Naval Research Laboratory

Washington, DC 20375-5320

NRL/MR/6110--14-9552

TNT Degradation by Natural Microbial Assemblages at Frontal Boundaries Between Water Masses in Coastal Ecosystems (ER-2124 Interim Report)

Michael T. Montgomery
Thomas J. Boyd
Richard B. Coffin

Chemical Dynamics and Diagnostics Branch
Chemistry Division

Lisa A. Drake

Center for Corrosion Science and Engineering
Chemistry Division

Lauren T. Hansen
Christopher L. Osburn

North Carolina State University
Raleigh, North Carolina

June 26, 2014

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
26-06-2014	Memorandum	01 January 2013 – 31 December 2013

4. TITLE AND SUBTITLE

TNT Degradation by Natural Microbial Assemblages at Frontal Boundaries Between Water Masses in Coastal Ecosystems (ER-2124 Interim Report)

5a. CONTRACT NUMBER
61-4540-0-3

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Michael T. Montgomery, Thomas J. Boyd, Richard B. Coffin, Lisa A. Drake, Lauren T. Hansen,* and Christopher L. Osburn*

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER
61-4540-0-3

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Naval Research Laboratory, Code 6114
4555 Overlook Avenue, SW
Washington, DC 20375-5344

8. PERFORMING ORGANIZATION REPORT NUMBER

NRL/MR/6110--14-9552

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Strategic Environmental Research and Development Program
P.O.C. Andrea Leeson
4800 Mark Center Drive, Suite 17D08
Alexandria, Virginia 22350-3605

10. SPONSOR / MONITOR'S ACRONYM(S)

SERDP

11. SPONSOR / MONITOR'S REPORT NUMBER(S)

ER-2124

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

*Marine, Earth, and Atmospheric Sciences, North Carolina State University, Raleigh, NC 27695

14. ABSTRACT

This limited scope, three year SERDP project involves determining the primary biogeochemical factors that control TNT metabolism by natural microbial assemblages in coastal systems. By correlating standard water quality measurements with degradation rates, we can predict turnover times for energetics released into hydrodynamically similar, UXO-impacted ecosystems where access to site samples may be limited. During this first year of sampling in the Florida Keys, USA, we found that mixing experiments between mangrove lagoon water and open ocean Gulf of Mexico water resulted in more rapid rates of bacterial growth and aromatic contaminant mineralization (i.e., TNT, RDX, HMX and phenanthrene) than would have been predicted by interpolation of unmixed end members. This line of evidence supports the hypothesis that coastal mixing zones may lead to more rapid energetic and PAH biodegradation than would be expected using standard measures and techniques. Surveys of energetic and PAH mineralization rates in areas adjacent to DoD sites in the Key West area suggest that contaminants in surface runoff from shoreside areas would be rapidly metabolized (i.e., hours to weeks) in the adjacent seawater and surface sediment. Rapid intrinsic biodegradation rates of such contaminants would potentially mitigate the ecological risk associated with exposure of marine biota to these compounds. In addition, as part of these surveys, the most rapid RDX and HMX mineralization rates associated with any natural assemblage to date were found in the tropical sediment at some stations around Key West. Taken together, this work supports a site conceptual model where PAH and energetics would be rapidly biodegraded by natural microbial assemblages were they to migrate from mangrove-dominated lagoon systems to adjacent coastal waterways in tropical ecosystems. Understanding the relationships between biogeochemical parameters and aromatic contaminant biodegradation amongst various ecosystem (biome) types may allow the determination of attenuation rates even at DoD sites with limited access to water and sediment samples due to the presence of UXO.

15. SUBJECT TERMS

Bacteria	Biodegradation	DOC	Frontal boundary	Key West	Mineralization	Salt wedge
Bacterial production	Coastal	Energetics	HMX	Marine	PAHs	2,4,6-trinitrotoluene

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					Michael T. Montgomery
a. REPORT	b. ABSTRACT	c. THIS PAGE	Unclassified Unlimited	32	19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified			(202) 404-6419

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure 14. EEM fluorescence analyses of potential OM sources.

