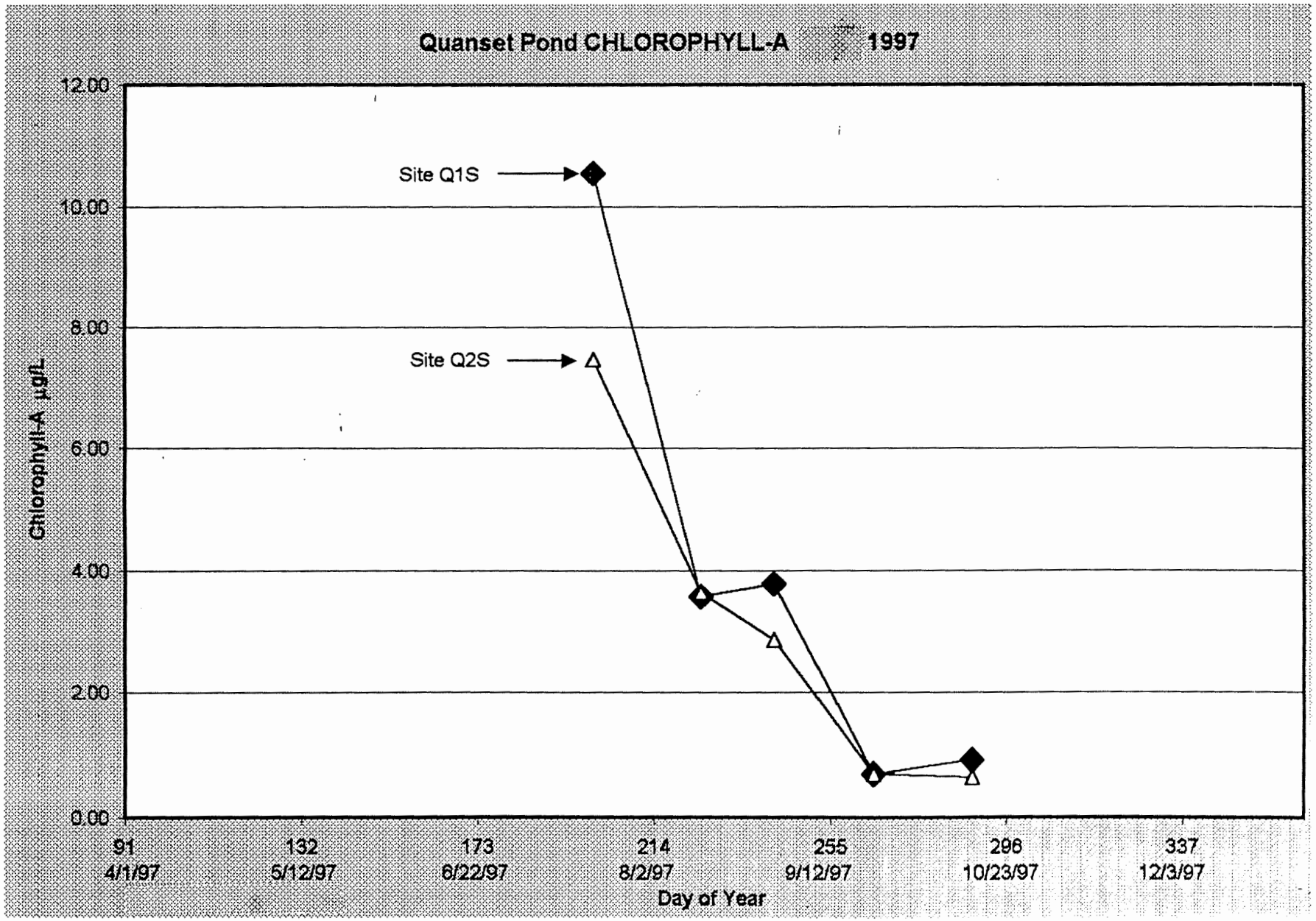
Quanset Pond CHLOROPHYLL-A 1997
Site Q1S
Site Q2S
Chlorophyll-A µg/L
12.00
10.00
8.00
6.00
4.00
2.00
0.00
91
4/1/97
132
5/12/97
173
6/22/97
214
8/2/97
255
9/12/97
296
10/23/97
337
12/3/97
Day of Year

Figure 22

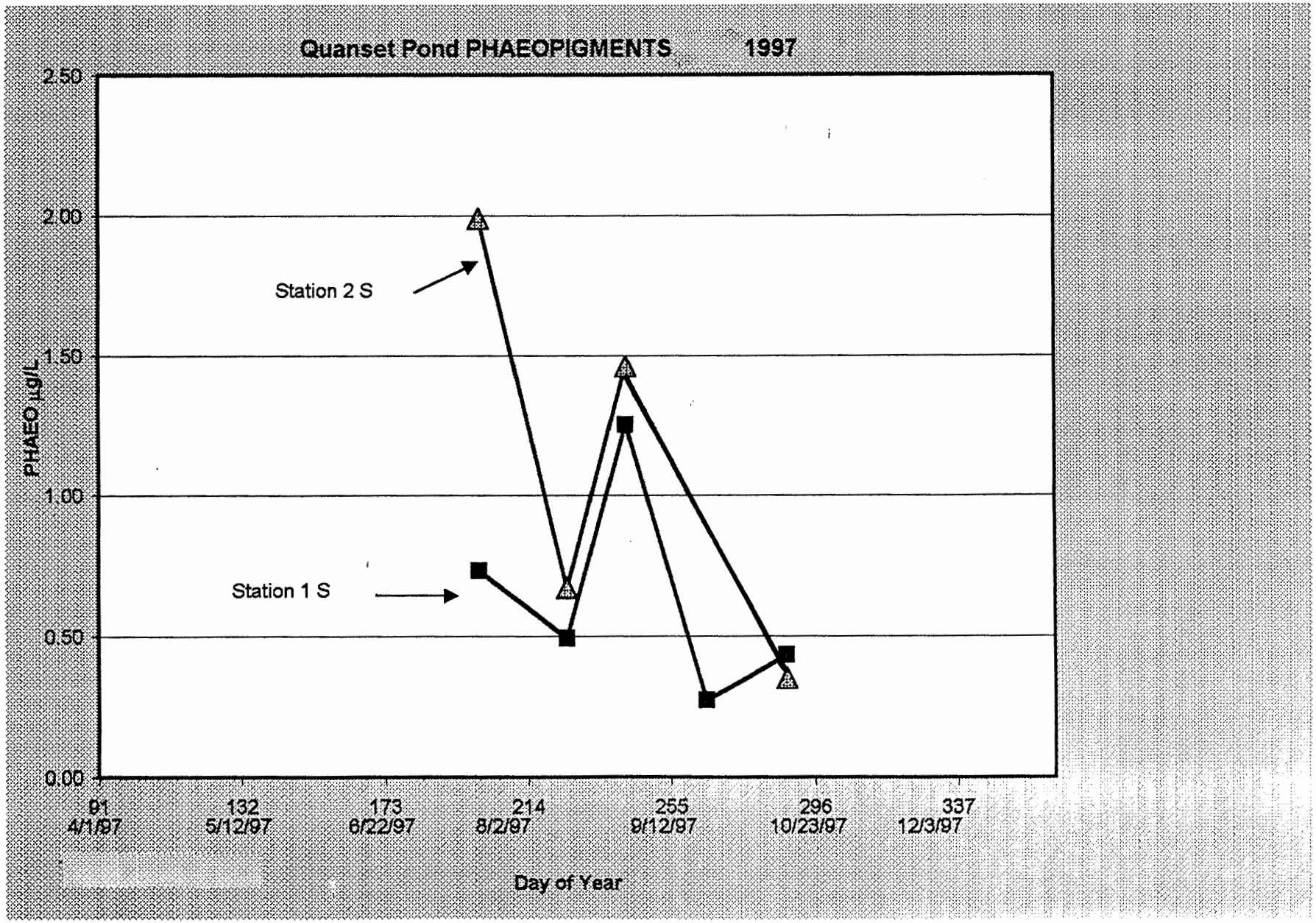
Quanset Pond PHAEOPIGMENTS 1997
2.50
2.00
1.50
1.00
0.50
0.00
PHAEO µg/L
Station 2 S
Station 1 S
91
4/1/97
132
5/12/97
173
6/22/97
214
8/2/97
255
9/12/97
296
10/23/97
337
12/3/97
Day of Year

Figure 23

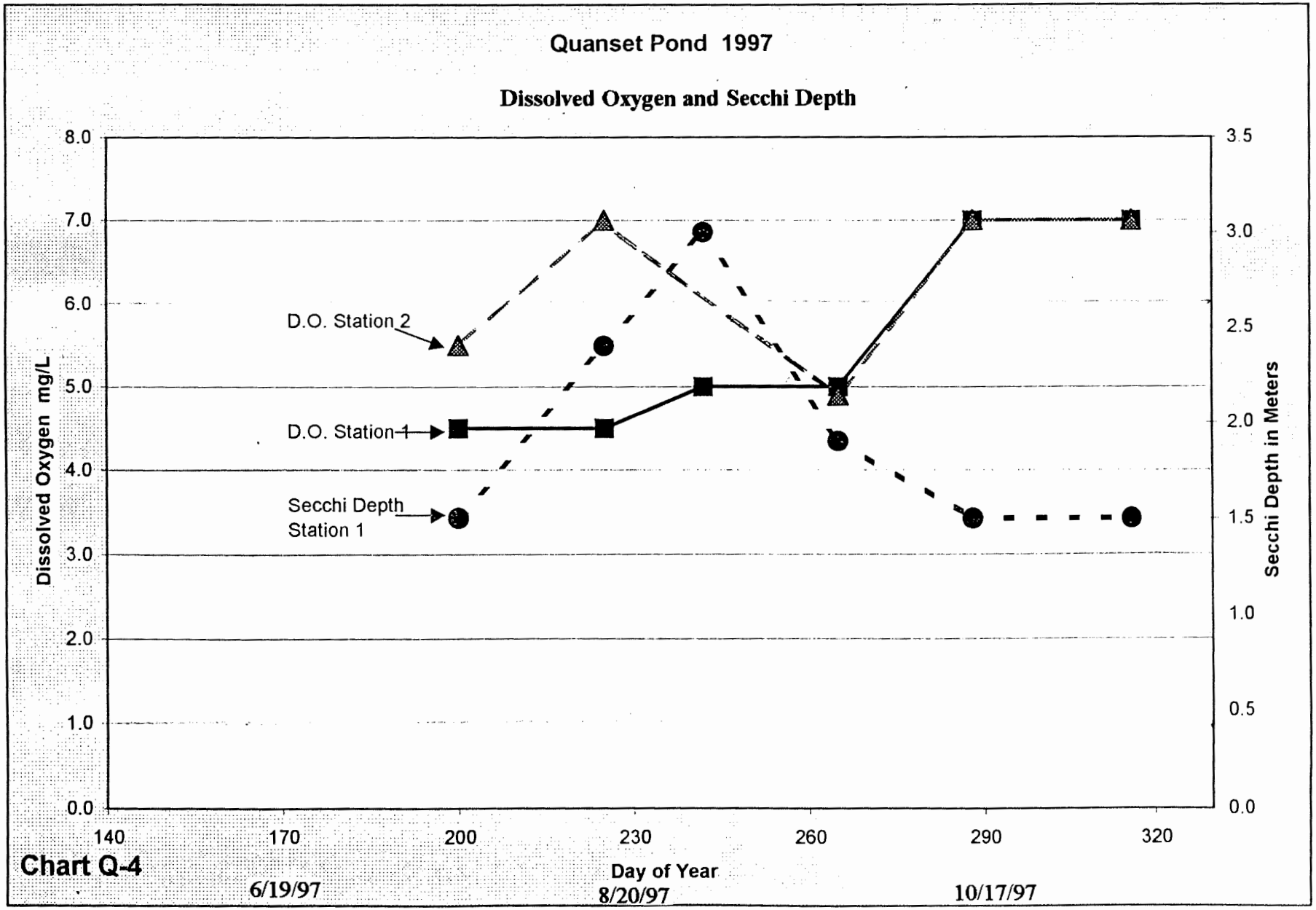
Quanset Pond 1997
Dissolved Oxygen and Secchi Depth
Dissolved Oxygen mg/L
Secchi Depth in Meters
D.O. Station 2
D.O. Station 1
Secchi Depth
Station 1
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
140
170
200
230
260
290
320
Day of Year
6/19/97
8/20/97
10/17/97
Chart Q-4

Figure 24

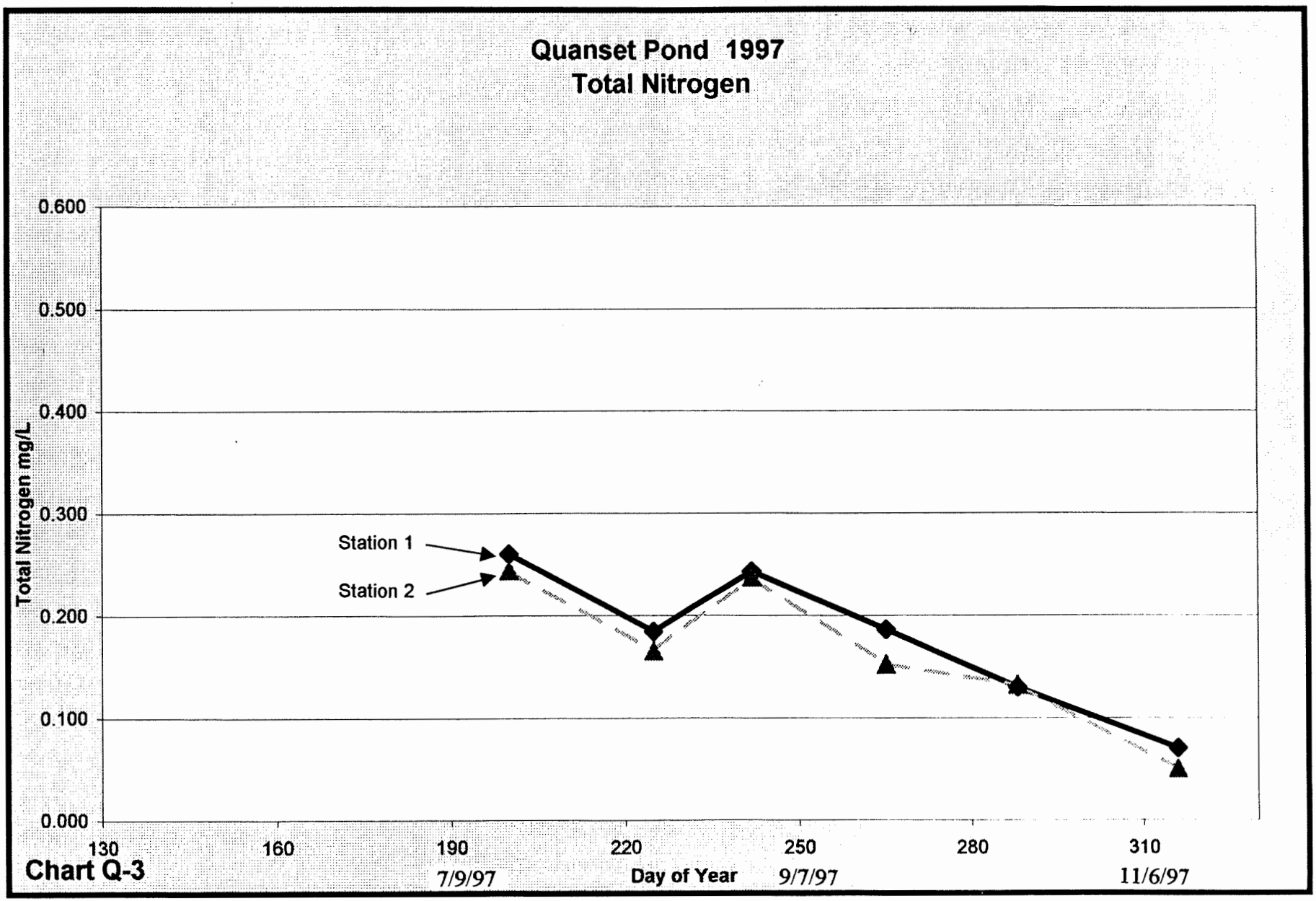
Quanset Pond 1997
Total Nitrogen
Total Nitrogen mg/L
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Station 1
Station 2
130
160
190
220
250
280
310
7/9/97
Day of Year
9/7/97
11/6/97
Chart Q-3

