First Progress Report
FY-2008 to 2009

MOTE / FWC-FWRI COOPERATIVE RED TIDE RESEARCH PROGRAM: MONITORING, PREDICTION AND MITIGATION OF FLORIDA RED TIDES

FWC AGREEMENT NO. 07041

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Introduction

This report describes the progress during the first quarter of the FY-2008-2009 cooperative program between the FWC-FWRI and Mote that is designed to focus on specific red tide research areas that are of critical concern to the State of Florida, in cooperation with other on-going HAB initiatives. The overall purpose is to determine and quantify the multiple causes of the Florida red tide initiation, development, transport and termination and to provide science-based information to assist resource managers and public health officials to make informed decisions directed toward reducing the adverse effects of red tide. The project components were developed around a series of three organizational goals:

Goal 1. Determine the role of natural and human-derived factors contributing to initiation, maintenance and termination of red tide blooms.

Goal 2. Provide supplemental studies in support of collaborative programs.

Goal 3. Implement communication and outreach programs for stakeholders.

SUMMARY OF PROGRESS TO DATE:

Goal 1. Determine the role of natural and human-derived factors contributing to initiation, maintenance and termination of red tide blooms.

Task 1 A. Field Monitoring:

a) Routine Transects

One-day ship surveys including the ECOHAB 'Sarasota Line' out to the 30m isobath were conducted on May 28, 2008, June 24, 2008, July 23, 2008, August 27, 2008 and September 8, 2008. Data were also collected at one station (ECO01 along the Sarasota transect line) on June 19, 2008 before mechanical failure on board the R/V Eugenie Clark forced an early termination of the survey. The current cruise track was established to assess conditions along the long-term observation line out from Sarasota and employed a different track for the outbound and inbound directions to maximize the coastal region sampled, passing close to the 'MB3-Venice' BreveBuster buoy established at the 10 m isobath offshore of the municipal pier adjacent to the Venice airport. Conductivity, temperature, and chlorophyll fluorescence measurements were collected continuously from surface waters, and dissolved nitrite and nitrate-nitrogen and dissolved inorganic phosphorus were monitored using the SubChemPak Analyzer. Vertical profiles were conducted using a Sea-Bird SBE 19 CTD for temperature, salinity and density measurements as well as relative chlorophyll fluorescence. Discrete station samples were collected for *K. brevis* presence and abundance determinations using optically-based instrumentation (BreveBuster). Underway hydrographic data from May to September 2008 are presented in **Appendix A**. Cross shelf vertical profiles are presented in **Appendix B**.

Hydrographic data indicate the near shore waters west of Sarasota, FL varied only slightly between May and September 2008. While water temperature displayed a typical seasonal warming throughout the summer months, salinity remained nearly constant with a slight freshening later in the study period (**Appendix A and B**). A well mixed water column was present in all surveys and increased relative chlorophyll fluorescence was present in only near shore and bottom waters.

i) Phytoplankton Distribution

Samples collected for HPLC pigment analyses during routine monitoring surveys have been processed and ChemTax® analyses were performed to asses the phytoplankton community composition. Results were incorporated into a GIS format (**Appendix C**). Pigment samples collected during the August and September 2008 surveys have not yet been processed. Data from these surveys will be reported during the next reporting period.

The general trend in phytoplankton distribution between May and July 2008 showed chlorophytes, diatoms and cyanophytes dominating the near shore communities while cyanophytes, haptophytes and prochlorophytes increased in relative abundance offshore. Standing stocks of phytoplankton, as judged by total chlorophyll *a*, appeared to be relatively low throughout the reporting period (high of 0.82 µg l^{-1} at the surface and 1.25 µg l^{-1} near bottom) and these values decreased with distance from shore.

ii) Water Quality – Hydrography and Nutrients

River flow records from Tampa Bay and Charlotte Harbor have increased beginning May 2008 as precipitation has increased. A more evident increase in flow has occurred in the Peace River (Charlotte Harbor) as a result of increased hurricane activity in the Gulf of Mexico affecting this area (**Figure 1**). Dates for the one day sampling trips are illustrated with dotted vertical lines, and the bioassay studies are illustrated with solid vertical lines (August 28, 2007, November 5, 2007, March 26, 2008, and August 12, 2008). Flow records are from USGS stations 02296750 (Peace River at Arcadia) and 02301500 (Alafia River at Lithia).

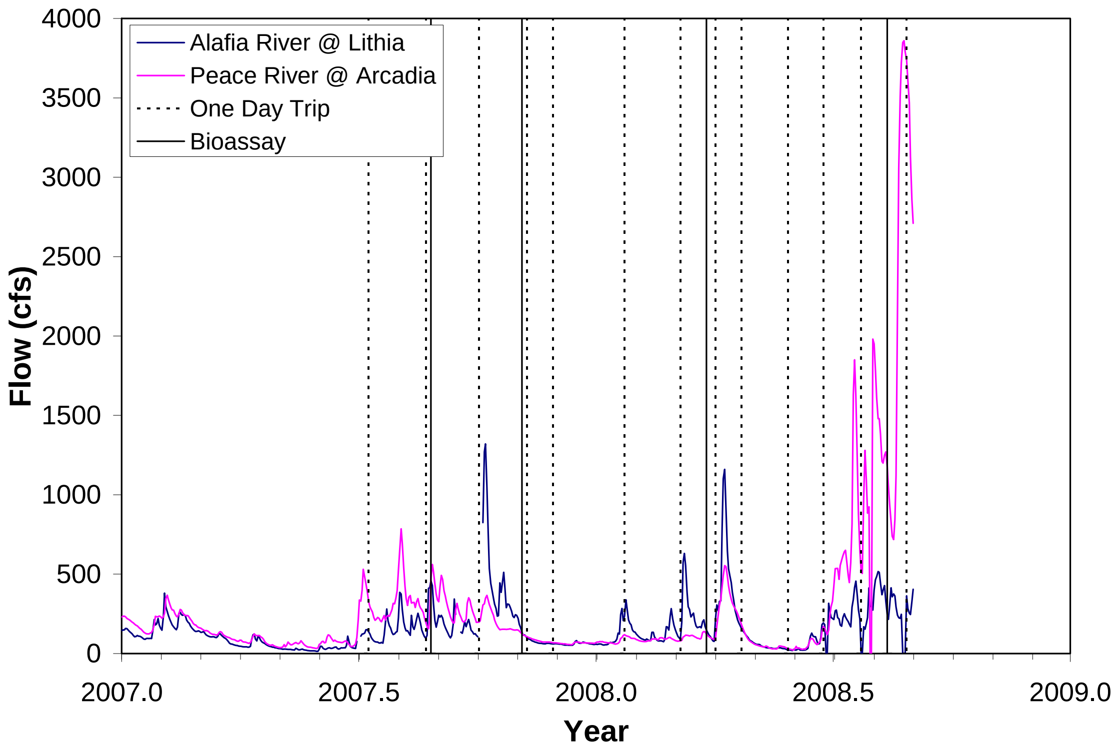

Figure 1. The daily mean flows of the Alafia River (blue) and the Peace River (purple) (ft^3/sec) Sampling dates are indicated by vertical dotted lines and bioassay studies are indicated by vertical solid lines.

The results of continuous and discrete sample analyses appear in **Appendix D**, **Appendix E**, and throughout the text, in which contours of surface data are mapped for salinity, fluorometric chlorophyll, absorption coefficients at 400nm (CDOM), DNH_4, DNO_x, DIN, DIP, DTP, DTN, DON, DOP, PP, PN, PC, TN, TP, Urea, $DSiO_2$, DIN:$DSiO_2$, DIN:DIP, PC:PN, PC:PP, PN:PP, and TN:TP. Methods of analyses are presented in Table 1. DIN, DIP, DON, and DOP are calculated values where DIN is equivalent to the sum of DNO_x and DNH_4, DIP is equivalent to DPO_4, DON is equivalent to the difference between DIN and DTN, and DOP is equivalent to the difference between DPO_4 and TDP. Contouring was accomplished with Surfer, using minimum curvature algorithms. Excessively convoluted contours were re-contoured using local polynomial, 3rd or 4th order. Parameter scales are maintained between months and can have up to three linear sections illustrated in order to accommodate high estuarine values which would otherwise visually obscure differences among coastal stations.

Overall water quality conditions are presented in **Appendix E**, and graphed together as a function of month, salinity, and *K. brevis* cell counts throughout the text. Data are cumulative, since July 2007. The LOWESS (Locally Weighted Scatterplot Smooth) lines in those figures are used to visually estimate central tendencies and are not a statistical test of significance. Graphically connecting the different samplings implies that the water is the same mass which has changed concentration or properties over time. Without physical or modeled estimates of water mass movement, the change between samplings could equally represent the influx of new water masses with differing constituents. Formal trend analyses were not conducted. The results of the bioassay studies are presented in **Appendix F** and graphical results are presented in **Appendix G**.

Table 1. Methods of analysis.

Parameter	**Method**
Chlorophyll *a*	EPA 445.0
Karenia brevis cell count	SM 10200F
Total phosphorus (DTP)	Solorzano and Sharp, 1980
Total nitrogen (DTN)	Bran Luebbe, 2005
Silicates ($DSiO_2$)	Bran Luebbe, 2004
Ammonia (DNH_4)	Bran Luebbe method G-177-96
Nitrite (DNO_2-N)	Grasshoff and Koroleff, 1983; Hanson, Jr. and Donaghay, 1998; Hanson, 2000
Nitrate plus nitrite (DNO_x-N)	Grasshoff and Koroleff, 1983; Hanson, 2000; Wood, Armstrong, and Richards, 1967
Phosphate (DPO_4-P)	Grasshoff and Koroleff, 1983; Murphy and Riley, 1962
CDOM Absorption at 400nm	Mitchell et al, 2003
Dissolved Urea	Grasshoff and Koroleff, 1983
Particulate phosphorus (PP)	Solorzano and Sharp, 1980
Particulate carbon (PC)	Thermo Electron, 2004
Particulate nitrogen (PN)	Thermo Electron, 2004

iii) Transect Sampling Events to Date

Sampling dates are planned for most months throughout the duration of the project. Brief summaries of the most recently added analyses and sampling events appear below. Contours of all available data are presented in **Appendix D**, and tables of all available data are presented in **Appendix E**.

May 28, 2008

River flows had been higher in previous months but were tapering off by the end of May. Salinity remained relatively high while nutrient concentrations remained low. DIN:DIP ratios continued to indicate a nitrogen-limited system (**Figure 2**), while bottom chlorophyll concentrations were slightly greater than surface chlorophyll concentrations (**Figure 2**).

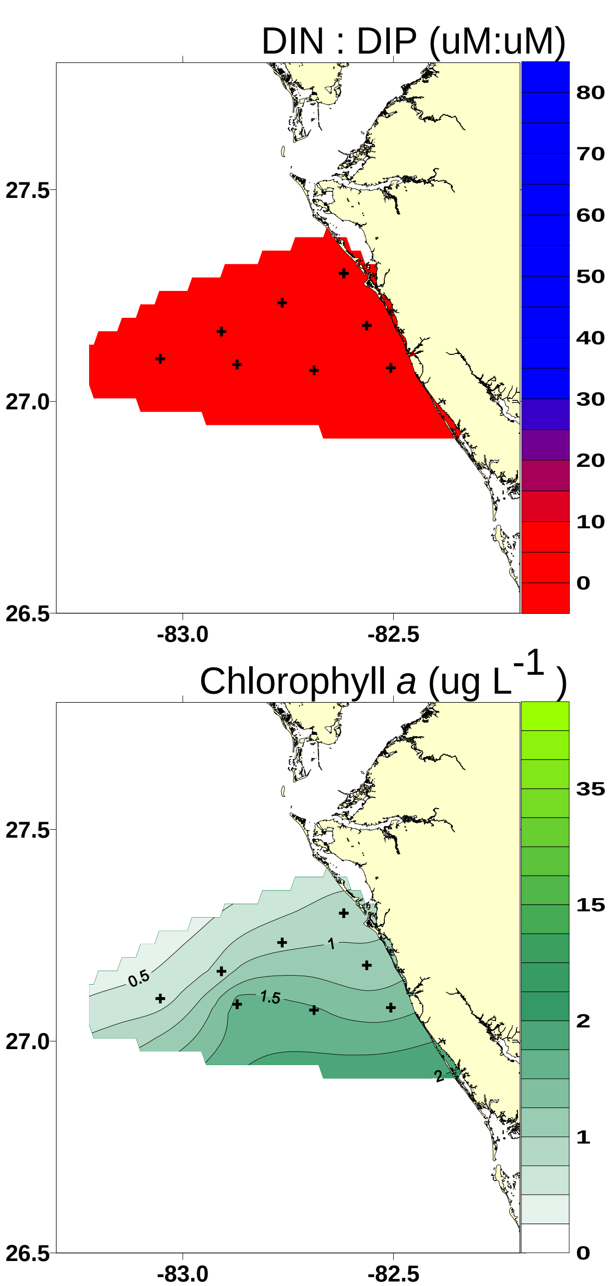

Figure 2. Surface measurement of DIN:DIP and chlorophyll *a* and bottom measurement of chlorophyll *a* from discrete station data, May 28, 2008. Locations of discrete stations are illustrated and size of station symbol is proportional to cell counts of *K. brevis*.

June 24, 2008

The June sampling trip showed a spike in silica just offshore of Sarasota County (**Figure 3**). River flows remained low, salinity remained high, and nutrient concentrations remained relatively low. The system was still nitrogen limited and there continued to be no *K. brevis* present.

