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Lobster Population Density and Distribution in Relation to Habitat Quality

Final Project Report

Submitted to the

Maine Department of Marine Resources

Lobster Research, Education and Development

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Introduction

The distribution of lobsters (*Homarus americanus*) in Casco Bay, Maine has changed significantly in the past five or six years (D. Millar, B. Tarbox, pers. comms.). Shallow water eelgrass beds and mud bottoms that formerly served as good habitat for molting lobsters in July and again in October now appear abandoned or poorly populated, based on catch and direct observations of the bottom (Tarbox, pers. observation). The majority of the catch of newly molted lobsters during the summer now occurs in fifteen to twenty fathoms of water in August and September (various anecdotal reports). This temporal and geographical shifting of the lobster population has led to crowding of gear, a breakdown of traditional territories, and friction between different groups of lobstermen. The small boat fishermen who used to make their living fishing in the shallow waters around Cousin's Island, the Falmouth and Freeport shores, Maquoit Bay and Middle Bay in Harpswell have been particularly hard hit.

Many factors have been implicated in this change in lobster behavior including: cooler water temperatures, release of chlorinated effluents from sewage treatment facilities, application of Dimilin and other pesticides, PCBs and other hydrocarbons, heavy metals, and localized eutrophication in shallow, inshore waters. Although most of the areas of Casco Bay are not subject to inputs of most of these pollutants and contaminants, many area fishermen are concerned that mass mortalities such as those seen in Western Long Island Sound (WLIS), New York may occur here although, to date, this has not been observed in Maine. Unfortunately, most of the water quality databases now existing for Casco Bay do not reflect conditions near or on the bottom or in the burrows. In addition, most of the information concerning lobster populations in the bay is anecdotal. Additionally, questions have been raised by lobster fishermen in the area concerning the possible role water temperature and salinity might be playing in the changes in lobster behavior and distribution.

It is unlikely that any single study could reveal the cause of the reported changes in lobster behavior and distribution. This project, therefore, sought to collect basic water quality information specific to lobster habitat and document lobster densities on formerly productive lobster bottom as well as currently productive bottom. The intent was to document the problem in a quantitative manner and try to either identify or rule out specific water quality parameters that may be causing a shift in lobster distribution.

To accomplish this objective, the project took a multifaceted approach of simultaneously measuring lobster population density in nearshore and offshore lobster harvesting areas and water and sediment quality of associated burrows. Additionally, measurements were made of water quality within the water column of both nearshore and offshore lobster harvesting areas with specific emphasis on water temperature and salinity differences between nearshore and offshore lobster harvesting areas.

To investigate possible stresses in the habitat, samples of burrow water (or in the absence of burrows, of near-bottom water) were tested for temperature, pH, dissolved oxygen, and ammonia ($\text{NH}_3\text{-N}$); sediment samples were taken immediately adjacent to burrows to determine evidence of hypoxic conditions. Water column profiles were also taken monthly to investigate the changes that may occur within the water column during the season and provide a periodic cross-sectional view of temperature and salinity from the nearshore to offshore area. These vertical profiles of temperature ($^{\circ}\text{C}$) and salinity (‰) were made using a YSI 6600 profiler at approximately 1-nautical mile (nm) intervals along an imaginary transect approximately 15 nm long running from the vicinity of Little Moshier Island, Freeport to about 6 nm miles south-southeast of Halfway Rock at a depth of approximately 300 ft., *i.e.* "edge of bottom". Additionally, HOBO Water Temp Pro (H20-001) temperature data loggers were installed in at least one trap in the vicinity of selected study sampling sites, as well as offshore locations, over the course of the study, *i.e.* June through October, to investigate the changes in bottom water temperature and supplement the monthly, single sampling date water column profiles

Sampling locations

The nearshore to offshore transect used for water column profiling and the Zones in which lobster population density, burrow water quality, and sediment quality were taken are shown in Figures 1 (Nearshore) and 2 (Offshore), where the zones are indicated by the transparent red circles and the approximate water column profile stations by the transparent blue circles.

Figure 1 Nearshore sampling transect, zones, and water column profile stations

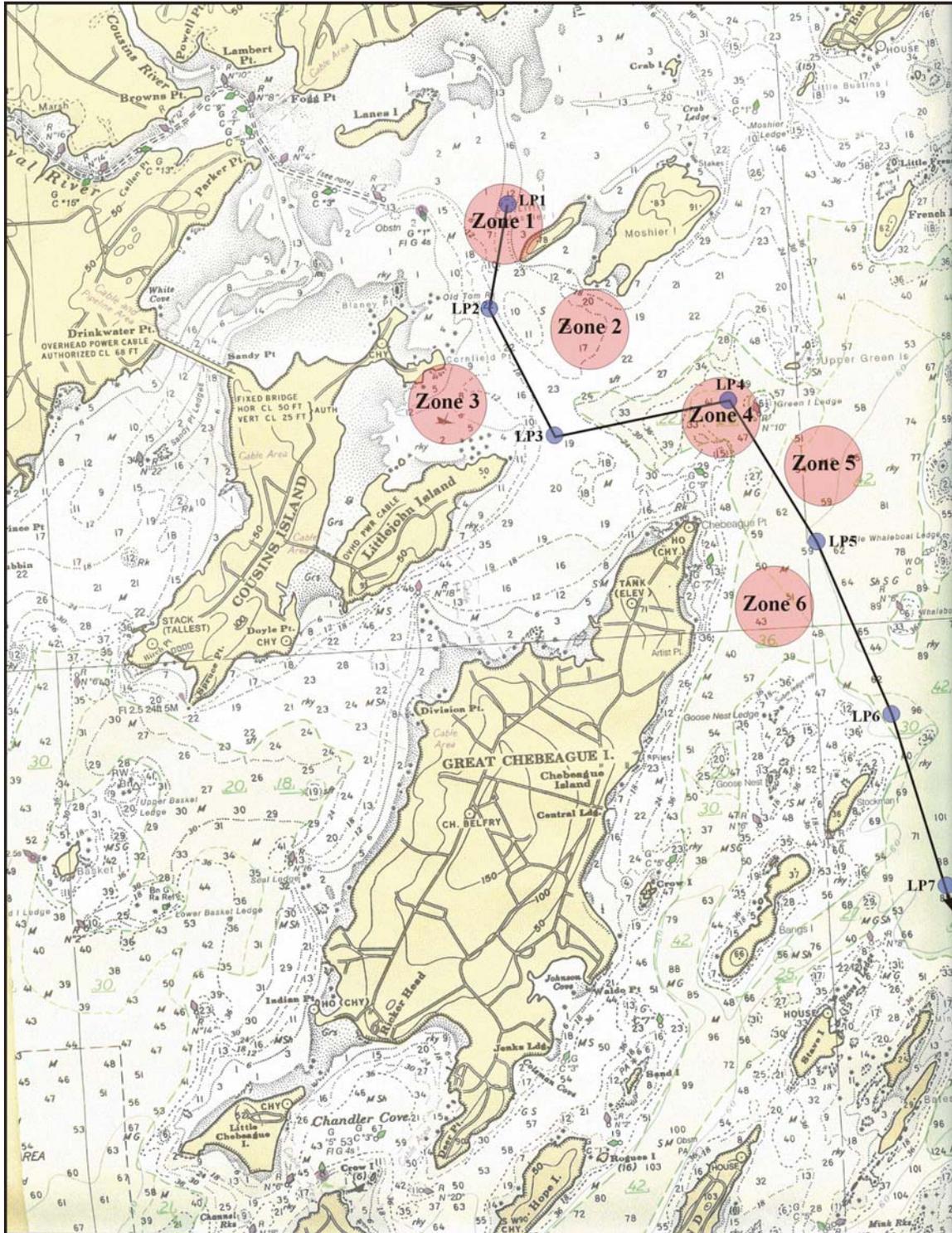
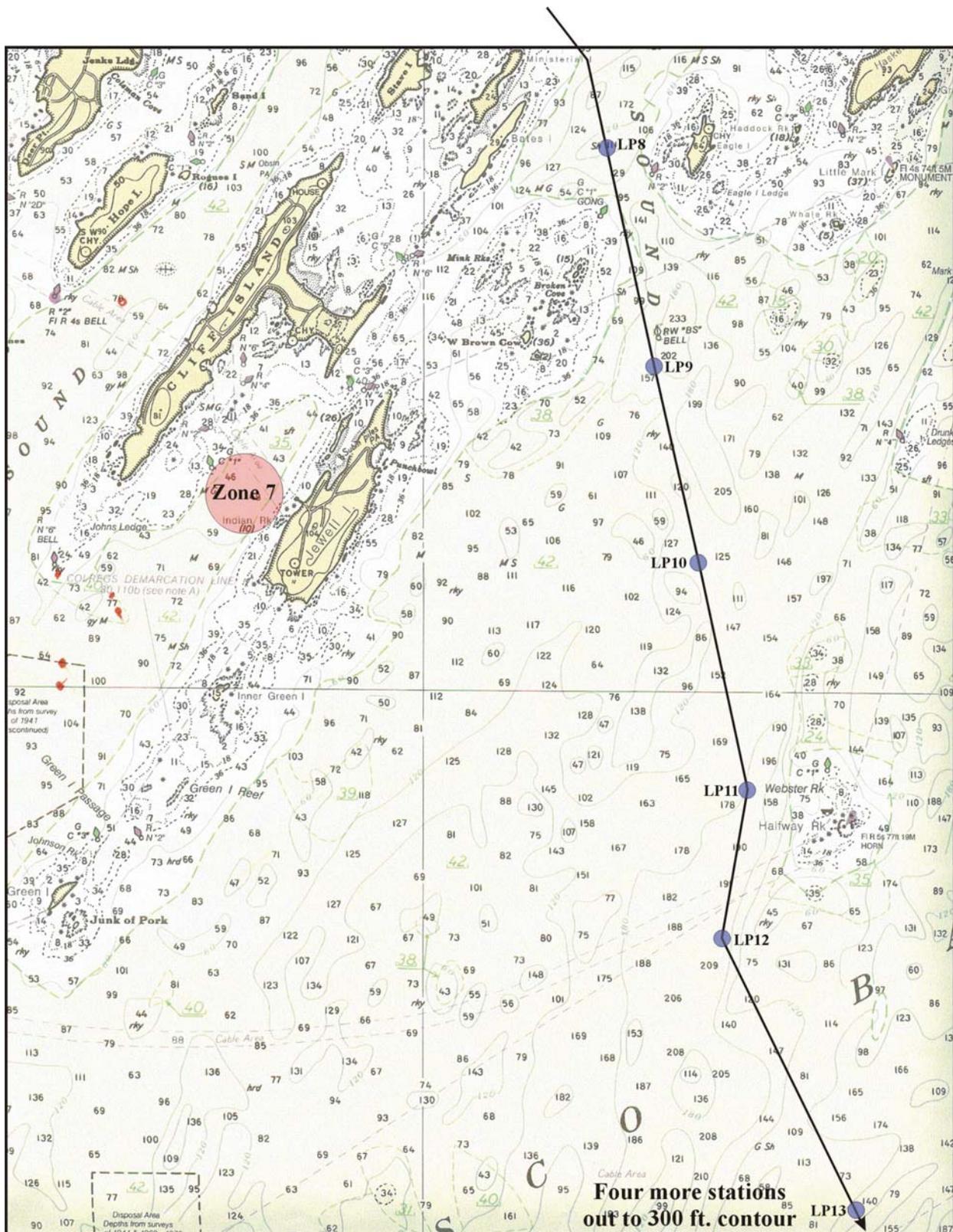


Figure 2 Offshore sampling transect, zones, and water column profile stations



Methods

Diver observations and video recordings along bottom-deployed transect lines for the estimation of lobster population density, collection of burrow water samples, and sediment coring within each zone and subsequent sample analysis took place over two 2-day periods (usually on four consecutive days) in each month between July and October. Sample processing and data input were done on the day following each field sampling date, thus resulting in a *field-lab-field-lab* sequence. Nearshore to offshore water column profiling along the ~16 nm transect was carried out on the fifth day following completion of the video recordings-laboratory work or as shortly before or after as possible, weather permitting.

Field sampling procedures

Population density

Lobster population density was estimated by the method previously described in Heinig and Cowperthwaite (1998) and Heinig and Tarbox (2000). Briefly, diver observations and simultaneous video recordings of lobsters and burrows were made within a 1-meter wide field of view along measured 100m transect lines deployed along the bottom in seven (7) pre-selected zones along an imaginary transect established from the shallow, nearshore area just west of Little Moshier Island (Zone 1) to a deeper, more offshore area along the western side of Jewell Island (Zone 7) as shown in Figures 1 and 2. For statistical analysis purposes, three (3) replicate 100m transects were deployed and observed/video recorded within each of the seven zones on each sampling date. Density, reported as lobsters/m² and burrows/m², were calculated by dividing the total number of lobsters and burrows observed within the 1-meter wide field of view by the length of the measured transect over which observations were made (*i.e.* 100m), both as reported by the diver and subsequently observed during video review.

Burrow water quality

Five (5) burrow water samples were collected along each of the three (3) 100m transects deployed at each of the seven (7) zones, thus fifteen (15) samples/zone. Samples of water for analyses were collected from occupied or unoccupied burrows, depending on presence of lobsters, using 140cc Kendall Monoject[®] syringes inserted into the burrow while minimally disturbing the resident lobsters to reduce the possibility of sediment resuspension by a rapidly moving or escaping lobster.

Water samples were preferentially collected from burrows containing lobsters, however, if no burrows with lobsters could be located, samples were taken from unoccupied burrows; where no burrows were present, water was drawn from as close to the sediment/water interface as possible, usually within 3-5 cm of the bottom, while avoiding disturbance to the sediment surface.

Approximately 140cc of water was collected from each sampling location to allow analysis for dissolved oxygen (DO), ammonia (NH₃), and pH. As soon as the samples were returned to the surface, 10 ml of sample were poured into a 10 ml HACH 1" round glass sample cell (HACH Prod. #2427606), capped, and set aside as a "blank"; an additional 50 ml were poured into a 125 ml Nalgene[®] wide mouth plastic container and the temperature and pH measured using a Fisher Scientific Accumet[®] AP63 portable pH/mV/Ion meter equipped with a Combination pH Electrode with built-in ATC probe (Fisher Prod. #ZZ-35925-50).

Dissolved oxygen (DO) was measured using HACH High Range Dissolved Oxygen (HRDO) AccuVac[®] Ampuls which were filled by breaking the tip of the ampule while submerged in the 50 ml of seawater in the Nalgene container; once filled, the ampule was maintained tip side down and the end capped with a specifically designed water-filled rubber cap (HACH Prod. #173125). The samples were subsequently treated and measured in the field using a HACH 890 Spectrophotometer as described in HACH Method 8166 attached hereto as Appendix I.