Figure 25

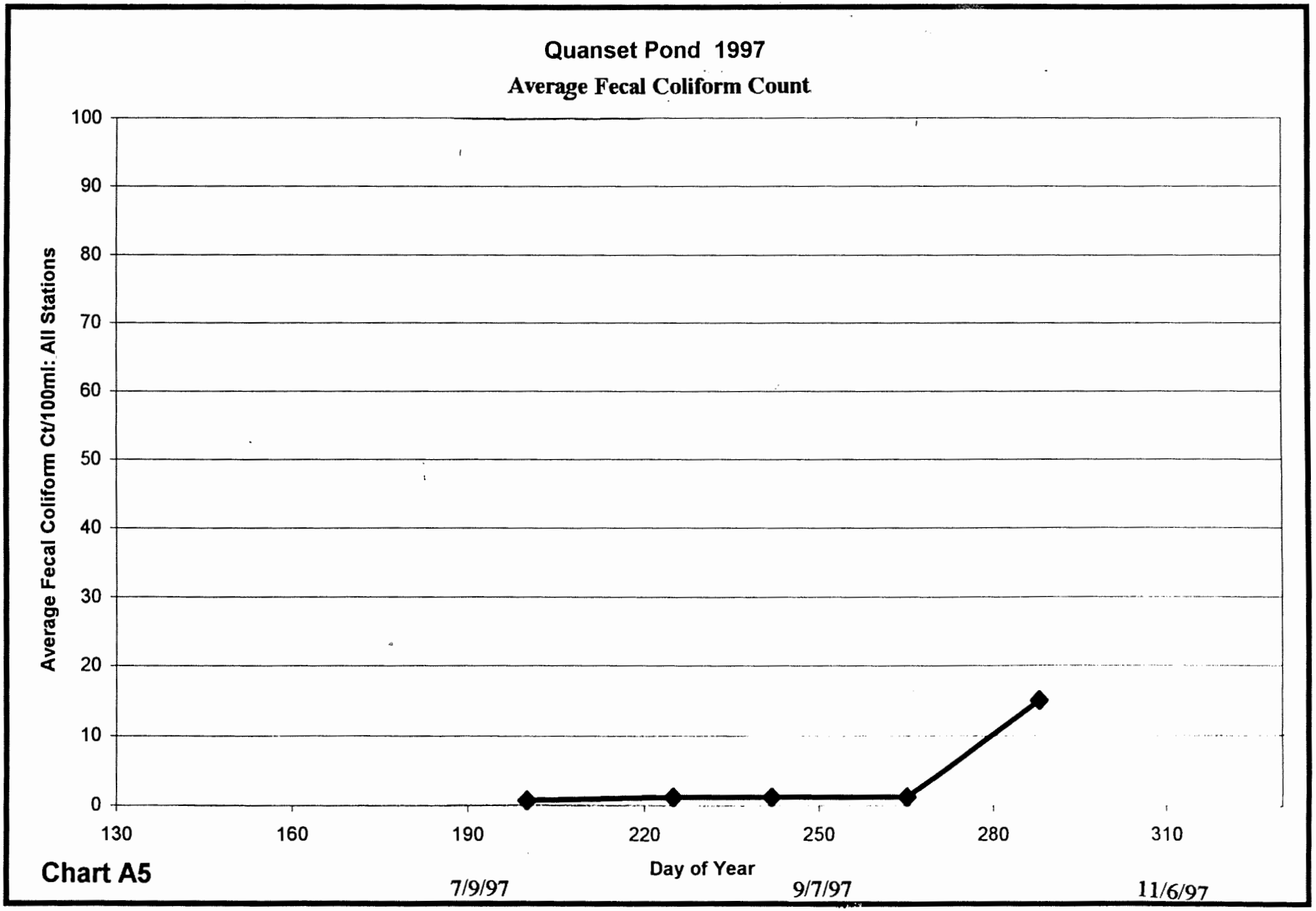
Quanset Pond 1997
Average Fecal Coliform Count
Average Fecal Coliform Ct/100ml: All Stations
100
90
80
70
60
50
40
30
20
10
0
130
160
190
220
250
280
310
Day of Year
7/9/97
9/7/97
11/6/97
Chart A5

Figure 26

CONCLUSIONS AND RECOMMENDATIONS FROM THE 1997 STUDY

CONCLUSIONS

1. The Orleans salt water ponds of the upper reaches of Pleasant Bay show definite signs of eutrophication, most likely due to excess nitrate nitrogen.

2. Kescayo Gansett, and Areys ponds have water quality characteristic of eutrophic conditions as measured by the "Buzzards Bay" Eutrophication Index. Meeting House pond is barely into the fair water quality range.

3. Paw Wah and Quanset Ponds are near the top of the fair water quality range by the same Eutrophication Index, probably because of being closer to the main Bay with more favorable tidal flushing.

4. Fecal coliform counts show that with the exception of Areys Pond, the other four ponds rarely exceeded the shellfish limit. All ponds were at safe levels for swimming in all of our tests.

RECOMMENDATIONS

1. Continued monitoring of water quality is essential to define the extent of the problems, and the effectiveness of any remediation steps.

2. We strongly recommend that remediation steps be planned and promptly carried out. The nitrogen loading from existing development will continue to increase the eutrophic conditions for all of the ponds.

Remediation steps have previously been discussed briefly in the section on Background on Estuaries and their Problems (p.2). See references 17, 21 and 24. The Pleasant Bay Resource Management Plan (Ref.8) discusses options for nitrogen management, and recommends the development of a nitrogen management program within 12 months of the plan's adoption. Segments of such a program involve waste water disposal practices (on-site denitrifying systems, cluster systems, sewering high density areas, etc.) open space acquisition, and land-care practices. Other essential elements are: Fertilizer and pesticide use, storm water management, and public education. Two publications of the *ad hoc* Task Force for Decentralized Waste Water Management (references 22 & 23) should be consulted for additional detail. The most practical and complete description of decentralized wastewater management is given by Hoover (ref 25). Fortunately the Town of Orleans already has a good start on storm water management remediation.

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Statement of Sandra Macfarlane on Conclusions of the Study

a. Estimates of the general "health" of the pond waters:

This report covers the sampling for one year. John Portnoy analyzed nitrogen suite samples at the National Park Service laboratory. Duplicate samples done in the field and at the Orleans Laboratory were not found to be satisfactory because of problems with the test kits. Based on the sampling to date, the waters of the ponds tested for nutrient analysis indicate a cause for concern. Both nitrate nitrogen and ammonia were consistently less than 1 mg/l. and PON was almost always less than 1 but dissolved inorganic nitrogen and the particulate nitrogen were high. The total nitrogen has not been calculated. There was considerable variability with the data for POC and Chlorophyll a. However, with all studies such as this, one year of data, especially the first year, does not tell the whole story and it is only with repeated sampling for several years that a trend would be evident. Using Costa's Eutrophication Index, even with the variability noted above, there is enough data in Table 4 to indicate that three of the ponds, Arey's, Meetinghouse and Kescayogansett fall into the category of eutrophic conditions.

b. Precautionary comments re: water quality for the future:

As part of the Pleasant Bay Management Plan, a flushing analysis was performed for the area tested by the nutrient project. The flushing study showed that the flushing rates of the Pleasant Bay system were faster than had been anticipated. For the entire Pleasant Bay system, the residence time is approximately 1 day. However, if one looked only at the residence time of the ponds, one finds that because of the depth of the ponds and the shallow depth of the rivers connecting the ponds to larger water bodies, the residence time is much greater. If one looks at the residence time of the ponds to the inlet, the time is substantially greater. Therefore, one must be cautious in interpreting residence times for complex systems such as Pleasant Bay. The location of the inlet in Chatham has helped delay the signs of eutrophication over the past 10 years but the data shows that there is reason for concern for the future.

Pleasant Bay is certainly not a static system. It is governed by barrier beach dynamics which, in this system, have been estimated to be part of a 150-year cycle. In 1987, we witnessed day one of that cycle. The breech in Chatham in that year produced some of the most dramatic changes in the hydrodynamics of this system that had been witnessed in the recent past. Tidal range increased, flow of water increased, flushing rates increased. Prior to 1987, upper reaches of the bay had exhibited signs of poor flushing and nutrient enrichment. Algal mats covered large portions of the ponds being studied as part of the current program. Mono-specific blooms of phytoplankton, especially dinoflagellates turned the water color a brownish-red due to the magnitude of the blooms. These visible signs of eutrophication were cause for concern and their existence now is cause for concern for the future. For, as the barrier beach in Chatham migrates south again, the amount of incoming oceanic water will be decreased and the nutrients from the land will again be a cause for concern.

Through the nutrient sampling as well as observations of floating seaweed and "colored water", we can assess the problems inherent in this part of the bay for the future. A concerted effort will be needed to address the problems of nutrient enrichment for the foreseeable future as the inlet is not likely to migrate north, a move that would bring oceanic water closer to the upper ponds.

Statement of Sandra Macfarlane on Conclusions of the Study (cont'd)

c. Recommendations for water quality management, especially for nitrogen loading:

1. Monitoring the waters of Orleans is the single most important activity that the town can do to ward off adverse effects of land use activities. The data collected for 1997 provides a baseline for future reference.
2. Use the groundwater flow maps to greatest advantage in planning for the nutrients getting to the waterbodies. Nutrient-rich groundwater travels at about 1 foot per day in these soils. Nutrients in the groundwater from houses built in the last 20 years, whose method of sanitary waste is the individual septic system, are expected to result in increased amounts of nutrients in the waters.
3. Calculate the amount of nitrogen entering the estuary now based on models that are available. As we continue to develop the land, the amount of nutrients eventually getting to Pleasant Bay increases.
4. Investigate alternatives to the present situation. The additional nutrients coupled with anticipated slower flushing through the barrier beach dynamics would begin to show increased amounts of either phytoplankton blooms or macroscopic algal intensities in the ponds.
5. Keep track of observed changes in water quality in the ponds and attempt to quantify the observations. During the summer of 1998, increased seaweed was observed in Arey's and Kescayogansett. If increased nutrients can be detected as a trend, land use decisions will be easier to support on a broad-based basis. As difficult as it is for resource managers to look at data from one year and want to initiate corrective action, it is unwise to do so without additional data. Planning for the future using the first year data is never a waste of time because it is a precautionary step. If the data from year 2 are similar and match the observations, then planning for remediation is wise. If samples from year 3 are also similar, then implementation of remediation is imperative. On-going monitoring is the best way to ensure that decisions made by the populace are based on sound reasoning.

d. As indicated above, the need for on-going monitoring is extremely important for the future health of the estuaries. The Town of Orleans has consistently monitored fecal coliform bacteria since 1987 in accordance with National Shellfish Sanitation Program guidelines. The addition of nutrient analyses will add immeasurably to our understanding of the processes at work within the estuaries and the land surrounding them. It is extremely important to the Town of Orleans that we continue with this program.

CAPE COD COMMISSION

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February 3, 1999

Bob Wineman
PO Box 306
E. Orleans, MA 02643

RE: Comments on 1997 Nutrient Data from the Orleans Marine Water Quality Task Force

Dear Mr. Wineman:

Thank you for providing me with a copy of the 1997 draft report on the water quality sampling by the Orleans Marine Water Quality Task Force. After reading through the report and reviewing some of the data, which was graciously provided to me by Joe McCarthy, I sending this letter to provide some clarification of the methods used in the Pleasant Bay Nitrogen Loading Study and how the type of analysis in the Study relates to water quality information. I would also like to reiterate that the Commission recognized the limitations (and benefits) of the analysis completed in the Loading Study and this in large part led to the main recommendation of the Loading Study to conduct water quality analyses.

The Nitrogen Loading Study provides an assessment of steady-state nitrogen loads reaching the various subembayments of Pleasant Bay. A steady-state analysis does not account for groundwater time lags in nitrogen discharging along the coast; this analysis assumes that once nitrogen reaches the groundwater anywhere in the watershed, it is discharged into the subembayment. This suggests that nitrogen loads within relatively small watersheds, with shorter flows to the coast, should better match the observed average concentrations than larger watersheds once corrections are made for background concentrations and tidal flushing characteristics. Based on this portion of the assessment the nitrogen loads predicted by the Study should overestimate the nitrogen concentrations observed in the various subembayments.

If one compares the average DIN concentrations with the nitrogen concentrations based on the results from the Nitrogen Loading Study while just considering the sizes of the watershed, the match is fairly good (Figure 1). Average dissolved inorganic nitrogen (DIN) concentrations in Meetinghouse and Kescayogansett Ponds, which have relatively small watersheds, matched the predicted existing nitrogen loading concentrations from the flushing study. Average DIN

concentrations in larger watersheds with longer flowpaths to the coast, Areys and Quanset Ponds, are approximately half of predicted. Only Quanset concentrations do not easily fit within this pattern.

Since DIN is only one portion of the total nitrogen within the embayment, but the predominant form of nitrogen coming from the watershed, the predicted loads within the nitrogen loading study should also account for any of the DIN that has been converted into particulate organic nitrogen (PON) within the embayment. The difficulty is that PON concentrations may also be reflective of regeneration of nitrogen from the sediments within the subembayments. Since interior portions of embayments are often depositional areas for particles from the main portion of the embayment, largely due to diminished tidal flushing, sediments in subembayments can be substantial sources of regenerated nitrogen. Following this reasoning, the comparison of combined DIN + PON concentrations to both the DIN and predicted nitrogen concentration should provide some idea of the potential for sediment regeneration within each subembayment.

So in Meetinghouse and Kescayogansett Pond, the good agreement between the predicted concentrations and the DIN concentrations suggests that a nitrogen load equivalent to the amount coming in from the watershed is being regenerated from the sediments within these embayments. In Quanset, Areys, and Paw Wah Ponds, the groundwater lag time makes this comparison somewhat more difficult. Of course, more refined sampling could help to explore these hypotheses and better calibrate the nitrogen loading analysis in the Nitrogen Loading Study.

Evaluation of the water quality data itself also leads to addition questions for further review. The values in Table 1 present the data from Figure 1 along with the standard deviations from determining the average concentrations. The standard deviations for both DIN and PON at almost all the sites are within a similar range except for the PON concentrations at Areys Pond (stdev > mean). The variability of the PON readings suggest the Task Force may want to consider refined water quality analysis in Areys Pond to help resolve this variability.