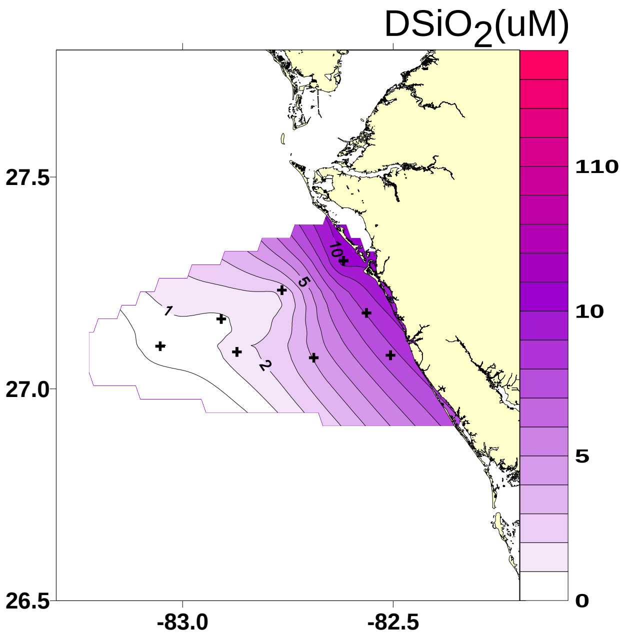

Figure 3. Surface measurement of silica from discrete station data, June 24, 2008. Locations of discrete stations are illustrated and size of station symbol is proportional to cell counts of *K. brevis*.

July 23, 2008

River flows had started to increase by mid/late July, yet salinity continued to remain high (**Figure 4**). A spike of silica was present nearshore just south of Sarasota (**Figure 4**). The rest of the nutrient (particulate and dissolved) remained comparable to previous months (**Appendix D**).

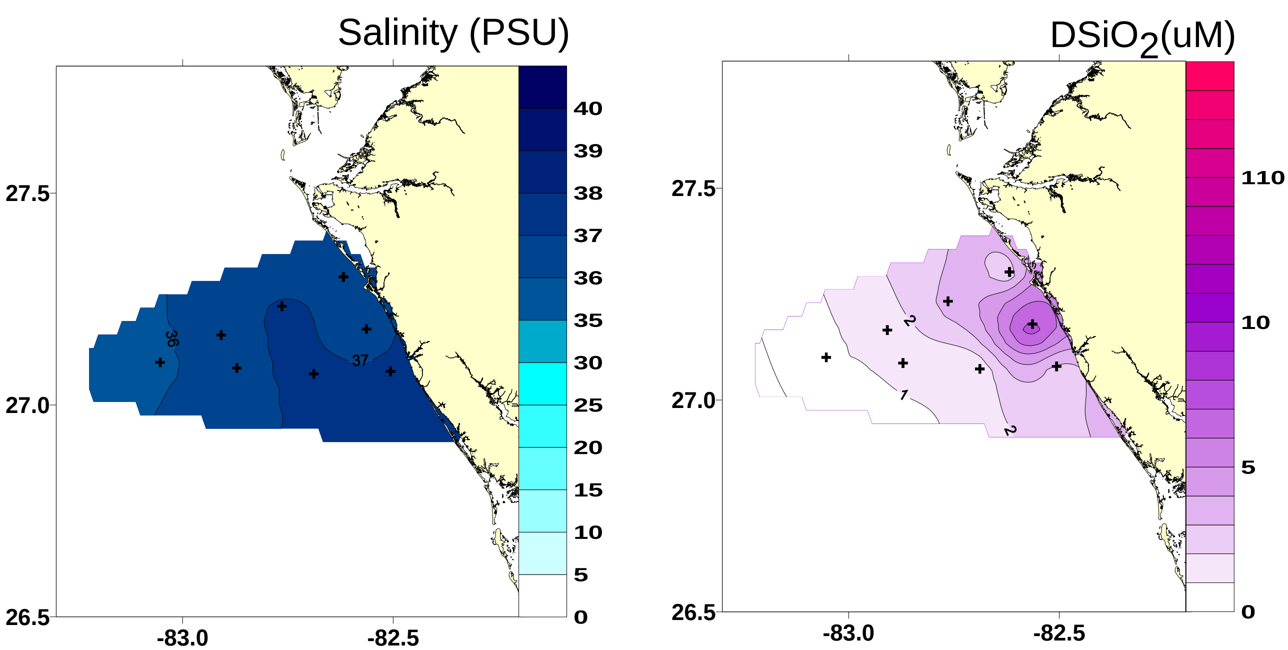

Figure 4. Surface measurement of salinity and silica from discrete station data, July 23, 2008. Locations of discrete stations are illustrated and size of station symbol is proportional to cell counts of *K. brevis*.

August 27, 2008
River flows had increased significantly by August, especially in the Peace River (**Figure 1**). There were several tropical storms and hurricanes that had passed through the area bringing copious amounts of rain. However, even with the high amount of precipitation, the only nutrient that has been analyzed and that has showed an increase in concentrations was DNOx (**Figure 5**). The remaining nutrient analyses for this month will be presented in a later report.

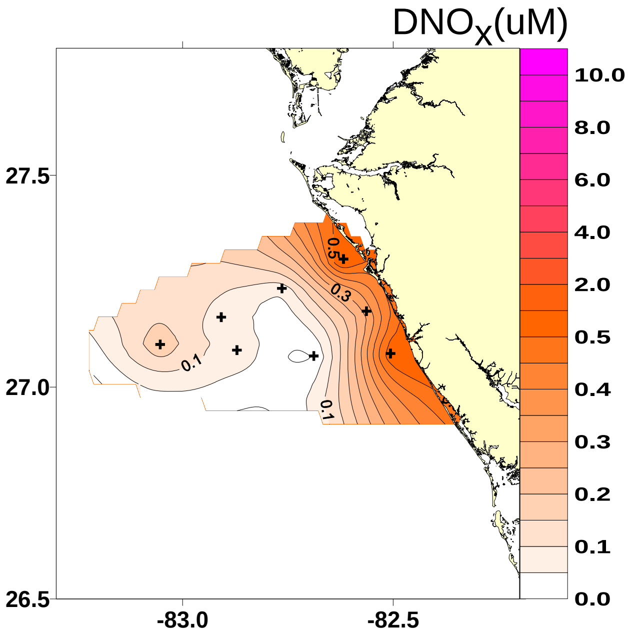

Figure 5. **S**urface measurement of DNOx from discrete station data, August 27, 2008. Locations of discrete stations are illustrated and size of station symbol is proportional to cell counts of *K. brevis*.

iv) Overall Water Quality Conditions

Available data from July, 2007 through August, 2008 are included in this report, and are presented as a table in **Appendix E**.

Concentrations of DIN, DIP, and $DSiO_2$, and DTP were decreasing in late 2007/early 2008 and have remained relatively constant throughout 2008; however the August 2008 sampling trip shows some higher concentrations (**Figure 6**). River flows in the Alafia and Peace Rivers were low in the end of 2007/beginning of 2008, which likely contributed to the lower DIN, DIP, and $DSiO_2$ concentrations, whereas in mid to late 2008, flows have begun increasing again. DIN ranged from below detection to approximately 1.5µM and DIP ranged from below detection to approximately 0.3µM (**Figure 7**). Dissolved silica concentrations were highest during the same sampling trip when DIN was lowest. Ratios of DIN:DIP continue to show that the system has been nitrogen limited (DIN:DIP<16) during the samplings, and low DIN:$DSiO_2$ ratios indicate nitrogen limitation for diatoms (**Figures 8 and 9**). Dissolved NH4N, total nitrogen (DTN), and urea concentrations have remained consistently low (**Figures 10** and **11**). *Karenia brevis* was only present at one station (M4S1) during the October 2007 sampling event and at one station (M4S3) during the late November 2007 sampling event.

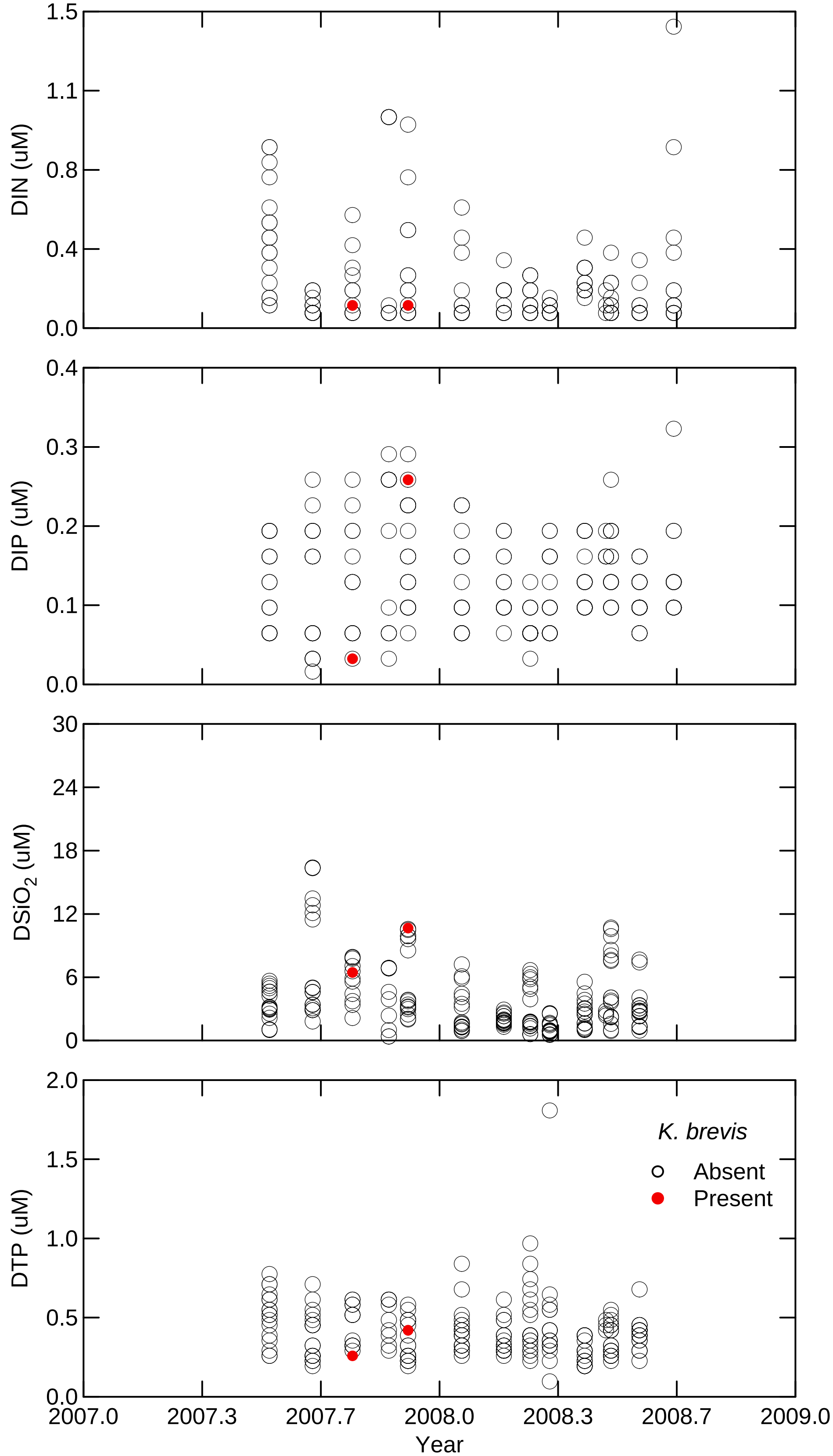

Figure 6. DIN, DIP, $DSiO_2$, and DTP in coastal stations over the course of the study. Data are characterized by presence (>1000 cell L^{-1})/absence of *K. brevis*.

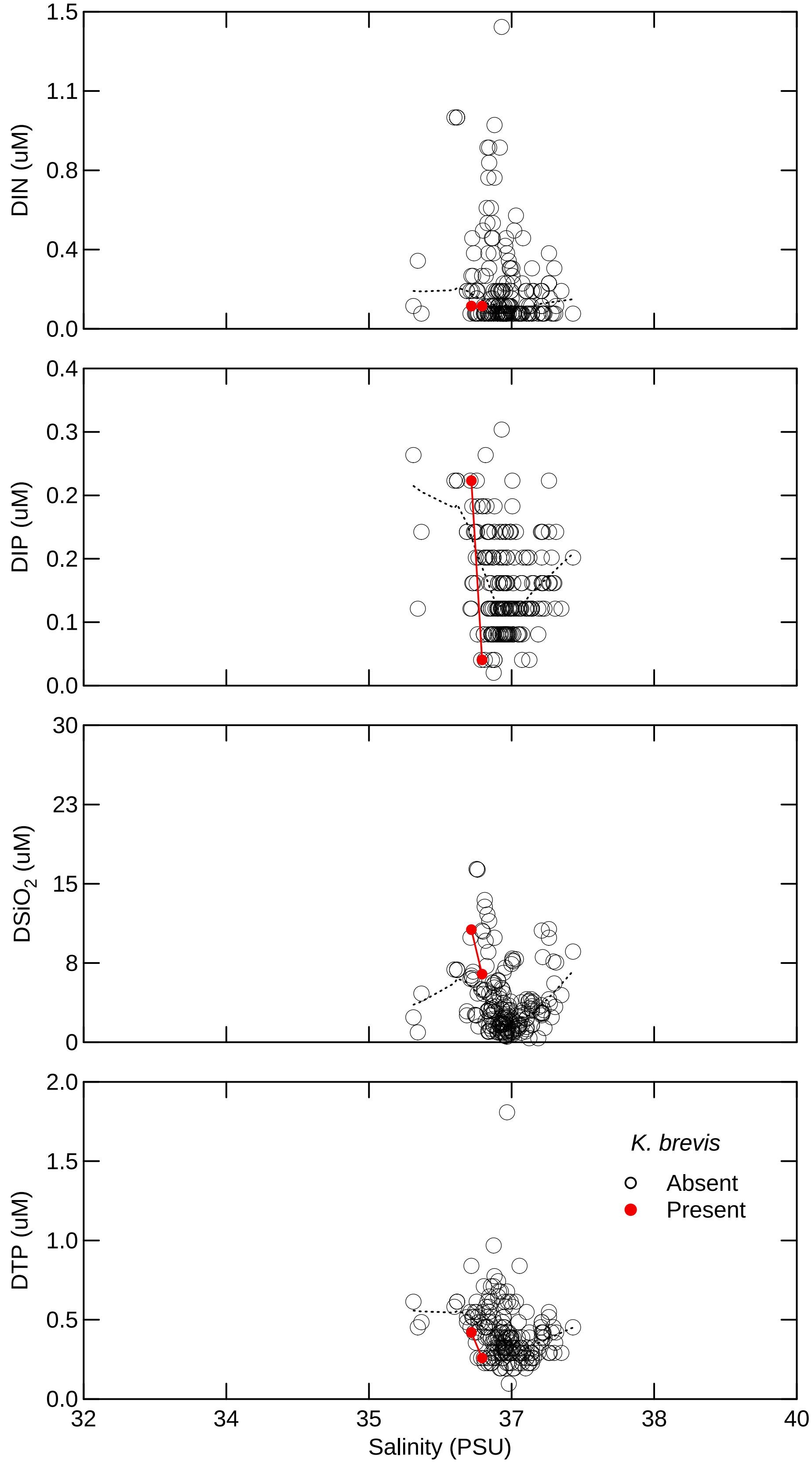

Figure 7. DIN, DIP, $DSiO_2$, and DTP in coastal stations as a function of salinity. Data are characterized by presence (>1000 cell L^{-1})/absence of *K. brevis*.