The remainder of the sample water was bulk vacuum filtered in the field to remove potentially analysis-interfering turbidity before being sub-sampled for specific analysis using a Pall Life Sciences (Gelman) magnetic filter holder (HACH Prod. #1352900) holding a 1.5 μm glass fiber filter paper (HACH Prod. #253000), fitted on a Pyrex 500 ml vacuum filtration flask; vacuum was pulled using a Nalgene[®] Mityvac[®] hand vacuum pump (HACH Prod. # 1428300); the 1.5 μm glass fiber filter paper was changed for each transect or more frequently, depending on rate of clogging. Following filtration, an additional 60 ml of sample was poured into a 60 ml pre-labeled sample bottle containing three (3) drops of concentrated sulfuric acid (HACH Cat. # 979-49) to reduce the pH of the seawater to <2.0 for temporary storage (<48 hrs). Immediately after collection, samples were placed on ice in a cooler to keep the samples at $\leq 4^{\circ}\text{C}$ until arrival at the lab where the samples were placed in a freezer.

Sediment chemistry

Single sediment cores for sediment chemistry analyses, including redox potential (Eh) and sulfide (S_2), were taken immediately adjacent to each sampled burrow; where no burrows were present, cores were taken of representative sediment material. Cores were collected using 4 in. diameter PVC pipe coring devices inserted into the bottom to a sufficient depth to allow undisturbed collection of the top 2 cm of bottom sediment; no samples were taken where sediment coarseness exceeded the threshold of these tests. After collection, sediment cores were removed from the corers by allowing the sediment column to slide out of the plastic corer so as not to disturb the sediment surface.

Sediment redox (Eh) and sulfide (S_2) sampling and measurements were done according to the methods of Wildish *et al.*, 1999. A portion of the core surface material was removed down to a depth of 2 cm and the sediment placed in a small 125 ml plastic container and thoroughly mixed with a plastic spoon for approximately 1-2 minutes. Following mixing, the redox potential was measured using an Accumet[®] AP63 pH/mV/Ion meter equipped with a Thermo Orion model 9678BN Combination Redox electrode filled with Thermo Orion Ag/AgCl Reference Electrode Filling Solution (900011) by immersing the electrode into the mixed sediment and waiting for the reading to stabilize while gently mixing the sediment with the electrode. Meter mV values were corrected by applying an appropriate correction factor for temperature, *e.g.* +214 @ 10°C (Thermo Orion Platinum Redox Electrode Instruction Manual, Model 96-78-00, 2001, p. 5).

After redox measurement, a 5 ml portion of the mixed sediment was removed with a modified 5 ml plastic syringe with the needle attachment end removed to form an open cylinder; the open end was immersed into the mixed sediment slurry and the sample extracted by pulling back on the plunger, thus obtaining a sample containing no bubbles. Immediately after obtaining the sample, the open end of the syringe was covered with plastic wrap insuring no air was trapped beneath the wrap. Aluminum foil was then placed over the end of the syringe to secure the plastic wrap in place. The syringe was then placed in a cooler with ice to maintain a temperature of $\leq 4^{\circ}\text{C}$ during transport to the laboratory for sulfide (S_2) analysis within ≤ 72 hrs. of sample collection.

Nearshore to offshore water column temperature and salinity

Water column profiles were collected at each of 17 locations indicated in Figures 1 and 2 as LP#. Weather permitting, this sampling was conducted immediately after completion of the lobster density and burrow water sampling within the seven zones, usually on the fifth day of the sampling sequence. Profiles were collected using a YSI 6600 series sonde equipped with a pressure sensor to measure depth (m), a temperature-conductivity ($^{\circ}\text{C}$, ‰) sensor, a dissolved oxygen sensor (mg/L, % saturation), a pH sensor, and a self-wiping turbidity probe (NTU). All data was stored on the onboard YSI sonde memory as individual station files; the files were downloaded to a PC at the end of each run day and exported into an Excel[®] spreadsheet to allow further analysis and graphing. Copies of MER's SOPs for use of the YSI 6600 profiler and data logging procedures are included here as Appendix II.

Bottom temperature

Bottom temperature was recorded using Onset Computer Corporation, HOBO[®] Water Temp Pro sensors. The sensors were wire-tied into the second or third mesh from the bottom of wire lobster traps fished at various locations within and just offshore of Casco Bay by three participating fishermen, B. Tarbox (Lannes Is., north Chebeague Is.), D. Millar (Broad Sound bell buoy, south of Half Way Rock), and B. Dyer (“offshore”). The six (6) HOBO[®] Water Temp Pro were programmed for deployment using Onset Computer Corporation BoxCar[®] Pro Ver. 4.3 (v4.3.1.1) software to begin recording at 18:00:00 6/24/2005 and set at a 15 min sample recording interval. The units were individually recovered at sometime between 8/19/2005 and 8/27/2005 for interim data downloading and individually redeployed at sometime between 8/23/2005 and 8/27/2005; all were recovered on 11/07/2005 for final data download.

Laboratory procedures

Ammonia nitrogen (NH₃-N)

Ammonia was measured in the laboratory, usually within 24 hrs. following sample collection, using a HACH 890 Spectrophotometer and HACH 8155 Salicylate Method attached hereto as Appendix III. A calibration check of the HACH 890 was carried out prior to each measurement series using a minimum of 3-replicate 0.16 mg/L standard prepared from a 10 mg/L HACH Ammonia Nitrogen Standard (HACH Prod. # 15349) using HACH Deionized water (HACH Prod. # 27256); calibration check results were dated and recorded in laboratory bound notebook. Due to the number of samples processed each day, samples were processed in two lots to avoid sample warming while awaiting processing. All data were recorded on the respective sampling date field sheet and subsequently entered into an Excel[®] spreadsheet for statistical analysis.

Sulfide (S₂)

Once at the lab, all syringes were allowed to warm to room temperature ($\approx 20^{\circ}\text{C}$) before analysis. The Accumet[®] AP63 pH/mV/Ion meter equipped with a Thermo Orion model 9616BN Combination Silver/Sulfide electrode filled with Thermo Orion Ionplus B Optimum Results[™] Reference Electrode Filling Solution (900062) was standardized using standards prepared according to Wildish *et al.*, 1999. The meter was standardized at 1.00 (100 μM), 10.0 (1,000 μM), and 100 (10,000 μM). All samples were analyzed within a maximum of 3 hrs. Following analysis of all samples, measurements of the three standards were retaken and recorded on the calibration sheets. All data were recorded on the respective sampling date field sheet and subsequently entered into an Excel[®] spreadsheet for statistical analysis; actual S₂ μM values were calculated by multiplying the meter readings by 100.

Nitrate (NO₃)

Nitrate was measured in the laboratory, usually on the same day as collection or within 24 hrs. following sample collection, using a HACH 890 Spectrophotometer and HACH 8192 Cadmium Reduction Method attached hereto as Appendix IV. A calibration check of the HACH 890 was carried out prior to each measurement series using a minimum of 3-replicate 0.16 mg/L standard prepared from a 10 mg/L HACH Nitrate Nitrogen Standard (HACH Prod. # 30749) using HACH Deionized water (HACH Prod. # 27256); calibration check results were dated and recorded in laboratory bound notebook. Due to salinity interference with the method, a 3% (30‰) NaCl solution was prepared by dissolving 3.0 g NaCl (Fisher Prod. # S271-1) to 100ml (volumetric) using the 0.16 mg/L ammonia standard solution; this consistently yielded HACH 890 spectrophotometer readings of 0.04mg/L. Attempts to standardize the HACH 890 using the salinity adjusted standard solution failed because the adjusted solution was below the standardization detection level of the unit; all samples were therefore run as straight samples without unit-adjusted curve. All data were recorded on the respective sampling date field sheet and subsequently entered into an Excel[®] spreadsheet for statistical analysis.

Results

Transect Data Analysis

A multiple correlation analysis was used to look for correlation between diver observed lobster density of lobsters and water quality parameters using Systat 11[®]. The parameters used were: sediment reduction-oxidation (redox) potential expressed as Eh, sediment sulfide (S₂) expressed in micromoles, bottom temperature in °C taken with a YSI 6600 sonde, pH, dissolved oxygen (DO) in mg/l, salinity (psu) and ammonia nitrogen in mg/l. Table 1a. shows the lobster and burrow data expressed as monthly averages (based on 3 transects/zone) for each zone as entered into Systat 11[®] for analysis; Table 1b. shows the water and sediment chemistry parametric monthly averages for each zone, again as entered into Systat 11[®] for analysis.

Table 1a. Monthly averages for diver and video observed lobsters and burrows for each zone

Date	Zone	Diver # lobs.	Diver Lob. Density	Video # lobs	Video Lob. Density	Diver # burrows	Diver Burr. Density	Video # burrows	Video Burr. Density
7/11/2005	Zone 1	2.7	0.03	1.3	0.01	5.0	0.05	18.0	0.18
7/11/2005	Zone 2	3.0	0.03	3.3	0.03	2.7	0.03	10.7	0.11
7/11/2005	Zone 3	1.3	0.01	0.3	0.00	2.7	0.03	17.3	0.17
7/11/2005	Zone 4	1.0	0.01	1.0	0.01	0.3	0.00	7.3	0.07
7/13/2005	Zone 5	3.3	0.03	1.7	0.02	6.3	0.06	22.3	0.22
7/13/2005	Zone 6	4.7	0.05	3.7	0.04	5.0	0.05	15.7	0.16
7/13/2005	Zone 7	7.0	0.07	6.3	0.06	1.7	0.02	19.3	0.19
8/15/2005	Zone 1	4.0	0.04	2.7	0.03	1.0	0.01	19.0	0.19
8/15/2005	Zone 2	3.0	0.03	3.0	0.03	0.0	0.00	12.7	0.13
8/15/2005	Zone 3	0.0	0.00	0.0	0.00	0.0	0.00	8.0	0.08
8/15/2005	Zone 4	2.0	0.02	1.7	0.02	0.0	0.00	15.7	0.16
8/19/2005	Zone 5	5.0	0.05	2.3	0.02	0.0	0.00	16.7	0.17
8/19/2005	Zone 6	3.3	0.03	2.7	0.03	0.0	0.00	20.3	0.20
8/19/2005	Zone 7	10.0	0.10	8.0	0.08	0.0	0.00	19.3	0.19
9/10/2005	Zone 1	4.7	0.05	3.0	0.03	10.3	0.10	18.7	0.19
9/10/2005	Zone 2	2.3	0.02	1.0	0.01	6.0	0.06	10.7	0.11
9/10/2005	Zone 3	0.0	0.00	0.0	0.00	4.0	0.04	14.7	0.15
9/10/2005	Zone 4	----	----	0.0	0.00	----	----	9.0	0.09
9/14/2005	Zone 5	2.7	0.03	0.7	0.01	7.3	0.07	14.7	0.15
9/14/2005	Zone 6	3.0	0.03	0.3	0.00	9.0	0.09	16.0	0.16
9/14/2005	Zone 7	2.0	0.02	1.0	0.01	5.3	0.05	13.7	0.14
10/19/2005	Zone 1	0.0	0.00	0.7	0.01	3.3	0.03	9.7	0.10
10/19/2005	Zone 2	1.0	0.01	1.3	0.01	4.0	0.04	4.0	0.04
10/19/2005	Zone 3	0.7	0.01	1.3	0.01	6.7	0.07	16.0	0.16
10/19/2005	Zone 4	1.0	0.01	1.0	0.01	6.0	0.06	3.0	0.03
10/20/2005	Zone 5	0.0	0.00	0.0	0.00	5.0	0.05	15.3	0.15
10/20/2005	Zone 6	0.0	0.00	0.0	0.00	4.0	0.04	6.0	0.06
10/20/2005	Zone 7	1.0	0.01	0.3	0.00	9.7	0.10	23.0	0.23

Table 1b. Monthly averages for water and sediment chemistry parameters for each zone

Date	Zone	Eh	S ₂ μM	YSI T°C	DO T°C	pH	DO mg/l	Sal. psu	DO %sat	NH ₃ -N mg/L
7/11/2005	Zone 1	-28.2	820.0	17.1	17.2	8.03	7.47	29.80	92.93	0.011
7/11/2005	Zone 2	-36.6	566.5	15.5	17.2	7.94	6.77	29.60	84.22	0.025
7/11/2005	Zone 3	-24.1	443.7	13.3	20.0	7.96	6.82	29.90	89.42	0.003
7/11/2005	Zone 4	8.5	150.3	12.5	18.7	7.91	6.73	30.20	86.08	0.021
7/13/2005	Zone 5	23.4	772.9	10.7	16.3	7.95	7.48	30.20	91.45	0.061
7/13/2005	Zone 6	27.9	562.1	10.5	14.4	7.92	7.99	30.30	93.93	0.051
7/13/2005	Zone 7	-14.6	909.3	9.2	13.9	7.87	7.35	30.90	85.58	0.073
8/15/2005	Zone 1	-36.0	920.1	16.0	18.2	7.94	6.88	30.68	87.67	0.048
8/15/2005	Zone 2	-4.5	1163.3	15.7	17.0	7.94	7.10	30.61	88.37	0.062
8/15/2005	Zone 3	-21.3	1016.5	15.6	18.8	7.81	7.13	30.72	91.87	0.086
8/15/2005	Zone 4	7.4	927.7	13.7	12.7	7.87	7.81	30.80	89.30	0.065
8/19/2005	Zone 5	21.9	1022.1	12.6	17.6	7.82	7.66	30.90	96.62	0.137
8/19/2005	Zone 6	66.7	636.3	11.3	16.7	7.84	7.51	31.00	93.11	0.265
8/19/2005	Zone 7	-8.9	1121.2	9.6	13.7	7.86	8.07	31.18	94.42	0.438
9/10/2005	Zone 1	-41.2	481.2	16.5	19.0	7.88	7.60	31.10	98.57	0.053
9/10/2005	Zone 2	-32.1	490.3	16.1	16.8	7.93	7.53	31.20	93.66	0.031
9/10/2005	Zone 3	-25.0	497.7	14.1	18.8	7.95	7.24	31.30	93.66	0.049
9/10/2005	Zone 4	-6.1	707.7	12.6	16.2	7.88	8.13	31.40	100.06	0.038
9/14/2005	Zone 5	53.8	804.1	11.2	16.6	8.17	7.04	31.60	87.42	0.061
9/14/2005	Zone 6	59.6	330.1	10.3	15.6	8.14	7.79	31.70	95.00	0.063
9/14/2005	Zone 7	43.5	485.6	9.6	13.0	8.06	7.50	31.80	86.79	0.071
10/19/2005	Zone 1	-8.0	620.3	10.4	12.3	7.84	7.12	30.10	80.29	0.095
10/19/2005	Zone 2	0.5	559.6	10.4	12.2	7.78	7.42	30.30	83.65	0.069
10/19/2005	Zone 3	25.4	685.9	10.5	12.9	7.71	8.73	30.40	99.98	0.077
10/19/2005	Zone 4	39.8	661.1	10.6	11.9	7.82	8.20	30.70	92.09	0.079
10/20/2005	Zone 5	62.3	670.1	10.6	12.0	7.91	6.95	31.20	78.43	0.023
10/20/2005	Zone 6	34.9	288.6	10.7	11.3	7.83	7.13	31.30	79.40	0.039
10/20/2005	Zone 7	-5.9	905.9	10.6	9.4	7.82	7.41	31.80	79.43	0.040