TABLE 1. Orleans 1997 Avg. N Concentrations

	DIN	Existing	DIN StDev
Meetinghouse	0.13	0.13	0.05
Kescayogansett	0.14	0.12	0.08
Areys	0.10	0.26	0.04
Quanset	0.07	0.13	0.02
Paw Wah	0.08	0.13	0.03
	PON	Existing	PON StDev
Meetinghouse	0.16	0.13	0.07
Kescayogansett	0.17	0.12	0.04
Areys	0.42	0.26	0.43
Quanset	0.12	0.13	0.04
Paw Wah	0.10	0.13	0.04
	DIN + PON	Existing	
Meetinghouse	0.29	0.13	
Kescayogansett	0.30	0.12	
Areys	0.52	0.26	
Quanset	0.19	0.13	
Paw Wah	0.17	0.13	

More information in environmental assessments often leads to more questions. The water quality information gathered so far suggests that additional study of sediment regeneration should be pursued, as well as consideration of more intensive sampling of Areys Pond to resolve the

variability of the PON concentrations. More data is necessary to evaluate the predictive value of the analyses in the Nitrogen Loading Study.

Also enclosed is a map of sampling sites that you had asked me to prepare. Please let me know if you have any recommended changes. I encourage you and the rest of the Task Force to continue your work and your regular review of the data. I hope these comments are helpful. Please let me know if the Commission can assist the Task Force in the future.

Sincerely,

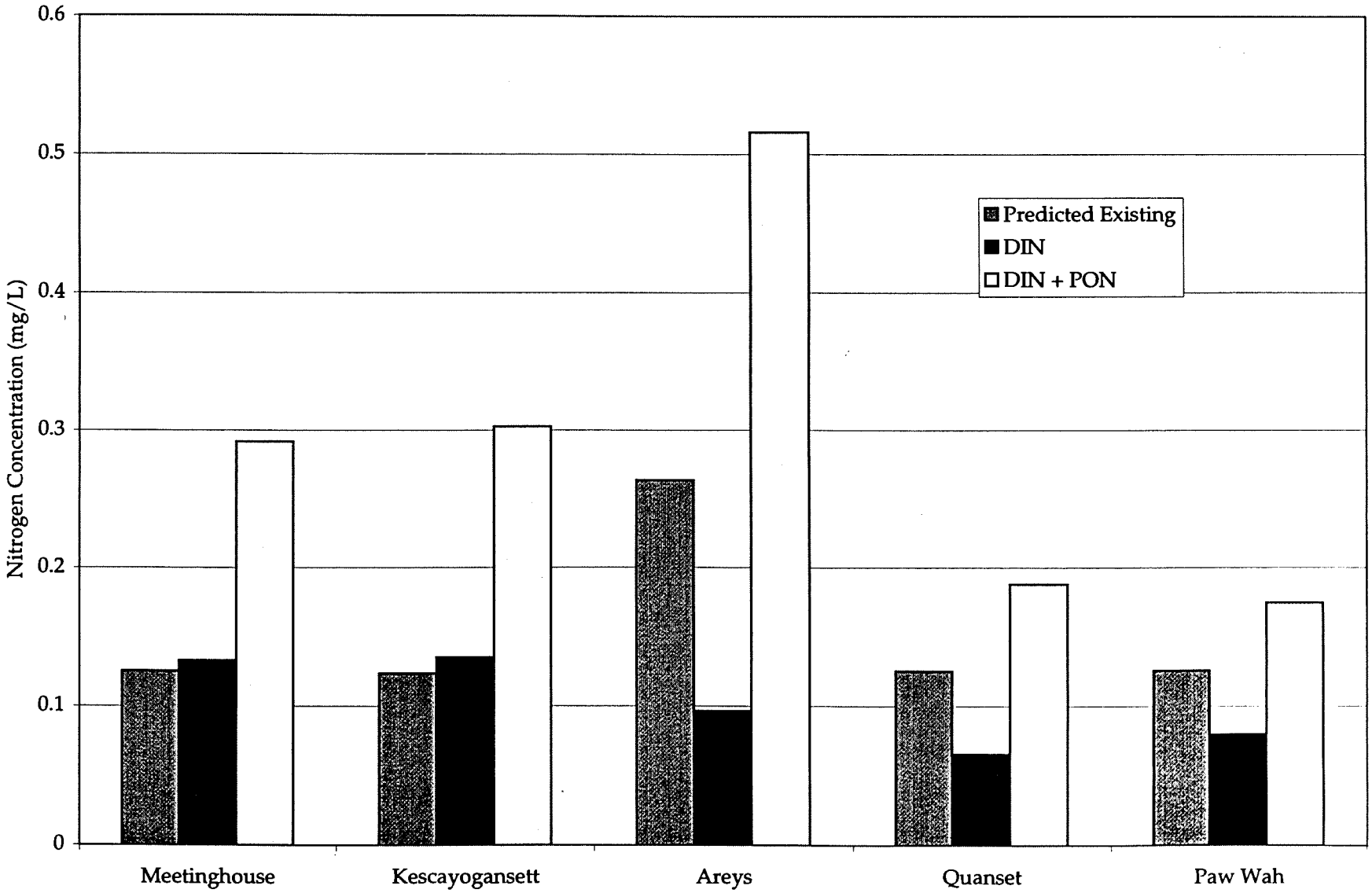
Eduard M. Eichner
Water Scientist

cc: Herb Olsen, Orleans CCC representative

Figure 1.

Orleans Marine Water Quality Task Force

1997 Nitrogen Data

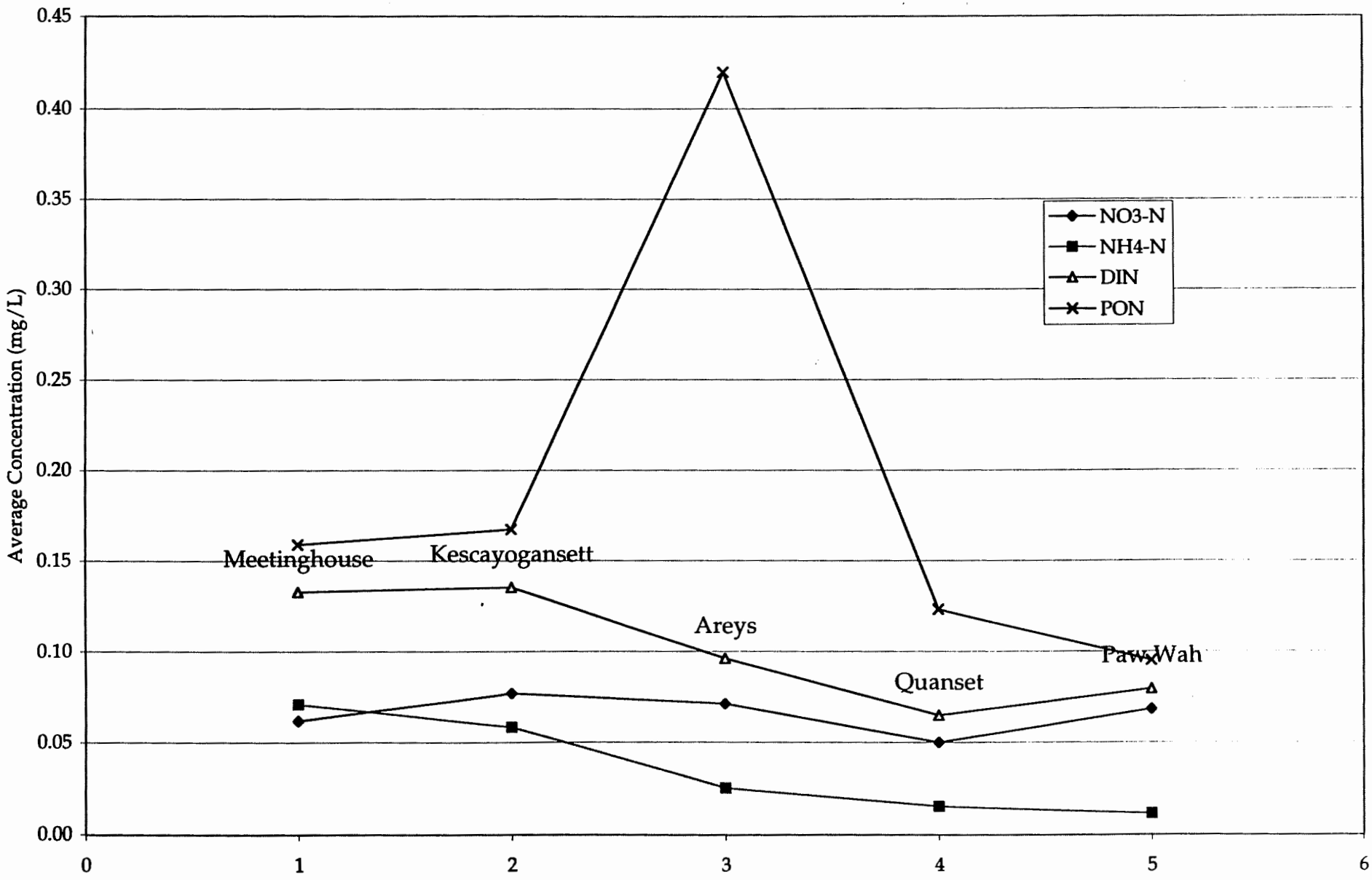


Predicted Existing concentrations based on Cape Cod Commission Pleasant Bay Nitrogen Loading Study
predicted concentrations assume 0.1 ppm background N concentration

by Ed Eichner, Cape Cod Commission
2/3/99

Orleans Marine Water Quality Task Force

1997 Average Nitrogen Concentrations



Assumptions: Averages represent data at both stations; less than detection limit treated as half of limit

analysis by Ed Eichner
Cape Cod Commission

Comments of John Portnoy, Ph.D., Research Ecologist, Cape Cod Nat'l. Seashore

Subject: 1997 Report 1/14/99

Bob and co-workers have produced a very well-written and comprehensive report on the 1997 survey. Although I don't have much time to review it before your 15 January meeting, here are a few comments:

Have you produced and documented a set of standard operating procedures? E.g. for occupying your sampling stations, do you use GPS to ensure boat is in the same place each time? Document in detail how sample bottles are cleaned and stored, how reagents are added, safety considerations, etc. You hear a lot about QC/QA and QAPP's these days, and it is important to standardize and document how you got your data. Perhaps you have already done this as a separate document.

Confusion persists about what to include in "total" nitrogen. Costa includes dissolved organic N (DON), but the Howes group does not, considering it unavailable. Both include particulate forms; however, determining the particulate fraction by combustion and CHN analysis is costly. Although the determination of total N by persulfate digestion may not be as accurate, it may be a fair compromise and adequate as an index for long-term monitoring of nitrogen availability in the water column.

Your data suggest that these ponds are much more sensitive to nutrient inputs than the current modeling and estimated flushing rates suggest. Therefore, the field monitoring is extremely important for pointing out potentially inaccurate assumptions and improving future modeling, as well as for monitoring directly the variables affecting estuarine organisms (including people).

Although this report doesn't address submerged aquatic vegetation, tracking historic eelgrass cover using existing aerial photography sounded promising and I hope that someone can pursue it. Perhaps MCZM can help as a clearing house for remote sensing data and expertise. NPS can also get involved but we need to define a "project".

John

NUTRIENT PROJECT
1997 REPORT

APPENDIX 1: TABULAR DATA

Meeting House Pond

Date Samp. Coll	Day of Yr	Sta. Code	Coll Hr.	Water Temp °C.	Sal inity ppt	Act. Deep m.	Secchi Deep m.	D.O. mg/L	pH	F.C. Ct. / 100ml	NO_3N mg/L	NH_4N mg/L	PO_4P mg/L	PON µg/L	PON mg/L (Cal)	Tot N mg/L. (Cal)	Chl-a µg/L	PHAEO µg/L
5/18/97	138	M1D	1400	13.0		7.5	2.3	5.0	8.0	0								
5/18/97	138	M1S	1500	15.0				7.0	8.2	0								
5/18/97	138	M2D	1600	15.0	28	2.0	1.7	5.0	8.1	0								
5/18/97	138	M2S	1600	15.5	30			8.0	8.2	0								
6/11/97	162	M1D		17.5	27	7.0	1.8	4.6	7.8	0								
6/11/97	162	M1S		19.0	28			5.0	7.9	4								
6/11/97	162	M2D		19.0	26	2.0	1.5	5.6	8.3	0								
6/11/97	162	M2S		19.5	26			5.5	8.1	4								
7/9/97	190	M1D	0800	25.0	30	6.5	1.1	3.0	7.8	0								
7/9/97	190	M1S	0800	25.0	30			5.0	7.8	0	0.06		0.09	331	0.331	0.391	15.96	5.67
7/9/97	190	M2D	0850	25.5	30	2.0	1.2	4.2	7.9	0								
7/9/97	190	M2S	0850	25.5	30			4.0	7.8	0	0.03		0.12			0.030	15.62	2.62
7/23/97	204	M1D	0800	24.0	30		1.8	3.6	7.8	0								
7/23/97	204	M1S	0800	24.0	28			3.9	7.9	0	0.05	0.13	0.19	151	0.151	0.331	8.38	0.26
7/23/97	204	M2D	0800	24.0	28		2.0	3.0	7.9	0								
7/23/97	204	M2S	0800	24.0	27			3.0	7.8	0	0.08		0.19	126	0.126	0.206	7.05	0.39
8/8/97	220	M1D	0800	23.2	30		1.5	3.4	7.7	10								
8/8/97	220	M1S		24.0	31			4.5	8.1	0	0.07	0.21	0.22	221	0.221	0.501	12.99	
8/8/97	220	M2D	0900	24.0	30	2.5	1.5	2,6	7.9	10								
8/8/97	220	M2S		24.0	30			3.4	7.9	5	0.07	0.06	0.16	138	0.138	0.268	10.02	0.91
8/25/97	237	M1D	1000	21.5	27	5.5	1.7	3.8	8.0	5								
8/25/97	237	M1S	1000	22.0	27			3.7	8.0	5	0.09	0.03	0.14	180	0.180	0.300	9.63	3.41
8/25/97	237	M2D	1030	22.0	30	2.0	1.5	3.9	8.0	0								
8/25/97	237	M2S	1030	22.0	29			3.8	8.0	0	0.07	0.04	0.14	144	0.144	0.254	7.39	2.80
9/15/97	258	M1D	1100	21.0	35	5.5	2.2	3.8	7.9	0								
9/15/97	258	M1S	1100	21.0	34			3.6	7.9	0	0.06	0.11	0.18	113	0.113	0.283	0.53	0.22
9/15/97	258	M2D	1130	22.0	32	2.0	2.0	3.8		15								
9/15/97	258	M2S	1130	22.0	32			3.8	8.0	0	0.07	0.12	0.16	100	0.100	0.290	0.64	0.49
9/26/97	269	M1D		18.5	31	6.5	3.2	3.8	7.9	5								
9/26/97	269	M1S		18.0	34			5.2	7.7	0	0.04	0.14	0.10	83	0.083	0.263	0.48	0.45
9/26/97	269	M2D		17.5	34	2.5	2.5	4.6	8.1	10								
9/26/97	269	M2S		17.5	33			4.9	8.1	5	0.04	0.14	0.10			0.180	0.15	0.10
10/9/97	282	M1D	1115	16.5	31	5.5	2.8	5.4	8.1	10								
10/9/97	282	M1S	1115	16.5	31			5.9	8.0	0	0.08		0.03			0.080	0.30	0.10
10/9/97	282	M2D	1115	16.5	31	1.5	1.5		8.3	3								
10/9/97	282	M2S	1115	16.5	37			7.4	8.2	5	0.05	0.08	0.03			0.130	0.21	0.11

Meeting House Pond

11/6/97	310	M1D	11.5	32		6.6	8.1	5							
11/6/97	310	M1S	11.0			6.4	8.2	0	0.08	0.05	0.01			0.130	
11/6/97	310	M2D	11.5	32		10.0	8.1	0							
11/6/97	310	M2S	11.0	34		8.7	8.1	5	0.05		0.005			0.050	