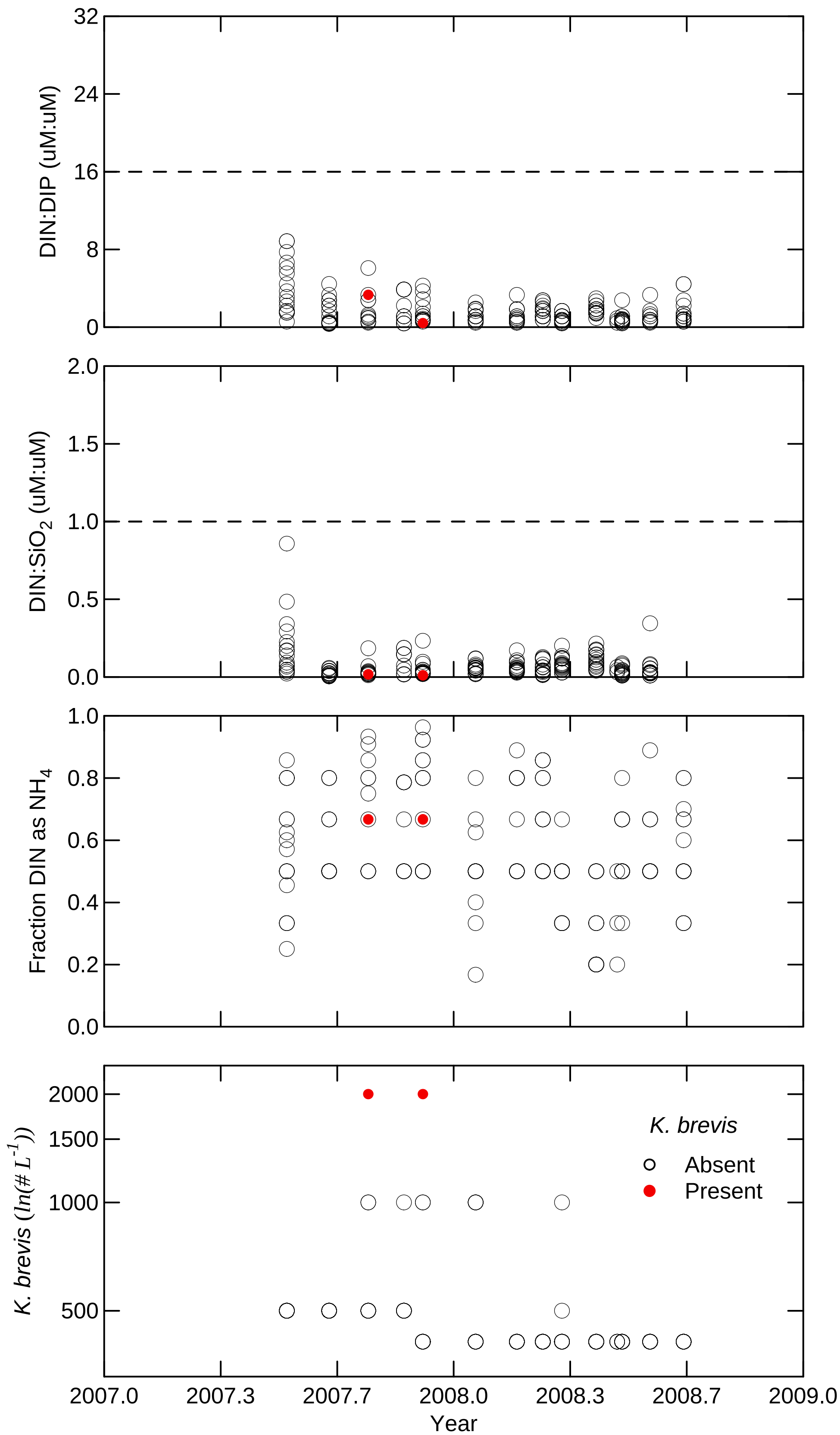

Figure 8. DIN:DIP, DIN:SiO_2, and fraction of DIN as NH_4 in coastal stations over the course of the study. Data are characterized by presence (>1000 cell L^{-1})/absence of *K. brevis*.

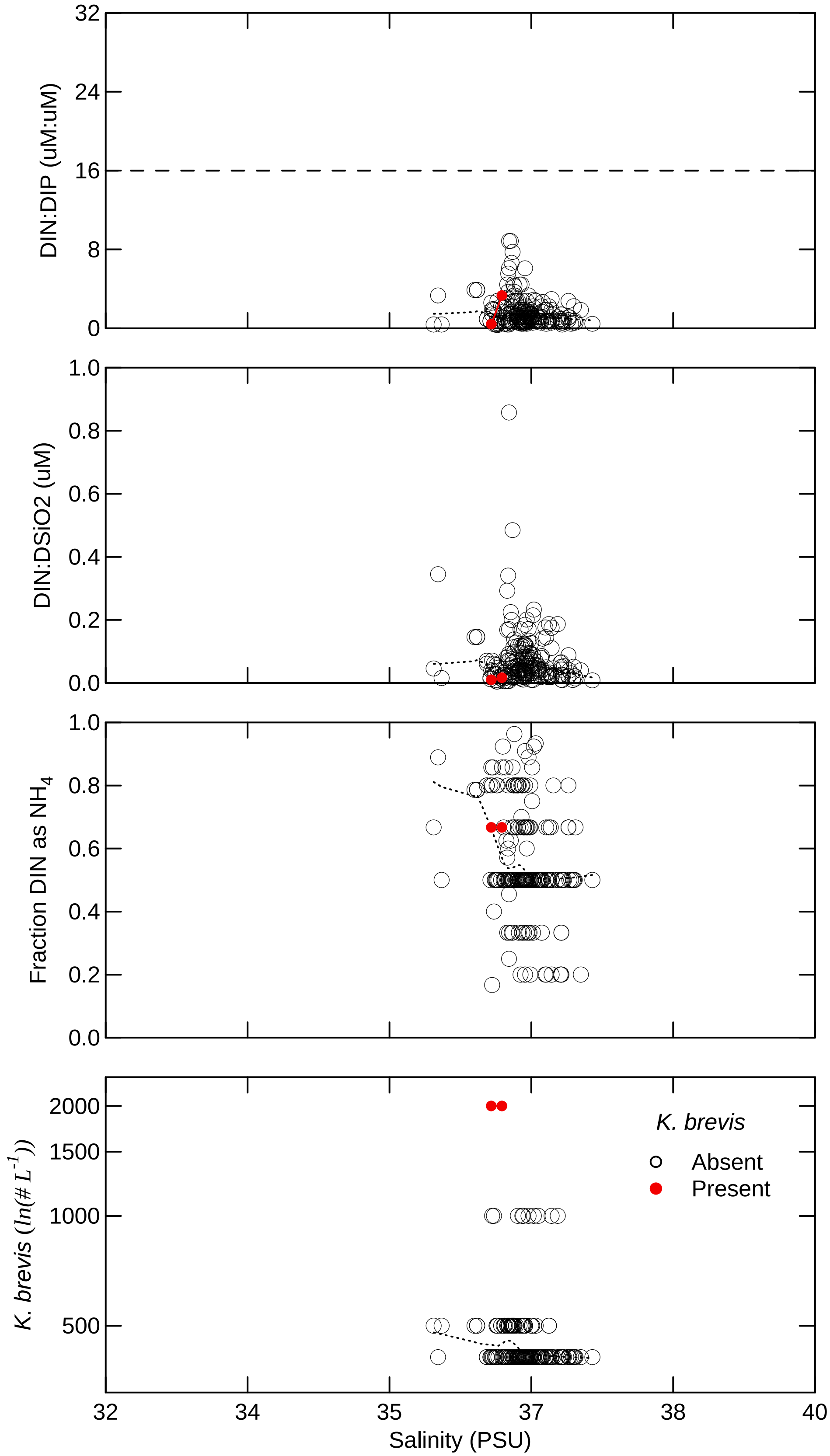

Figure 9. DIN:DIP, DIN:SiO_2, and fraction of DIN as NH_4 in coastal stations as a function of salinity. Data are characterized by presence (>1000 cell L^{-1})/absence of *K. brevis*.

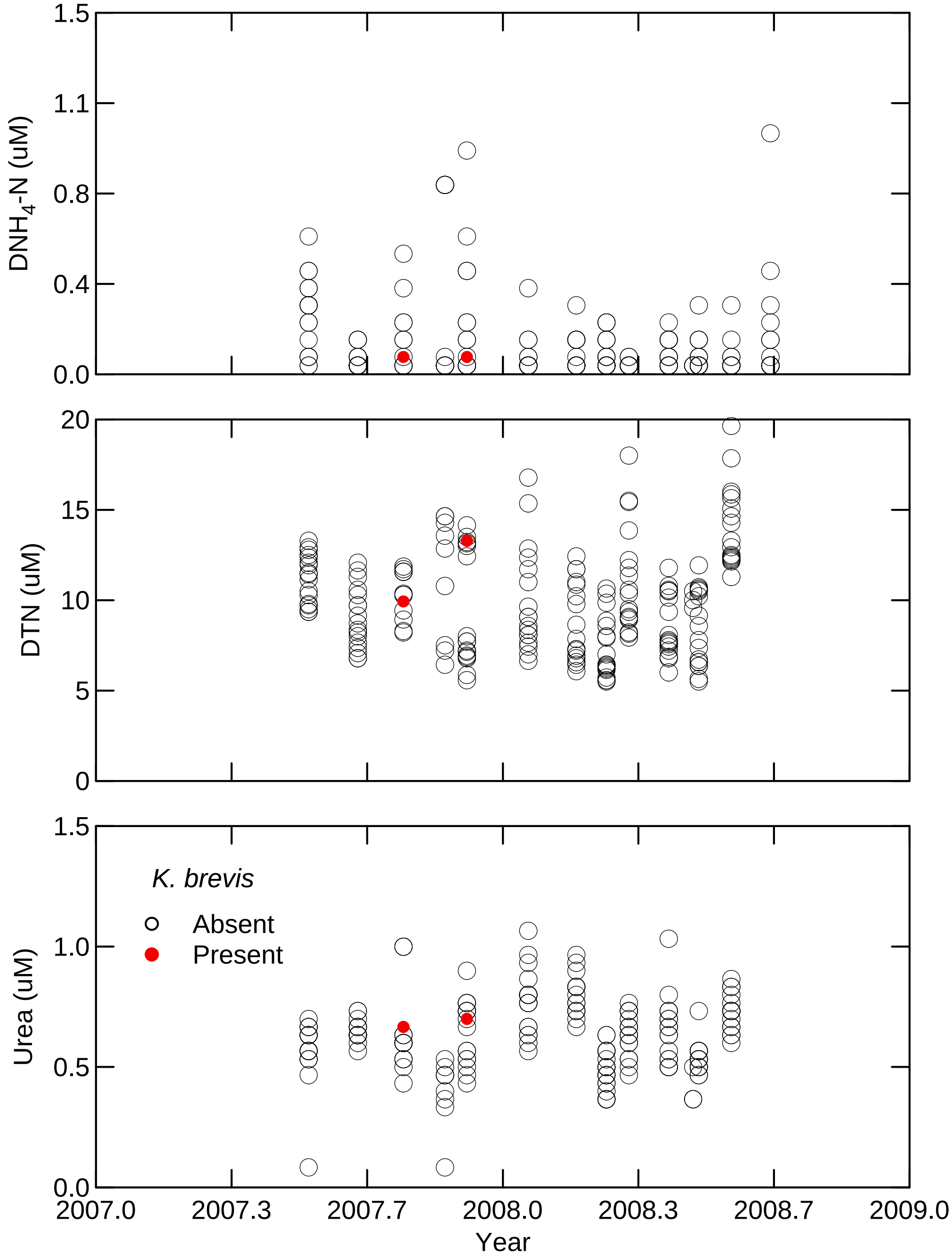

Figure 10. DNH_4N, DTN, and urea in coastal stations over the course of the study. Data are characterized by presence (>1000 cell L^{-1})/absence of *K. brevis*.