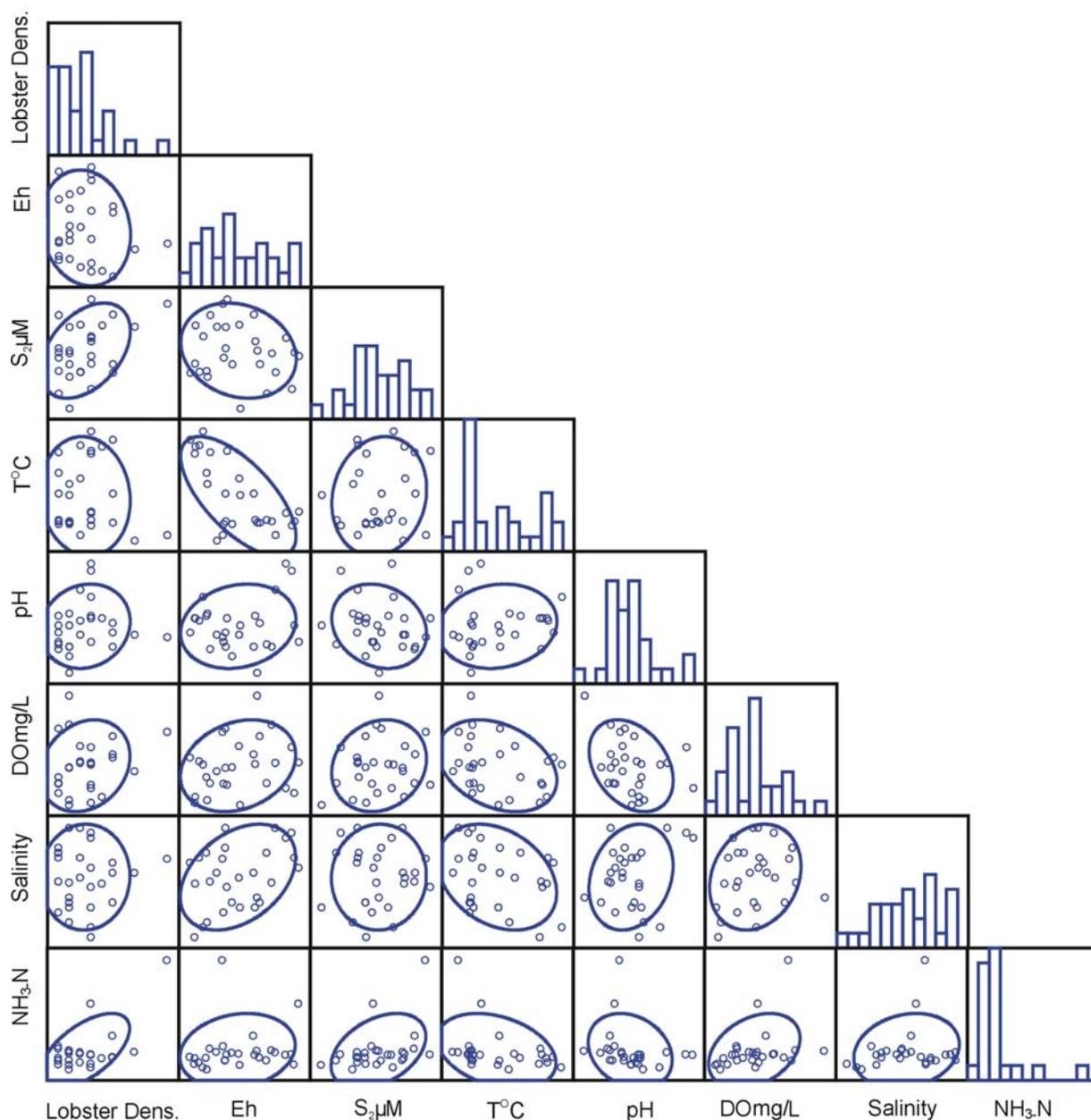
Table 2 gives the matrix of Pearson product-moment correlation coefficients.

Table 2 Pearson correlation matrix

	Diver Density	EH	S ₂ μM	YSI °C	pH	DO mg/L	Salinity	NH ₃ -N
Diver Density	1.000							
EH	-0.124	1.000						
S ₂ μM	0.415	-0.176	1.000					
YSI°C	-0.075	-0.670	0.123	1.000				
pH	0.116	0.163	-0.184	0.120	1.000			
DO mg/L	0.209	0.237	0.156	-0.315	-0.319	1.000		
Salinity	0.021	0.374	0.025	-0.299	0.215	0.200	1.000	
NH ₃ -N	0.602	0.165	0.393	-0.323	-0.232	0.339	0.181	1.000

The largest correlation coefficient was between total ammonia nitrogen and observed density of lobsters at 0.602. We attribute this high correlation to several outliers in the ammonia nitrogen data in Zones 5, 6 and 7 in the August data; the samples from these zones were inadvertently not acidified before storage and are therefore suspect and likely not representative. The outliers can be seen in the correlation plot of NH₃-N vs. Diver [lobster] density shown in the individual parametric correlation plots in Figure 3, below. The rest of the correlation coefficients in Table 2 are small and the individual parametric correlation plots shown in Figure 3 do not show significant trends in the data.

Figure 3. Correlation plots for diver observed lobster density and water and sediment chemistry parameters



Multiple regression was used to determine causal relationships between diver observed lobster density and water and sediment quality parameters. In this analysis, the diver observed density of lobsters on the bottom was considered to be the dependent variable that was determined by the water quality parameters which were treated as independent variables. The data used were the same as used for the multiple correlation analysis; the results are given in Table 3.

Table 3. Multiple regression analysis and Analysis of Variance of causal relationship between diver observed lobster density and water and sediment quality parameters.

Multiple regression analysis

Dep Var: Lobster Dens N: 28 Multiple R: 0.771 **Squared multiple R: 0.594**
 Adjusted squared multiple R: 0.452 Standard error of estimate: 0.018

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	-0.728	0.332	0.000	.	-2.194	0.040
EH	-0.000	0.000	-0.396	0.464	-1.894	0.073
S2µM	0.000	0.000	0.201	0.752	1.224	0.235
YSI°C	-0.002	0.002	-0.216	0.451	-1.018	0.321
pH	0.109	0.039	0.470	0.713	2.785	0.011
SALINITY	-0.006	0.006	-0.145	0.776	-0.894	0.382
DO mg/L	0.009	0.008	0.181	0.739	1.093	0.287
NH₃N	0.163	0.048	0.592	0.669	3.398	0.003

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	0.009	7	0.001	4.182	0.005
Residual	0.006	20	0.000		

*** WARNING ***

Case 7 is an outlier (Studentized Residual = 2.801)
 Case 14 has large leverage (Leverage = 0.807)

Durbin-Watson D Statistic: 1.351
 First Order Autocorrelation: 0.320

The squared multiple regression coefficient was 0.594, implying that the linear model only accounts for 59 percent of the observed variation in lobster density. The analysis of variance indicates that the regression coefficient is greater than zero at a significance of 0.005 but the partial regression coefficients vary between 0.000 and 0.163. The largest partial correlation coefficient (0.163) was for ammonia nitrogen which is suspect due to the outliers in August. Further, the t test for significance of the partial regression coefficients shows that the only coefficients that were significant at 0.01 or less were the coefficients for pH and ammonia nitrogen. The partial regression coefficient for pH was only 0.109 however and the Pearson correlation coefficient for correlation between pH and Diver observed density was only 0.116.

Monthly changes in lobster density between zones due to bottom temperature were tested by simple linear regression using Excel[®]. The dependent variable was diver observed lobster density for each transect in each zone and the independent variable was bottom water temperature in °C for each zone measured with a YSI sonde. An analysis was done for each month and the regression data are given in Table 4.

Table 4 Results of linear regression data for diver observed lobster density and temperature

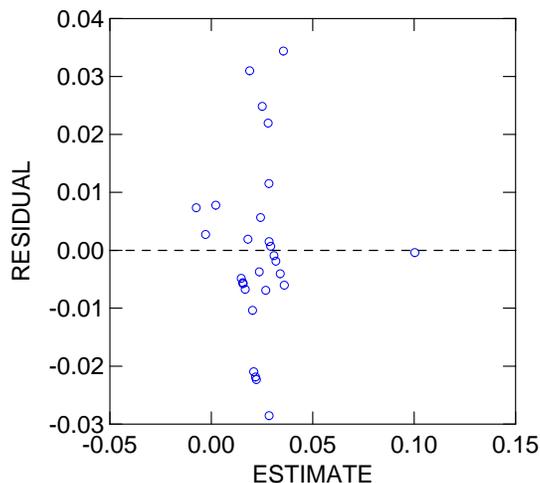
Month	Slope	Intercept	R ²	F	DF
July	-0.004	0.086	0.196	4.639	19
Aug	-0.009	0.164	0.390	12.155	19 (significant at 0.01)
September	0.001	0.011	0.019	0.317	16
Oct	-0.007	0.083	0.011	0.217	19

Although there is a slightly negative slope to the relation between diver observed densities of lobsters and water temperature with higher densities and lower water temperatures observed in Zones 6 and 7, the correlation coefficients are very low (0.083 – 0.390) and the only slope that is significantly different from zero is for the August data.

The plot of residuals against predicted values for the analysis are shown in Figure 4.

Figure 4

Plot of residuals against predicted values



Nearshore to offshore water column temperature and salinity

The YSI profile data was exported into Excel, formatted as an Ocean Data View (ODV) (Schlitzer, R., 2004) spreadsheet and converted to an ASCII text file for import into ODV. The volume of data involved makes tabulation of the data here or in the appendix impractical, but a copy of the Excel file containing all data for all stations for each sampling data is included on a Data CD provided with the report.

The temperature and salinity profiles for each monthly sampling are shown in the following sets of cross-sectional graphs, the shallower, shoreward side on the left, the deeper, offshore stations to the right. Ranges for all parameters, including distance (km), depth (m), temperature (°C), and salinity (psu), are maintained constant throughout the graph series to enhance differences over time. Although each profile was started at the same GPS coordinates on each sampling date, since deep profiles required some time to reach bottom, the slight differences in the bathymetric profile simply reflect slightly different locations at final depth resulting from drift due to tide or wind; shallow area difference are also due to differences in tide elevation.