Kescayogansett Pond

Date Samp. Coll	Day of Yr	Sta. Code	Coll Hr.	Water Temp °C.	Sal inity ppt	Act. Deep m.	Secchi Deep m.	D.O. mg/L	pH	F.C. Ct. / 100ml	NO_3N mg/L	NH_4N mg/L	PO_4P mg/L	PON µg/L	PON mg/L (Cal)	Tot N mg/L. (Cal)	Chl-a µg/L	PHAEO µg/L
5/27/97	147	K1D		16.5	27	4.5	1.2	4.2	8.1	0								
5/27/97	147	K1S		18.5	25			6.5	8.0	4								
5/27/97	147	K2D		17.0	26	2.5	1.5	5.2	8.1	0								
5/27/97	147	K2S		18.0	27			6.2	8.1	0								
7/3/97	184	K1D	1800	24.5	31	4.4	1.0	2.3	8.0	0								
7/3/97	184	K1S	1800	25.0	33			6.0	8.1	0	0.03	0.24	0.12	197	0.197	0.467	10.49	1.49
7/3/97	184	K2D	1645	24.5	32	3.0	1.2	2.5	8.1	0								
7/3/97	184	K2S	1645	25.0	31			3.8	8.2	0	0.36		0.12	126	0.126	0.486	8.04	1.93
7/31/97	212	K1D	1530	24.0	29	4.5	1.1	6.4	8.5	10								
7/31/97	212	K1S	1530	24.5	30			6.7	8.6	0	0.06	0.03	0.14	181	0.181	0.271	14.55	
7/31/97	212	K2D	1500	24.0	30	3.5	1.5	6.7	8.4	10								
7/31/97	212	K2S	1500	25.7	30			6.5	8.5	10	0.08	0.23	0.10			0.310		
8/15/97	227	K1D	1347	24.0	32	4.3	1.3	4.3	8.0	5								
8/15/97	227	K1S		25.0	31			5.8	8.4	30	0.06	0.17	0.10	205	0.205	0.435	12.58	0.57
8/15/97	227	K2D	1347	24.0	31	2.9	1.5		8.1	75								
8/15/97	227	K2S	1347	25.0	30			5.4	8.3	0	0.09	0.01	0.14	188	0.188	0.288	12.10	0.12
8/28/97	240	K1D	1507	22.5	33	4.1	1.6	4.6	8.1	0								
8/28/97	240	K1S	1507	23.5	33			4.8	8.2	5	0.06		0.15	200	0.200	0.260	12.39	
8/28/97	240	K2D	1420	22.0	33	3.1	1.7	4.3	8.0	0								
8/28/97	240	K2S	1420	23.0	33			5.6	8.0	0	0.07		0.14	179	0.179	0.249	8.51	0.06
9/16/97	259	K1D	1620	21.0	34	4.6	1.4	3.8	8.1	5								
9/16/97	259	K1S	1620	21.5	35			4.4	8.1	5	0.05	0.08	0.15	211	0.211	0.341	2.12	0.30
9/16/97	259	K2D	1532	22.5	34	3.8	1.5	3.6	7.8	5								
9/16/97	259	K2S	1532	22.5	34			5.0	8.1	0	0.05		0.16	164	0.164	0.214	1.33	0.60
9/30/97	273	K1D	1530	17.0	34	5.0	1.7	4.6	8.1	0								
9/30/97	273	K1S	1530	17.5	34			5.2	8.1	0	0.04	0.01	0.10			0.050	0.45	0.22
9/30/97	273	K2D	1530	17.5	36	3.7	2.3	4.7	8.1	0								
9/30/97	273	K2S	1530	17.5	32			5.1	8.0	0	0.04	0.07	0.10			0.110	0.38	0.21
10/14/97	287	K1D	1610		36	4.0	2.4	6.0										
10/14/97	287	K1S	1610	15.5	36			5.4	8.2	5	0.04		0.02	92	0.092	0.132	1.14	0.50
10/14/97	287	K2D	1610	16.0	37	3.2	3.2	5.1	8.1									
10/14/97	287	K2S	1610	15.5	37			5.4	8.1	5	0.09		0.02	95	0.095	0.185	1.14	0.61
11/13/97	317	K1D		9.5	38	4.4	2.5	7.2	8.1	0								
11/13/97	317	K1S		10.5	36			7.0	8.3	0	0.04	0.01	0.007			0.050		
11/13/97	317	K2D		7.5	36	3.0	2.0	7.0	8.3	0								
11/13/97	317	K2S		8.0	37			6.9	8.3	0	0.07	0.01	0.007			0.080		

NUTRIENT PROJECT
1997 REPORT

APPENDIX 2: LABORATORY PROCEDURES FOR REMEDIATION AND NUTRIENT PROJECTS (REVISED FOR 1998)

AP

CONTENTS

LABORATORY PROTOCOL

Wash hands when entering and before leaving the laboratory.

Wipe off with cleaning solution the bench surface to be used.

Check temperature of thermometers in the incubator(44.5 C.)and the refrigerator(4-8 C.)

ALWAYS enter your name and date of visit in the PINK spiral record book. Record your activities. Note any needs or deficiencies.
Keep BLACK Procedures Book, RED Lab Report Book, and the PINK Spiral Record Book in far right top drawer. **Please do not remove these notebooks from the lab.**

Return all lab equipment to its proper place(see labels), while working or before leaving.

SAFETY

Normal precautions should be exercised in handling all laboratory solutions and chemicals. When in doubt about the hazardous nature of a material, assume the worst until someone with the proper knowledge can be contacted.

The incubation of microorganisms is associated with certain risk factors and normal micro biological safety procedures should be practiced. Follow directions and see washing and sterilization procedures (pp.1,2,&2A). Special precautions should be taken in handling incubated culture dishes. The fecal coliform method in use in our laboratory(M-FC Agar) is designed to maximize the growth of fecal coliform bacteria and minimize the growth of other organisms. This is accomplished by the nutrient composition of the media and the specific incubation temperature. While it is unlikely, there is however a risk of incubating unknown organisms, possibly pathogens. Extreme care should be exercised when counting colonies and in sterilization of the dishes before they are discarded. **Please wear gloves and eye protection when handling cultured Petri dishes.** Dishes should be opened only when necessary. Counting is preferably done with the lid in place. If it is necessary to open the Petri dishes to see colonies clearly, keep face as far away as possible. Avoid breathing in the aerosols that can be produced simply by removing the lid. Hands should be thoroughly washed after handling open dishes, and they should not touch eyes, mouth, or face as a routine procedure.

Common sense safety precautions should be followed in all cases.

Food or beverage intake is prohibited in the laboratory.

WASHING AND STERILIZING GLASSWARE

Cleanse all glassware thoroughly with detergent and hot water. Rinse(three times, at least) with hot water to remove all traces of residual washing compound, and finally rinse with distilled water. Drain to dry and store on cabinet shelf.

Sterilize glassware in the autoclave at 121 degrees C. for 15 min. (See directions below).

STERILIZING COLLECTION BOTTLES

Bottles to be washed and sterilized are stored under the sink. Remove **all labels** from lids and bottles **except** the collection bottle numbers (Orl. plus 3 digits on the side of the bottle, for example: Orl. 875, Orl. 201, etc.). When present, remove small white labels which have letters and numbers, such as: M-1-D, or A-2-S, etc., which are temporary identification for the Nutrient Project.

Scrub with brush, inside and out, bottles and lids in hot soapy water (VERY strong soap use only a few drops). Invert on the drain board to drain. Then rinse 3 times in tap water, BE SURE ALL SOAP IS REMOVED! Invert on drain board. Attach firmly about 1 1/4" of autoclave-sensitive tape to each bottle lid and screw lids onto the bottles one turn. **Be sure lids are loose enough to let steam pass freely in, but not ready to fall off !)**

Remove the two-handled aluminum bottle holder from the big kettle. Occasionally, clean out the bottom of the kettle, if needed, and re-fill the bottom of kettle with distilled water, just to cover the top of the thermostat shield (small horizontal pipe visible at the bottom of the kettle). Do not put water in the aluminum bottle holder. Plug in the pressure cooker, switch to "ON" (red light), and turn dial to "high". Place clean, loosely capped bottles upright in the aluminum holder and position it so that the slot on the inside wall is 90 degrees to the right from front of kettle. Then position the kettle lid so that the flexible tube fits into the slot on the inside wall. Line up the two arrows. Put one of the lid screw clamps into its slot and turn until it is slightly tight. Go to the screw clamp directly opposite and tighten it similarly. Screw all clamps down, alternating sides, so the lid will be uniformly adjusted. Repeat until completely tight. **See that the steam vent is in open position** (vertical)!
When the water inside reaches boiling, you will see steam from the vent. Wait until there is a good flow of steam and then allow it to flow five more minutes to evacuate kettle before closing vent, by pushing it over at a 90 degree angle (**Hot! Use pot holder**).

(CONTINUED--NEXT PAGE)

Allow considerable time for the kettle to reach 15 pounds pressure on the dial (121 C.) When it does, set the timer for 15 minutes and adjust the dial to maintain the pressure at 15 pounds (frequent adjustment is usually necessary). After 15 minutes, turn off the pressure cooker, unplug it, and allow it to cool so the pressure falls to 5 pounds.(DO NOT ATTEMPT TO OPEN THE COOKER UNTIL COOL). If pressure is reduced rapidly, plastic bottles will collapse.

At this point, the pressure cooker can be left overnight or longer with the vent closed, prior to unpacking. Leave the sterilizer electrical cord (temporarily) in the far right-hand drawer.

When the pressure cooker is completely cooled, the lid screw clamps can be unscrewed, alternately across the kettle. Twist the lid and raise it to release the vacuum. This may require insertion of the large screw driver (from tool drawer) in between the kettle-base and the lid to pry the lid up. Lift the lid off. Then, **be sure to screw each bottle lid down tightly**, as it is removed from the sterilizer. Put sterile bottles in a plastic bag and return to the sampling coordinator, (Joe McCarthy: 2554648).

Store the sterilizer electrical cord inside the kettle, so it doesn't get lost.

Record activities in the Pink Laboratory Record Book. Date and Sign.

STOCK SOLUTIONS AND DILUTION BUFFER

General instructions: For preparation of stock solutions, use sterilized large plastic bottles with screw caps (top shelf, far right upper cabinet).
Shelf Life: Discard any buffer solution which looks cloudy, or has precipitate. Discard all solutions at the end of the laboratory "season".

STOCK SOLUTIONS

Safety note: Use special care and eye protection (goggles and gloves recommended) when handling concentrated hydrochloric acid (HCl) and sodium hydroxide (NaOH). THEY CAN CAUSE BURNS! NEVER ADD WATER TO CONCENTRATED NaOH.

To prepare STOCK POTASSIUM PHOSPHATE solution:
Wash the stainless steel tray on the Ohaus weigh scale, an dry thoroughly. Adjust tare.
*Rinse a clean 1L (1000 ml.) TALL glass beaker with distilled water (three times). Measure 500 ml distilled water into the beaker. Then dissolve in it 17.0 grams of potassium dihydrogen phosphate (KH_2PO_4). Store this stock solution in a sterile plastic reagent bottle (top shelf). Label bottle with "stock potassium phosphate solution" with date and your initials. Keep lid tightly closed.

To prepare STOCK MAGNESIUM CHLORIDE solution:
Proceed, as above*, to rinse the TALL 1L beaker and measure 500 ml distilled water into it. Then dissolve in it 40.5 grams of magnesium chloride hydrate ($MgCl_2.6H_2O$). Store this stock solution in a sterile plastic reagent bottle. Label bottle with "stock magnesium chloride solution" with date and your initials. Keep lid tightly closed.

DILUTION BUFFER:

For pH adjustment, you will need to calibrate the pH Meter, following the accompanying printed instructions.
Then rinse a clean 1500 ml. beaker with distilled water (three times). Measure 1L (1000 ml.) distilled water into the beaker. Using a sterile graduated 10-ml. plastic pipette, add 1.25 ml of stock potassium phosphate solution (discard the pipette). Using another sterile plastic pipette, add 5.0 ml of stock magnesium chloride solution, and use this pipette to stir your solution in the following step: USING EYE PROTECTION AND GREAT CARE, adjust the pH to 7.2, plus or minus 0.5, drop by drop (use plastic transfer dropper) with 1 N sodium hydroxide (NaOH), (1 or 2 drops should be sufficient). Rinse the pH meter and wipe with Kimwipes between uses. Store this dilution buffer solution in a large sterile plastic bottle. Label bottle "Dilution buffer" with date and your initials. Keep lid tightly closed.
Record activities in the PINK Lab Record Book with names of lab workers. Date and sign.

MEDIA PREPARATION

Rinse thoroughly with distilled water, a 500 ml. or larger Erlenmeyer flask. Using the large graduated cylinder (labeled "for distilled. water only"), kept in the far-right middle drawer, measure 200 ml of distilled water into the flask.

Place a weighing paper on the Ohaus scale and adjust scale for the tare weight.

Weigh out 3.0 grams of M-FC agar (from the small glass screw-top jar, so labeled) and add it to the water in the flask. Swirl the flask to dissolve the agar.

Place the flask on a hot plate (setting 2 to 3 is suggested, so as not to boil over) and bring just to a low rolling boil. **Safety Note: Use pot holder or gloves to handle the hot flask.** Remove from the heat, and allow to cool for a few minutes, swirling occasionally.

Retrieve (2nd from right drawer) the digital Celsius thermometer with steel probe (in white box). Remove the sheath and turn the right-hand thermometer switch to "ON". Left-hand switch should read degrees C. Lower the probe (CAREFULLY) into the liquid in the flask, and allow to cool to 45-47 degrees centigrade.

While the agar is cooling, line up 35 to 40 small Petri dishes on the bench, and loosen all their lids so they can be raised with one hand.
When the agar reaches 45- 47 degrees C., remove lids and pour each Petri dish about half full of media, and replace the lids. **Note:** leave the plates flat on bench without moving until the agar hardens.
Then place culture dishes in rack marked "A-1,2,`3,4" or "B-1,2,3,4". Store in the refrigerator.
Wash the flask with hot water (if necessary, heat water in the flask on the electric plate) to remove any congealed agar. Then rinse thoroughly with tap water, then with distilled. Drain to dry.
Wipe the thermometer probe with alcohol to clean, replace sheath. **Be sure to turn switch** to "OFF" before returning thermometer to the white box.

Replace all equipment in its storage location and clean the Lab bench.

Record date, number of dishes in each stack, and your initials on sheet on refrigerator door. The plates can be used up to two weeks after preparation.

Briefly record the media preparation (no. of dishes, etc.) in the PINK Spiral Laboratory Record Book. Date and sign.

RUNNING FECAL COLIFORM TESTS

Retrieve Black Procedures Book, Red Lab Report Book and, Pink Lab Record Book.

In the new RED Lab Report Book, on the WHITE LAB REPORT SHEET (using the CARDBOARD DIVIDER and BALL-POINT PEN), press down heavily and record WITH CARE the Collection Sample Bottle Numbers, in the order they appear on the Field Report Sheet. Label two empty lines for "Dupes" and one for "Blank". If any sample needs to be run in three dilutions, the Coordinator will note this on the Sample Report Sheet, otherwise our standard procedure is to run each sample two dilutions,: 0.1 ml. and 10 ml., plus 1 Blank, and two "Dupes" each testing session.
Be sure to fill in the names of the testers (top of Report Sheet) and record observations (see bottom of Report Sheet)

Safety Note: UV light for the Millipore sterilizer can damage your eyes, Use care not to look at it as the lid is opened or closed.

Check and record temperature of refrigerator (4-8 C.)and incubator (44.5 C, +0.2 C.) Clean the bench top before starting the procedure.