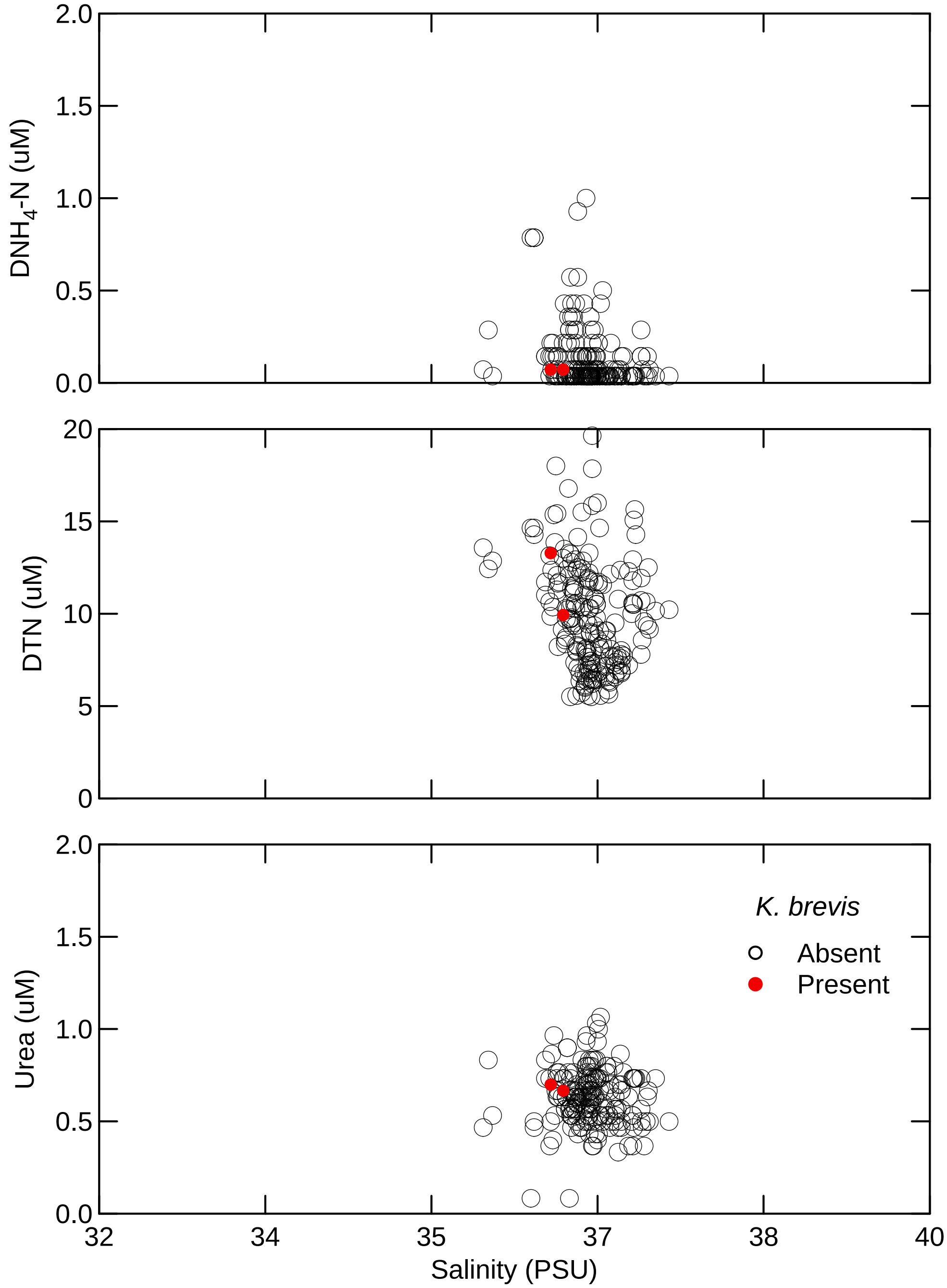

Figure 11. DNH_4N, DTN, and urea in coastal stations as a function of salinity. Data are characterized by presence (>1000 cell L^{-1})/absence of *K.brevis*.

The fraction of DTP (total dissolved phosphorus) as DIP (dissolved inorganic phosphorus) and as a function of DTP is presented in **Figure 12.** DTP continues to consist of mostly organic phosphorus, and organic phosphorus remains typically greater than inorganic phosphorus at salinities greater than 30 PSU.

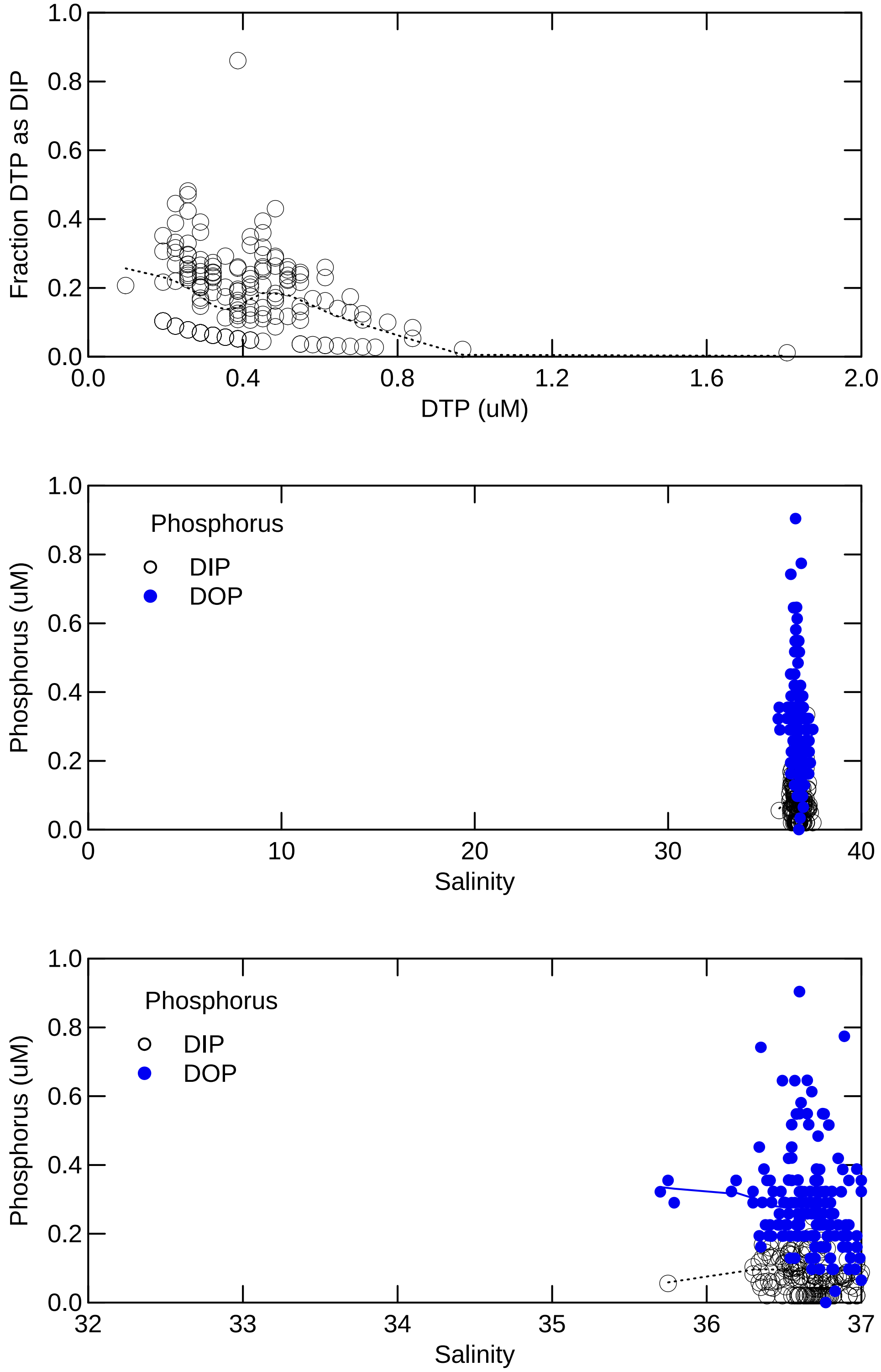

Figure 12. Dissolved inorganic and organic phosphorus as a function of salinity.

Chlorophyll *a*, fluorescence, and sigma-t data are presented in **Figure 13**. Chlorophyll *a* concentrations have been increasing throughout 2008 while fluorescence has been decreasing.

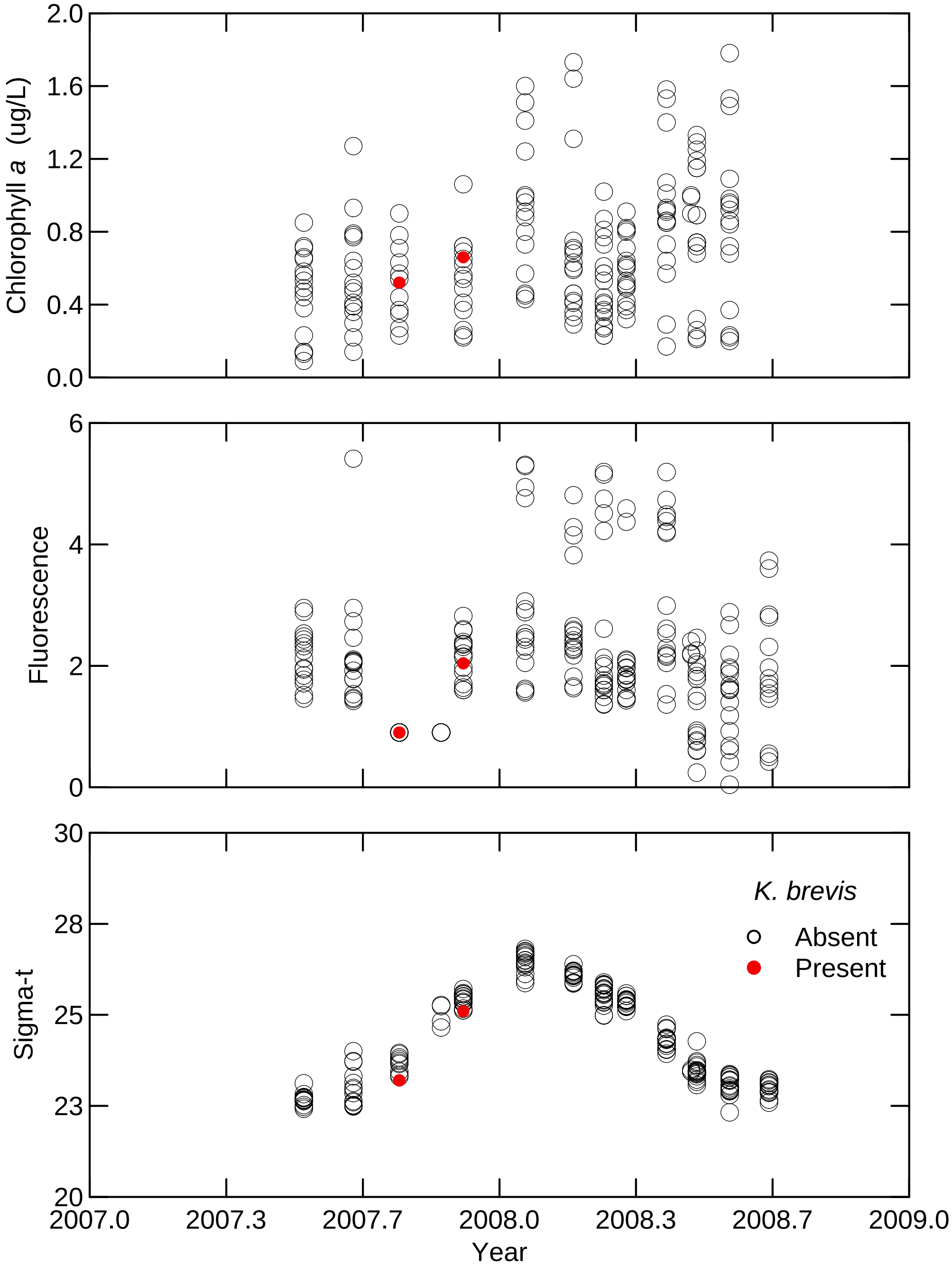

Figure 13. Chlorophyll *a*, fluorescence, and sigma-t in coastal areas over the course of the study. Data are characterized by presence (>1000 cell L^{-1})/absence of *K. brevis*.

Particulate nutrients (PP, PN, and PC) were also relatively consistent throughout the study, with the highest concentrations in March, 2008 (**Figures 14 and 15**). Ratios of PC:PN showed that most of the areas sampled were not carbon limited. PN:PP ratios were evenly distributed except during the October and early November 2007 sampling events where nitrogen was limiting (**Figures 16 and 17**).