Figure 5. June 12, 2005

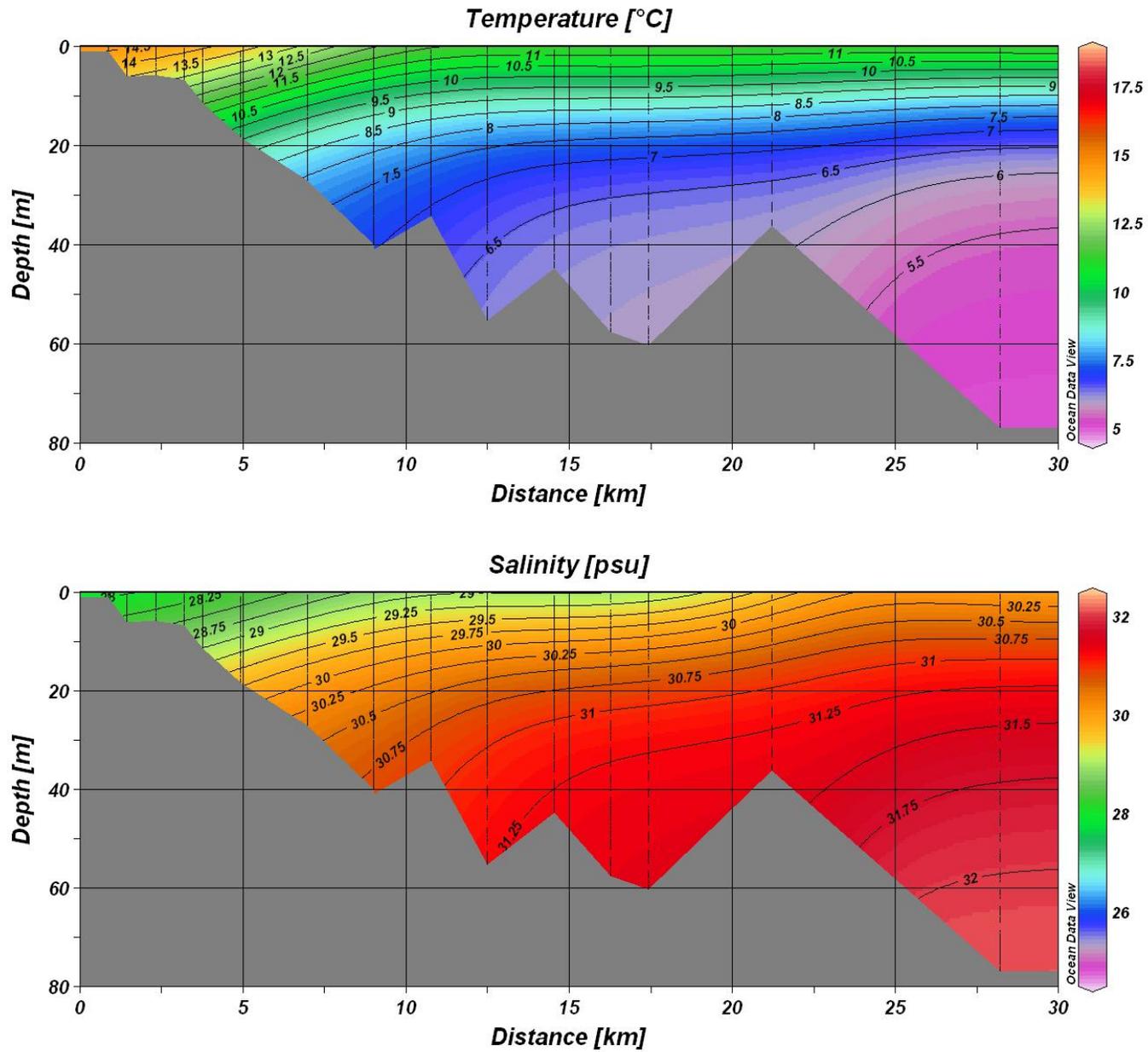


Figure 6. July 15, 2005

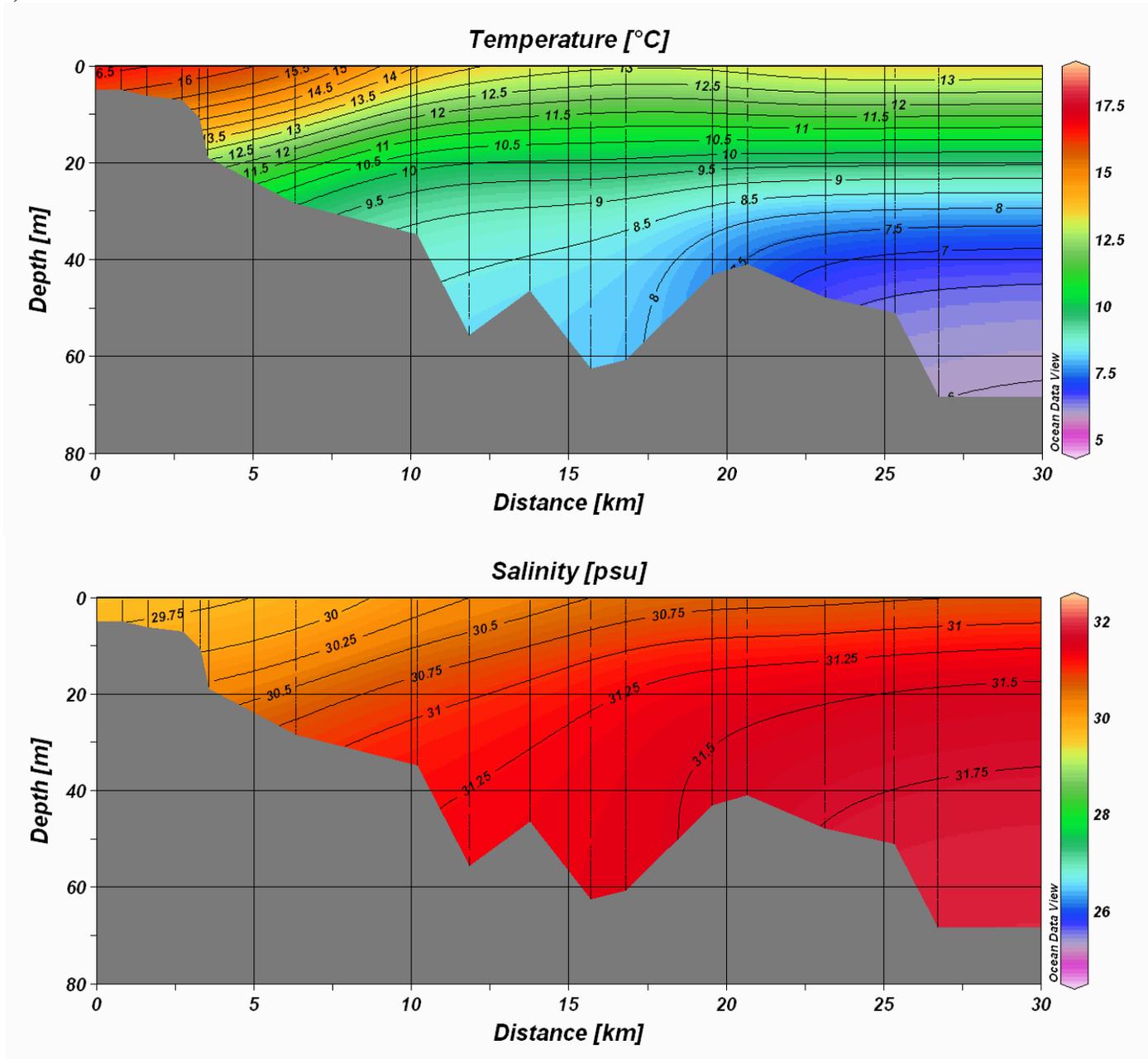


Figure 7. August 19, 2005

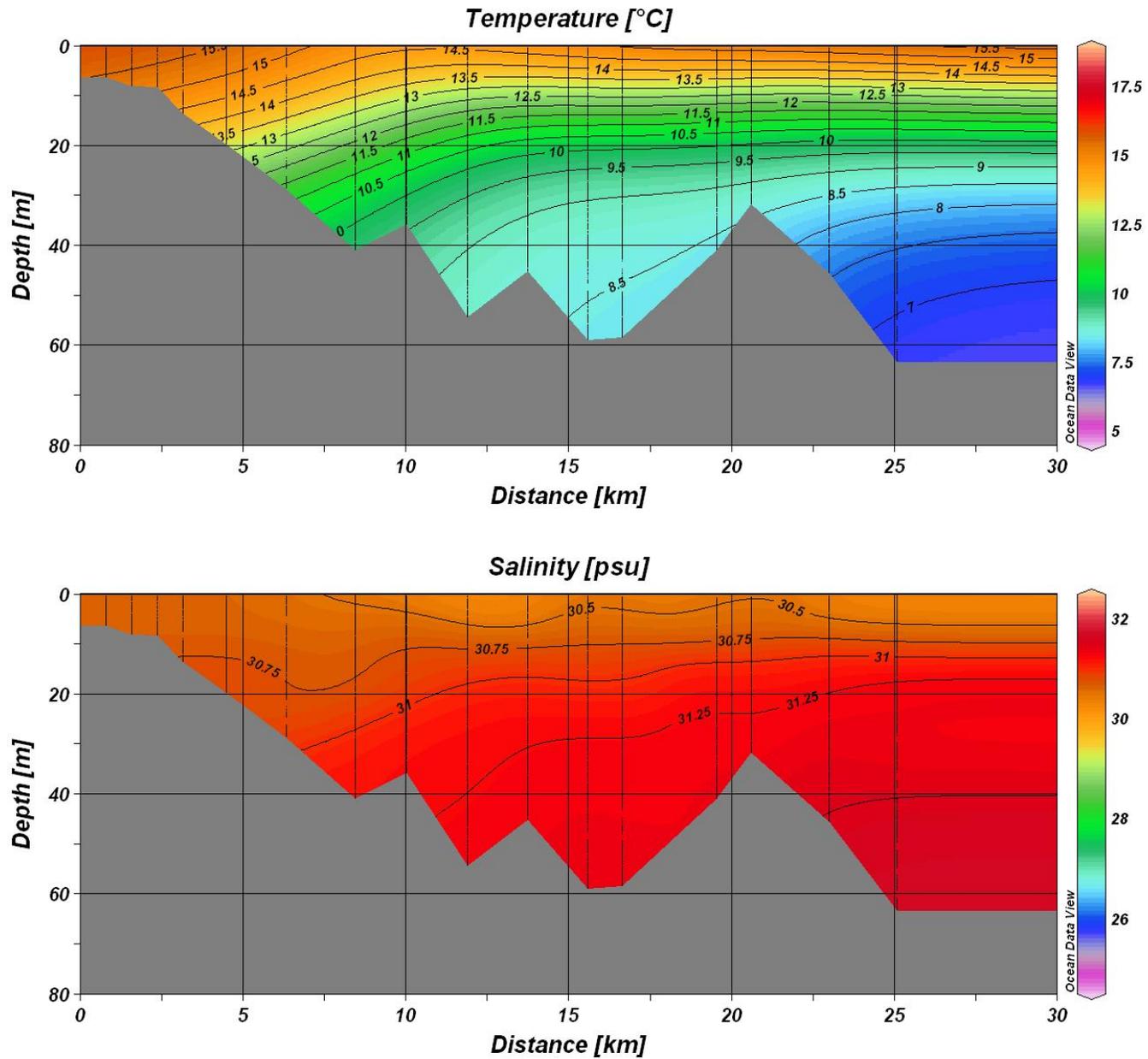


Figure 8. September 14, 2005

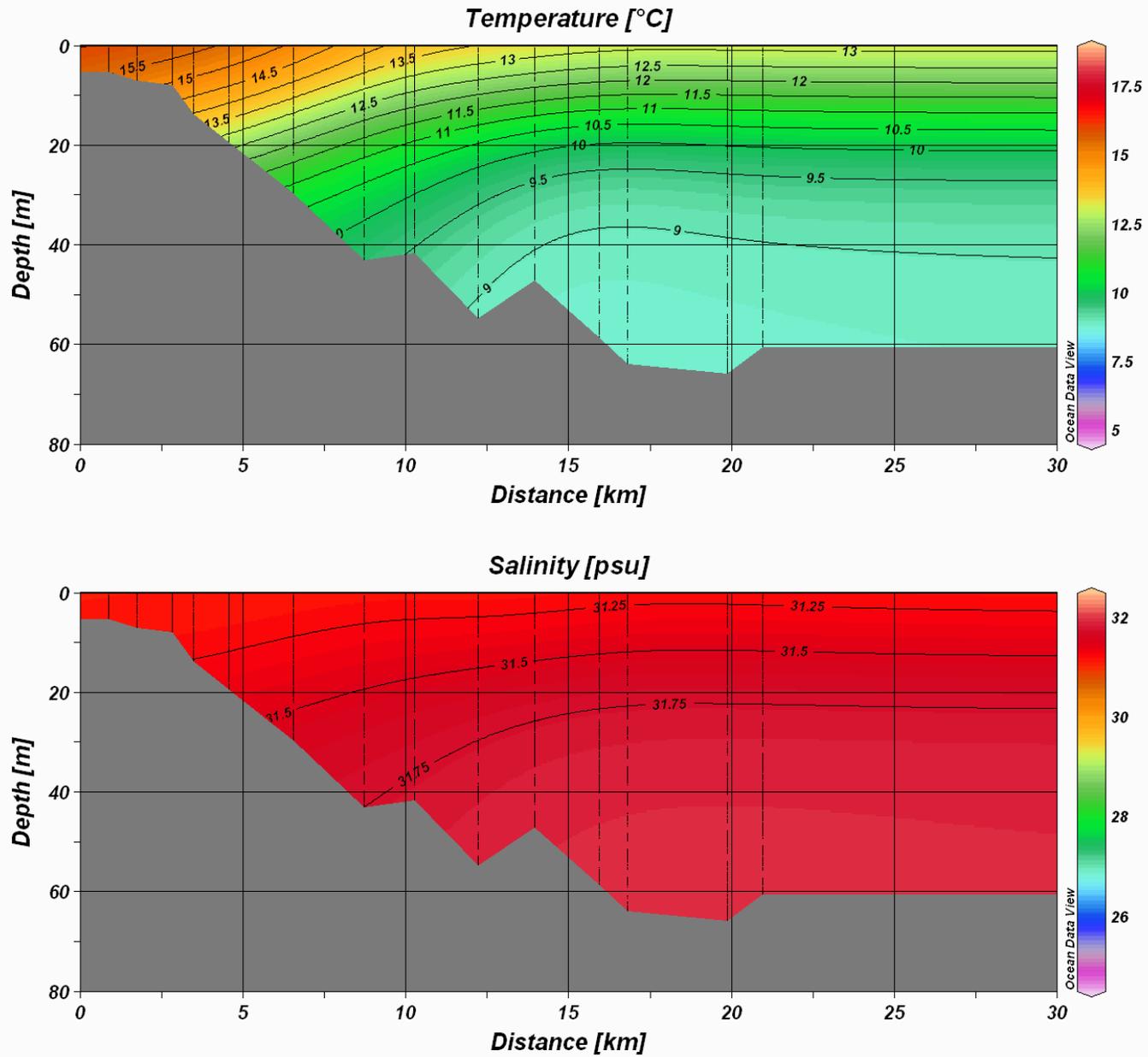
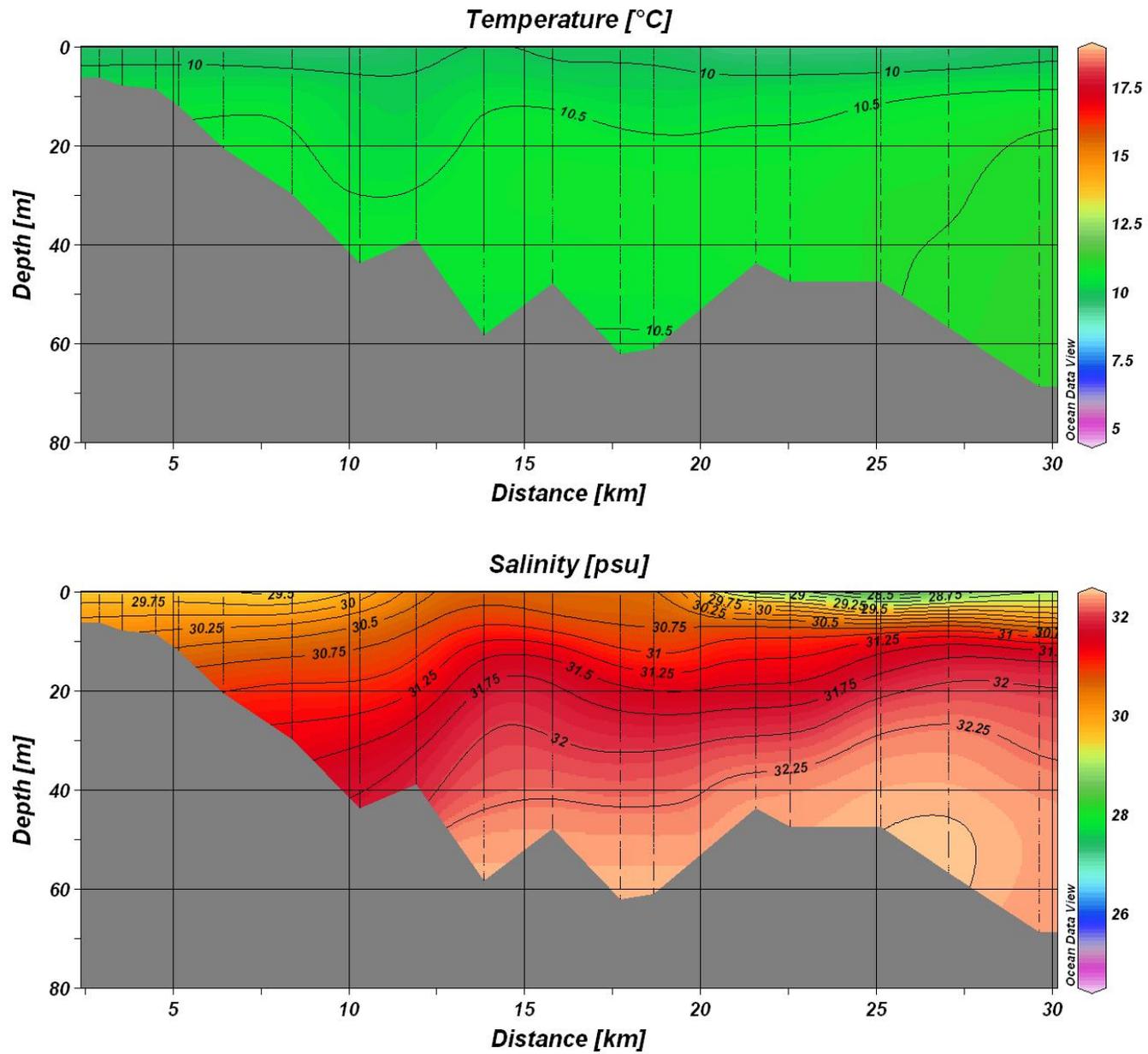