LIST OF ACRONYMS

AUV: Autonomous Underwater Vehicle
CDOM: Colored Dissolved Organic Matter
CO_2: Carbon Dioxide
CTD: Conductivity-Temperature-Depth water sampling device
CuO: Cupric Oxide
d^{-1}: Per Day
DAT: 2,4-Diaminotoluene
DNT: 2,4-Dinitrotoluene
DO: Dissolved Oxygen
DOC: Dissolved Organic Carbon
DoD: Department of Defense
DOM: Dissolved Organic Matter
EEM: Excitation-Emission Matrix
FID: Flame Ionization Detection
GC/MS: Gas Chromatography/Mass Spectrometry
H_2SO_4: Sulfuric Acid
HMX: Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
kg^{-1}: Per Kilogram
L^{-1}: Per Liter
mCi: Millicurie
mL: Milliliter
MNA: Monitored Natural Attenuation
MRP: Munitions Response Program
NAVFAC: Naval Facilities Engineering Command
NaOH: Sodium Hydroxide
NFA: No Further Action
NG: Nitroglycerine
nm: Nanometers
NRL: Naval Research Laboratory
OCONUS: Outside the Continental United States
OM: Organic Matter
PAH: Polycyclic Aromatic Hydrocarbons
PARAFAC: Parallel Factor Analyses
POC: Particulate Organic Carbon
PSU: Practical Salinity Units
QSE: Quinine Sulfate Equivalents
RDX: 1,3,5-Trinitroperhydro-1,3,5-triazine
RPM: Remedial Program Manager
SUVA: Specific Ultraviolet Absorption
SERDP: Strategic Environmental Research and Development Program
TCA: Trichloroacetic Acid
TBD: To Be Determined
TNT: 2,4,6,-Trinitrotoluene
μg: Micrograms
UL-: Uniformly Labeled
USN: United States Navy
UUV: Unmanned Underwater Vehicle
UXO: Unexploded Ordnance

ACKNOWLEDGMENTS

In addition to the SERDP Environmental Restoration program (Program Manager: Andrea Leeson), thanks to the scientists, engineers and support staff at NRL-Key West Code 6136 (Section Head Diane Lysogorski) including Matt Krupa, Matt First, Stephanie Robbins-Wamsley, and Scott Riley, and NAS-Key West including Glenn Hayes, Ed Donohue, and Ed Barham. Also thanks to Dana Hayworth and Brian Syme at NAVFAC SE for Navy site coordination and information, Jane Castelline for sampling, and Dave Kirchman and Matt Cottrell for sample processing. Environmental sampling guidance was provided by Joanne Delaney at NOAA (National Marine Sanctuary Permit# FKNMS-2013-061) and Nancy Finley at U.S. Fish and Wildlife Service (Special Use Permit#2013-016).

ABSTRACT

This limited scope, three year SERDP project involves determining the primary biogeochemical factors that control TNT metabolism by natural microbial assemblages in coastal systems. By correlating standard water quality measurements with degradation rates, we can predict turnover times for energetics released into hydrodynamically similar, UXO-impacted ecosystems where access to site samples may be limited. During this first year of sampling in the Florida Keys, USA, we found that mixing experiments between mangrove lagoon water and open ocean Gulf of Mexico water resulted in more rapid rates of bacterial growth and aromatic contaminant mineralization (*i.e.,* TNT, RDX, HMX and phenanthrene) than would have been predicted by interpolation of unmixed end members. This line of evidence supports the hypothesis that coastal mixing zones may lead to more rapid energetic and PAH biodegradation than would be expected using standard measures and techniques. Surveys of energetic and PAH mineralization rates in areas adjacent to DoD sites in the Key West area suggest that contaminants in surface runoff from shoreside areas would be rapidly metabolized (*i.e.,* hours to weeks) in the adjacent seawater and surface sediment. Rapid intrinsic biodegradation rates of such contaminants would potentially mitigate the ecological risk associated with exposure of marine biota to these compounds. In addition, as part of these surveys, the most rapid RDX and HMX mineralization rates associated with any natural assemblage to date were found in the tropical sediment at some stations around Key West. Taken together, this work supports a site conceptual model where PAH and energetics would be rapidly biodegraded by natural microbial assemblages were they to migrate from mangrove-dominated lagoon systems to adjacent coastal waterways in tropical ecosystems. Understanding the relationships between biogeochemical parameters and aromatic contaminant biodegradation amongst various ecosystem (biome) types may allow the determination of attenuation rates even at DoD sites with limited access to water and sediment samples due to the presence of UXO.