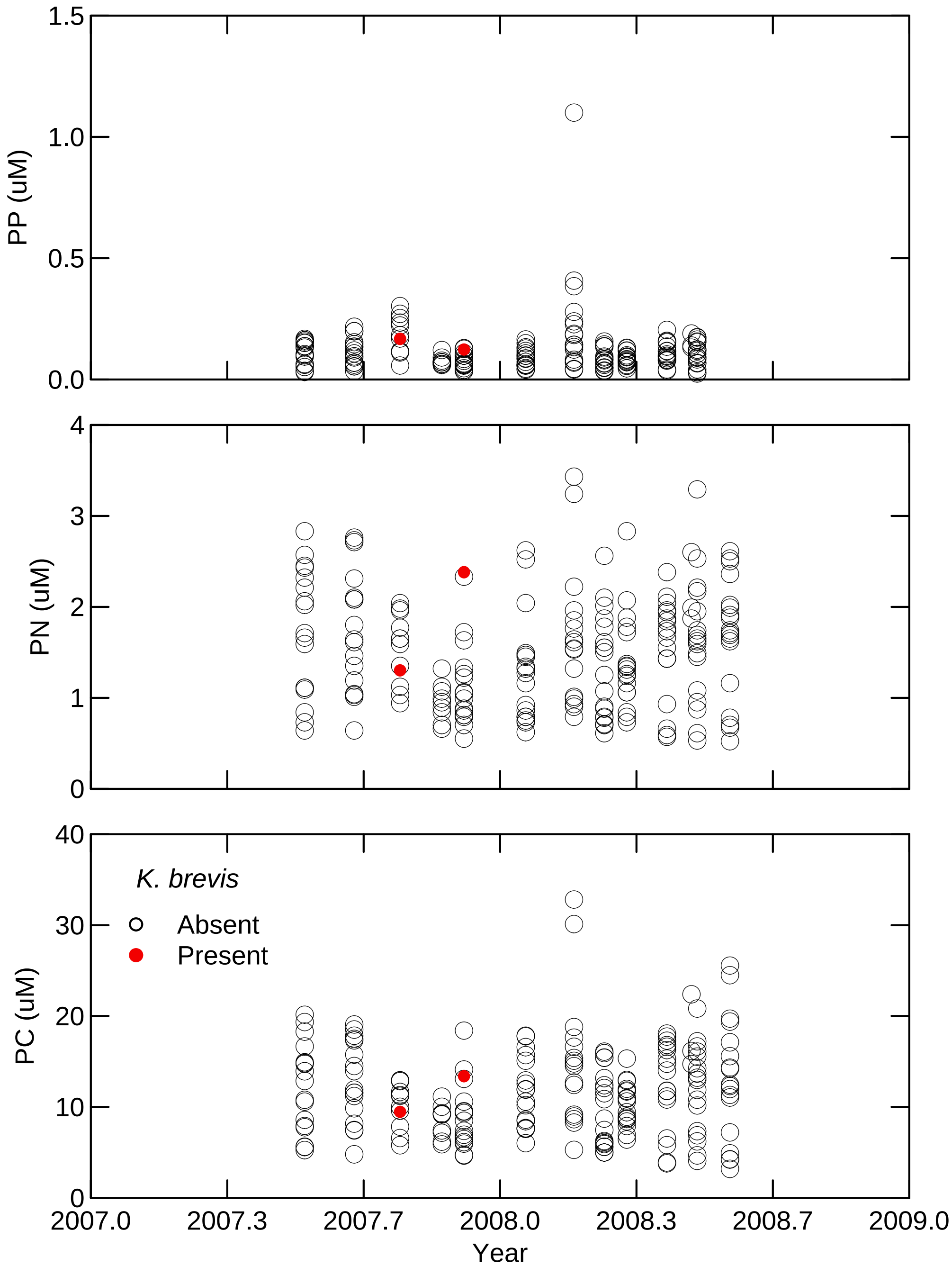

Figure 14. PP, PN, and PC in coastal stations over the course of the study. Data are characterized by presence (>1000 cell L^{-1})/absence of *K. brevis*.

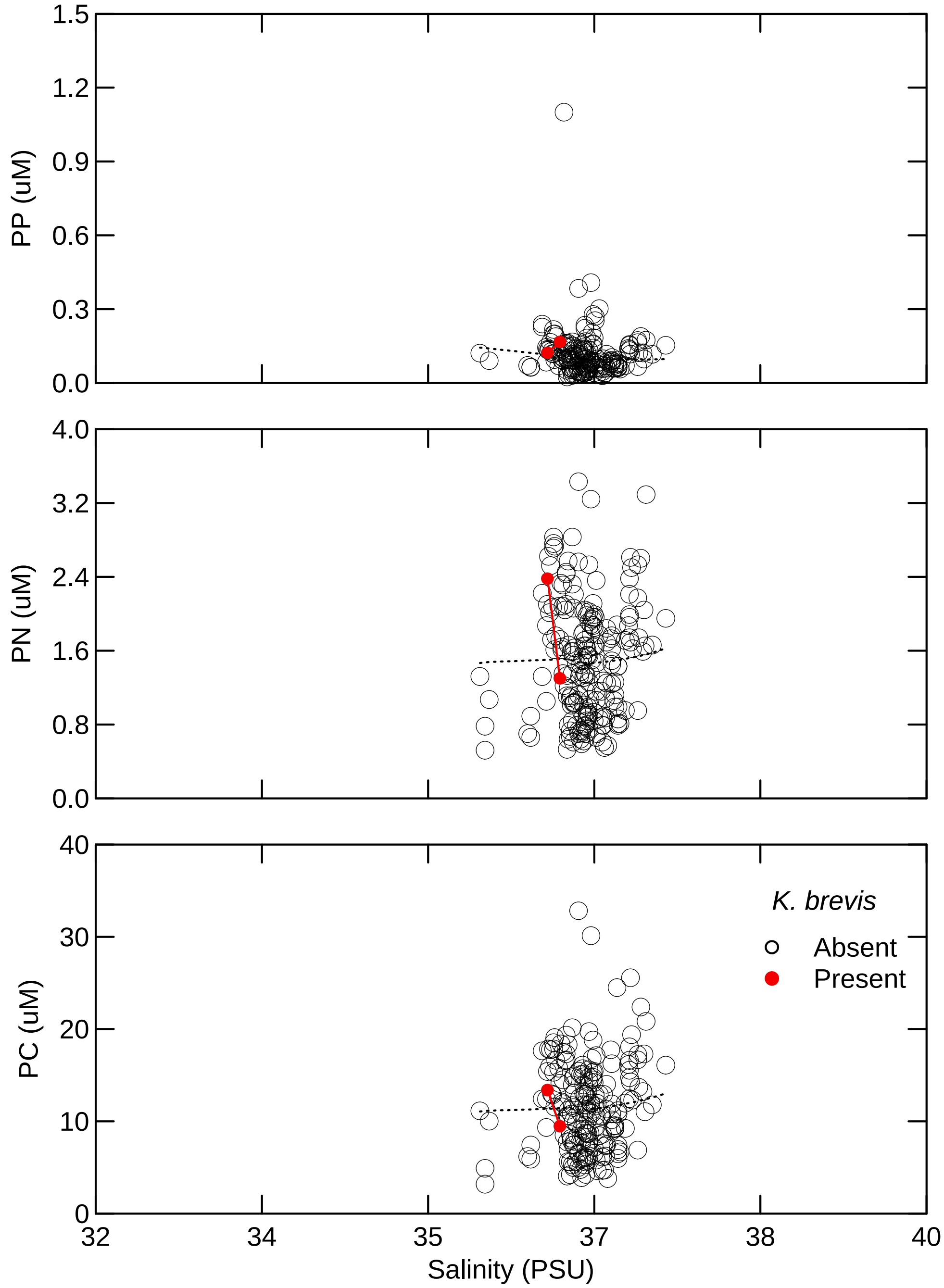

Figure 15. PP, PN, and PC in coastal stations as a function of salinity. Data are characterized by presence (>1000 cell L^{-1})/absence of *K. brevis*.

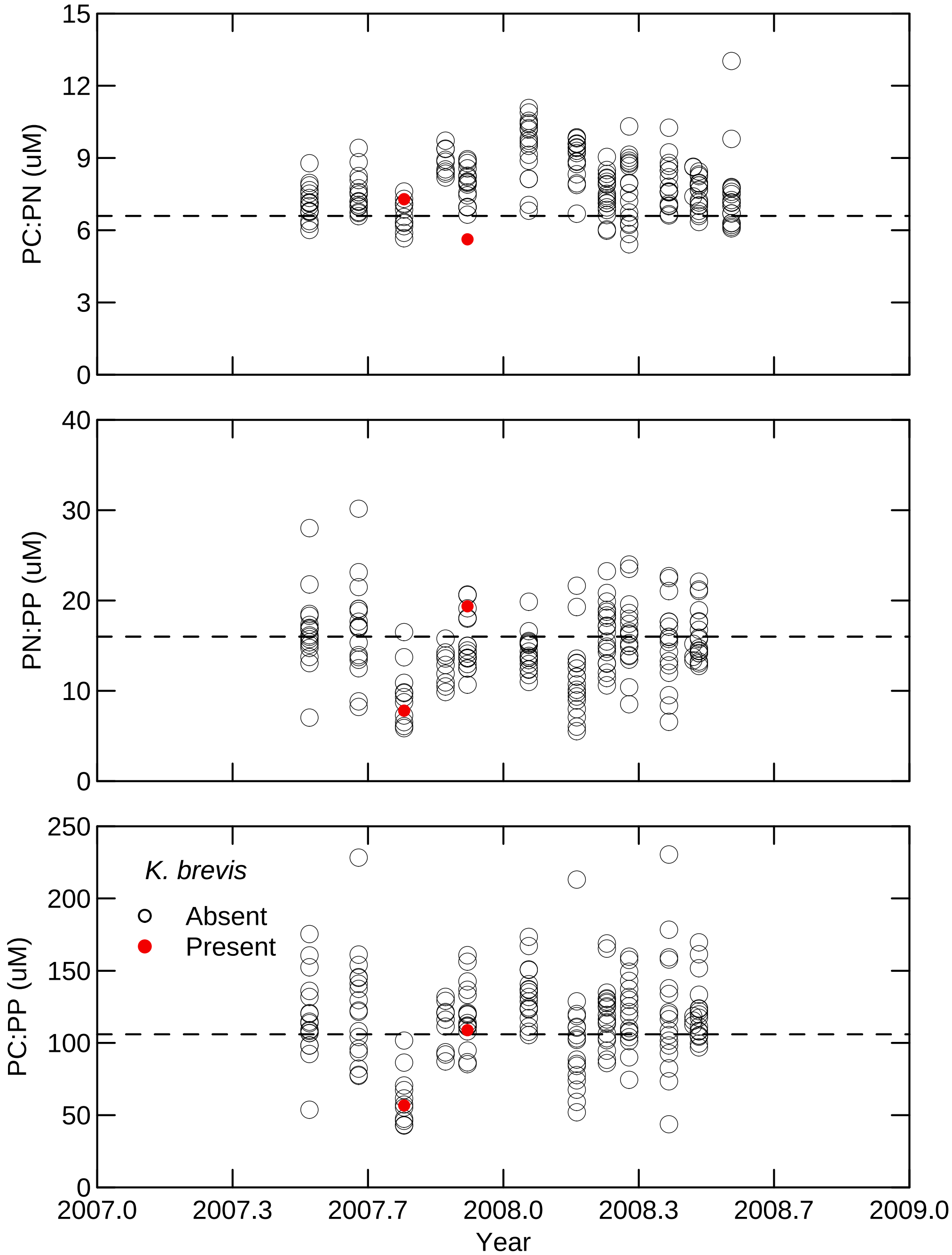

Figure 16. PC:PN, PN:PP, and PC:PP ratios in coastal areas over the course of the study. Data are characterized by presence (>1000 cell L^{-1})/absence of *K. brevis*.

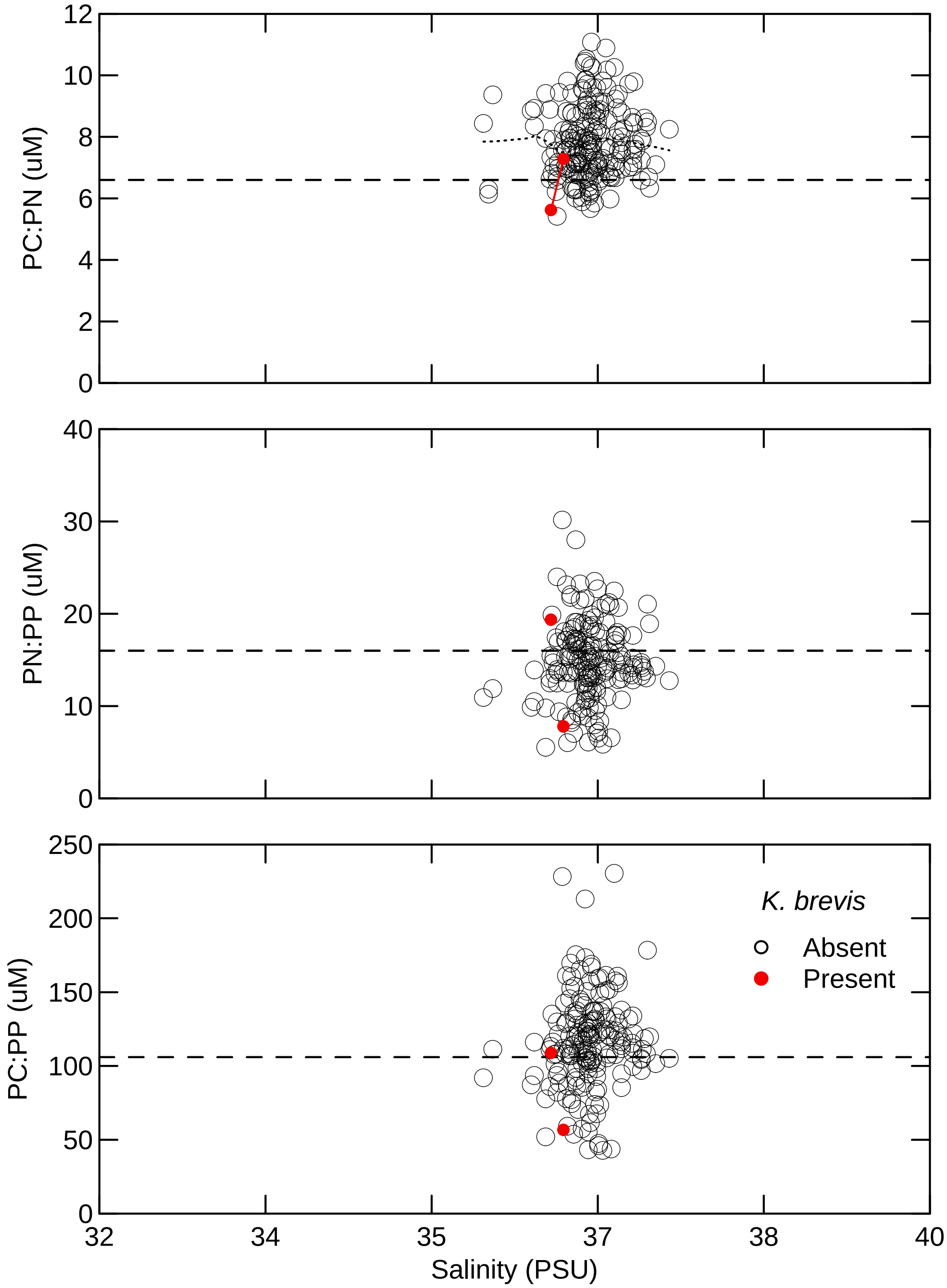

Figure 17. PC:PN, PN:PP, and PC:PP ratios in coastal stations as a function of salinity. Data are characterized by presence (>1000 cell L^{-1})/absence of *K. brevis*.