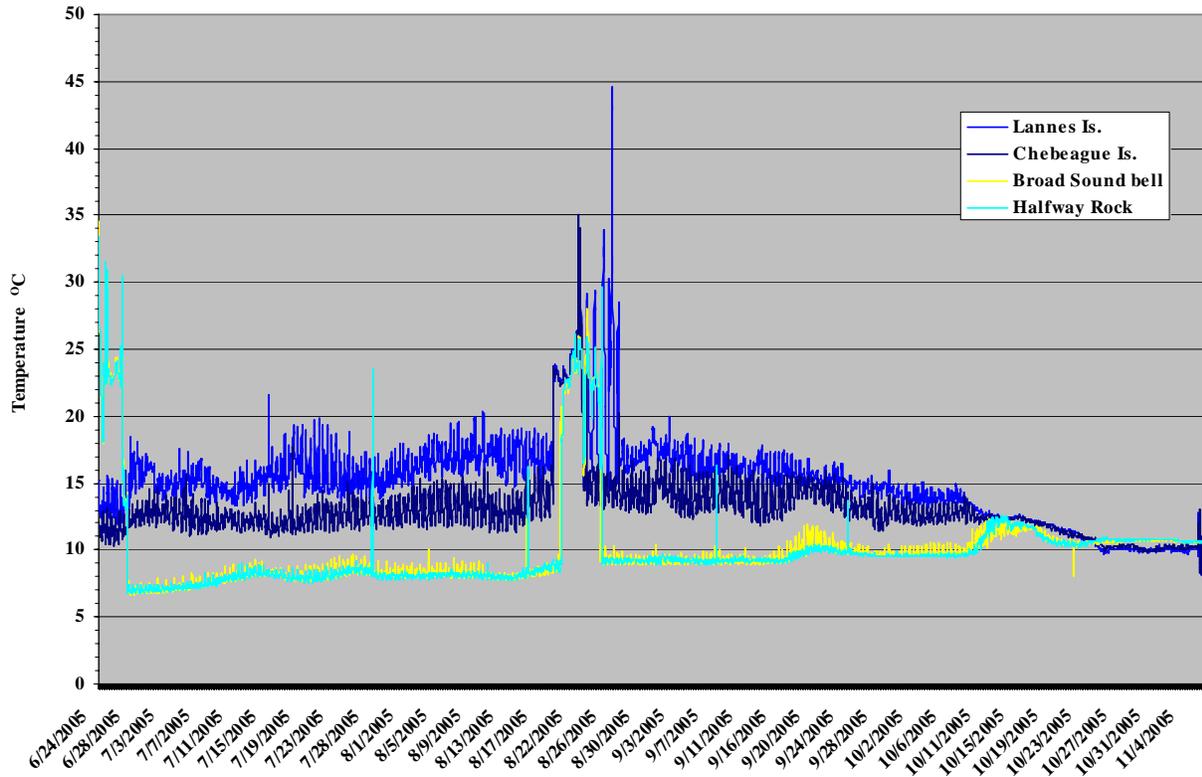
Figure 9. October 28, 2005



Bottom Temperature

The dataset for each HOBO® Water Temp Pro sensor contains approximately 13,060 recordings taken over the deployment period and are therefore not tabulated here or in the Appendix; however, all data are provided on the Data CD accompanying this report. For purposes of presentation, the data are summarized graphically in Figure 10, below, using Excel® and data exported from BoxCar®.

Figure 10. Bottom temperature at selected locations in Casco Bay, 6/24/2005 through 11/7/2005



The elevated temperatures for both the Broad Sound bell buoy and Halfway Rock early in the dataset represent a delay between pre-programmed activation and actual deployment of the units. Similarly, the elevated temperatures recorded between 8/20/2005 and 8/27/2005 represent the interim data download interval during which the units were removed from the water; the occasional spikes simply represent recordings made while the trap carrying the unit was on-board during routine tending.

Depth at Lannes Is. (LI) at low water (LW) is approximately 4m, Chebeague Is. (CI) location approximately 10m, and the Broad Sound bell buoy (BS) and Halfway Rock (HWR) locations approximately 45m. Temperature is predictably warmer in the shallower locations, but the consistency in temperatures at the BS and HWR locations is remarkable although the BS location does show a greater degree of fluctuation than HWR. Of greatest interest, however, is the rapid, simultaneous increase in temperature of 2-3°C at both BS and HWR from about 9.6/9.7°C to nearly 12.0°C over the course of just three days between 10/08/2005 and 10/11/2005 resulting in a nearly thermally mixed water mass from surface to bottom from inshore to offshore, consistent with the results of the 16 nm transect profile work carried out on 10/28/2005; this is followed shortly after by a temperature inversion where the surface water becomes cooler than the bottom water as fall sets in toward the end of October/beginning on November.

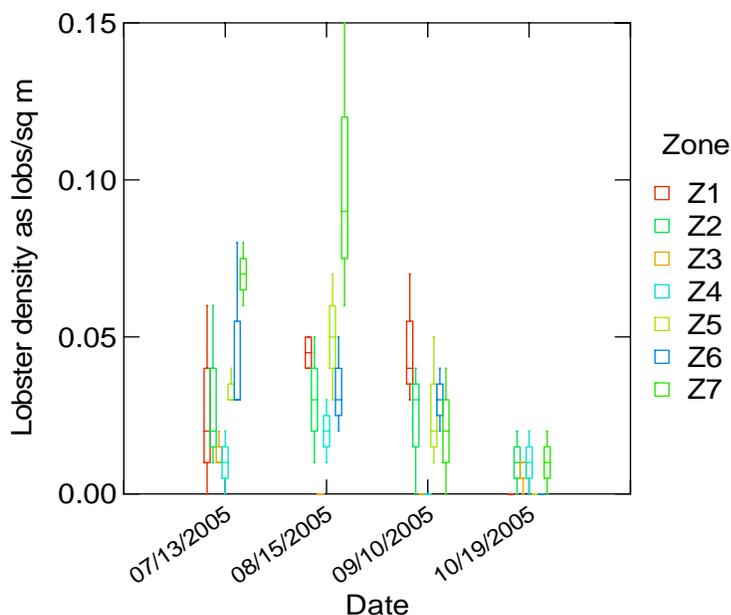
Discussion

The lobster catch in the shallow, inner areas of Casco Bay has decreased in the past several years (B. Tarbox, M. Nappi, M. Kimball, Pers. comm.). The intent of this study was to determine if water quality parameters used to indicate environmental degradation, *e.g.* eutrophication, are affecting the distribution and abundance of lobsters in shallow portions of Casco Bay. Similar water and sediment quality parameters, along with usual temperature and salinity, were used here as were used to indicate eutrophication and presumed to be linked to lobster mortalities in Long Island Sound, New York (Cuomo, *et al.*, 2005), specifically, dissolved oxygen (DO) and ammonia (NH₃); sulfide (S₂) was also analyzed, however sulfide reported here is sediment sulfide while that reported by Cuomo, *et al.* (2005) was dissolved hydrogen sulfide in bottom and near-bottom water.

Lobster density

As shown in Figure 11, lobster density was well below the Maine DMR guideline of 0.1 lobsters/m², indicative of important lobster habitat, at all study areas with exception of Zone 7 in August. Initially, lobster density appeared to be generally greater further from shore (Zone 7); this general trend appeared to continue through August, but began to breakdown in September until all zones yielded essentially the same, low densities by October.

Figure 11. Box plot diagram of lobster density by zone and date



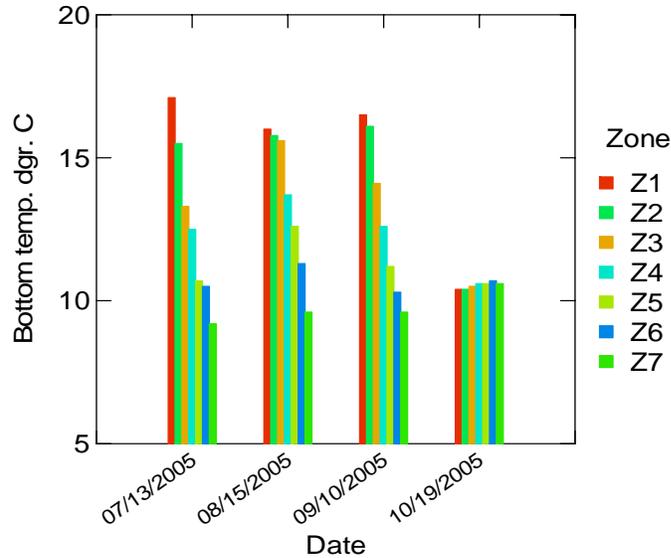
The decline in observed lobsters (lobster density) from August through October, both by the diver and subsequently by video review of the recording, may have been a direct result of harvesting in the areas, and as discussed later, does not appear to have been related to any environmental degradation. The sharp decline in October may also have resulted from a change in the diver/observer which had remained consistent through September.

It is also worth noting that fishermen fishing the areas sampled during the study, particularly the shallower areas of Zones 1-3, anecdotally reported that catch jumped dramatically toward the end of October and beginning of November, about the time that the bay appears to have become thermally mixed and homogeneous. Unfortunately, the final project sampling occurred just prior to this occurrence and the increase in lobsters was not empirically documented.

Environmental Data Results

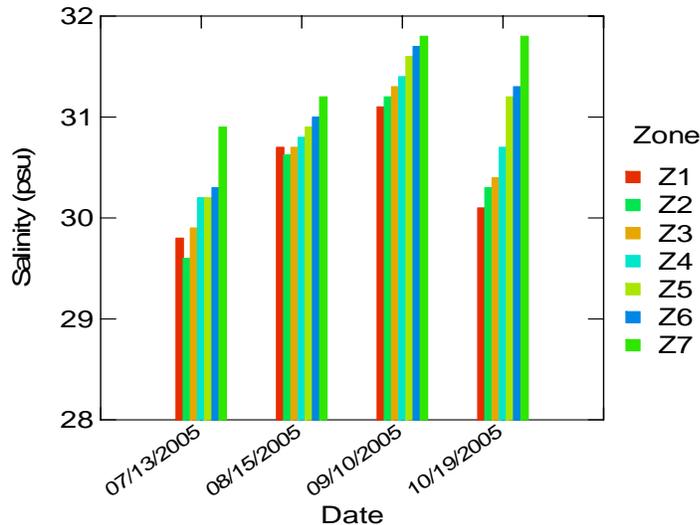
The lobster’s normal habitat temperature ranges from 5°C to 20°C (Aiken and Waddy, 1986). The temperature tolerance of lobsters ranges widely from -1°C to 30.5°C and they are also capable of tolerating abrupt temperature increases of 16°C and decreases of 20°C (Harding, 1992). As Figure 10, above, and Figure 12, below, show, the bottom temperature throughout the study area and period remained within the lobster’s normal habitat range and only rarely approached the upper limit of the range, even at the shallowest study site.

Figure 12. Bottom temperature for each study zone by sampling date



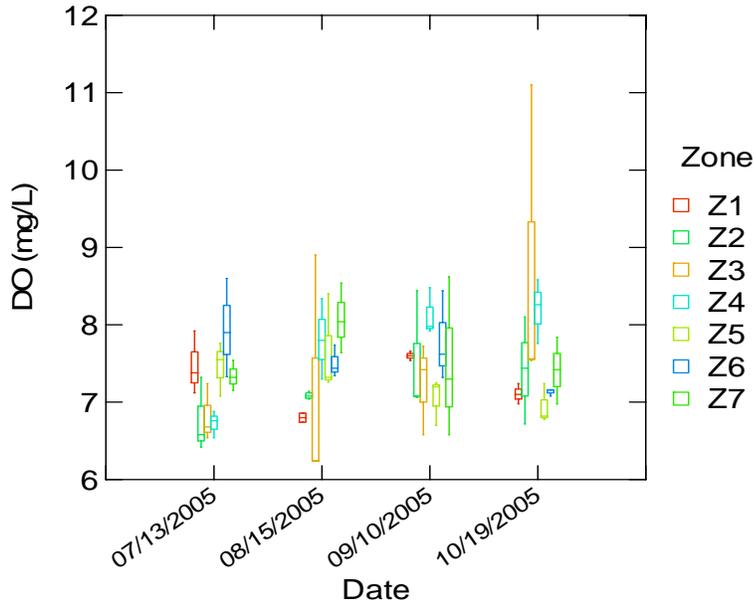
Lobsters typically inhabit areas where salinity is >25 ppt (psu) (Dall, 1970); salinities ≤10 ppt are extremely stressful and the lethal limit varies between 8-14 ppt, depending on temperature, oxygen, and acclimation (McLeese, 1956). Salinities varied between 29.5 and 31.8 psu during the study with increasing proximity to open ocean, as shown in Figure 13; at no point during the study did salinity drop below the levels in which lobsters are typically found.

Figure 13. Mean salinity for each study zone by sampling date



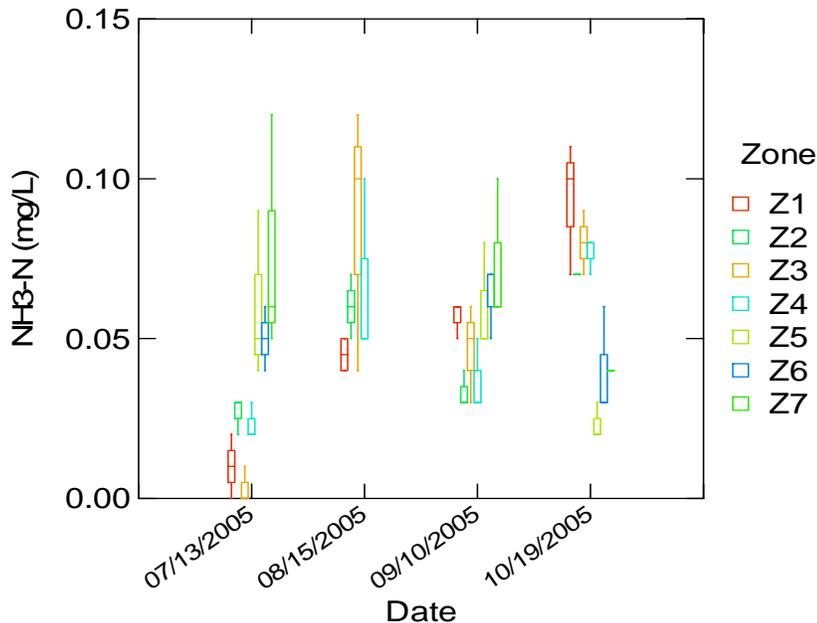
Lobsters can tolerate low dissolved oxygen (DO) levels with their lower lethal limits ranging from 0.2 mg/L at 5°C to 1.2 mg/L at 25°C at a salinity of 30 ppt (Harding, 1992). Cuomo, *et. al.*, (2005) reported hypoxic conditions (<3.0 mg/L DO) in near-bottom (1 m above bottom) and bottom (5 cm above the bottom) waters in Western Long Island Sound (WLIS) persisting for 64 days between August and September 2002. Burrow water DO at all sites in this study remained above 6.0 mg/L throughout the study period, thus never represented stressful conditions, as shown in Figure 14.

Figure 14. Box plot diagram of dissolved oxygen by zone and date



Un-ionized ammonia (NH₃-N) from burrow water samples is presented in Figure 15 for each zone by sampling date.

Figure 15. Box plot diagram of un-ionized (NH₃-N) ammonia by zone and date



According to Olst *et al.* in Cobb and Phillips (1980), the 192 hr incipient median lethal concentration (LC₅₀) of un-ionized ammonia nitrogen for stage IV lobsters is 1.4 mg/L (Delistraty *et al.*, 1977) and the 24 hr LC₅₀ for stage IV lobsters is 8.2 mg/L (Sastry and Laczak, 1975); the 96 hr LC₅₀ for 1.0 g wet body weight lobsters was found to be 1.2 mg/L (L. Gleye, personal communication to Olst *et al.*). As the plot in Figure 14 shows, the values found in this study are an order of magnitude less than the LC₅₀ values reported in Cobb and Phillips (1980). It should be noted that, for purposes of presentation, the August outliers resulting from failure to acidify the samples from Zones 5, 6, and 7 have been omitted from the plot.