Start each testing session with two new, sterile, 10-ml pipettes (pipette drawer) which can thereafter be sterilized in the UV Box for remainder of the day's testing. When testing is complete discard these pipettes (this is to prevent chloride build-up).

Place the tops and bottom portions of three glass filter holders in the Millipore UV sterilizer box. Also place the two new pipettes (above), two forceps, a 10 ml graduated cylinder, and a ten ml dilution vial into the sterilizer. (If filters are dirty wash with soap and water, drain, immerse in clean water, then rinse three times with tap water-get all soap out!- and a final rinse with distilled water.) Turn the power on the sterilizer unit and shut the lid. Set the electric timer for the three minute sterilization cycle. When timer rings, open sterilizer and remove bottoms of two or three filters, depending on the number of sample dilutions being run, and place them in the filter manifold. Open the manifold valves on the positions in use. Connect the hoses from the manifold to the drain flask and the vacuum pump.

Safety Note: Place alcohol burner where its location will not be a burn hazard, and use special care to avoid burning your skin or clothing!

(CONTINUED--NEXT PAGE)

Place one set of forceps in a 90% + isopropyl alcohol bath, use mechanical lighter to ignite alcohol burner, using isopropyl alcohol for fuel as needed. Remove forceps from the alcohol and pass through the burner flame. Open a sterile filter package. Using sterile technique, use the sterile forceps to remove the filter from its package, and place it GRID SIDE UP onto a funnel base. Carefully place matched funnel unit over receptacle, and the clamp tightly ON THE RIM ONLY (TO PREVENT LEAKAGE). Prepare the other funnels with filters in the same way.

Using a wash bottle, pour buffered water up to the lip of the bottom of the funnel, and filter under partial vacuum. (Note: When filtering bacteria suspensions, adjust the right hand valve so as to limit the vacuum applied to the filter to approximately 5-15 inches of mercury, or 1/3 to 1/2 an atmosphere, or 33- 50 kPa, or 5-7.5 psi, to avoid damage to the bacteria cells.) Check for leaks at the join of the funnel and the filter base by sucking a small amount of distilled water through the filter. You may need to back-off the filter clamp to hold the rim only. Shake the sample bottle at least twenty times to get a good distribution of the bacteria, and any sediment in the sample which may have adhering bacteria. (Be sure to record observations of sediments, etc. on the Report Sheet).

Attach a sterile pipette to a pipette pump. NOTE: When emptying a pipette, do not PUMP the liquid all the way out. Release
by removing the pump and allowing the liquid to drain out.
Draw up 11 ml of shaken sample into the pipette, release 1 ml of sample into one filter and 10 ml into a second filter. Filter samples under partial vacuum. Rinse* down walls of funnel using 5-10 ml of buffer solution from the wash bottle of buffered water. Repeat the* rinse two more times. Filter the rinses under partial vacuum.
Turn vacuum pump on only when it is needed for filtration, then shut it off, and release the vacuum GRADUALLY.
Note: If high counts are expected and you wish to run a 1/10 ml sample (as well as the 1.0 and 10 ml), the procedure is as follows: Set up a third filter. Pipette 1 ml of sample into a dilution vial from the sterilizer. Add 9 ml of dilution buffer from a sterile 10 ml graduate, cap and shake. Using another sterile pipette, draw up some of this 10 to 1 dilution sample from the vial, and release 1 ml into a third filter, rinsing as described above*.

Set out the required number of prepared Petri dishes. Turn the dishes over and label the bottom of each dish with the code for the sample location and the volume of sample filtered through each filter. Use a blue or black marking pen and write clearly on the "etched" portion of the bottom of the plate. Example: BNF 10, is the label for a 10 ml sample from the Barley Neck Float site. (Take care to label Petri dishes correctly!)

(CONTINUED---NEXT PAGE)

Remove the top of the first funnel. Remove the cover of the first Petri dish. Remove the forceps from the alcohol and pass them through the alcohol flame. Using sterile technique, remove the filter membrane from the filter base and place it on the agar plate, grid side up, with a rolling motion to avoid entrapment of air. Carefully remove any air bubbles observed between the membrane and medium surface. Replace the dish cover.

Handle the other dishes from the same sample in a similar manner. Most frequently a 1 ml and a 10 ml sample are run on each site. If high counts are expected a 1/10 sample is run by the dilution of a 1 ml sample to 10 ml as described above. If low counts are expected a 20 ml sample might be used, instead of the 10 ml..

Note: One "blank" sample, using approximately 10 ml of dilution buffer, should be run at each testing session to insure that our reagents are not contaminated; plus, please run duplicate tests on two samples, chosen at random (EPA quality control).

Check to make sure that the incubator has been turned on and the temperature is at 44.5 degrees C plus or minus 0.2 degrees C. When all Petri dishes from the same sample have been prepared, the dishes are placed **up side down** (i.e. gel-side up)in the incubator.

Store remaining samples in the refrigerator for pH and salinity testing. Empty any pipettes or dilution bottles used and place them in the UV Sterilizer along with filter tops and bottoms, Close the lid and set timer for 3 minutes to sterilize all components.

Select the next sample to be run using the same procedures.

Precaution: Always wash hands after handling samples.

When all samples have been run, rinse thoroughly, the filter tops and bases, and dilution equipment. Shake off all excess water (so as not to drip on the lamps) and replace all equipment used in the UV sterilizer. Close the sterilizer and set timer for 3 min. to sterilize. Empty the suction flask connected to the vacuum pump, rinse with tap water, and replace. Turn off all equipment (except for the incubator and refrigerator!), and remove electrical plugs. Replace equipment in its storage location and clean the laboratory bench.

In the Pink Spiral Lab Record Book, please make a record of the date, number of samples run, no. of Dupes, Blank, etc. Include the names of the testers, as well as comments, suggestions or needs. Please sign this record.

The culture dishes are to be incubated for 24, plus or minus 2, hours, before counting. PLEASE notify the person designated to do the counts the following day as to the time when all the culture dishes were in the incubator.

COUNTING

NOTE: Before beginning the counting, please remove the water samples from the refrigerator, and place on the bench in the order of the tests, to allow them to reach room temp. by the time you wish to test pH, etc.

SAFETY NOTE: Please review the safety precautions (Page 1) on the handling of incubated culture dishes. WEAR GLOVES AND EYE PROTECTION when handling incubated Petri dishes.

Colonies produced by fecal coliform bacteria on M-FC medium are various shades of blue, are discrete dots, or larger, and are usually round. Do not count large light-blue areas, lacking a discrete colony. Do not count non-fecal coliform colonies which may be gray to cream colored. Normally, few non-fecal colonies will be observed. Take care to distinguish small particulates from colonies, especially if sediment is observed in the sample.

Count colonies with a low-power (10 to 15 magnifications) binocular wide-field dissecting microscope. A hand-held magnifier is available in the middle drawer, if it helps.

MICROSCOPE

The binocular microscope is a fairly rugged instrument, but can be damaged if carelessly handled. It has two separate light sources, one above the specimen stage and one below. Both sources are controlled by a single switch and the switch has two different brightness positions for each source. In counting cultured colonies the lower light source is used. In using the microscope, the focusing knob is turned clockwise to the lowest position, making sure that nothing can contact the objective lens. The unopened Petri dish to be counted is placed on the glass stage and the microscope body slowly raised by turning the knobs counter-clockwise until the membrane grids are in focus. After counting, turn the light switch to the off position and unplug the cord. Replace the cord and return microscope to lower cabinet.

SPECIAL PROCEDURE FOR LIQUID IN PETRI DISHES

Place a large (1 L.) glass beaker on bench next to the incubator. Fill to 500 ml. with tap water.

Remove ONE cultured Petri dish from the incubator WITHOUT TURNING IT OVER (i.e.gel-side up, as it was cultured). Examine the dish for liquid accumulation in the lid (that is, at the bottom). If liquid is present, CAREFULLY open the dish and drain the liquid into the beaker of water (this will be sterilized later). Then replace lid and turn dish over to count. Examine each cultured dish as you remove it from refrigerator.

(CONTINUED---NEXT PAGE)

CALCULATION OF FECAL COLIFORM DENSITY

The desired range of colonies for optimum accuracy is to have 20-60 fecal coliform colonies on the plate. Also it is recommended not to have more than 200 colonies of all types per membrane.
With water of good quality, the occurrence of fecal coliforms generally will be minimal. Therefore, count all fecal coliform colonies (disregarding the lower limit of 20 cited above)

It is preferable to make counts with the lids closed, but at times condensation under the lids requires that the lids be raised. Follow safety precautions.

Count the blue colonies, ~~row~~ by row, using the grid on the filter paper. If colonies are numerous use the manual counter (middle drawer) to keep track of the count. Ask partner to re-count, as a check (average counts if you disagree, or re-count). Then record the count in the RED Lab Report Book on the WHITE Report Sheet (Use CARDBOARD DIVIDER and BALL-POINT PEN and press firmly). Be sure to follow the Code numbers carefully, and to use the correct column for the dilution.

If confluent growth occurs, that is growth covering either the entire filtration area or a portion thereof, and colonies are not discrete, report results on the Report Sheet Observations as "confluent growth with (or without) fecal coliforms". If the total number of colonies exceeds 200 per membrane, report results as > 200. For either "confluent growth", or >200, write a request on the Report sheet for a sample from the same location, with more appropriate volumes to be filtered per membrane, for the next round of testing.

Compute fecal coliform density by the following equation:

$$\text{(Total) fecal coliform colonies / 100 ml} = \frac{\text{fecal coliform colonies counted X 100}}{\text{ml sample filtered}}$$

For the usual dilutions(0.1, 1.0, and 10), this formula is equivalent to multiplication, as follows: Number of colonies in the 10 ml. column x 10 = colonies / 100 ml
" " " " " 1.0 ml " x 100 = " "
" " " " " 0.1 ml " x 1000 = " "

If there are conflicting results, from different dilutions of the same sample, place greater reliance on the larger, or largest sample filtered, unless the upper limits for accurate counting are exceeded by the larger sample. In the latter case, use the lesser dilution.

(CONTINUED--NEXT PAGE)

RECORDING DATA

Record all counts on the Laboratory Report Sheet (RED Lab. Report Book). Be sure the cardboard divider is in place, and use ball-point pen. ALSO CALCULATE AND RECORD the total fecal coliform colonies/ 100 ml. Check each sample for sediment, particulates, color, oil, turbidity, etc. and record in the "Comments" column. Data entries should be double-checked by the lab partner and both names should appear at the top of sheet.

After counting is finished, discard any Petri dishes which showed no colonies of any type directly into the laboratory trash container. Place all dishes with colonies in the large glass beaker for sterilizing, as follows:

CLEAN-UP AND DISPOSAL

Safety Note: Use eye and hand protection when handling sodium hypochlorite (bleach) solutions.

Bleach is kept in the cabinet under the sink. Bring the large glass beaker, used for disposal of liquid from Petri dishes, and place in the sink. With CAUTION add 50 ml of concentrated bleach to the water in the beaker and stir carefully, using the stainless steel tongs (from the tool drawer). Move the plates around, removing lids, so the entire load contacts the bleach. Allow to stand for 5-10 min., then carefully pour the liquid down the sink, rinse the dishes, etc. with tap water, drain, and discard in the trash. Wash tongs and beaker and leave to drain, then dry and store.

Clean any counter space used.

Proceed to the determination of pH and Salinity.

SALINITY MEASUREMENT

Your portable refractometer (black snap case, left middle drawer) is a precision optical instrument, designed to measure the concentration of salt in aqueous solutions. The larger the concentration of salt the higher the reading.

Start with a micro-beaker (left middle drawer) to hold samples for testing. Work over a 250 ml glass beaker ("clean glass" shelf, upper cabinet) to catch drips and used sample water. Obtain 3 or 4 new wiping cloths (zip-baggie, left middle drawer), and the distilled water squeeze-bottle from left cabinet, for rinsing.
Remove the refractometer and small plastic pipette from the case.

To operate the refractometer:

1. Pour water from the first sample bottle into the micro-beaker. With the plastic pipette, place several drops of first sample on the prism and close the prism cover plate so the solution spreads evenly. If the blue area is not covered completely, open the cover and add more drops of sample.

2. Keeping the instrument as level as possible, aim the front of the refractometer toward the Laboratory ceiling lights. Adjust the eyepiece for clearest focus on the boundary between the light and dark hemispheres (where the blue ends). Read the right-hand scale (each division =1).

3. The boundary of light and dark will indicate the ppt (parts per thousand) salt of the sample. Report salinity ppt on Lab Report Sheet (RED Laboratory Report Book). Be sure to use the CARDBOARD divider and BALL-POINT). Have each determination checked by Lab partner.

4. After each use, clean prisms with a few drops of distilled water from the distilled water squeeze bottle (cabinet shelf), and wipe with a cloth. (DO NOT use a paper towel, etc., it will scratch the surface.) When a wiping cloth becomes too wet, discard in wastebasket, and get a new one. DO NOT run the refractometer unit under water, or allow water to seep into the internal sections.
Discard the tested sample into the glass beaker, and shake out the micro-beaker, thoroughly. It is now ready for the next sample. Squeeze out the pipette, thoroughly and SHAKE the water out of the tip. It may now be used for next sample.

Proceed to the next sample.

(CONTINUED NEXT PAGE)

When all samples have been tested for salinity, rinse the pipette with distilled water three times: Squeeze dist. water in, shake pipette up-side-down, then squeeze dist. water out and shake the residual from the tip. Replace pipette in the black case.
Be sure to clean the prism of the refractometer as in step #4 above, using a clean cloth.
Replace unit in its case and return to left middle drawer.
Refill the distilled water squeeze bottle and return it to the cabinet shelf.

Proceed to pH, page 13.

MEASUREMENT OF pH

Calibration of the pH meter: You will need Kimwipes and a plastic micro-beaker (from cardboard tube in left middle drawer) filled about 3/4 full with distilled water for rinsing. Take from upper cabinet a 250 ml glass beaker for collecting used sample water during testing, for later disposal in the sink.

Remove the pH 7 Buffer (yellow liquid, second shelf of upper cabinet) with labeled micro-beaker on top. Pour a small amount of the buffer solution into the micro-beaker.

Take the pH meter from its box, and remove the protective cap from the electrode. Rinse the electrode tip in the distilled water., and wipe "dry" with a Kimwipe tissue.
Turn the pH meter switch ON. Immerse the electrode in the pH 7 buffer. Swirl the buffer slightly, then allow the reading to stabilize. If the meter reads pH 7.0 +/- 0.3, rinse electrode in distilled water, wipe with tissue and proceed to measure the pH of each sample, as described below.

If the pH meter reads outside the range above after the reading has stabilized, adjust the meter reading to pH 7.0 by adjusting the left hand brass screw on the top of the meter with the small screw driver provided. To the left lowers; to the right raises. Remove meter from the buffer, rinse with distilled water, wipe with tissue, and proceed with measurements of the samples.

To measure the pH of each sample: Pour some of sample into a small disposable beaker to about half full. Immerse pH meter electrode in the sample and swirl gently. When reading stabilizes, record pH to nearest 0.1 unit, on Report sheet and in the RED Laboratory Report Book. (Remember the DIVIDER and BALL-POINT). Always have the reporting checked by the Lab partner, as the determinations are made.

Remove the electrode, wipe with Kimwipe, and rinse it in distilled water. It is now ready to use for the next sample. Discard the tested sample water in the glass beaker and shake the micro-beaker out thoroughly. It can then be used for the next sample. Continue, as above, until all samples have been tested for pH.