b) Red Tide Event Response

No red tide events occurred during the report period.

c) Brevetoxin Composition:

i) Routine "Sharks Tooth" Transects:

Water samples were collected for brevetoxin analysis during the April 22nd, May 28th, June 24th, July 23rd, August 27th, and September 8th 2008 "Shark Tooth" transects. Results from samples collected in April, May and June are listed in **table 2**. No detectable toxin or cells were observed in any of the samples collected in April, May or June. Analyses of the remaining samples are underway.

Table 2. Brevetoxin Concentration and Cell Counts from "Shark Tooth" Transects.

Sample	Depth	Date	*K. brev* cells/L	Pbtx-1 ng/L	Pbtx-2 ng/L	Pbtx-3 ng/L	PbTx-CA ng/L	Brevenal ng/L
ECO01	Sur	4/22/08	<1000	<10	<10	<10	<10	<10
ECO01	Bot	4/22/08	<1000	<10	<10	<10	<10	<10
ECO01	Sur rep	4/22/08	<1000	<10	<10	<10	<10	<10
ECO10	Sur	4/22/08	<1000	<10	<10	<10	<10	<10
ECO10	Bot	4/22/08	<1000	<10	<10	<10	<10	<10
ECO20	Sur	4/22/08	<1000	<10	<10	<10	<10	<10
ECO20	Bot	4/22/08	<1000	<10	<10	<10	<10	<10
ECO30	Sur	4/22/08	<1000	<10	<10	<10	<10	<10
ECO30	Bot	4/22/08	<1000	<10	<10	<10	<10	<10
M4S1	Sur	4/22/08	<1000	<10	<10	<10	<10	<10
M4S1	Bot	4/22/08	<1000	<10	<10	<10	<10	<10
M4S2	Sur	4/22/08	<1000	<10	<10	<10	<10	<10
M4S2	Bot	4/22/08	<1000	<10	<10	<10	<10	<10
M3D03	Sur	4/22/08	<1000	<10	<10	<10	<10	<10
M3D03	Bot	4/22/08	<1000	<10	<10	<10	<10	<10
M4S3	Sur	4/22/08	<1000	<10	<10	<10	<10	<10
M4S3	Bot	4/22/08	<1000	<10	<10	<10	<10	<10
ECO01	Sur	5/28/08	<1000	<10	<10	<10	<10	<10
ECO01	Bot	5/28/08	<1000	<10	<10	<10	<10	<10
ECO01	Sur rep	5/28/08	<1000	<10	<10	<10	<10	<10
ECO10	Sur	5/28/08	<1000	<10	<10	<10	<10	<10
ECO10	Bot	5/28/08	<1000	<10	<10	<10	<10	<10
ECO20	Sur	5/28/08	<1000	<10	<10	<10	<10	<10
ECO20	Bot	5/28/08	<1000	<10	<10	<10	<10	<10
ECO30	Sur	5/28/08	<1000	<10	<10	<10	<10	<10
ECO30	Bot	5/28/08	<1000	<10	<10	<10	<10	<10
M4S1	Sur	5/28/08	<1000	<10	<10	<10	<10	<10
M4S1	Bot	5/28/08	<1000	<10	<10	<10	<10	<10
M4S2	Sur	5/28/08	<1000	<10	<10	<10	<10	<10
M4S2	Bot	5/28/08	<1000	<10	<10	<10	<10	<10
M3D03	Sur	5/28/08	<1000	<10	<10	<10	<10	<10
M3D03	Bot	5/28/08	<1000	<10	<10	<10	<10	<10
M4S3	Sur	5/28/08	<1000	<10	<10	<10	<10	<10
M4S3	Bot	5/28/08	<1000	<10	<10	<10	<10	<10
ECO01	Sur	6/24/08	<1000	<10	<10	<10	<10	<10
ECO01	Bot	6/24/08	<1000	<10	<10	<10	<10	<10
ECO01	Sur rep	6/24/08	<1000	<10	<10	<10	<10	<10
ECO10	Sur	6/24/08	<1000	<10	<10	<10	<10	<10
ECO10	Bot	6/24/08	<1000	<10	<10	<10	<10	<10
ECO20	Sur	6/24/08	<1000	<10	<10	<10	<10	<10
ECO20	Bot	6/24/08	<1000	<10	<10	<10	<10	<10
ECO30	Sur	6/24/08	<1000	<10	<10	<10	<10	<10

ECO30	Bot	6/24/08	<1000	<10	<10	<10	<10	<10
M4S1	Sur	6/24/08	<1000	<10	<10	<10	<10	<10
M4S1	Bot	6/24/08	<1000	<10	<10	<10	<10	<10
M4S2	Sur	6/24/08	<1000	<10	<10	<10	<10	<10
M4S2	Bot	6/24/08	<1000	<10	<10	<10	<10	<10
M3D03	Sur	6/24/08	<1000	<10	<10	<10	<10	<10
M3D03	Bot	6/24/08	<1000	<10	<10	<10	<10	<10
M4S3	Sur	6/24/08	<1000	<10	<10	<10	<10	<10
M4S3	Bot	6/24/08	<1000	<10	<10	<10	<10	<10

ii) Water samples from the Florida Keys transects were also analyzed for brevetoxins. Results from the samples collected on May 6th, June 10th, and July 2nd 2008 are presented in **Table 3.** No samples were collected in August due to tropical storm Fay.

Table 3. Results Brevetoxin analyses of Water Samples from Florida Keys Transects.

Sample	Date	*K. brev* Cells/L	Pbtx-1 ng/L	Pbtx-2 ng/L	Pbtx-3 ng/L	Pbtx-CA ng/L	Brevenal ng/L
RT08	5/6/08	<1000	<10	<10	<10	<10	<10
RT23	5/6/08	<1000	<10	<10	<10	<10	<10
RT19	5/6/08	<1000	<10	<10	<10	<10	<10
RT21	5/6/08	<1000	<10	<10	<10	<10	<10
RT17	5/6/08	<1000	<10	<10	<10	<10	<10
RT14	5/6/08	<1000	<10	<10	<10	<10	<10
RT12	5/6/08	<1000	<10	<10	<10	<10	<10
RT08	6/10/08	<1000	<10	<10	<10	<10	<10
RT23	6/10/08	<1000	<10	<10	<10	<10	<10
RT19	6/10/08	<1000	<10	<10	<10	<10	<10
RT17	6/10/08	<1000	<10	<10	<10	<10	<10
RT14	6/10/08	<1000	<10	<10	<10	<10	<10
RT12	6/10/08	<1000	<10	<10	<10	<10	<10
RT08	7/2/08	<1000	<10	<10	<10	<10	<10
RT23	7/2/08	<1000	<10	<10	<10	<10	<10
RT19	7/2/08	<1000	<10	<10	<10	<10	<10
RT21	7/2/08	<1000	<10	<10	<10	<10	<10
RT17	7/2/08	<1000	<10	<10	<10	<10	<10
RT14	7/2/08	<1000	<10	<10	<10	<10	<10
RT12	7/2/08	<1000	<10	<10	<10	<10	<10

iii) Red Tide Event Response:

No red tide blooms were observed during this period. Therefore, no red tide events surveys were undertaken.

d) Florida Keys Transects

Methods

Previously established stations along the gulf-side of the Lower Keys and through Key West Harbor to the reef tract were routinely monitored on a monthly basis (**Figure 18**). Water samples were collected and stained for cell counts and algae identification, along withand additional bulk water samples were collected and which were filtered/extracted in preparation for brevetoxin analysis. Water quality data was collected at each station including temperature, salinity, and dissolved oxygen. Cell counts and filtration/extraction of samples were performed by Mote staff in the Florida Keys. Extracted samples were shipped to Sarasota for brevetoxin analysis by Mote personnel.

A volunteer opportunistic sampling program was also continued through several separately funded programs aimed at coordinating community monitoring efforts in the Florida Keys. Results from samples collected for cell counts and identification through these separately funded volunteer monitoring efforts are also included in this report due to their relevance. These volunteer opportunistic sampling programs utilized commercial fishermen, dive operators backcountry guides, researchers, and state and federal agency personnel to collect additional samples for cell counts and algal identification, and provided extended coverage along the areas north of the Lower and Middle Keys to Cape Sable, as well as along the entire offshore reef tract. When either routine or volunteer sampling efforts, either routine or volunteer, or satellite imagery indicated the potential for *Karenia. brevis* bloom development, a response sampling effort was was initiated to investigate. Response sampling typically included collection of stained samples for cell counts and algae identification, bulk water samples as needed for brevetoxin analysis, water quality measurements, and any additional samples requested by other researchers.

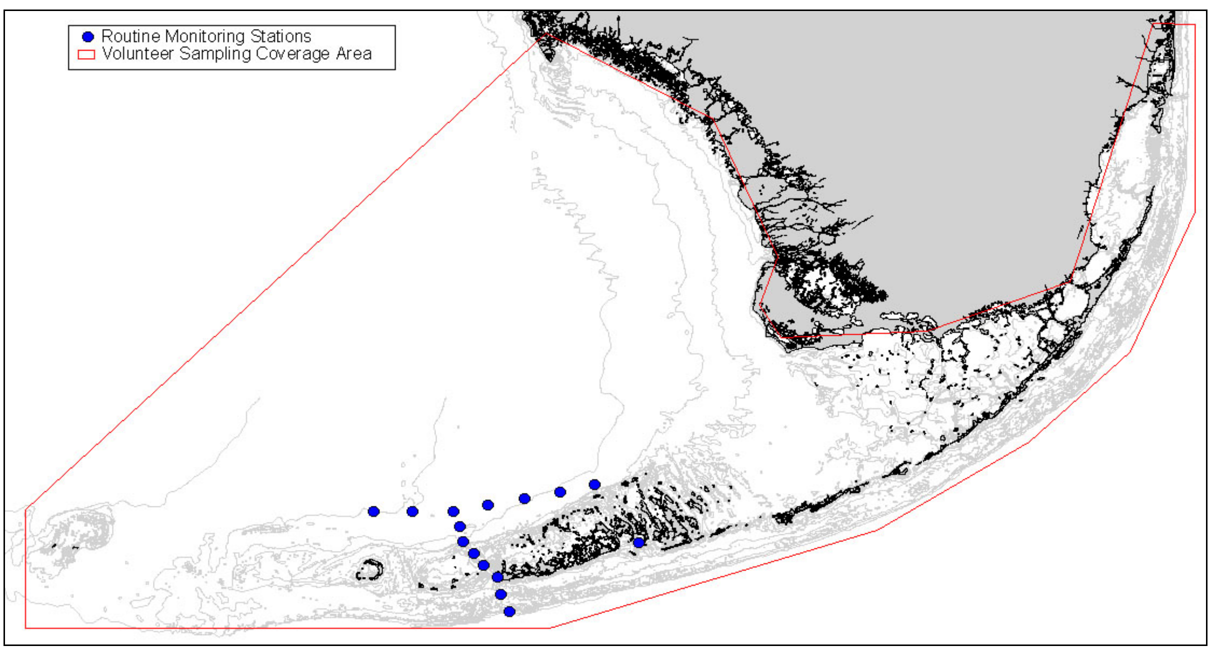

Figure 18 Map of Mote Marine Laboratory's Florida Keys routine monitoring stations and opportunistic volunteer sampling coverage area (July 1 – September 15, 2008)

Results

Only one routine monitoring cruise was conducted during this report period, and 15 samples were collected for cell counts and algae identification during routine monitoring cruises and response efforts (**Appendix H**). An additional 7 bulk water samples were collected during routine monitoring cruises and response efforts and extracted for later brevetoxin analysis. Water quality measurements, including temperature, salinity, and dissolved oxygen, were also logged at each station. Toxin analysis results are reported separately, because while collections and initial extractions were conducted by Mote staff in the Florida Keys, final analysis of extracted samples was conducted by Mote staff in Sarasota. Volunteer opportunistic sampling efforts, coordinated in collaboration with a separately funded program but reported here due to their relevance, provided an additional 81 samples for cell counts and algae identification (results also included in **Appendix H**.