OBJECTIVE

This work involves determining the primary biogeochemical factors that control TNT metabolism by natural microbial assemblages in coastal systems. By correlating standard water quality measurements with degradation rates, we can predict turnover times for energetics released into hydrodynamically similar UXO-impacted ecosystems where access to site samples may be limited. Three data gaps are being addressed as part on an integrated sampling scheme of DoD-relevant field sites: 1) determining energetic metabolism rates for ecosystem types where we currently lack data; 2) correlating biogeochemical water quality data with biodegradation rates; and, 3) measuring TNT incorporation rates into bacterial DNA using sediment samples. By seasonally repeating these survey measurements and mixing experiments across DoD-relevant ecosystems and further identifying the specific areas and conditions associated with energetic degradation, we will increase the likelihood of public and regulatory acceptance of natural attenuation as a viable risk reduction alternative. Documenting and validating such natural pollution abatement mechanisms supports continued use of active DoD ranges.

BACKGROUND

Although SERDP, Army and Air Force have sponsored much work on energetic transformation in terrestrial and groundwater systems, relatively little information is available on the attenuation or transport of energetics in coastal aquatic systems. In lieu of evidence that these materials are rapidly degraded to nontoxic substances or metabolized by microbial assemblages, DoD is being pressured to recover all UXO from coastal waters and decontaminate the surrounding media due to presumed ecological risk (*e.g.,* CH2M HILL 2006). Historically, TNT and other energetics were believed to be

Manuscript approved February 16, 2014.

recalcitrant to biodegradation to harmless products (*e.g.,* bacterial biomass, CO_2; Hawari et al. 2000, Spain et al. 2000, Claus 2014). However, these conclusions were largely based on bacterial laboratory culture and field work on terrestrial and groundwater sites (*e.g.,* Myers et al. 1998, Travis et al. 2008) that are unlikely to apply to natural assemblages in coastal estuarine and marine systems (Rappe and Giovannoni 2001). Most energetic compounds that are currently the subject of DoD site investigations are nitrogen-containing organics and organic nitrogen itself is typically scavenged by natural bacteria in coastal ecosystems (Pomeroy 1970, Paerl and Piehler 2008). Anecdotally, energetic organics are not typically found during surveys of UXO-impacted coastal water and sediment which suggests that they may be more transient than the laboratory findings indicate (*e.g.,* CH2M HILL 2000, NOAA 2006, Simmons 2007). A limited amount of further investigation will likely reconcile these disparate findings resulting in cost savings by eliminating unnecessary cleanup and restoration of coastal sediment that is perceived to be (but not actually) impacted by energetics for a length of time that would pose an unacceptable ecological or human health risk.

Recent work by our group and others has focused on determining energetic degradation rates and biogeochemical control of these rates amongst water and sediment of aquatic ecosystems (Conder 2002, Douglas et al. 2009, Zheng et al. 2009, Chappelle et al. 2011, Fahrenfeld et al. 2013). As part of our previous project, ER-1431 (Montgomery et al. 2008), we concluded that TNT biodegrades and photodegrades on ecologically relevant time scales (*e.g.* hours to months) but that other abiotic chemical processes (*e.g.,* hydrolysis) may be too slow. Biodegradation rates of TNT in nitrogen-limited coastal waters are surprisingly rapid compared to rates in terrestrial systems (which are usually phosphorus-limited) and may be the dominant removal pathway for estuarine and marine ecosystems (Montgomery et al. 2011b). TNT mineralization has been reported in many studies to range from 1 to 1000 $\mu g\ kg^{-1}\ d^{-1}$ once data are converted into common units (Montgomery et al. 2011a and references therein). These rates are often similar to those of other natural and anthropogenic aromatic organics with half lives of days to months (*e.g.,* PAHs; Boyd et al. 2008).

TNT ring carbon is incorporated into bacterial macromolecules at rates in water that are 10-100 times higher than mineralization rates (complete conversion to CO_2; Montgomery et al. 2013) though incorporation rates have yet to be measured in sediment assemblages because of technical reasons (*i.e.,* high background due to abiotic binding to humic). Adding in TNT incorporation rates as part of total metabolism reduces half lives to hours to weeks (*i.e.,* similar to those rapid rates for amino acids, Kirchman 1994). TNT incorporates into bacterial macromolecules including DNA which enables identification of bacterial genotypes involved in complete TNT biodegradation in natural samples and is relatively unequivocal evidence that TNT ring carbon is fully metabolized amongst natural sedimentary assemblages (Gallagher et al. 2010). Because TNT is metabolized and the ring carbon incorporated at high efficiency (*i.e.* low amount of carbon diverted to CO_2), it is very unlikely that toxic intermediates are produced as part of this metabolic process. This contrasts with what has been shown in soil and groundwater when high concentrations of TNT are used with relatively low bacterial production (*e.g.,* dissimilatory metabolism with TNT used as an alternate electron acceptor verses assimilatory TNT metabolism for biomass production in aerobic coastal waters; Clark and Boopathy 2007, Kubota et al. 2008). Because of the high TNT incorporation efficiency measured in most coastal waters, it is likely that the TNT mineralization rates are actually re-mineralization of radiolabelled bacteria by protozoan grazers (Caron et al. 1988) rather than the use of TNT ring carbon for energy rather than biomass (Montgomery et al. 2013).