After testing is complete, turn the pH meter OFF. Pour a few drops of buffer into the pH meter electrode cap. Replace cap, and wipe exterior of meter with a Kimwipe tissue. Place meter in its box along with screw driver, and the box in the drawer.
Discard the used pH buffer and the used sample water in the sink. Return the bottle of buffer to the cabinet with the labeled beaker on top. Wash out the glass beaker ,rinse thoroughly, drain to dry, and replace in cabinet.

Proceed to ~~determination of salinity of the samples~~ REPORTING AND RECORDS, page 14

REPORT SHEETS AND LABORATORY RECORDS

Open the Red Report Book rings and remove the WHITE Report Sheet ONLY. Please leave the yellow and pink sheets in the Report Book. The Laboratory Report Sheet and the Sampling Report Sheet are to be delivered to the Coordinator (Joe McCarthy, 255-4648).

Please record, briefly, your day's activities in the PINK Laboratory Record Book, plus any comments or needs. SIGN and DATE.

When counting, salinity,
and pH testing has been completed, dispose of all Field Samples by pouring down the sink and rinsing the bottle and cap with tap water. Place bottles and caps in the yellow plastic dishpan and store in the cabinet under the sink to be washed and sterilized.

Always wash hands after handling samples. Clean any bench space used.

Check that all equipment is replaced in its storage location. Store all Books in the far right-hand drawer.

To All Laboratory Personnel: If you have corrections, comments, or suggestions regarding these "Procedures", your input would be greatly appreciated.
Please call Peg Wineman, (255-3491) or Michelle Hague, 255-1821.

Nutrient Project Data
Data by Pond 1997

Areys Pond

Printed March 15, 1999

Date Samp. Coll	Day of Yr	Sta. Code	Coll Hr.	Water Temp °C.	Sal inity ppt	Act. Deep m.	Secchi Deep m.	D.O. mg/L	pH	F.C. Ct. / 100ml	NO_3N mg/L	NH_4N mg/L	PO_4P mg/L	PON µg/L	PON mg/L (Cal)	Tot N mg/L. (Cal)	Chl-a µg/L	PHAEO µg/L
7/10/97	191	A1D	1100	24.5	33	3.6	1.2	1.9	8.0	5								
7/10/97	191	A1S	1100	25.0	32			3.5	8.0	0	0.03		0.12	448	0.448	0.478	18.27	6.17
7/10/97	191	A2D	1115	25.0	31	2.7	1.2	2.0	8.0	8								
7/10/97	191	A2S	1115	25.0	31			3.8	8.0	13	0.03		0.18	388	0.388	0.418	25.94	0.58
7/27/97	208	A1D	1100	22.0	26		3.0		7.9	0								
7/27/97	208	A1S	1100	22.0	23			3.2	7.6	0	0.07	0.08	0.16	128	0.128	0.278	1.36	2.53
7/27/97	208	A2D	1115	22.0	23	2.2	1.8	2.5	7.7	3								
7/27/97	208	A2S	1115	22.0	25			3.5	7.8	3	0.09	0.07	0.16	93	0.093	0.253	1.36	2.24
8/11/97	223	A1D	1120	24.5	33	3.1	1.2	1.5	8.0	25								
8/11/97	223	A1S	1120	24.0	32			5.7	8.3	0	0.06		0.14	270	0.270	0.330	16.37	0.10
8/11/97	223	A2D	1030	24.0	34	1.2	1.1	3.5	8.3	295								
8/11/97	223	A2S	1030	24.0	30			5.9	8.4	55	0.08	0.02	0.14	200	0.200	0.300	15.10	
8/26/97	238	A1D	1200		31	3.9	0.6	4.0	7.9	0								
8/26/97	238	A1S	1200		32			10.0	8.7	5	0.09		0.13	505	0.505	0.595	36.95	
8/26/97	238	A2D	1115	23.0	31	1.8	1.1	10.8	7.9	10								
8/26/97	238	A2S	1115		31			8.3	8.3	0	0.08		0.14	307	0.307	0.387	24.63	
9/7/97	250	A1D	0845	21.0	34	4.0	0.5		7.9	0								
9/7/97	250	A1S	0845	22.0	34			8.0	8.4	0	0.11	.02	0.15	1585	1.585	1.715	100.99	
9/7/97	250	A2D	0800	22.0	35	1.6	1.3	2.8	8.2	0								
9/7/97	250	A2S	0845	22.0	34			5.0	8.0	0	0.09		0.14	277	0.277	0.367	6.59	
9/28/97	271	A1D		19.0	35	4.0	1.8	4.5	8.1	40								
9/28/97	271	A1S		19.0	35			5.5	8.0	20	0.04	0.06	0.10			0.100	1.04	0.24
9/28/97	271	A2D		18.0	35	3.0	1.8	4.5	8.1	30								
9/28/97	271	A2S		19.0	35			5.5	8.1	15	0.07	0.10	0.10			0.170	0.74	0.18
10/11/97	284	A1D	1215	16.0	37	4.0	1.8	9.5	8.3	8								
10/11/97	284	A1S	1215	16.0	37			6.8	8.4	5	0.06		0.03			0.060	0.19	0.07
10/11/97	284	A2D	1215	16.0	37	2.6	1.9	6.0	8.3	3								
10/11/97	284	A2S	1215	16.0				5.8	8.4	5	0.09		0.02			0.090	0.38	0.12
11/5/97	309	A1D		12.0	33	4	1.8	5.4	7.8	0								
11/5/97	309	A1S		12.0	32			5.8	7.9	18	0.07	0.01	0.01			0.080		
11/5/97	309	A2D		12.0	31	1.4	1.2	5.8	8.0	5								
11/5/97	309	A2S		12.0	32			5.5	8.0	15	0.08					0.080		

Paw Wah Pond

Date Samp. Coll	Day of Yr	Sta. Code	Coll Hr.	Water Temp °C.	Sal inity ppt	Act. Deep m.	Secchi Deep m.	D.O. mg/L	pH	F.C. Ct. / 100ml	NO_3N mg/L	NH_4N mg/L	PO_4P mg/L	PON µg/L	PON mg/L (Cal)	Tot N mg/L. (Cal)	Chl-a µg/L	PHAEO µg/L
7/28/97	209	P1D	1030	22.5	30	4.7	2.70	5.0	8.1	5								
7/28/97	209	P1S	1045	24.0	30			4.5	8.1	15	0.07	0.01	0.09	42	0.042	0.122	0.94	1.33
7/28/97	209	P2D	1200	24.0	31	1.7	1.7	4.3	8.1	0								
7/28/97	209	P2S	1200	24.0	30			4.0	8.0	5	0.06	0.01	0.10	52	0.052	0.122	1.20	1.49
8/16/97	228	P1D	1515	24.5	32	4.3	3.00	4.0	8.0	0								
8/16/97	228	P1S		26.0	32			6.0	8.5	10	0.06	0.01	0.11	84	0.084	0.154	2.32	0.46
8/16/97	228	P2D		26.5	31	1.5	1.5	6.5	8.7	0								
8/16/97	228	P2S		26.5	33			7.0	8.7	10	0.06		0.13	109	0.109	0.169	5.51	
8/29/97	241	P1D	1450	21.0	34	4.3	1.70	4.0	8.2	0								
8/29/97	241	P1S	1450	21.5	32			5.0	8.5	0	0.07	0.05	0.12	120	0.120	0.240	4.57	1.07
8/29/97	241	P2D	1550	21.0	33	1.5	1.5	4.5	8.4	0								
8/29/97	241	P2S	1550	21.5	33			5.0	8.5	0	0.07		0.13	160	0.160	0.230	59.22	
9/11/97	254	P1D	1245	20.0	35	4.1	1.80	3.0	8.0	0								
9/11/97	254	P1S	1245	20.5	33			3.6	8.1	5	0.11		0.11	131	0.131	0.241	0.25	0.11
9/11/97	254	P2D	1345	20.5	32	1.6	1.6	4.2	8.2	10								
9/11/97	254	P2S	1345	20.0	34			4.5	8.2	10	0.07	0.03	1.31	153	0.153	0.253	0.78	0.11
9/28/97	271	P1D		17.0	35	4.3	2.50	6.4	8.5	0								
9/28/97	271	P1S		16.5	34			6.2	8.4	0	0.06	0.03	0.07			0.090	0.68	0.22
9/28/97	271	P2D		16.5	33	1.5	1.5	6.2	8.5	10								
9/28/97	271	P2S		16.5	36			6.2	8.5	0	0.03	0.03	0.07			0.060	0.13	0.07
10/12/97	285	P1D	1415	15.0	34	4.0	2.50	6.4	8.5	0								
10/12/97	285	P1S	1415	16.0	34			6.0	8.5	5	0.08		0.003			0.080	0.91	0.19
10/12/97	285	P2D	1415	16.5	35	1.0	1.0	6.8	8.5	5								
10/12/97	285	P2S	1415	16.5				6.8	8.5	3	0.04		0.004			0.040	1.41	0.35
11/16/97	320	P1D		6.5	36	4.3	2.50	9.0	8.2	15								
11/16/97	320	P1S		6.5	37			8.5	8.1	10	0.12	0.10	0.001			0.220		
11/16/97	320	P2D		6.5	38	1.9	1.9	7.5	8.3	5								
11/16/97	320	P2S		6.0	39			8.0	8.3	5	0.06					0.060		

Quanset Pond

Date Samp. Coll	Day of Yr	Sta. Code	Coll Hr.	Water Temp °C.	Sal inity ppt	Act. Deep m.	Secchi Deep m.	D.O. mg/L	pH	F.C. Ct. / 100ml	NO_3N mg/L	NH_4N mg/L	PO_4P mg/L	PON µg/L	PON mg/L (Cal)	Tot N mg/L. (Cal)	Chl-a µg/L	PHAEO µg/L
7/19/97	200	Q1D		22.5	30	4.1	1.5	4.5	8.6	0								
7/19/97	200	Q1S		23.0	30			5.5	8.6	0	0.06		0.08	190	0.190	0.250	10.54	0.73
7/19/97	200	Q2D		21.5	30	1.5	1.3	5.5	8.6	3								
7/19/97	200	Q2S		22.0	30			7.0	8.6	0	0.06		0.09	174	0.174	0.234	7.46	1.99
8/13/97	225	Q1D	1115	24.0	29	4.0	2.4	4.5	8.4	0								
8/13/97	225	Q1S		24.0	30			6.0	8.4	0	0.07		0.08	104	0.104	0.174	3.56	0.49
8/13/97	225	Q2D	1215	24.0	30	1.4	1.4	7.0	8.5	5								
8/13/97	225	Q2S	1215	24.5	30			5.0	8.5	0	0.06		0.08	95	0.095	0.155	3.63	0.67
8/30/97	242	Q1D	1530	22.0	32	3.8	3.0	5.0										
8/30/97	242	Q1S	1530	23.0	30			5.0	8.4	0	0.06	0.03	0.11	153	0.153	0.243	3.78	1.25
8/30/97	242	Q2D	1630	23.0	31	3.0	1.0			0								
8/30/97	242	Q2S	1630	22.0	31			5.0	8.4	5	0.07	0.04	0.12	127	0.127	0.237	2.86	1.46
9/22/97	265	Q1D		21.0	34	3.6	1.9	5.0	8.3	0								
9/22/97	265	Q1S		21.0	33				8.1	0	0.03	0.05	0.19	106	0.106	0.186	0.70	0.27
9/22/97	265	Q2D		20.0	34	2.6	1.2	4.9	8.3	0								
9/22/97	265	Q2S		20.0	34				8.3	5	0.03	0.02	0.13	102	0.102	0.152	0.70	
10/15/97	288	Q1D		14.0	35	4.5	1.5	7.0	8.4	10								
10/15/97	288	Q1S			35			5.0	8.4	10	0.03			90	0.090	0.120	0.92	0.43
10/15/97	288	Q2D	1610	14.0	35	2.0	1.5	7.0	8.4	30								
10/15/97	288	Q2S		14.0	35			5.5	8.5	10	0.03			92	0.092	0.122	0.65	0.35
11/12/97	316	Q1D		8.0	35	4.8	1.5	7.0	8.4	5								
11/12/97	316	Q1S		8.0	35				8.4	0	0.06					0.060		
11/12/97	316	Q2D		8.1	34	1.0	1.0	7.0	8.4	0								
11/12/97	316	Q2S		8.1	34				8.4	5	0.04					0.040		

NUTRIENT PROJECT
1997 REPORT

APPENDIX 3: SPECIAL LABORATORY PROCEDURES FOR NUTRIENT PROJECT AND FIELD TEST PROCEDURES

ORLEANS MARINE WATER QUALITY TASK FORCE

NUTRIENT PROJECT

LABORATORY PROCEDURES

PRE-SAMPLING

PREPARATION OF VIALS FOR NATIONAL PARK SERVICE SAMPLES

For each round of samples, two samples from each pond are drawn, only one from each station at the shallow depth of 0.5 meters.

For this purpose, each kit must have available two or more black capped small glass vials (16 ml) which contain 2 drops of 1 normal hydrochloric acid to lower the pH to the range of 2-3, thus stabilizing the sample for refrigerated storage.

Periodically, on an as needed basis, the laboratory volunteer will prepare a supply of 10-12 acidified, labeled vials. Call 255-3491 if you run out. New vials and caps are stored in the left hand lower cabinet, one normal hydrochloric acid is stored in the right hand lower cabinet. The addition of two drops of HCl, is most conveniently made by using a small plastic transfer pipette. New vials and caps are used without any pre-rinsing or other cleaning. Vials are also labeled with a blank label. The sample collector will write the date, and site code during collection.

POST-SAMPLING

FECAL COLIFORM TESTS

As part of the Nutrient Project, samples are taken at two depths at each site in each pond for Fecal Coliform testing. Since the concentration of coliforms in the bulk water of the ponds is expected to be quite low, we are using larger samples for this test than we have been using for the remediation project. Currently our practice is to conduct the tests as follows: For each sample, a 20 ml aliquot is tested, labeled for the date sampled, the site code, the depth, and "20" for the volume filtered. In addition, one duplicate sample and one reagent blank is run for each series. For example, at Quanset Pond site 2, the label might read: 7/15/97, Q-2 - S or D, 20 or 20 dupe. Thus, for each pond there would be 2 sites, two depths, and one duplicate sample, for five samples plus one blank.

FECAL COLIFORM TESTS (CON'T)

The set up for the membrane filtration is the same as we have always used. See the Black Laboratory Procedures Notebook for details,(top far right drawer). The difference in the filtration procedure is only in the volume of sample filtered. To filter the 20 ml sample, use two fillings of the "standard" 11 ml pipette to the 10 ml mark and allow each to drain into the filter receptacle. Once the filtration has been completed, rinsed with buffer solution, and sucked dry, the gridded membrane filter is transferred to the Petri dish using sterile forceps, and centered carefully on the media, leaving no air bubbles. The cover of the dish is replaced, and the Petri dish is placed in the 44.5 degree incubator. After 24 hours the plates are read for the number of blue fecal coliform colonies which develop. The site code and the data are recorded the Nutrient Project Laboratory Data Report Sheets (triple duplicator sheets - please use the attached white divider sheet very carefully).

The counting, and data recording are done as described on pages 8-9 of the black Laboratory Procedures book, except that the data are kept Nutrient Project Laboratory Report Sheets and in the blue Nutrient Project Record Book.

Cleanup and disposal are handled as specified on page 13 of the Black Laboratory Procedures Notebook.

MEASUREMENT OF pH

These measurements are carried out as described on page 13 of the Black Laboratory Procedures Notebook, kept in the far right hand drawer of the bench.

PORTNOY SAMPLES

Vials containing the Portnoy samples are placed in the refrigerator in a cardboard box, on the lower back shelf. Periodically, these will be transported to Truro to the National Park Service laboratory for analysis for Ammonia Nitrogen, Nitrate nitrogen, and Phosphate Phosphorus. No processing is required by the Orleans lab.