July 1 - 31, 2008 (Figure 19):

- No elevated levels of *K. brevis* were noted in routine monitoring samples.
- No elevated levels of *K. brevis* were noted in volunteer samples.
- No response efforts were initiated.

August 1 - 31, 2008 (Figure 20):

- No routine monitoring cruise was conducted.
- No elevated levels of *K. brevis* were noted in volunteer samples.
- No response efforts were initiated.

September 1- 15, 2008 (Figure 21):

- No routine monitoring cruise was conducted.
- No elevated levels of *K. brevis* were noted in volunteer samples.
- No response efforts were initiated.

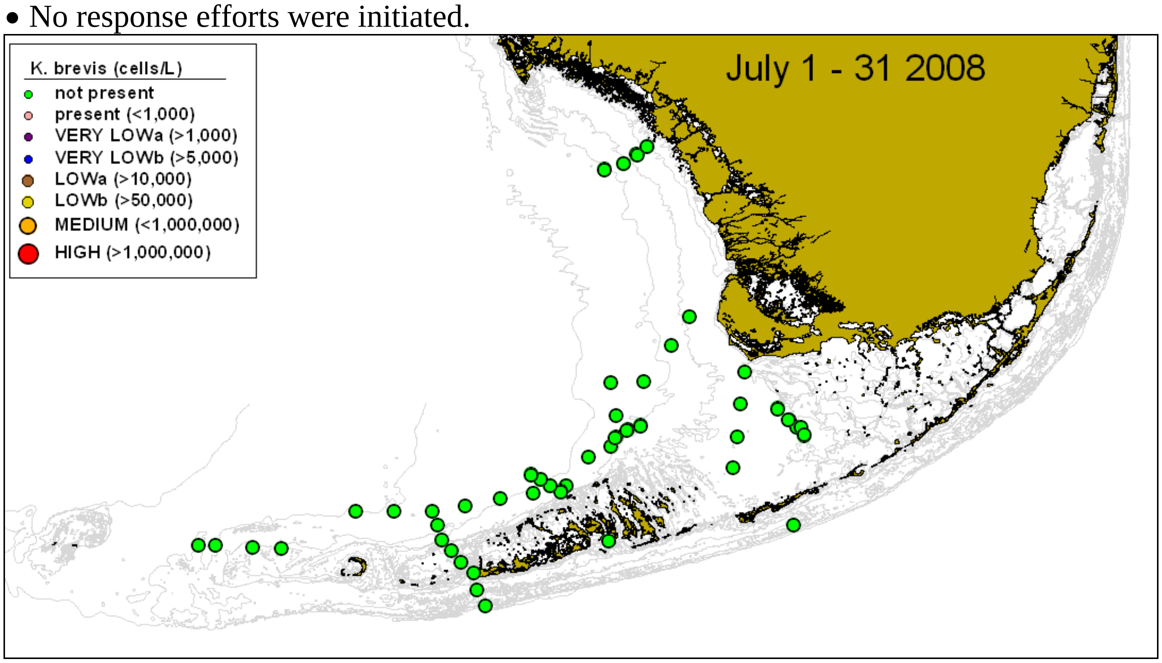

Figure 19 Map of *K. brevis* cell counts for routine monitoring, response, and volunteer samples in the Florida Keys (July 1-31, 2008).

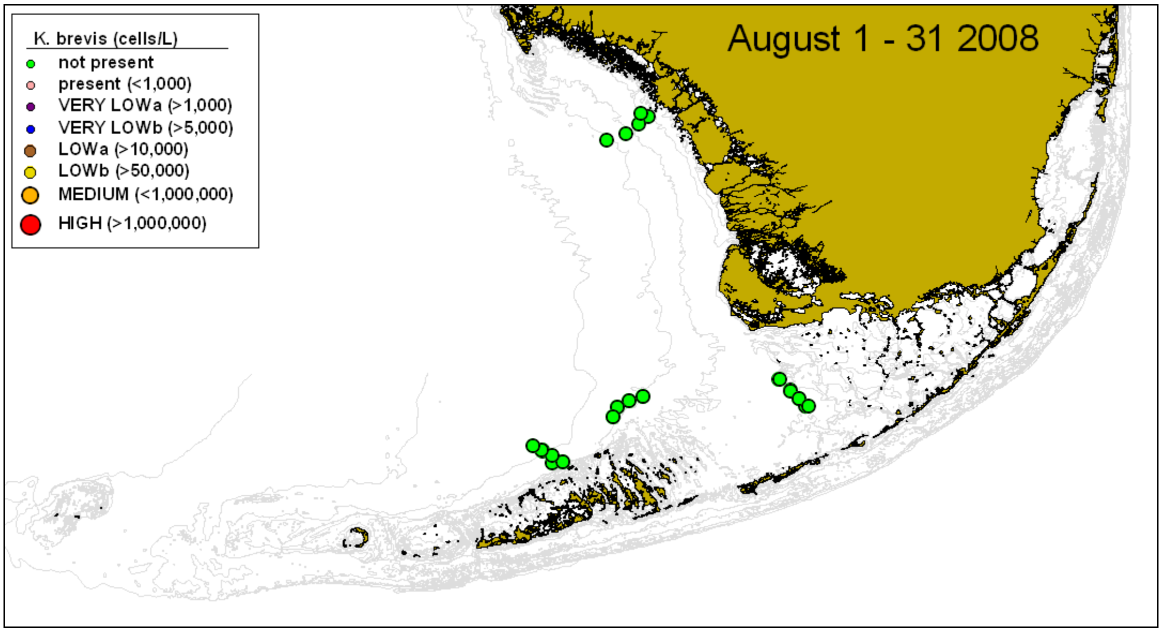

Figure 20 Map of *K. brevis* cell counts for routine monitoring, response, and volunteer samples in the Florida Keys (August 1-31, 2008).

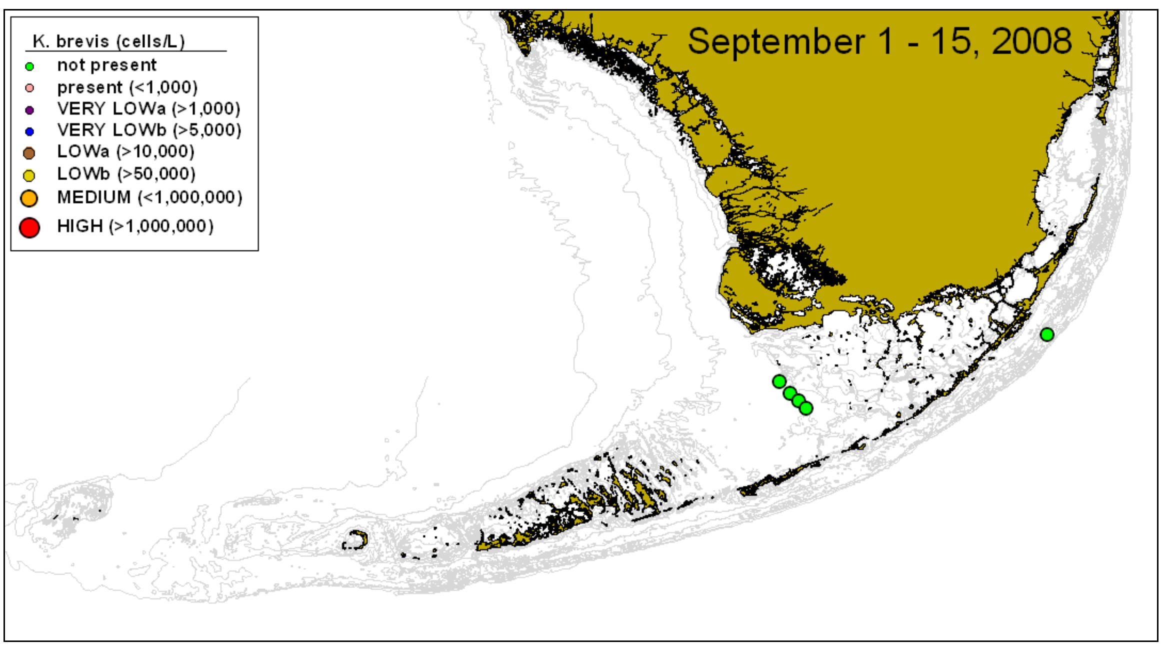

Figure 21 Map of *K. brevis* cell counts for routine monitoring, response, and volunteer samples in the Florida Keys (September 1-15, 2008).

Task 1.B. Implement automated technology to provide continuous, real-time monitoring of conditions where red tides develop and propagate.

a) Automated Red Tide Sensors:

At the beginning of this reporting period there were BreveBuster deployed on the Venice buoy (MB3 – Venice), on the Anna Maria buoy (MB3 – Anna Maria) and on the C15 buoy offshore of New Pass. The Venice BreveBuster sampled and reported every two hours during the entire reporting period (**Fig. 22**). The Anna Maria BreveBuster sampled and reported every two hours until July 13 when a power failure ended 66 days of operation. The BreveBuster on the C15 buoy operated until July 22 when it was taken out of service during a buoy swap operation. A freshly serviced BreveBuster was installed on the Anna Maria buoy on August 31 and has sampled and reported every two hours since that date. The Venice installation is 5 km offshore of the Venice Pier on the 10 m isobath and is therefore somewhat isolated from the outflow from Tampa Bay and Charlotte Harbor.

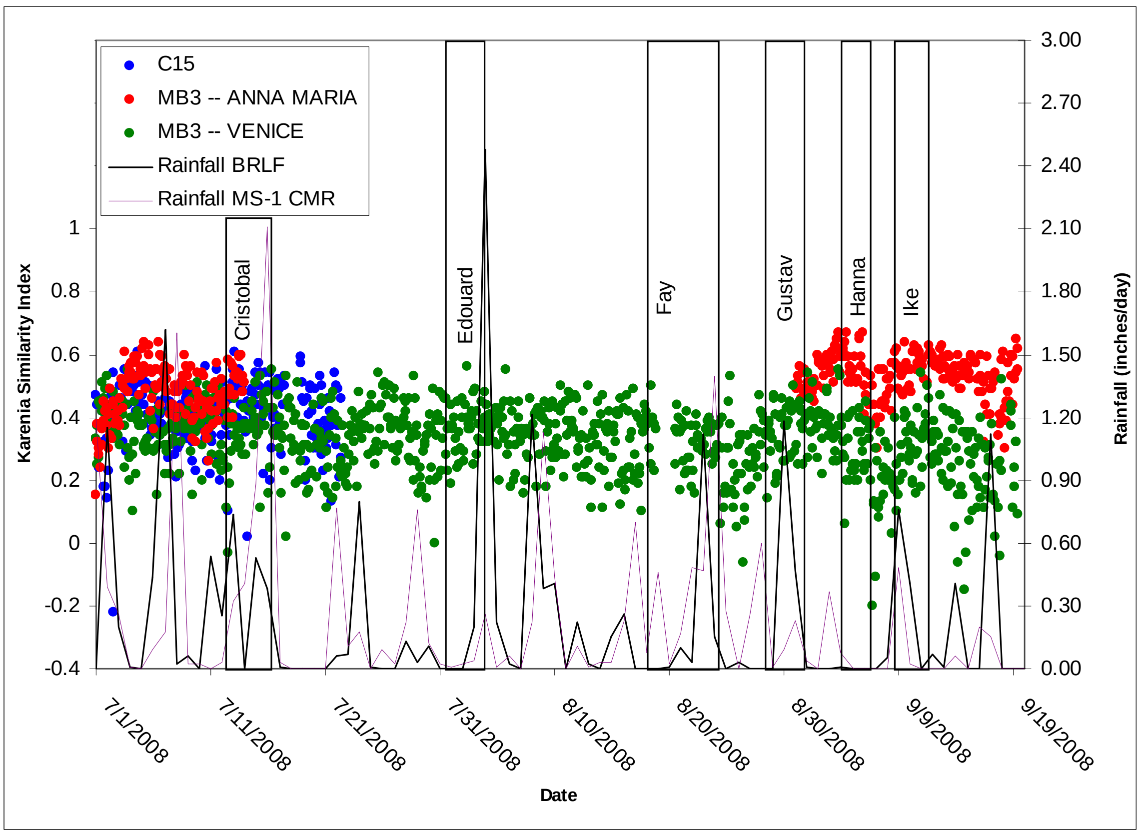

Figure 22 Time series from BreveBusters on the C15 buoy, the Anna Maria buoy and the Venice buoy (instruments #007, #010, #012) located at one meter depth. Most *Karenia* sp. similarity indexes were below the presence threshold of 0.6 between July 1, 2008 and September 3, 2008. Beginning early in September there were numerous indications at the Anna Maria buoy of low *Karenia* sp biomass fraction. Periods of tropical storm and hurricane influence are shown as stippled rectangles. Daily rainfall accumulation is reported from two sites in Sarasota County.