Organic carbon metabolism rates (including TNT and all other organic energetics) are generally bounded by those of total heterotrophic bacterial metabolism (secondary production; Kirchman et al. 1985). Static, unbioturbated sediment and slow moving, stratified, anaerobic waters will limit biodegradation as bacterial production will likewise be limited. Transitional coastal areas such as bioturbated, surface sediment (Montgomery et al. 2008), fronts between water masses (Borsheim 1990, Floodgate et al. 1981,

Josefson and Conley 1997), tidal salt marshes (Weston et al. 2011) and intertidal zones (Rocha 2008) will often have the highest rates of carbon metabolism. Within the three order of magnitude range of total bacterial production, biogeochemical factors that control TNT biodegradation (and other nitrogenous energetics like RDX and HMX) will likely be those that influence rate of nitrogen metabolism (*e.g.,* nitrogen demand by the assemblage; availability of more labile nitrogen sources) including parameters like salinity, temperature, and dissolved oxygen. In ER-1431, we found that there appeared to be some influence of salinity on energetic degradation. Upon more in depth examination in ER-2124, we found that it was actually areas of rapid salinity change that were associated with enhanced TNT degradation (Montgomery et al. 2012). Salinity change was indicative of the location of these important frontal boundary regions rather than being a controlling biogeochemical property itself. These are important distinctions when trying to model energetic fate in a natural ecosystem.

Also within the upper bound of total bacterial metabolism are likely factors that preferentially influence aromatic organic carbon degradation. Based on our earlier work, TNT metabolism (also aromatics like DNT, DAT) appears to be influenced by parameters that control degradation of the aromatic component of dissolved organic carbon (DOC; *e.g.,* lignin, PAHs) in the water column and particulate organic carbon (POC) in sediment (Montgomery et al. 2004). These biogeochemical features may include presence of more labile carbon (*e.g.,* simple sugars) that would outcompete energetics as a bacterial carbon source.

This research extends our previous SERDP work by examining biogeochemical control of degradation rates in studies of DoD relevant ecosystems where we have a paucity of data. Three data gaps are being addressed as part on an integrated sampling scheme of DoD relevant field sites: determining energetic metabolism rates for *ecosystem types* where we currently lack data; correlating biogeochemical water and *organic matter quality* data with areas of elevated degradation rates; and, measuring *TNT incorporation rates* into bacterial DNA using sediment samples (the latter to be performed Year 3):

Ecosystem Types: Though there are site specific variables that affect each controlling factor at a given site, there are general features that can be used to bound these variables for a given ecosystem type (biome). Navy underwater coastal UXO sites can be categorized for Remedial Program Managers (RPMs) according to expected range of each variable based on published data (Table 1), though in some cases, for historically restricted-access areas, or OCONUS sites of limited published study, empirical data may need to be collected. Published data for similar or nearby ecosystems might be transferable to sites of interest that lack data (Figure 1) or may be used to estimate expected degradation rates involving a known or hypothetical amount of munitions breached at a coastal site (Figure 2).

Organic Matter Quality: It is likely that establishing a correlation between metabolism of TNT and aromatic organic carbon may be the best predictor of TNT removal by natural bacteria in surface water and sediment. Unfortunately it is also the most difficult to establish directly because there are few radiotracers for natural aromatic organics (*e.g.,* ^{14}C-lignin monomers > \$60K per experiment). The best way to examine this may be statistical analyses of changes in DOC quality or character (*i.e.,* aromaticity, humification) as measured by fluorescence (Osburn et al. 2012). If this correlation can be established, it may be possible to model TNT metabolism rates of large sites using in situ instrumentation deployed by personnel or by remote observatories (*e.g.,* AUV, UUV) while minimizing the need to sample submerged sites amongst hazardous UXO and obviating expensive chemical analyses.