WOODS HOLE SAMPLES

PARTICULATE CARBON AND NITROGEN

During the sampling, a one liter sample for this determination was collected, brought to the laboratory in a cooler, and placed in the laboratory refrigerator. Both particulate carbon and nitrogen are determined by an elemental analysis involving combustion of the particulate solids contained in the sample. Each sample must be prefiltered and dried to permit its stable storage until it can be sent to Woods Hole and analyzed. The solids collected on the filters, are the items of interest.

The procedure for the filtration given to us by Woods Hole follows. Some of the terms used may require definition. For example we use a special small "Swinex " filter holder, connected by tubing to a plastic funnel for the filtration. The filter holder is attached to our standard vacuum manifold by a rubber stopper. The filters used are pre-combusted glass fiber filters, abbreviated GFF. We use our usual, new, empty 47 mm Petri dishes for containers for the filters and adhering solids after filtration. For drying the filters and the filter cake we are using an overnight or longer placement in our 44.5 degree oven. To measure the volume filtered, after shaking the sample bottle,we collect 200 ml in a clear plastic 250 ml graduate, and feed the filter from the graduate. The vacuum pump is normally adjusted for the Fecal coliform testing to provide a limited vacuum of between 5-10 inches. For the PON/ POC filtration and that for Chlorophyll a, it is desirable to use a stronger vacuum of about 25 inches. This is done by opening the right hand valve on the vacuum pump and observing the vacuum gauge with the manifold vents all closed or containing filters. When removing the filter paper from the opened holder, pick it up with forceps on the edge, which is free of solids. The dried filters are transferred to a closed can (currently a Baking Powder can), in which they can be kept at room temperature, in their individual Petri dishes. IMPORTANT NOTE: During the filtration it is essential to empty the 500 ml suction flask periodically to prevent water from going into the pump, which will ruin it!

Results are reported to us in micromoles of particulate organic carbon and nitrogen per liter.

CHLOROPHYLL a FILTRATION

The concentration of photosynthetic pigments is used to estimate phytoplankton biomass. All green plants contain chlorophyll a, which is roughly 1-2 % of the dry weight of planktonic algae. All work with chlorophyll extracts must be carried out in subdued light to avoid degradation of the pigments. For this reason samples are collected in brown bottles and kept cool to minimize degradation. Additionally, when pigments are filtered from the sample the handling and filtration must be carried out in the laboratory using only subdued green light from a single 25 watt green glass bulb. The filtration process uses a special 47mm Nucleopore filter in the same filtration apparatus we use for fecal coliform determinations. The filter papers are placed in pre-labeled dram vial, which are stored in the dark (by taping wrapping the vials with paper and taping with green tape) in the freezer compartment of the laboratory refrigerator until they can be shipped in a cooler pack to Woods Hole. Label sample vials with the date, the site code, and the volume of sample filtered. At Woods Hole samples are ground, extracted with a aqueous acetone, and the Chlorophyll a determined fluorometrically. Results are reported as grams/liter of Chlorophyll a. As in the filtration for PON/C, the sample bottle is shaken, and 200 ml or so is transferred to the clear plastic graduate to measure the volume filtered. As in the former filtration it is necessary to monitor carefully the level of filtrate in the suction flask and empty it as needed.

Detailed instructions follow:

SAMPLING PROTOCOL

The goal is to collect representative water column samples without disturbing the bottom sediments (this is very important, especially if you are standing nearby in the water) and to avoid direct collection of surface water which may have additional particles floating on the surface. POC samples are collected in white or brown 1l bottles, Chla in the brown 1l bottles. Keep samples cold and dark until processing.

Procedures:

- POC Filtration:

a) Using forceps, place pre-combusted 25mm GFF filters on filter holder; attach filter funnel (DO NOT ALLOW FILTERS TO TOUCH ANYTHING BUT FORCEPS, FILTRATION APPARATUS AND PETRI DISH).

b) Pre-lable petri dish with date, station and space for volume filtered lable on bottom of dish (widest part). This allows you to keep the filters identified when tops are ajar while air drying.

c) Turn on vaccume pump.

d) Shake one liter bottle and pour 100-200 mls into graduate cylinder; pour into filter funnel.

e) Add additional aliquots of sample as necessary in 100-200 ml increments until filter starts to slow, then add at 50 ml intervals (depending on how productive the water column is, can be anywhere from 200-500 mls. Filters and petri dishes have been provided for reps if you want, lable them a and b. KEEP TRACK OF HOW MUCH SAMPLE GOES THROUGH FILTER AND BE VERY ACCURATE IN MEASUREMENT. ONCE SAMPLE IS ADDED TO FUNNEL IT CANNOT BE WITHDRAWN AS PARTICLES MAY SETTLE!!!

f) Allow filter to suck dry, rinse funnel with a squirt of distilled water, allow to suck dry.

g) Write filtered volume for each sample on petri dish lable and data sheet.

h) Unscrew funnel, remove filter with forceps and place in petri dish.

i) When done, crack tops of petri dishes and allow filters to air dry for several hours in warm location. It's best in a box or pan where foil can be placed over top to prevent additional dust or particles from settling on filter.

j) Store petri dishes in stack; KEEP UPRIGHT.

- Chla Filtration; brown bottles

NOTE: These filters cannot go dry until the end of filtration after the addition of $MgCO_3$.

a) Must be done in the **DARK**; use a green light bulb if possible to see. A tiny crack of light might be OK if samples all from euphotic zone but really should be avoided. Pre-lable dram vials for each sample.

b) Using forceps, place 47 mm Nucleopore filter on filter holder and clamp on funnel. Shake bottle, measure out 100-200 mls then add at 50 ml increments. Again, once sample has been added it cannot be withdrawn.

c) After sufficient sample has been filtered to go really slow and just before last of sample passes through filter, add 3 drops of saturated $MgCO_3$ (1 gram in 100 mls distilled water) with Pasteur Pipette or eyedropper around surface of filter. This should be added while there is still a few mm of water still on the surface. (swirl $MgCO_3$ to keep in suspension when pipetting)

d) Remove filter, rest on kimwipe and fold in half without touching filter (kimwipe will blot excess water). Fold again and place into pre-labeled dram vial. Store filters in dark box (coffee can, any dark container) and freeze. If frozen conditions cannot be insured until return to Woods Hole, keep very cold (far preferable to freeze, however).

ORLEANS MARINE WATER QUALITY TASK FORCE

FIELD PROCEDURES

GETTING STARTED

Choose A Sampling Day

Samples should be taken at scheduled intervals since evenly spaced samples give a more accurate picture of water conditions throughout the year. Sampling is to be done at half-tide, going out, plus or minus one hour (in our ponds this is roughly five hours after high tide on the outside).

Your Site

Each of our sites is marked by a buoy, labeled with the site abbreviation, such an M1, for site 1 of Meeting House Pond. For each pond site 1 is located in approximately the deepest part of the pond, while site 2 is in a shallower part of the pond usually where it will intercept flow from an adjacent marsh or other fresh water stream. For our project, all samples are taken from a boat. See the next section for sample collection.

Recording Your Test Results

It is important to record your data on a standardized form.

The first step in sampling is filling out the preliminary section of the data sheet, i.e., the general observations and identification sections.

Always record the test results as you go along. **Don't rely on your memory.**

Do not leave a site until all tests have been made and all blanks in the data record are filled in. Be sure that the date and sampling time for the site have been recorded. Observations about the site such as the presence of birds, fish, or animals, any algal blooms, recent notable weather, and any problems you had should be noted in the comments section.

When you have completed your testing, sign and date where indicated.

II. HOW TO DO THESE TESTS

The text of these instructions has been derived from several sources including extensive quotations from some, particularly the training manual for volunteer monitors for Chesapeake Bay, as well as others such as The instructions from Woods Hole Oceanographic Laboratory, prepared by Dale Goehringer and others. John Portnoy of the National Park Service provided the verbal instructions for sampling for nutrients to be run in his Laboratory.

COLLECTING THE WATER SAMPLES

Note: It is assumed that all sample collection for this project will be made from a boat.

First, it is necessary to identify the exact location of the sampling site to be sampled. All sites are marked with an orange buoy, labeled "Orleans Shellfish Dept", and have a unique letter number code.

Second, it is necessary to maneuver the boat so that it can come to anchor on its own anchor, and will come to "rest", adjacent to the chosen marker buoy. You should be able to suspend lines from the boat directly to the bottom very near the marker, but without entangling its mooring line.

One water sample is taken for all purposes except for dissolved oxygen, for which a special sample is to be drawn. Directions will be given first for the general sample. For the general sample we use either the LaMotte Water Sampling Bottle (3-0026), or a Water-Mark Water Sampler, each of which allows about a 450 ml. sample to be collected, at specified depths. For uniformity, samples are drawn at a "Deep" location (defined as 0.5 meters off the bottom), and a "Shalow" location (defined as 0.5 meters below the surface).

PROCEDURE

1.Using the lead line with the calibrated line, measure the depth from the bottom to the surface of the water several times, and estimate the average depth to the nearest 0.1 meter as your boat swings. Record the depth.

2. Remove the sampling bottle from the case, spread out the lines, and attach the two pound weight (its already attached to the Water-Mark Sampler). If the bottle is open, close it by replacing the lid and for the LaMotte snap the wire retainer into the groves in the lid.

3. Subtract 0.5 meters from the depth you measured, the difference is the amount of line to allow out when the bottle is lowered for the filling holes to be 0.5 meters above the bottom, for sampling the "Deep" water sample.

4. For the LaMotte, press the plastic stopper securely into the center inlet hole. For the Water-Mark bottle, press the two rubber stoppers into the holes in the lid. Lower the water sampling bottle to the desired depth.

5. Collect the water sample by removing the stopper(s) from the inlet hole(s) with a quick jerk of the stopper line for the LaMotte, and the suspension line for the Water-Mark. Note: As air is displaced by water entering the sampler, bubbles will be observed rising to the surface. When the sampler is filled, bubbles no longer appear. Filling takes 45-60 seconds, or longer.

6. Use a steady hand over hand motion to retrieve the sampler. Place on a flat surface. Open the sampler, and pour the sample of water for the Orleans Laboratory test for Fecal Coliform (and other tests) into a sterile plastic sample bottle. Label the bottle with the date, site code and the depth. Store samples for the Fecal Coliform test, (and any other Orleans Laboratory test) in the cooler. Bring all samples to the Orleans Laboratory and leave in the laboratory refridgerator,

7. Use the remaining sample for determining water temperature, salinity and for the NPS sample, required only for the "shallow" samples. See page 12 for additional detail on the NPS sample. See other instructions that follow, for other tests and the sample and testing for dissolved oxygen.

8. After all samples have been obtained, discard remaining water, rinse sampler with tap water and close sampler for next use.

WATER TEMPERATURE

Although temperature may be one of the easiest measurements to perform, it is probably one of the most important parameters to be considered. It affects the rates of chemical and biochemical reaction within the water. Many biological, physical and chemical princiiples are temperature dependent. Among the most common of these are the solubility of compounds in sea water, distribution and abundance of organisms living in the estuary, rates of chemical reactions, density, inversions and mixing, and currents. Because the estuary is so shallow, its capacity to store heat over time is relatively small. As a result, water temperature fluctuates considerably.

The temperatures of surface and subsurface water usually differ. With increase in depth the water generally becomes colder. This results in thermal stratification of deeper water and can lead to density differences. During the spring and summer months,the surface waters are warmer than the deeper waters. In the fall, the warming radiation of the sun diminishes. As the surface water cools, it increases in density, becoming heavier. Once the surface water becomes colder and denser than the water toward the bottom, it begins to sink and vertical mixing occurs. Wind may speed up the process. This mixing action can bring nutrients, materials essential to the growth of organisms, up from the bottom and into higher water levels. The turn-over makes the nutrients available to phytoplankton and other organisms inhabiting the upper water levels. During the winter, the water temperature becomes relatively constant from surface to bottom until March, when the process of surface warming begins again.

Temperature is reported in degrees Celsius (Centigrade).

PROCEDURE

1. Determine air temperature by reading the thermometer after its reading in air has stabilized, approximately two minutes. From tag on thermometer, note whether any correction is necessary. Record corrected temperature on data sheet.

2. For water sample temperature, place the thermometer in the sampler water and allow to stabilize for about two minutes. From tag on thermometer, note whether any correction is necessary. Record corrected temperature on data sheet.

MEASURING TURBIDITY

Although the most accurate means of measuring turbidity is with a nephelometer which electronically measures light scatter, many monitoring programs rely on the Secchi disk. Easy to use, inexpensive, and consistently accurate, this simple weighted disk is used by volunteers to measure water depth at which the disk just disappears from view- the Secchi depth. Most programs find that the Secchi disk gives sufficiently good clarity readings. The Secchi disk is 20 centimeters in diameter and divided into alternate black and white quadrants to enhance visibility and contrast. Optimum conditions for recording Secchi disk readings are clear sky, sun overhead but disk should be lowered in shade or shadow, and on the side of the boat with minimal waves or ripples.

PROCEDURE

1. Keep the reel of the line in-board to avoid losing it overboard.

2. Take a Secchi disk reading, by lowering the disk into the water until the disk just disappears from sight. Record the amount of cord submerged to the nearest one-tenth meter. Raise the disk slowly until the disk just becomes visible and record this depth as well. Average the two recorded values. This average is called the limit of visibility. If the disk hits the bottom before dropping out of sight, note this observation and record the bottom depth.

SALINITY

Salinity is a measure of the amount of salts dissolved in water. An estuary exhibits a change in salinity throughout its length as fresh water flowing from tributaries mixes with sea water moving in from the ocean. Even at a single site, salinity will fluctuate with tidal movement, dilution by fresh water and mixing by the wind. Salinity levels control, to a large degree, the types of plants and animals that can live in different zones of the estuary.

Salinity is usualy expressed in parts per thousand (ppt). Seawater has about 35 parts of salt per 1000 parts of water, and drinking water has under 0.5 ppt. Salinity can be measured by a variety of methods. This project uses refractivity of seawater. The refractive index is a measure of the relative change in the direction of a light beam as it passes from air into water. Salinity and temperature affect the index.

PROCEDURE

Your portable refractometer is a precision optical instrument, designed to measure the concentration of salt in aqueous solutions. The larger the concentration of salt the higher the reading.

1. With the plastic pipette, place enough drops of sample from the water sampler on the oval prism to cover it with no bubbles. Close the prism cover plate so the solution spreads evenly.

2. Aim the front of the refractometer toward a light source (avoid direct sunlight, but aim at white clouds away from direct sun). Adjust the eyepiece for clearest focus on the boundary between the light and dark hemispheres, (where the blue ends).

3. The boundary of light and dark will indicate the ppt (parts per thousand) salt of the sample. Record the reading.

4. After use, clean prisms with a few drops of distilled water and wipe with a cloth. (Do not use a paper towel, etc, it will scratch the surface.) Discard wet cloths.

5. Do not dip or run refractometer under water, or allow water to seep into the internal sections.

DISSOLVED OXYGEN

Of all of the parameters that characterize an estuary, the level of dissolved oxygen (DO) in the water is one of the best indicators of the estuary's health. An estuary with little or no oxygen in its waters cannot support healty levels of animal or plant life. Unlike many of the problems of estuaries, the consequences of a rapid decline in DO occur quickly, and animals must move to areas with higher levels of oxygen or perish. This immediate impact makes measuring the level of DO an important means of assessing water quality.