The Maine Department of Environmental Protection (DEP) uses sediment redox and sulfide levels as indicators of environmental degradation in its permitting requirements for salmon aquaculture (MEG13000). Accordingly, a redox value of <0 mV is considered “warning” level and <-100 mV the “impact” limit (violation level); sediment sulfide of 1300 μM is considered “warning” level and 6000 μM the “impact” limit. The sediment redox and sulfide levels recorded during the study are presented in Figures 16 and 17, respectively.

The sediment redox levels recorded in this study generally remained above or just below the 0 mV level in Zones 4-7; Zones 1-3 consistently showed redox levels below 0 mV during the warmer months indicating mildly hypoxic conditions within the sediment, but improved in October; no readings <-100 mV indicating severe hypoxia or anoxia were seen. The low levels at these zones are likely attributable to higher organic content of the sediment resulting from the adjacent or nearby eelgrass beds.

Similarly, the sulfide levels generally remained below the 1300 μM with the exception of a few readings above this level in August as shown in Figure 16; all sulfide levels recorded were well below the 6000 μM “impact” level indicative of severely hypoxic or anoxic conditions. These results indicate only mildly hypoxic sediment conditions in August but otherwise normal sulfide levels and little, if any, sulfide-related stress on the benthos, including lobsters.

Figure 16. Box plot diagram of sediment redox (Eh) by zone and date

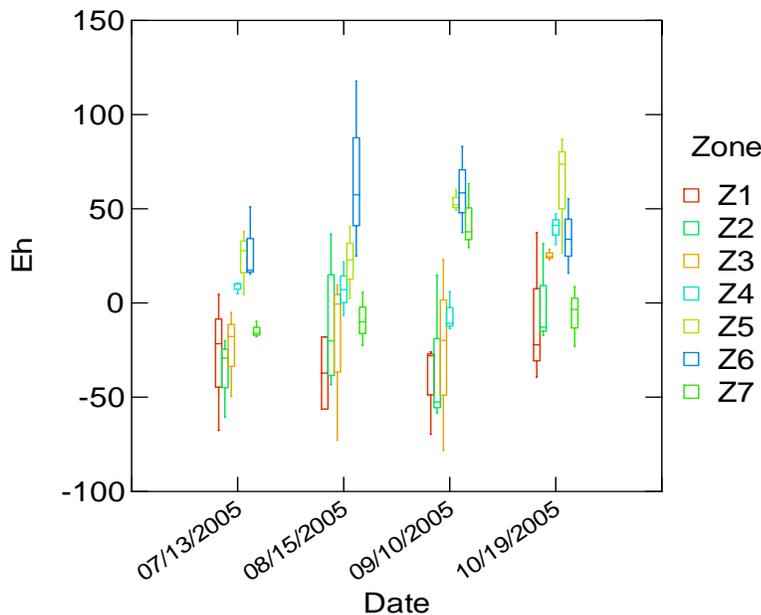
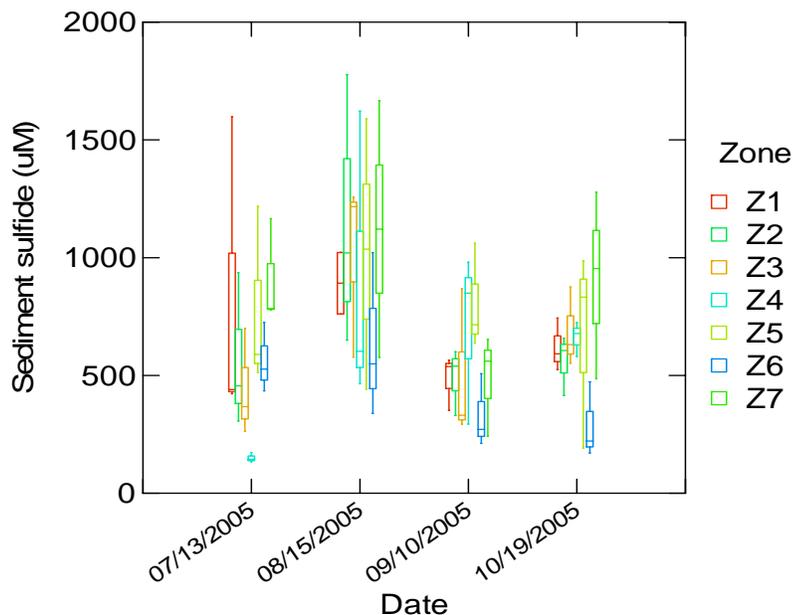


Figure 17. Box plot diagram of sediment sulfide by zone and date



As previously noted, the measurement of nitrate was confounded by the inability to properly standardize the HACH 890 spectrophotometer because the salinity adjusted 0.16 mg/L standardizing solution was below the detection limit. However, although nitrate values would have been valuable in confirming the existence of eutrophic conditions, none of the other measured parameters, particularly dissolved oxygen and sediment sulfide, suggest that substantive eutrophication is occurring, at least at the sites studied.

Conclusions

Based on the results and analysis presented here, there appears to be no significant relationship between water and sediment quality and the density of lobsters, as observed by divers, at any of the areas covered by the study. Furthermore, the individual parametric results indicate that conditions in all areas are well within the tolerable limits of lobsters.

As stated at the outset, the intent of this study was to document the anecdotally reported lower abundance of lobsters in shallower areas of Casco Bay compared to deeper, more offshore areas in a quantitative manner and try to either identify or rule out specific water quality parameters that may be causing a shift in lobster distribution. Although the results of the study show no evidence of environmental degradation or significant relationship between environmental conditions and lobster distribution, this study represents but a single year and is limited to the parameters tested. There is evidence, however, that changes are occurring in at least a portion of the area studied.

A comparison of aerial photographs taken in 1993 and 2001 of the area just to the north of Cousins Island (Zones 1, 2, and 3 of this study) show rather dramatic changes in the distribution of eelgrass, *Zostera marina*, over the intervening 8-year period. Figure 18 is the aerial photograph of the area taken in 1993 and Figure 19 is the same photograph with a percent coverage overlay (Seth Barker, DMR). Figure 20 is the same area as it appeared in 2001 and Figure 21 shows the percent coverage for that year.

Figure 18. Aerial photograph of the area just north of Cousins Island in 1993 (Source, Seth barker, DMR)



Figure 19. Aerial photograph of the area just north of Cousins Island in 1993 with percent eelgrass coverage overlay (Source, Seth barker, DMR)

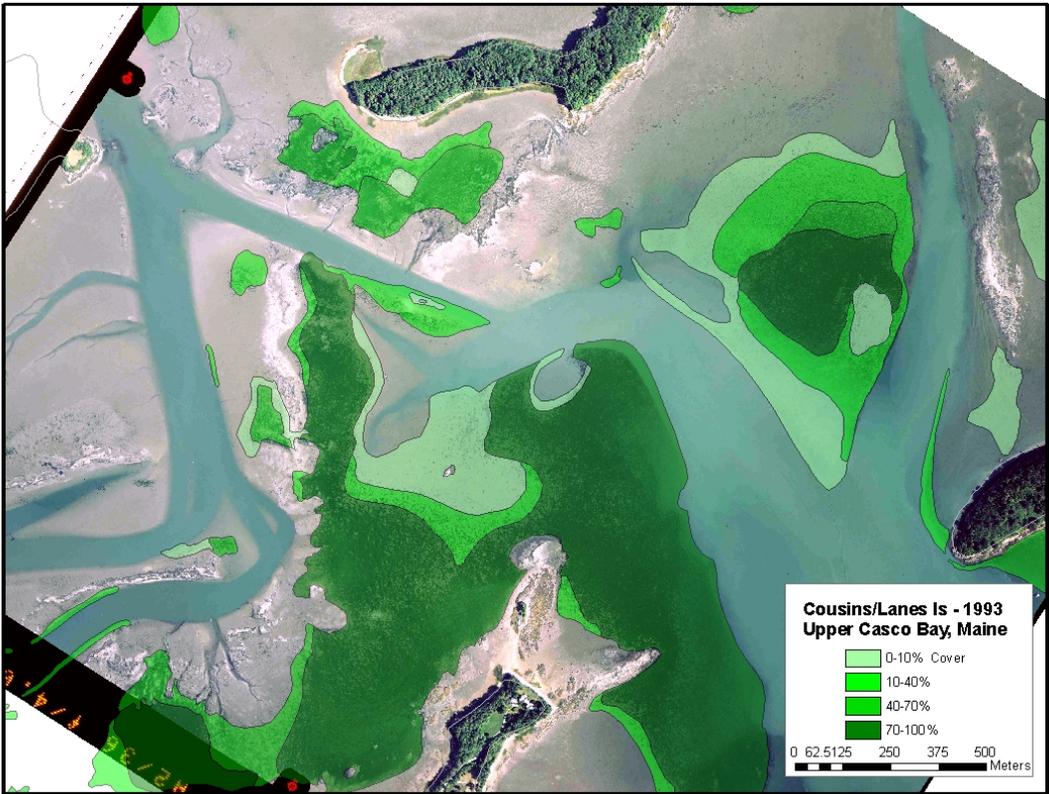


Figure 20. Aerial photograph of the area just north of Cousins Island in 2001 (Source, Seth barker, DMR)

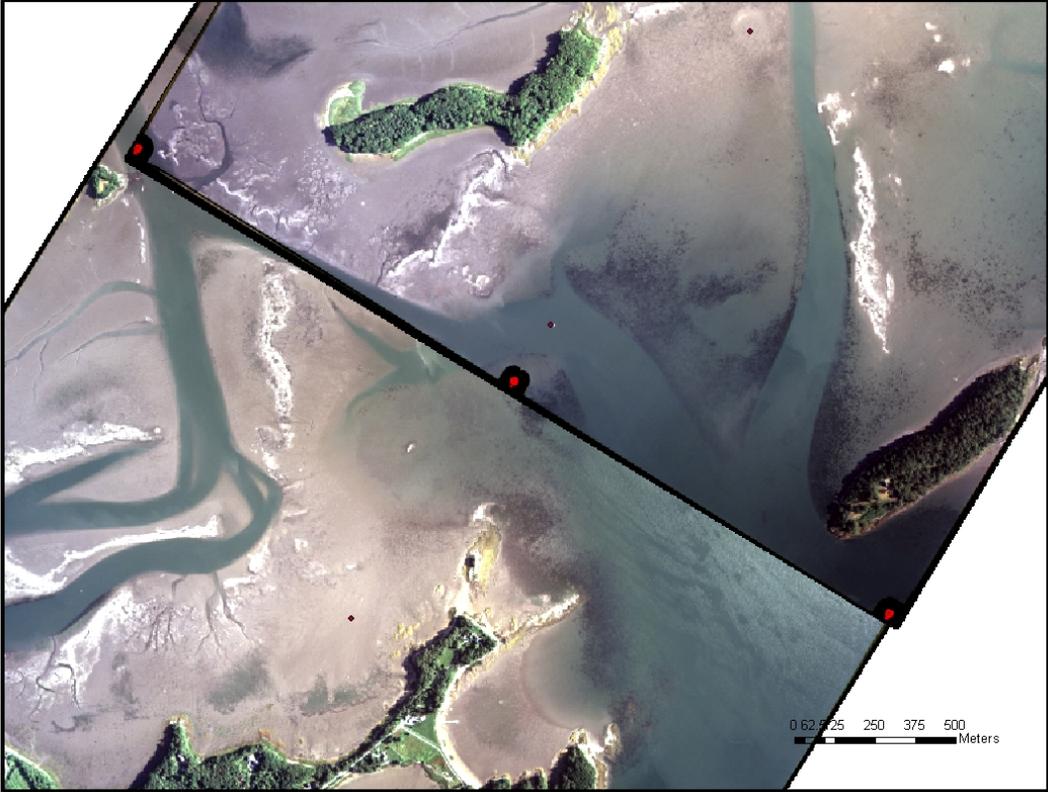
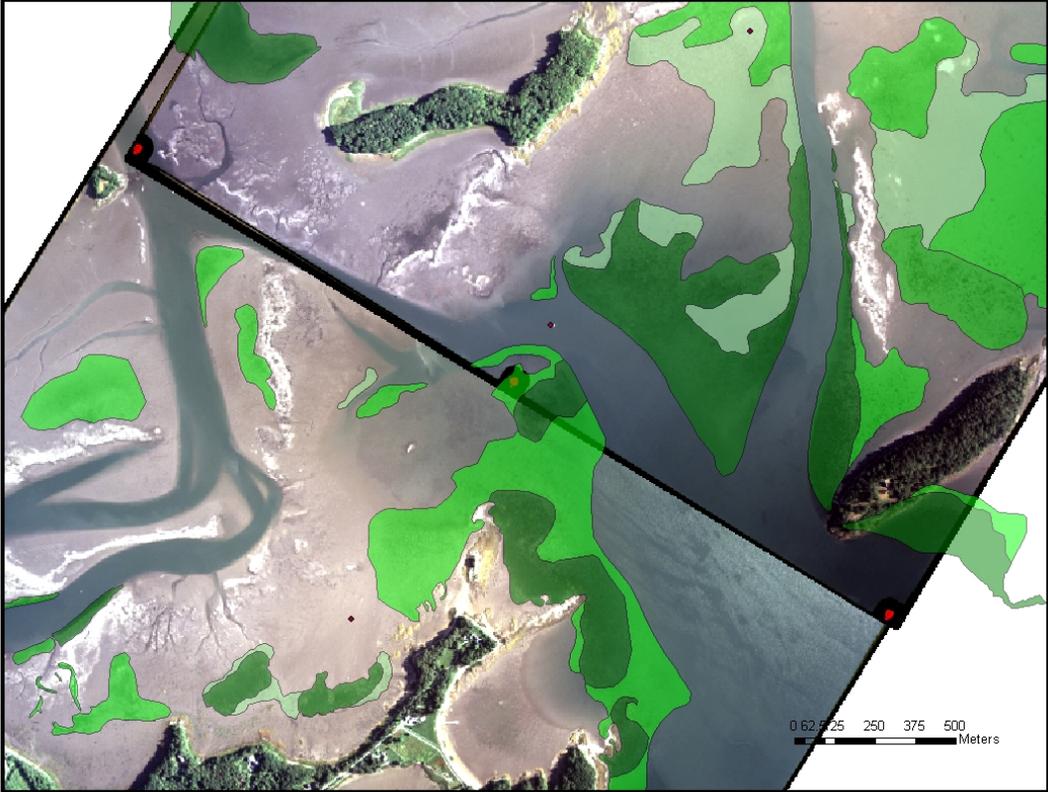


Figure 21. Aerial photograph of the area just north of Cousins Island in 2001 with percent eelgrass coverage overlay (Source, Seth barker, DMR)



The changes in both density and spatial distribution of the eelgrass between 1993 and 2001 are rather clear but the reasons for these changes are far less clear, suggesting that other factors may be at work in the area that may either directly or indirectly affect lobster distribution, although not necessary consistently. Indeed, the 2006 season appears to have been quite different from the 2005 season with numerous shedder lobsters being reported very early in the season. Clearly, therefore, additional work would be necessary to determine if the 2005 season and the results of this study truly reflect the norm, or whether the anecdotally reported changes in lobster behavior are merely temporary.