Table 1. Range of values of biogeochemical factors that influence energetic degradation may be classified according to ecosystem. This may help an RPM classify their site and identify candidates for Monitored Natural Attenuation (MNA) or No Further Action (NFA).

Coastal Ecosystem	Characteristics			Biogeochemical Factors						States/ Territories	Coastal Sites
	Temperature	Rainfall	Sediment Type	Salinity	Sediment Load	DO	DOC	Nitrogen Demand	Bacterial Metabolism		
Arctic	low	high	silt/gravel	---	---	---	---	---	---	AK	Adak, AK-01, AK-2, AK-04, AK-12
Northern Coniferous	low	high	silt/clay	---	---	---	---	---	---	WA, OR, No CA	Ostrich Bay, Jackson Park, Puget Sound, El Toro, Mare Is., Treasure Is., Moffett Field, Crow's Landing, Concord, WA-X01, CA-X01
Northern Temperate	variable	moderate	silt/clay	---	---	---	---	---	---	ME, MA, NH, RI, NY, CT	Portsmouth, Nomans Is., South Weymouth, RI-01, Fort Constitution Range Complex, Fort Stark Range Complex, Duck Is.
Southern Temperate	variable	moderate	silt/clay	---	---	---	---	---	---	NC, MD, VA, DE	White Oak, Fort Miles, Blossom Point, Camp LeJeune, Cherry Point, Dare County, Dalgren, Wood/Cat Island, Long Shoal, NAAS Edenton, Bull Bay, Drummond Point, Corolla, Duck, Southern Shores, Jockey's Ridge
Subtropical (dry)	moderate	low	sand/silt	---	---	---	---	---	---	So CA	San Diego Bay, Seal Beach, CA-12, CA-03
Subtropical (wet)	moderate/high	high	silt/clay	---	---	---	---	---	---	LA, AL, MS	Offshore LA-X01, LA-X02, LA-02, AL-X01, MS-X01, AL-01
Tropical (dry)	high	low	carbonaceous/ sand	---	---	---	---	---	---	HI (leeward)	Pearl Harbor, Ordnance Reef, Leeward Offshore Disposal Areas HI-01, HI-02, HI-05
Tropical (wet)	high	high	carbonaceous/ sand	---	---	---	---	---	---	PR, FL, HI (windward)	Vieques, Fleming Key, Trumbo Point, Sigsbee Park, North Boca, Dry Tortugas

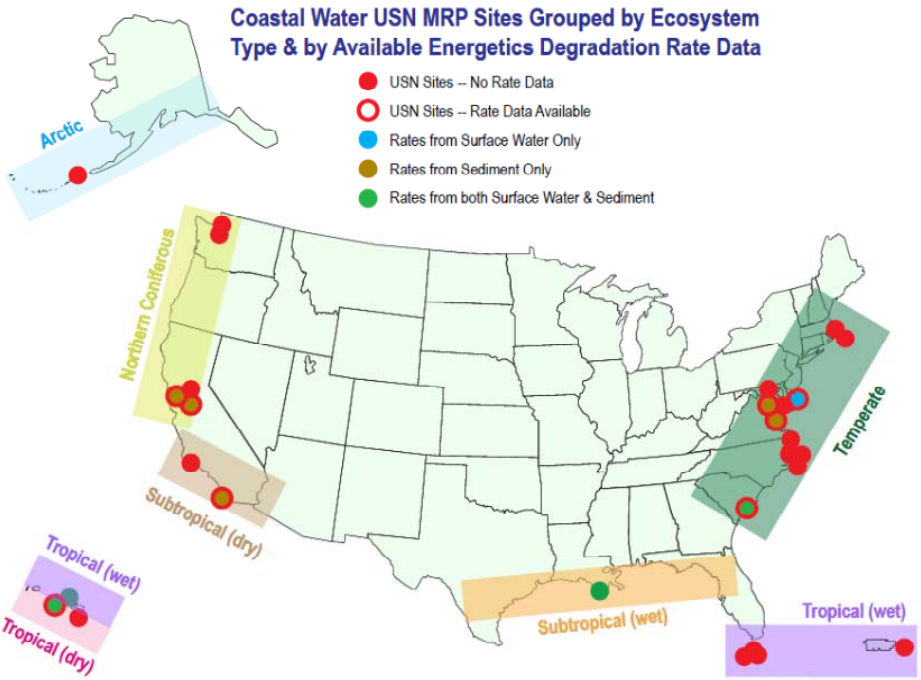
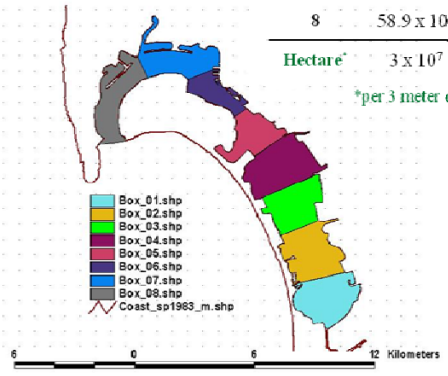