Throughout most of the reporting period *Karenia* sp. similarity indexes were below the presence threshold of 0.6 between. Except for a few samples in early July the period from July 1, 2008 to September 3, 2008 was devoid of *Karenia* sp according to these instruments. However, beginning early in September there have been numerous indications at the Anna Maria buoy of low *Karenia* sp biomass fraction. We have not been successful verifying these observations by collecting water samples at the Anna Maria buoy that turn out to be coincident with BreveBuster samples that show similarity indexes above 0.6. It is not possible to plan a boat trip a priori to an unknown event! We are considering deployment of an automated water sampler to try to collect verification samples

Colored dissolved organic material absorption measured by the BreveBusters deployed at those three buoys was generally very low prior to July 2008. This would indicate that the input of CDOM laden terrestrial runoff was low and/or there was a strong influence by oceanic waters, characteristically low in CDOM. As the summer progressed and rainfall accumulated the CDOM absorption increased gradually **(Figure 23)** until hurricanes Fay and Gustav dumped

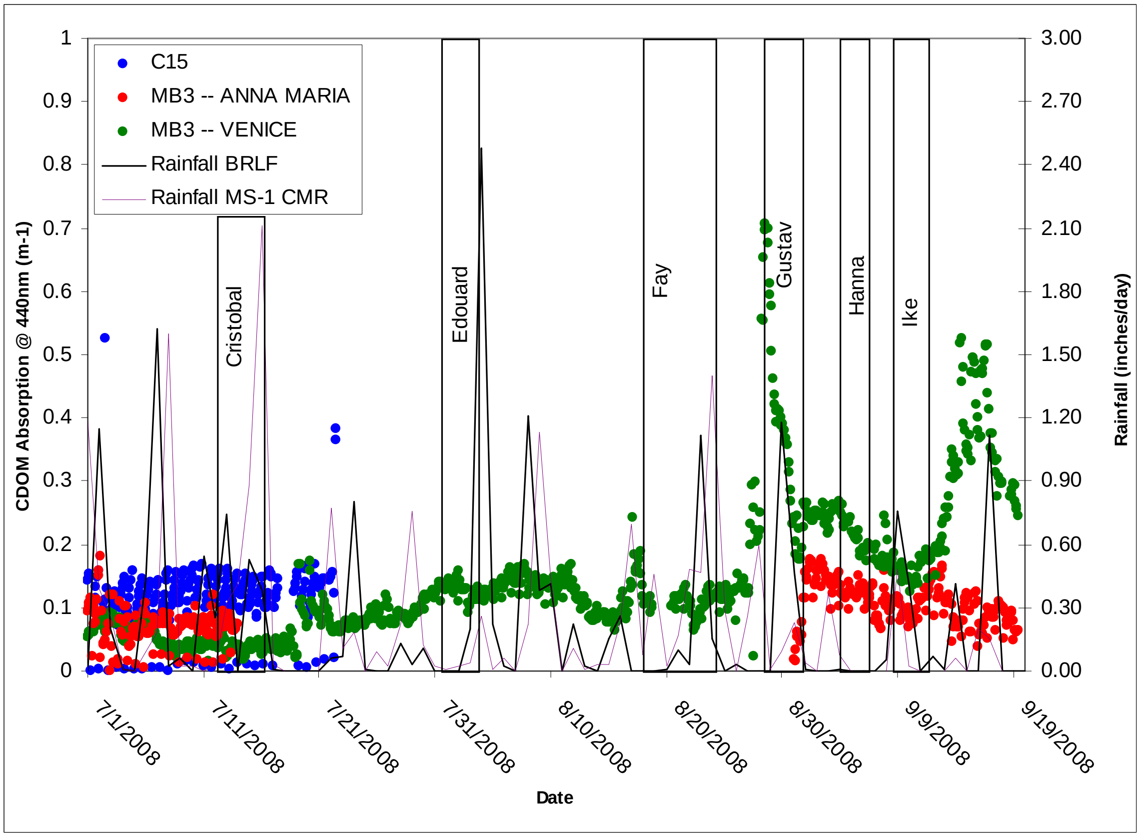

Figure 23 Time series from BreveBusters on the C15 buoy, the Anna Maria buoy and the Venice buoy (instruments #007, #010, #012) located at one meter depth. The CDOM values shown in this figure are absorption values (1/m) at 440 nanometers. As rainfall has increased through the summer the CDOM has increased at the Anna Maria and Venice buoys. Periods of tropical storm and hurricane influence are shown as stippled rectangles. Daily rainfall accumulation is reported from two sites in Sarasota County. Note the large increases in CDOM absorption following the passage of Fay and during Gustav and then again following the passage of Ike.

large amounts of rain and mixed the estuarine outflow along the coast. It is interesting to note that during the last two weeks of data presented here the CDOM absorption has declined at the Anna Maria buoy (just south of the mouth of Tampa Bay), but the CDOM absorption has increased dramatically at the Venice buoy. This observation has been supported by satellite imagery (when available) that shows a CDOM plume skewed northward at Tampa Bay, away from the Anna Maria buoy and also skewed northward at Charlotte Harbor, toward the Venice buoy.

Two Slocum glider mission were conducted during this reporting period (**Figure 24)**. They ran from July 23 to August 4, 2008 and September 3 to September 6, 2008. The July-August mission ended when battery voltage dropped to a level where the BreveBuster was shutdown to conserve battery power for glider recovery. That mission was begun with a partially discharged battery, the result of previous missions that did not go to battery exhaustion. The September mission was cut short by the approach of Hurricane Ike. Similarity indexes collected during those missions showed no signs of *Karenia* sp.

Figure 24 The tracks followed by the Slocum glider Waldo (SN045) during missions July 23 to August 4, 2008 (red line) and September 3 - 6, 2008 (light blue line). Yellow hexagons indicate locations where BreveBusters were deployed on buoys.

b) Sarasota Operations Coastal Oceans Observing Laboratory (SO COOL)
Data streams from deployed BreveBusters have been received and processed by the data servers at the SO COOL during the past reporting period. Portions of those data are also processed through an IOOS data portal for dissemination through the GCOOS network. The acquisition process has begun for additional data storage capacity to improve archival reliability. A one-terabyte disk array is being specified to act as archival backup. Improvements are being planned for research vessel tracking and at-sea data recovery. The goal is to have a capability to actively track more than one research vessel and telemeter the data acquired by those vessels directly to the SO COOL in real-time. This capability will help to provide context for deployed BreveBusters. Currently, remote sensing imagery is relied upon to help develop glider mission tracks. This works well when the skies are clear, but suffers in the summer when cloud cover is common. Incorporating real-time boat observations will provide some cloudy day guidance. Data received from the deployed BreveBusters were presented on the SO COOL website either as time series plots for fixed position installations or Google surface plots of findings reported during surfacing events of the Slocum gliders. Work continues to adapt the Google visualization system to provide 3-D display including depth distribution.

c) Development and implementation of Brevetoxin detectors:
This portion of the project was undertaken by NSF-REU Student Intern, Laura Heyl, of Bowdoin College, Brunswick, ME, during her summer internship at Mote. Ms. Heyl worked on this project under the direction of Michael Henry, from July through August, 2008.

The objectives of this project were to determine the optimal techniques, specifications, and parameters for the extraction module and the spectrophotometer setup for use in the proposed detector.

Objective 1 – Extraction Module
Asses the efficiency and reproducibility of the solid-phase extraction method in comparison to known techniques.

Objective 2 – Spectrophotometer Setup
Adapt the existing BreveBuster spectrophotometer and deuterium-tungsten light source setup as well as the capillary wave-guide system for the identification and analysis of brevetoxins by their distinctive UV absorption spectra.

Extraction Module
K. brevis cultures of 1.5×10^7 and 2.3×10^7 cells/L were each diluted to approximately 8.3×10^6 cells/L and 6.3×10^6 cells/L respectively. Each original culture was extracted with C-18 discs to verify the original toxin content. The diluted culture was extracted using C-18 discs, Strata-X polymeric-based cartridges, and Strata C-18 silica-based cartridge to obtain comparisons of the various extraction methods.

Results of the extraction module tests indicated that all 3 methods provided adequate recovery of PbTx-2 and 3. However, the SPE cartridges were more efficient at extraction recovery of brevenal and PbTx-1 than the C-18 discs.

Spectrophotometer Setup

Existing BreveBuster technology was used in the spectrophotometer setup. A euterium-tungsten light source (World Precision Instruments) and a charge coupled device (Ocean Optics) were connected to a 28cm liquid waveguide capillary cell (World Precision Instruments) with included fiber optic cables. PTx-2 standards of 4.2, 8.4, 16.8, and 25.2 ng per ml were prepared and verified by L/MSMS analyzed. Absorption spectra from the capillary wave guide were collected with the OOIBase32 software provided by Ocean Optics.

Results of the spectrophotometer setup exhibited a positive exponential relationship between absorbance and concentration of toxin within the range of 4.2ng/ml and 25.2 ng/ml.

Task 1.C. Regular monitoring of airborne brevetoxin at the Mote Marine Laboratory New Pass Dock

Initial phases of this task were completed last year. The next phase for collecting brevetoxins will be completed when the next red tide occurs in Sarasota Bay.

Task 1.D. Assess K. brevis response to different types of nutrient inputs and different source waters

One bioassay study, in addition to the three conducted last year, was conducted August 12-14, 2008. Bulk water was collected approximately 4 km off of New Pass near Sarasota, FL and *Karenia brevis* was not present. Two new sets of treatments were added to this experiment including L-glutamine (AA3) and a treatment of nitrogen and phosphorus (urea and phosphate).

Ambient coastal water was collected and apportioned into eleven sets of incubation chambers. Treatments were applied of inorganic nitrogen (ammonia and nitrate, separately), inorganic phosphorus, urea, three different amino acids, silica, combined urea and phosphate and filtered estuarine water collected from the mouths of Tampa Bay (TB), Charlotte Harbor (CH), and San Carlos Bay/Caloosahatchee River (CR). Treatment levels for estuarine waters approximated a 1:25 dilution of estuarine water with coastal water, while the addition of inorganic single nutrients was similarly scaled, based on existing information of typical concentrations in estuarine outflow. Incubations took place at the Mote facility under ambient conditions and near surface. Pertinent physical data were recorded (water temperature, solar radiation, water clarity).

Treatments were sampled at initiation and at 24 and 48 hours post-treatment. Analyses from all samplings included *Karenia* sp. cell counts, total dissolved nitrogen and phosphorus, dissolved inorganic nutrients (ammonia, nitrate-nitrite-nitrogen, orthophosphorus), particulate nutrients (C, N, and P), urea, chlorophyll *a*, and photopigments via HPLC analyses. Brevetoxin analyses were performed on bulk water with replication at initiation and on all treatments at completion.

Bioassay Results

A fourth bioassay study was completed in August 2008. Two new sets of treatments were added to this experiment including L-glutamine (AA3) and a treatment of nitrogen and phosphorus (urea and phosphate). Results of that study continue to be processed. Available data are presented in **Appendix F and G**. *Karenia brevis* was not present during this bioassay experiment.

Control Study:
A bioassay was conducted from August 12th to 14th 2008 consisting of 42 different nutrient treatments. Analysis of these samples for brevetoxin are underway.

Goal 2. Supplemental studies in support of collaborative programs.

Task 2.A. Field program in support of NOAA-ECOHAB:Karenia Nutrient Dynamics

No ECOHAB related surveys were conducted during this reporting period. Surveys are scheduled for September 23 and November 4, 2008.

Task 2.B. Sarasota Department of Health Beach Monitoring
Karenia sp have been microscopically enumerated in twelve sets of 16 water samples for the Sarasota Department of Health Beach Monitoring Program during this reporting period. Samples were collected every Monday and The results have been reported to FWRI as they were collected.

Task 2.C. Sarasota County Ambient Monitoring Program

Karenia sp. cell counts were provided in conjunction with monthly water quality measurements in Sarasota Bay performed by Mote Marine Laboratory for the Sarasota County Ambient Monitoring Program. No *Karenia brevis* cells were observed in any of these samples, therefore, no brevetoxin analyses were performed on the water samples.

Goal 3. Implement communication and outreach programs for stakeholders.

Task 3.A. Community and media relations.
a) Web site and Phone Information.
Due to the lack of an active bloom, Mote's red tide website and phone line have been updated once a week.

b) On August 22, 2008, a small group of scientists and communication experts to discuss the current methods of red tide information and discuss improvements/ways to work better together and achieve even better results. In attendance were Cindy Heil and Wendy Quigley (FWRI), Frank Alcock (MML Policy Institute), Jill Copeland and Charlotte Richardson (START), Martha Wells (Mote VP of Communications) and Barbara Kirkpatrick and Kate Nierenberg (Mote/Environmental Health). The abbreviated minutes of the meeting are attached (**APPENDIX I)**. It should be noted that the minutes from the meeting were also shared with the FWC Control and Mitigation grant addressing outreach strategies, led by Dr. Sherry Larkin at the University of Florida, to inform that group of the activities currently in place in Florida.

c) A second meeting by conference call was held on September 10th, 2008. Participating on the call was Martha Wells, Nadine Slimak, and Barbara Kirkpatrick from Mote and Wendy Quigley and Carli Segelson. The prime point of discussion was to tighten and/or further improve the communications between the 2 organizations during Florida red tide blooms. Increased coordination would improve accurate and timely messaging to the public. Another outcome from the discussion was for both organizations to review their Florida red tide web pages for antiquated information.

d) The scheduling of the town hall meeting is in the planning stages. Discussion with Mote's communication department has occurred regarding the best time for the event. Ideally, the 2 times, not mutually exclusive, would be during a red tide bloom and/or during the height of the tourist season- both to assure good attendance.

e) On August 27, 2008, Jeff Paternoster conducted a training session for the NOAA sponsored Phytoplankton Monitoring Network (http://www.chbr.noaa.gov/PMN/) in Sarasota. Approximately 15 people attended the session, includ8ing several local high school science teachers and members of the Florida Paddling Trails Association. Space for the training was provided by Mote, Mr. Paternoster brought all needed supplies and materials to complete the training.

Task 3.B. Information and Outreach to the General Public.

In conjunction with funding from FL Department of Health, Dr. Barbara Kirkpatrick continued her outreach and education activities to the local medical community.

a) Presentations to the general public have included:
   a. Riverview High School, Aug 29, 2008, K. Nierenberg
   b. Manatee County Chamber of Commerce, Sept 9, 2008, B. Kirkpatrick

b) Media interviews:
   WFGCU radio- July 18, 2008, B. Kirkpatrick

Total number of Public presentations: 2

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