Oxygen enters an estuary's waters from the atmosphere and through aquatic plant and phytoplankton photosynthesis. Currents and wind generated waves boost the amount of oxygen entering the water by putting more water in contact with the atmosphere. Oxygen solubility in water is poor, and even well-aerated, cold fresh water can only hold 14.2 milligrams per liter (mg/L) of oxygen when fully saturated. Salt water can absorb even less. Warm water holds less oxygen than cold.

Most animals and plants can grow and reproduce unimpaired when DO levels exceed 5 mg/L. When levels drop to 3-5 mg/L, living organisms often become stressed. If levels fall under 3 mg/L, a condition known as hypoxia, many species will move elsewhere and non-mobile species may die. A second condition, known as anoxia, occurs when water becomes totally depleted of oxygen (under 0.5 mg/L) and results in the dath of any organism that requires oxygen for survival.

Oxygen levels may change sharply in a matter of hours, making it difficlult to assess the significance of any single DO value. At the surface on an estuary, the water at mid-day is often close to oxygen saturation due both to mixing with air and the production of oxygen by plant photosynthesis. As night falls, photosynthesis ceases, and animals consume available oxygen, forcing DO levels to decline. DO levels in deeper parts of the estuary fluctuate according to diffusion, mixing, currents, and other factors.

MEASURING DISSOLVED OXYGEN

For measurement of dissolved oxygen we use a Hach Ampul Sampler (#24051) to collect the water sample at the designated depth, into an ampul pre-loaded with the chemicals to provide a colorimetric test, whose color density is proportional to the dissolved oxygen of the water. The collection is done by the vacuum in the ampul, which avoids additional exposure to atmospheric oxygen.

Carry out the following steps:

1. Push the plastic piston, flat end first into the bottom of the aluminum cup.

2. Hold the piston cup with the open side up, and insert the flat end of an AccuVac ampul (Hach #25150-25) into the opening. Blue foam containers hold 5 ampules, for 4 measurements plus an extra, in case of accidental breakage.

3. Place the open end of the plastic ampul snapper over the ampul and twist to the left to lock into place.

4. Hold the rubber bulb end of the tubing and note the depth marks on the tubing, to select the proper depth. For "surface" samples this will be 0.5 meters; for "deep" samples this will be the full length of the tubing, (3.5-4.0 meters) or if the depth is less than the length of the tubing, the sample should be taken 0.5 meters above the bottom. Position the sampler at the desired depth.

5. Squeeze the rubber bulb firmly to snap the ampul.

6. Retrieve sampler assembly. First, note whether ampul has broken, and filled with sample as expected. If not, see that the ampul is positioned properly, lower to depth, and re-squeeze rubber bulb. If sampler tip is broken, hold the aluminum cup so that the broken tip of the ampul is upright. Remove plastic "breaker" by twisting to the right, and removing. Place plastic breaker on its flat bottom to retain the broken tip, and bits of reagent. Using blue ruibber cap, cover the broken end of the ampul. Remove the capped ampul from the aluminum cup. If ampul liquid is black, accidental breakage has probably occurred: discard in toxic waste, and repeat the procedure using the extra ampul.

7. Holding the ampul bottom and the rubber cap, shake ampul up and down for 30 seconds.

8. Place capped ampul on a flat surface, preferably in the shade or the dark, and allow color to develop for 2 minutes, then place the capped ampul in the right top opening of the color comparator.

9. Hold the comparator up to the sky (away from the sun), look through the openings in the front.

10. Rotate the color disk until the color matches in the two openings.

11. Read and record the mg/L dissolved oxygen from the scale window.

12. Note that the chemicals used for the dissolved oxygen test may be harmful, so the used ampul and contents are placed in a hazardous waste bottle, together with any broken tip or glass with adhering reagent. If necessary, rinse any external droplets into the same bottle with a small amount of tap water.

13. Replace equipment into the carrying case.

14. When you return to your base or the laboratory, clean, rinse and dry any used equipment, and replace in the case. Use care with the color comparator, and store away from heat and light.

ADDITIONAL INSTRUCTIONS FOR THE NPS (Portnoy) SAMPLES

Through the cooperation of the National Park Service (NPS) and Dr. John Portnoy, the Orleans Water Quality Task Force Nutrient Project is able to send our samples to the NPS laboratory in Truro for determination of three important nutrient constituents: nitrate nitrogen, ammonia nitrogen and phosphate phosphorus.

To preserve these samples, they must be filtered into a "pre-acidified" vial, which contain 2 drops of 1 Normal Hydrochloric acid. Do not spill. The water to be sampled is first drawn up into a large syringe (60 ml), which is then attached to a 25 mm. Swinnex filter, and filtered directly into the small black capped vial. Carry out the following steps:

1. The Swinnex filter should be examined to be sure there is an intact filter paper in it. Look at the top of the filter and note whether the full circumference of the filter is discernable. If in doubt, one can loosen the top of the filter and examine the paper, then reclose the filter. (We believe the filter can be used for 5 or more samplings without difficulty.)

2. Examine the large syringe. Remove protective cap on tip. The syringe should be clean and the plunger should operate smoothly. Immerse the tip in the sample and gently draw about 30 ml into the syringe.

3. Hold syringe upright (tip up), and expell any air. Attach filled syringe to filter by insertion into the top fitting of the filter.

Note: to preserve the filter paper and keep it flat, never pull a vacuum on the top of the filter.

4. SLOWLY expel a few ml of filtrate by pressing on the plunger, remove cap from vial and hold vial upright under the exit tubing of the filter and fill vial to within about 5 mm or 1/4 inch of the top.

5. Close vial tightly. Remove syringe from filter, and expel remaining sample overboard, rinse syringe by drawing up some tap water and expelling to waste a couple of times, finally, with residual tap water in the syringe, connect it to the filter, and filter a small amount of tap water through the filter to clean and rinse it.

6. Disconnect syringe, from filter and shake out residual rinse water, from both components, replace syringe cover, and return parts to the storage box.

7. Label the sample vial with the date, and site code. Keep in cooler.

8. Later, when storing the sample in the laboratory refrigerator, place sample in the small box labeled NPS samples.

Revised May 26, 1998

WQNPLP1.DOC

ORLEANS MARINE WATER QUALITY TASK FORCE

NUTRIENT PROJECT

CHECK LIST FOR SAMPLES AND "FIELD" TESTS AT EACH SITE

1. Measure actual depth, note any general observations.
2. Measure Secchi depth.
3. Obtain "deep" sample (.5 meters off the bottom) with La Motte sampler.
    a. Record water temperature, and *rinse thermometer with tap water.*
    b. Pour sample for Fecal coliform test into sterile plastic bottle.
    c. Pour 5 ml. sample for nitrate nitrogen test.
    d. Measure salinity with several drops of sampler water.
    e. Discard remaining sample, *and rinse sampler with tap water.*
    (this is a change in procedure to minimize any carry over of bacteria from one sample to the next)
4. Obtain "deep" (.5 meters off bottom, or end of tubing) sample for dissolved oxygen test.
5. Obtain "shallow" sample (.5 meters below surface) with *rinsed* La Motte sampler.
    a. Record water temperature, and *rinse thermometer with tap water.*
    b. Pour sample for Fecal coliform test into sterile plastic bottle.
    c. Pour 5 ml sample for nitrate nitrogen test.
    d. Measure salinity with several drops of sampler water.
    e. Obtain sample for NPS laboratory by filling syringe, and filtering sample into a 16 ml black capped vial.
    f. Discard remaining sample, *and rinse sampler with tap water.*
6. Obtain "shallow"sample (0.5 meters below surface) for dissolved oxygen test.
7. Obtain two one-liter "shallow" samples for Woods Hole using sampler for brown bottles.
8. Keep all samples in the cooler.
9. Before leaving site, check that all samples and tests for the site have been made, all data is recorded, and samples are labeled with date, site code and depth, and general observations were noted.

Please give any comments or corrections to Peg or Bob Wineman, 255-3491.

WQNPLP3

ORLEANS MARINE WATER QUALITY TASK FORCE
NUTRIENT PROJECT -- FIELD DATA REPORT

POND _____DATE_____SAMPLERS _____

OBSERVATIONS (CHECK): Sunny__ Partly Cloudy__ Overcast__ Rain__;
Calm__ Breeze__ Windy__; OTHER_____
(Note: presence of birds, animals, fish, blooms or coloration in the water, etc.)

SITE CODE _____ SAMPLE DEEP: TIME_____
DEPTH ACTUAL _____Meters; DEPTH SECCHI _____Meters;
AIR TEMP ____ C.; WATER TEMP____ C. (Rinse thermometer with tap water)
ORLEANS LAB SAMPLE: Fill plastic sample bottle to 1" from cap. Secure cap.
Label with Site Code, Depth, and Date.
DISSOLVED O ____mg/L.; SALINITY_____ppt.
(RINSE SAMPLER with tap water BETWEEN SAMPLINGS.)

SITE CODE_____ SAMPLE SHALLOW:
WATER TEMP_____ C. (Rinse thermometer)
ORLEANS LAB SAMPLE: (as above); NPS SAMPLE: Fill black-capped vial to 1/4"
from cap. Secure cap. Label with Site Code and Date.
DISSOLVED O _____mg/L.; SALINITY_____ppt. (RINSE SAMPLER)
STORE ALL LAB SAMPLES IN COOLER
Before leaving site, please check that all tests are completed and results recorded.

* * *

OTHER OBSERVATIONS_____

SITE CODE_____SAMPLE DEEP: TIME_____
DEPTH ACTUAL____Meters; DEPTH SECCHI____Meters
WATER TEMP_____ C. (Rinse thermometer with tap water)
ORLEANS LAB SAMPLE: Fill plastic sample bottle to 1" from cap. Secure cap. Label.
DISSOLVED O _____mg/L.; SALINITY_____PPT.
(Rinse sampler with tap water between samplings).

SITE CODE_____ SAMPLE SHALLOW:
WATER TEMP_____ C. (Rinse thermometer).
ORLEANS LAB SAMPLE: (As above); NPS LAB---Fill black-capped vial to 1/4" from cap. Secure cap. (label with site code and date)
DISSOLVED O_____mg/L.; SALINITY_____PPT
STORE ALL LAB SAMPLES IN COOLER
Before leaving site, please check that all tests are completed and results recorded.

NUTRIENT PROJECT TIDE CHART FY 1998

JULY

Date	Wk.Day	High Tide @ Nauset Beach	Ebb Tide **SAMPLE TIME**	-for upper reaches of Pleasant Bay
7/1	Wed.	5:45 AM	10:45 AM	
7/2	Thurs.	6:39 AM	11:30 AM	
7/3	Fri.	7:34 AM	12:30 PM	
7/4	Sat.	8:28 AM	1:30 PM	
7/5	Sun.	9:22 AM	2:30 PM	
7/6	Mon.	10:12 AM	3:15 PM	
7/7	Tues.	10:59 AM	4:00 PM	
7/13	Mon.	2:49 AM	7:45 AM	*tides to exceed 11'
7/14	Tues.	3:39 AM	8:30 AM	
7/15	Wed.	4:32 AM	9:30 AM	
7/16	Thurs.	5:18 AM	10:15 AM	
7/17	Fri.	6:28 AM	11:30 AM	
7/18	Sat.	7:30 AM	12:30 PM	
7/19	Sun.	8:30 AM	1:30 PM	
7/20	Mon.	9:36 AM	2:30 PM	
7/21	Tues.	10:35 AM	3:30 PM	
7/27	Mon.	2:49 AM	7:45 AM	
7/28	Tues.	3:33 AM	8:30 AM	
7/29	Wed.	4:19 AM	9:15 AM	
7/30	Thurs.	5:07 AM	10:00 AM	
7/31	Fri.	5:58 AM	11:00 AM	

NUTRIENT PROJECT TIDE CHART FY 1998

AUGUST

Date	Wk.Day	High Tide @ Nauset Beach	Ebb Tide **SAMPLE TIME**	
8/1	Sat.	6:51 AM	Noon	-for upper reaches of Pleasant Bay
8/2	Sun.	7:46 AM	12:45 PM	
8/3	Mon.	8:40 AM	1:45 PM	
8/4	Tues.	9:34 AM	2:30 PM	
8/5	Wed.	10:24 AM	3:30 PM	
8/12	Wed.	3:21 AM	8:30 AM	*tides to exceed 11'
8/13	Thurs.	4:15 AM	9:15 AM	
8/14	Fri.	5:12 AM	10:15 AM	
8/15	Sat.	6:12 AM	11:15 AM	
8/16	Sun.	7:16 AM	12:15 PM	
8/17	Mon.	8:22 AM	1:15 PM	
8/18	Tues.	9:25 AM	2:30 PM	
8/19	Wed.	10:25 AM	3:30 PM	
8/26	Wed.	3:03 AM	8:00 AM	
8/27	Thurs.	3:40 AM	8:45 AM	
8/28	Fri.	4:31 AM	9:30 AM	
8/29	Sat.	5:20 AM	10:15 AM	
8/30	Sun.	6:11 AM	11:00 AM	
8/31	Mon.	7:06 AM	Noon	

(over)

NUTRIENT PROJECT TIDE CHART FY 1998

SEPTEMBER

Date	Wk.Day	High Tide @ Nauset Beach	Ebb Tide **SAMPLE TIME**	-for upper reaches of Pleasant Bay
9/1	Tues.	8:00 AM	1:00 PM	
9/2	Wed.	8:56 AM	2:00 PM	
9/3	Thurs.	9:46 AM	2:45 PM	
9/4	Fri.	10:38 AM	3:30 PM	
9/10	Thurs.	3:03 AM	8:00 AM	*tides to exceed 11'
9/11	Fri.	3:57 AM	9:00 AM	
9/12	Sat.	4:56 AM	10:00 AM	
9/13	Sun.	5:58 AM	11:00 AM	
9/14	Mon.	7:03 AM	Noon	
9/15	Tues.	8:10 AM	1:00 PM	
9/16	Wed.	9:13 AM	2:15 PM	
9/17	Thurs.	10:11 AM	3:15 PM	
9/18	Fri.	11:00 AM	4:00 PM	
9/25	Fri.	3:16 AM	8:15 AM	
9/26	Sat.	3:59 AM	9:00 AM	
9/27	Sun.	4:46 AM	9:45 AM	
9/28	Mon.	5:36 AM	10:30 AM	
9/29	Tues.	6:30 AM	11:30 AM	
9/30	Wed.	7:26 AM	12:30 PM	

NUTRIENT PROJECT
1997 REPORT

APPENDIX 4: LATITUDE/LONGITUDE POSITIONS OF MONITORING BOUYS

ORLEANS MARINE WATER QUALITY TASK FORCE

NUTRIENT PROJECT

LISTING OF LATITUDE/LONGITUDE POSITIONS OF MONITORING BOUYS

POND	SITE	COORDINATES	SATS
MEETINGHOUSE POND	M-1	Lat. 41 46.83' N Lon. 069 57.86' W	7
	M-2	Lat. 41 46.88' N Lon. 069 58.01' W	7
KESCAYOGANSETT POND	K-1	Lat. 41 46.19' N Lon. 069 58.56' W	7
	K-2	Lat. 41 46.15' N Lon. 069 58.62' W	7
AREYS POND	A-1	Lat. 41 45.62' N Lon. 069 58.98' W	6
	A-2	Lat. 41 45.62' N Lon. 069 59.02' W	6
PAW WAH POND	P-1	Lat. 41 45.34' N Lon. 069 58.23' W	5
	P-2	Lat. 41 45.35' N Lon. 069 58.18' W	5
QUANSET POND	Q-1	Lat. 41 44.25' N Lon. 069 58.92' W	6
	Q-2	Lat. 41 44.28' N Lon. 069 58.78' W	6