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**Appendix I
HACH Method 8166
High Range Dissolved Oxygen**

Method 8166

HRDO Method

AccuVac® Ampuls

HR (0.3 to 15.0 mg/L O₂)

Scope and Application: For water and wastewater



Tips and Techniques

- Analyze samples on-site. Do not store for later analysis.

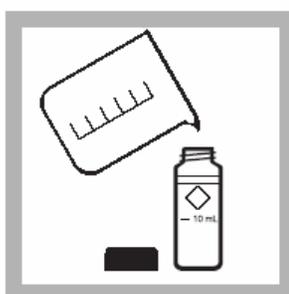


AccuVac Ampul

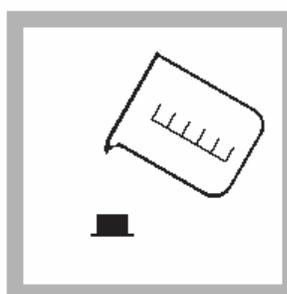
Method 8166



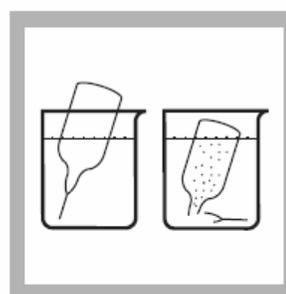
- Touch **Hach Programs**.
Select program
445 Oxygen Di. HR AV.
Touch **Start**.



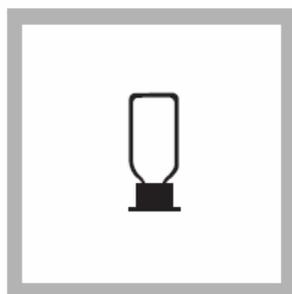
- Fill a round sample cell (the blank) with at least 10-mL of sample.



- Fill a blue ampule cap with sample.



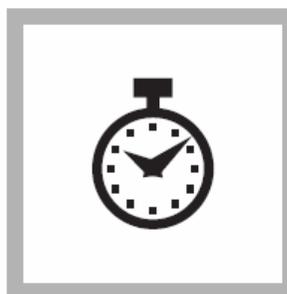
- Fill a High Range Dissolved Oxygen AccuVac Ampul with sample. Keep the tip immersed while the ampule fills completely.



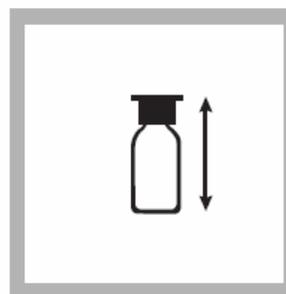
- Hold the ampule with the tip pointing down and immediately place the ampule into the ampule cap. The cap prevents contamination from atmospheric oxygen.



- Shake the ampule for 30 seconds. A small amount of undissolved reagent will not affect results.

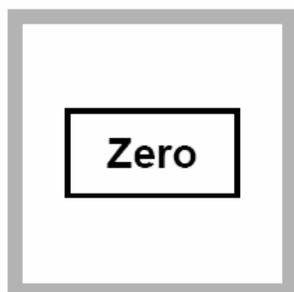


- Touch the timer icon. Touch **OK**. A two-minute reaction period will begin. This enables the oxygen that was degassed during aspiration to redissolve and react.



- When the timer beeps, shake the ampule for 30 seconds.

Oxygen, Dissolved



9. Place the blank in the cell holder.

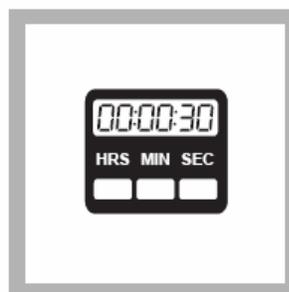
Touch **Zero**.

The display will show:

0.0 mg/L O₂



10. Place the ampule into the cell holder.



11. Wait approximately 30 seconds for the air bubbles to disperse from the light path.

Results will appear in mg/L O₂.

Interferences

Interfering Substance	Interference Levels and Treatments
Cr ³⁺	Greater than 10 mg/L
Cu ²⁺	Greater than 10 mg/L
Fe ²⁺	Greater than 10 mg/L
Mg ²⁺	Magnesium is commonly present in seawater and causes a negative interference. If the sample contains more than 50% seawater, the oxygen concentration obtained by this method will be 25% less than the true oxygen concentration. If the sample contains less than 50% seawater, the interference will be less than 5%.
Mn ²⁺	Greater than 10 mg/L
Ni ²⁺	Greater than 10 mg/L
NO ₂ ⁻	Greater than 10 mg/L

Sample Collection, Preservation, and Storage

The main consideration in sampling with the High Range Dissolved Oxygen Ampul is to prevent the sample from becoming contaminated with atmospheric oxygen between breaking open the ampule and reading the absorbance. This is accomplished by capping the ampule with an ampule cap. If the ampule is securely capped, the ampule should be safe from contamination for several hours. The absorbance will decrease by approximately 3% during the first hour and will not change significantly afterwards.

Sampling and sample handling are important considerations in obtaining meaningful results. The dissolved oxygen content of the water being tested may change with depth, turbulence, temperature, sludge deposits, light, microbial action, mixing, travel time, and other factors. A single dissolved oxygen test rarely reflects the accurate overall condition of a body of water. Several samples taken at different times, locations, and depths are recommended for most reliable results. Samples must be tested immediately upon collection, although only a small error results if the absorbance reading is taken several hours later.

Accuracy Check

The results of this procedure may be compared with the results of a titrimetric procedure (request Lit. Code 8042) or *sension*^{TM6} Dissolved Oxygen Meter (Cat. No. 51850-01).

Method Performance

Precision

Standard: 7.9 mg/L O₂

Program	95% Confidence Limits of Distribution
445	7.5–8.3 mg/L O ₂

See Section 3.4.3 Precision on page 53 for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.09 mg/L O ₂

See Section 3.4.5 Sensitivity on page 54 for more information.

Summary of Method

The High Range Dissolved Oxygen AccuVac Ampul contains reagent vacuum sealed in a 14-mL ampule. When the AccuVac Ampul is opened in a sample containing dissolved oxygen, it forms a yellow color which turns purple. The purple color development is proportional to the concentration of dissolved oxygen. Test results are measured at 535 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
High Range Dissolved Oxygen AccuVac® Ampuls, 0–10 mg/L with 2 reusable ampule caps.....	1 ampul.....	25/pkg.....	25150-25

Required Apparatus

Polypropylene Beaker, 50-mL, Low Form, with pour spout.....	each.....		1080-41
Sample Cells, 10-mL, w/cap.....	1.....	6/pkg.....	24276-06

Appendix II
MER SOP for YSI profiler deployment

**MER Assessment Corporation
Standard Operating Procedure (SOP) 005
YSI 6600 Water Column Profiler
Revision 0, 20 December 2005**

The YSI 6600 Environmental Monitoring System is a multi parameter water quality- measuring device. The parameters presently measured by the water column profiler are: temperature (°C.), depth (m), DO (%sat), DO (mg/L), salinity (psu), and pH; , the profiler is capable of having additional parameters added if necessary. Prior to use in the field our YSI 6600 unit under goes a series of different calibrations and inspections, as follows:

1. Once a year the YSI is returned to the factory for internal circuitry inspection and a factory electronic calibration. Documentation can be found in Appendix A.
2. Prior to deployment in the field, the YSI 6600 sonde undergoes a series of bench calibrations using standard solutions to insure that the electrodes are functioning properly electronically, and measuring properly (see next page). This procedure is also carried out immediately upon returning from the field to insure accuracy and reproducibility. All bench calibrations are conducted by strictly following the YSI Operator's Manual, Sec. 2.6 and using YSI's standard calibration solutions for conductivity and two-point pH measurements. A copy of MER's calibration performance sheet can be found on the following page.
3. a.) In the field the YSI is again checked to make sure all the electrodes are reading properly and are within normal operating ranges; particular attention is given to the membrane of the dissolve oxygen electrode to confirm there are no bubbles under the membrane or holes to insure accurate measurements. This procedure can be found in MER's YSI Field Procedure Guide, following the calibration sheet.

b.) All water column profiles are done in replicate. In addition, if requested, triplicate Winkler titrations for DO mg/L comparisons to insure the YSI is operating accurately can be included. These samples are run once before any profiles are taken and again after the profiles are completed, and at reference stations some distance from the area being profiled. Winkler samples are taken at a depth of 10m with a General Oceanic Niskin Sampling Bottle; the YSI is simultaneously deployed to a depth of 10m as well, so that the most accurate comparison can be made.
4. Periodically we conduct joint bench calibrations and/or field sampling with other organizations that have the same instrument.



Revision 0
Date: 20 December 2005

MER Assessment Corporation

MER YSI 6600 D Calibration Sheet

6600 D Ser. No.: 01A0870

650 MDS ID: 01A0851 AB

Date:

Pre-sampling calibration

Time:

Battery volts:

Temperature

NIST Therm. °C.:

YSI 6600 °C.:

Discrepancy.:

% discrp.:

Calibration: pass/fail

Dissolved oxygen

Atmospheric press. (mmHg) (25.4 mm/in.):

Temp. °C.

D.O. % sat.:

D.O. mg/l (6600):

D.O. mg/l (table):

Discrepancy.:

% discrp.:

D.O. charge: **(50 ±25 DO ch)**

Calibration: pass/fail

Salinity

Conductivity standard:

Conductivity YSI 6600:

Discrepancy.:

% discrp.:

Calibration: pass/fail

pH (2-point 7.0/10.0)

pH standard: 1) 2)

pH YSI 6600: 1) 2)

Discrepancy.: 1) 2)

% discrp.: 1) 2)

Calibration: pass/fail

Verification initials

Date:

Post-sampling calibration check

Time:

Battery volts:

Temperature

NIST Therm. °C.:

YSI 6600 °C.:

Discrepancy.:

% discrp.:

Calibration: pass/fail

Dissolved oxygen

Atmospheric press. (mmHg) (25.4 mm/in.):

Temp. °C.

D.O. % sat.:

D.O. mg/l (6600):

D.O. mg/l (table):

Discrepancy.:

% discrp.:

D.O. charge: **(50 ±25 DO ch)**

Calibration: pass/fail

Salinity

Conductivity standard:

Conductivity YSI 6600:

Discrepancy.:

% discrp.:

Calibration: pass/fail

pH

pH standard: 1) 2)

pH YSI 6600: 1) 2)

Discrepancy.: 1) 2)

% discrp.: 1) 2)

Calibration: pass/fail

Verification initials

MER Assessment Corporation
YSI Field Standard Operating Procedure (SOP) 005-B
Logging water column data set at single specific depth using YSI 6600 Water Column Profiler

Revision 0, 20 December 2005

1. Remove clear calibration cup taking care not to snag the O-ring on the dissolved oxygen probe;
2. D.O. membrane should have been replaced 12 hours prior to field deployment, however, inspect the probes for integrity and check D.O. membrane for integrity and any air bubbles;
3. Screw probe guard onto end of sonde taking care once again not to strike probes or D.O. "O" ring;
4. Remove yellow connection protectors from both ends of sonde cable;
5. Unscrew sonde connection protection cap and connect chrome end of sonde cable to sonde; connect other end to either the YSI handheld data logger (650MDS) or laptop for real time data recording;
6. Turn data recorder on by pressing green circle on 650 MDS, or turn laptop on
7. To proceed with 650 MDS go to 8; to proceed with laptop, go to 20;
8. Check battery level (icon at lower right will indicate whether data logger or sonde batteries are being checked);
9. Highlight "Sonde run" and press enter (symbol);
10. In Run screen check that date and time are accurate;
11. Ensure all desired parameters are being measured and all requisite probes are activated. Required parameters include: Temp. (^oC.), salinity (‰), D.O. (% sat.), D.O. (mg/L), and pH and depth. If a parameter is missing continue to 12, otherwise go to 20
12. Hit "Esc", highlight "Sonde menu" and hit enter; data recorder will connect to sonde as indicated by icon at lower right corner;
13. Highlight "Sensor" and hit enter; "Sensor enabled" screen will appear;
14. In "Sensor enabled" menu ensure that radio button next to desire parameter is darkened; if not, highlight parameter and hit enter;
15. If sensor is enabled but parameter does not appear on "Run" menu, hit "Esc" to return to Main menu
16. In Main menu select Report and enter; Report setup screen appears;
17. In "Report setup" menu, scroll down to ensure that the radio buttons for all required parameters are highlighted; if not, highlight parameter and hit enter; hit "Esc" to return to Sonde Main menu
18. Hit "Esc" again to return to 650 Main Menu and select Sonde run and hit enter; data logger will connect to sonde – sonde may go through momentary stabilization;
19. Review Run menu to ensure all required parameters are now being measured; if all parameters are being measured proceed to 21, otherwise return to 13;
20. Fill bucket with water, place sonde in bucket with data recorder on and allow sonde to fully stabilize, that is, fluctuations in parameter readings no greater than 0.1 for three scrolling lines on laptop or 10-15 seconds of data shown on data logger. In the meantime complete data sheet, including taking Secchi disk depth (refer to Appendix 2, Filling out datasheet);
21. Lower sonde to the surface of the water and ensure that depth reads 0.000m (± 0.002); if depth is correct, proceed to 27 ; otherwise calibrate depth by following 22;
22. Hit "Esc" to return to 650 Main Menu; highlight Sonde menu and enter;
23. Highlight Calibrate and press enter;

24. Highlight Pressure-Abs press enter;
25. In Calibrate menu enter the depth in meters as “0.000” and hit enter; 650 screen remains in Calibrate mode but parameters are displayed with Calibrate under the Sonde label highlighted (display will continue to show old depth); hit enter to accept calibration – depth should now read 0.000m (± 0.002); highlighted bar will now read Continue – press enter to return to Calibrate menu;
26. Press Esc to return to Main sonde menu screen
27. Highlight Run, press enter and select Discrete sample;
28. Scroll down to Close file and press enter – screen will switch to Open a file
29. In the Discrete sample menu select Site; in Site identifier type, for example, CASCOBAY; for work in CASCOBAY or CB092305 for Casco Bay work on 092305; press Enter to log in Site Id;
30. Scroll up to File=, press enter and begin entering filename as the station , date, or both (i.e. ,LP(or LP90923, etc), followed, if necessary, by the replicate (a, b or c), i.e. 0708021a for the first replicate at Station 1 on July 8, 2002 ;
31. Scroll up to Start sampling and press enter; data recorder will connect to sonde as indicated by icon at lower right corner;
32. Lower sonde into the water such that the probes are $< 0.5\text{m}$ below the surface;
33. Ensure data will be logged to the sonde – if Log one sample is highlighted under the 650, press the right ► arrow to highlight Log one sample under Sonde;
34. To log a constant profile of the water column at depth hit every 0.5. sec., press the ▼ arrow to highlight “Start Logging”;
35. Allow probes to stabilize; once stabilized press enter [make sure the message switches to **Stop logging**] to ensure data logging has begun;
36. Lower sonde through the water column at an appropriate, predetermined rate, constantly watching the display for any anomalous readings; allow sonde to continue logging through the full profile depth, i.e. sonde hits bottom and depth ceases to increase;
37. Once bottom is reached, hit Enter to change “**Stop Logging**” back to “**Start Logging**”;
38. If moving to a new station, Hit Esc once (1) time to return to Discrete sample menu; scroll down to **Close file** and hit enter; **Open a file** will appear; Scroll up to File= and enter the name of the new station; proceed from step 31; if data logging run is complete scroll down to **Close file** and hit enter to secure data;
39. Hit Esc 3 times to return to 650 Main Menu; to turn data recorder off, press green circle button.
40. Reverse first 6 steps to stow sonde.



Revision 0

Date: 20 December 2005

**Appendix III
HACH Method 8155
Nitrogen, Ammonia Salicylate Method**

Method 8155

Salicylate Method*

Powder Pillows

(0.01 to 0.50 mg/L NH₃-N)

Scope and Application: For water, wastewater, and seawater

* Adapted from *Clin. Chim. Acta.*, 14, 403 (1966)



Tips and Techniques

- A green color will develop if ammonia nitrogen is present.

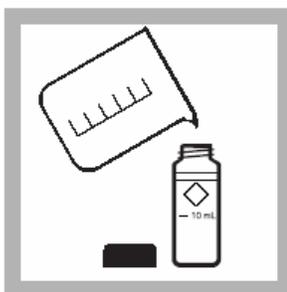


Powder Pillows

Method 8155



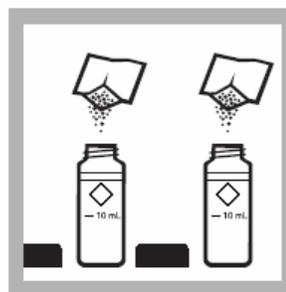
1. Touch **Hach Programs**.
Select program **385 N, Ammonia, Salic.**
Touch **Start**.



2. Fill a round sample cell to the 10-mL mark with sample.



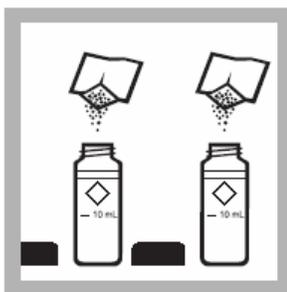
3. Fill another round sample cell to the 10-mL mark with deionized water (this is the blank).



4. Add the contents of one Ammonia Salicylate Powder Pillow to each cell. Stopper and shake to dissolve the powder.



5. Touch the timer icon.
Touch **OK**.
A three-minute reaction period will begin.



6. When the timer beeps, add the contents of one Ammonia Cyanurate Reagent Powder Pillow to each cell. Stopper and shake to dissolve the reagent.

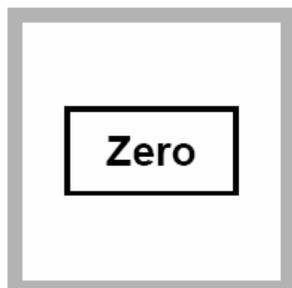


7. Touch the timer icon.
Touch **OK**.
A 15-minute reaction period will begin.
A green color will develop if ammonia-nitrogen is present.



8. When the timer beeps, place the blank into the cell holder.

Nitrogen, Ammonia



9. Touch Zero.
The display will show:
0.00 mg/L NH₃-N

10. Wipe the sample and place it into the cell holder.
Results will appear in mg/L NH₃-N.

Interferences

Interfering Substance	Interference Levels and Treatments
Calcium	Greater than 1000 mg/L as CaCO ₃
Iron	All levels. Correct for iron interference as follows: 1. Determine the amount of iron present in the sample by following one of the Iron, Total, procedures. 2. Add the same iron concentration to the ammonia-free water in step 3. The interference will be successfully blanked out.
Magnesium	Greater than 6000 mg/L as CaCO ₃
Nitrate	Greater than 100 mg/L as NO ₃ ⁻ -N
Nitrite	Greater than 12 mg/L as NO ₂ ⁻ -N
Phosphate	Greater than 100 mg/L as PO ₄ ³⁻ -P
Sulfate	Greater than 300 mg/L as SO ₄ ²⁻
Sulfide	Sulfide will intensify the color. Eliminate sulfide interference as follows: 1. Measure about 350 mL of sample in a 500-mL Erlenmeyer flask (Cat. No. 505-49). 2. Add the contents of one Sulfide Inhibitor Reagent Powder Pillow (Cat. No. 2418-99). Swirl to mix. 3. Filter the sample through a Folded Filter Paper (Cat. No. 1894-57) and Filter Funnel (Cat. No. 1083-67). 4. Use the filtered solution in step 3.
Other Substances	Less common interferences such as hydrazine and glycine will cause intensified colors in the prepared sample. Turbidity and color will give erroneous high values. Samples with severe interferences require distillation. Hach recommends the distillation procedure using the Hach General Purpose Distillation Set (Cat. No. 22653-00).

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Most reliable results are obtained when samples are analyzed as soon as possible after collection.

If chlorine is known to be present, the sample must be treated immediately with sodium thiosulfate. Add one drop of Sodium Thiosulfate Standard Solution, 0.1 N (Cat. No. 323-32), for each 0.3 mg of chlorine present in a one-liter sample.

For longer storage, adjust the pH to 2 or less with concentrated (about 2 mL per liter) Sulfuric Acid (Cat. No. 979-49). Store samples at 4 °C or less. Samples preserved in this manner can be stored up to 28 days. Just before testing the stored sample, warm to room temperature and neutralize with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-26). Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page 43.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Open an Ammonia Nitrogen Standard Solution, 10-mg/L as NH₃-N.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.2 mL, 0.4 mL and 0.6 mL of standard, respectively to the cylinders and mix each thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.2 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page 46 for more information.

Standard Solution Method

Prepare a 0.20 mg/L ammonia nitrogen standard as follows:

1. Diluting 2.00 mL of the Ammonia Nitrogen Standard Solution, 10-mg/L, to 100 mL with deionized water. Or, use the TenSette® Pipet to prepare a 0.20 mg/L ammonia nitrogen standard by diluting 0.4 mL of a Ammonia Nitrogen Voluette® Standard Solution, 50-mg/L as NH₃-N, to 100 mL with deionized water.
2. To adjust the calibration curve using the reading obtained with the 0.20-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page 49 for more information.

Nitrogen, Ammonia

Method Performance

Precision

Standard: 0.30 mg/L NH₃-N

Program	95% Confidence Limits of Distribution
385	0.25–0.35mg/L NH ₃ -N

See Section 3.4.3 Precision on page 53 for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.004 mg/L NH ₃ -N

See Section 3.4.5 Sensitivity on page 54 for more information.

Summary of Method

Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue-colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green-colored solution. Test results are measured at 655 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Ammonia Nitrogen Reagent Set, for 10-mL samples (100 tests)			26680-00
Includes:			
(2) Ammonia Cyanurate Reagent Powder Pillows	2	100/pkg	26531-99
(2) Ammonia Salicylate Reagent Powder Pillows	2	100/pkg	26532-99

Required Apparatus

Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
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Required Standards

Ammonia Nitrogen Standard Solution, 10-mg/L as NH ₃ -N	500 mL	153-49
Ammonia Nitrogen Standard, 2-mL PourRite® Ampule, 50-mg/L as NH ₃ -N	20/pkg	14791-20
Water, deionized	4 liters	272-56



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
 In the U.S.A. – Call toll-free 800-227-4224
 Outside the U.S.A. – Contact the HACH office or distributor serving you.
 On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
 WORLD HEADQUARTERS
 Telephone: (970) 669-3050
 FAX: (970) 669-2932

**Appendix IV
HACH Method 8192
Nitrate, Cadmium Reduction Method**

Method 8192

Cadmium Reduction Method

Powder Pillows

LR (0.01 to 0.50 mg/L NO₃⁻-N)

Scope and Application: For water, wastewater, and seawater



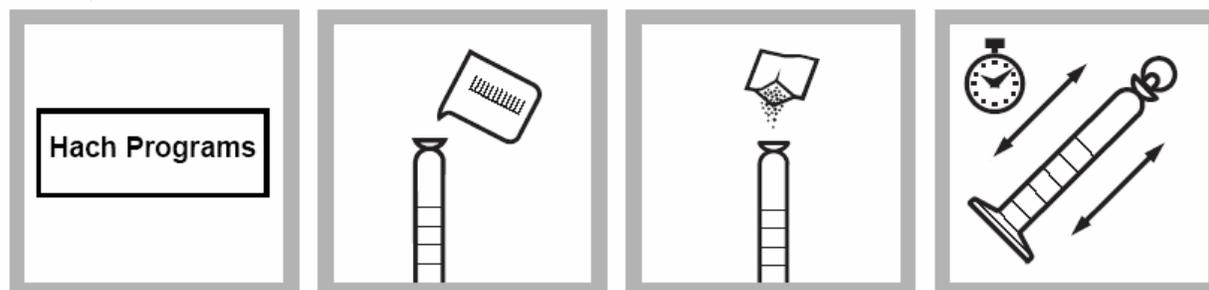
Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- A deposit of unoxidized metal will remain after the NitraVer® 6 dissolves. The deposit will not affect results.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Rinse the sample cell and mixing cylinder immediately after use to remove all cadmium particles.
- Properly dispose of the used sample. Prepared samples contain cadmium and must be disposed of according to Federal, State, and local hazardous waste regulations. For information on pollution prevention and waste management, see *Section 5* on page 79.
- Shaking time and technique influence color development. Analyze a standard solution several times and adjust the shaking time to obtain the correct result. Use this time for analyzing samples



Powder Pillows

Method 8192



1. Touch **Hach Programs**.
Select program **351 N, Nitrate LR**.
Touch **Start**.
2. Fill a 25-mL graduated mixing cylinder with 15 mL of sample.
3. Add the contents of one NitraVer 6 Reagent Powder Pillow to the cylinder. Stopper.
4. Touch the timer icon. Touch **OK**.
Shake the cylinder vigorously for three minutes.

Nitrate



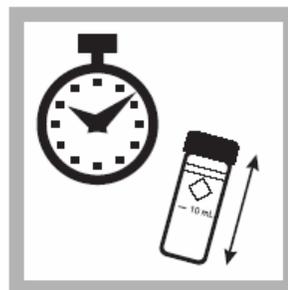
5. When the timer beeps, touch the timer icon again.
Touch **OK**.
A 2-minute reaction period will begin.



6. When the timer beeps, carefully pour 10 mL of the sample into a clean, round sample cell. Do not transfer any cadmium particles to the sample cell.



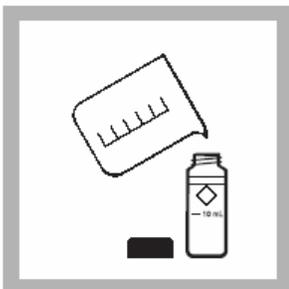
7. Add the contents of one NitriVer3 Nitrite Reagent Powder Pillow to the sample cell (this is the prepared sample).
Cap the sample cell.



8. Touch the timer icon.
Touch **OK**.
Shake the sample cell gently for 30 seconds.
A pink color will develop if nitrate is present.



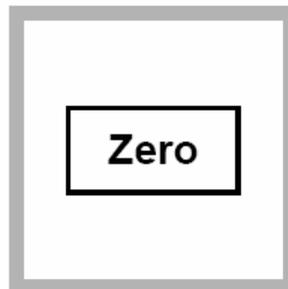
9. Touch the timer icon.
A 15-minute reaction period will begin.



10. When the timer beeps, fill a second sample cell with 10 mL of original sample (this is the blank).
Cap the sample cell.



11. Place the blank into the cell holder.



12. Touch **Zero**.
The display will show:
0.00 mg/L NO₃⁻-N



13. Place the prepared sample into the cell holder.
Results will appear in mg/L NO₃⁻-N.

Interferences

Nitrate

Page 2 of 6

Nitrate_PP_Low_CDR_Eng_Ody.fm

Interfering Substance	Interference Levels and Treatments
Calcium	100 mg/L
Chloride	Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride concentrations (seawater) but a calibration must be done using standards spiked to the same chloride concentration.
Ferric iron	All levels
Nitrite	All levels: This method measures both the nitrate and nitrite in the sample. If nitrite is present, the nitrite nitrogen test (Program #371) should be done on the sample. Pretreat the nitrate nitrogen sample with the following pretreatment. Then subtract the amount of nitrite found from the results of the LR nitrate nitrogen test. 1. Add 30-g/L Bromine Water (Cat. No. 2211-20) dropwise to the sample in <i>step 3</i> until a yellow color remains. Mix after each drop. 2. Add one drop of 30-g/L Phenol Solution (Cat. No. 2112-20) to destroy the color. 3. Proceed with the LR Nitrate procedure.
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Strong oxidizing and reducing substances	Interfere at all levels

Sample Collection, Storage and Preservation

More reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 48 hours at 4 °C. To preserve samples for longer periods, add 2 mL of Concentrated Sulfuric Acid (Cat. No. 979-49) per liter and store at 4 °C.

Before analysis, warm the sample to room temperature and adjust the pH to 7 with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-53). Do not use mercury compounds as preservatives. Correct the test result for volume additions by dividing the total volume (acid + base + sample) by the original sample volume and multiplying the test result by this factor.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Nitrate Nitrogen Voluette® Ampule Standard, 12.0-mg/L NO₃⁻-N (Cat. No. 14333-10).
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 15 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

Nitrate

- After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

See *Section 3.2.2 Standard Additions* on page 46 for more information.

Standard Solution Method

- To test accuracy, use a 0.20-mg/L NO_3^- -N standard in place of the sample and perform the procedure as described. Prepare this standard by diluting 2.00 mL of a 10-mg/L Nitrate Nitrogen Standard Solution to 100.0 mL with deionized water.
- To adjust the calibration curve using the reading obtained with the 0.20-mg/L nitrate nitrogen standard, touch **Options** on the current program menu. Touch **Standard Adjust**.
- Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page 49 for more information.

Method Performance

Precision

Standard: 0.2 mg/L NO_3^- -N

Program	95% Confidence Limits of Distribution
351	0.18–0.23 mg/L NO_3^- -N

See *Section 3.4.3 Precision* on page 53 for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	Δ Abs	Δ Concentration
Entire range	0.010	0.004 mg/L NO_3^- -N

See *Section 3.4.5 Sensitivity* on page 54 for more information.

Summary of Method

Cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with chromotropic acid to form a pink-colored product. Test results are measured at 507 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Low Range Nitrate Reagent Set (100 tests)			24298-00
Includes:			
NitraVer® 6 Nitrate Reagent Powder Pillows.....	1 pillow.....	100/pkg.....	21072-49
NitriVer® 3 Nitrite Reagent Powder Pillows	1 pillow.....	100/pkg.....	21071-69

Required Apparatus

Cylinder, graduated, mixing, 25-mL.....	1	each.....	20886-40
Sample Cells, 10-mL, w/cap.....	2	6/pkg.....	24276-06

Required Standards

Nitrate Nitrogen Standard Solution, 10.0-mg/L NO ₃ ⁻ -N.....		500 mL.....	307-49
Nitrate Nitrogen Standard Solution, Voluette® Ampule, 12-mg/L NO ₃ ⁻ -N.....		16/pkg.....	14333-10
Water, deionized		4 liters.....	272-56