Figure 1. Coastal water USN Munitions Response Program sites grouped by ecosystem type with designations for those that have no corresponding data for rates of energetics degradation ( ). Sites with data have a red border ( ) that is filled based on type of media for which the data available: surface water ( ); sediment (; or both surface water and sediment ( ). Additional sites where data are available but with no nearby MRP site are designated without the red outline.

Capacity of a water body to naturally biodegrade TNT suddenly released from a breached ordnance varies with ecosystem but can be substantial (e.g. size of SD Bay = 100-3800 shells yr^{-1})

No.	Water Volume (L)	TNT Mineralization Rate (AVG (SD), kg yr^{-1})			Capacity to Degrade Amount of TNT in Breached 24 inch Shells (AVG(SD), shells yr^{-1})		
		Temperate	Subtropical	Tropical	Temperate	Subtropical	Tropical
1	12.5×10^9	1333 (542)	42 (21)	625 (229)	171 (71)	5 (3)	75 (29)
2	22.1×10^9	2357 (958)	74 (37)	1105 (405)	302 (125)	9 (5)	133 (52)
3	26.9×10^9	2869 (1166)	90 (45)	1345 (493)	368 (152)	11 (6)	161 (63)
4	44.6×10^9	4757 (1933)	149 (74)	2230 (818)	610 (253)	18 (10)	268 (104)
5	28.4×10^9	3029 (1231)	95 (47)	1420 (521)	388 (161)	11 (7)	170 (66)
6	28.7×10^9	3061 (1244)	96 (48)	1435 (526)	392 (163)	11 (7)	172 (67)
7	55.4×10^9	5909 (2401)	185 (92)	2770 (1016)	757 (314)	22 (13)	332 (129)
8	58.9×10^9	6283 (2552)	196 (98)	2945 (1080)	805 (334)	24 (14)	353 (137)
Hectare*	3×10^7	3.2 (1.3)	0.10 (0.05)	1.5 (0.55)	0.41 (0.17)	0.012 (0.007)	0.18 (0.07)

*per 3 meter of water depth



Length (inches)	Diameter (inches)	Hole radius (mm)	MC Mass (kg)
24	8	20mm	8

San Diego Bay model from ER-1453 (Wang, Lao, George & Wild 2008).

Figure 2. TNT degradation rates can be presented in terms of the amount of time it would take to attenuate a breached munition in a coastal environment. Here is an example of using our TNT mineralization rates in a transport model developed in SERDP ER-1453 for San Diego Bay.

MATERIAL AND METHODS

Site and Sampling Description In Year 1 (CY13), a wet tropical reef and lagoon system in the Florida Keys was sampled three times during 13-16 MAY, 5-7 AUG and 13-14 NOV using a 22" Sea Chaser boat. The survey of coastal NAVFAC sites on Key West (Fleming Key, Trumbo Point, and Sigsbee Park) and Boca Chica Key (North Boca) was coupled with a study of lagoons on No Name Key (Figure 3) that are analogous to those found on Vieques Island, Puerto Rico (Figure 4). The survey, which includes four NAVFAC sites, was coordinated with the RPMs Dana Hayworth and Brian Syme at NAVFAC SE Atlantic IPT.

Also in CY13, a saltwater marsh mesocosm was sampled in coordination with ER-2122 (Dr. Craig Tobias: *Tracking the Uptake, Translocation, Cycling, and Metabolism of Munitions Compounds in Coastal Marine Ecosystems using Stable Isotopic Tracer*) at University of Connecticut. Later in CY14 or CY15, a temperate biome saltwater marsh field site will be sampled either in coordination with ER-2122 (UXO-impacted) or independently using a *Spartina* marsh site where we have historically collected aromatic contaminant degradation data (adjacent to the Ashley River, North Charleston, S'C; not UXO-impacted). These results will be compared with those from a saltwater marsh mesocosm.

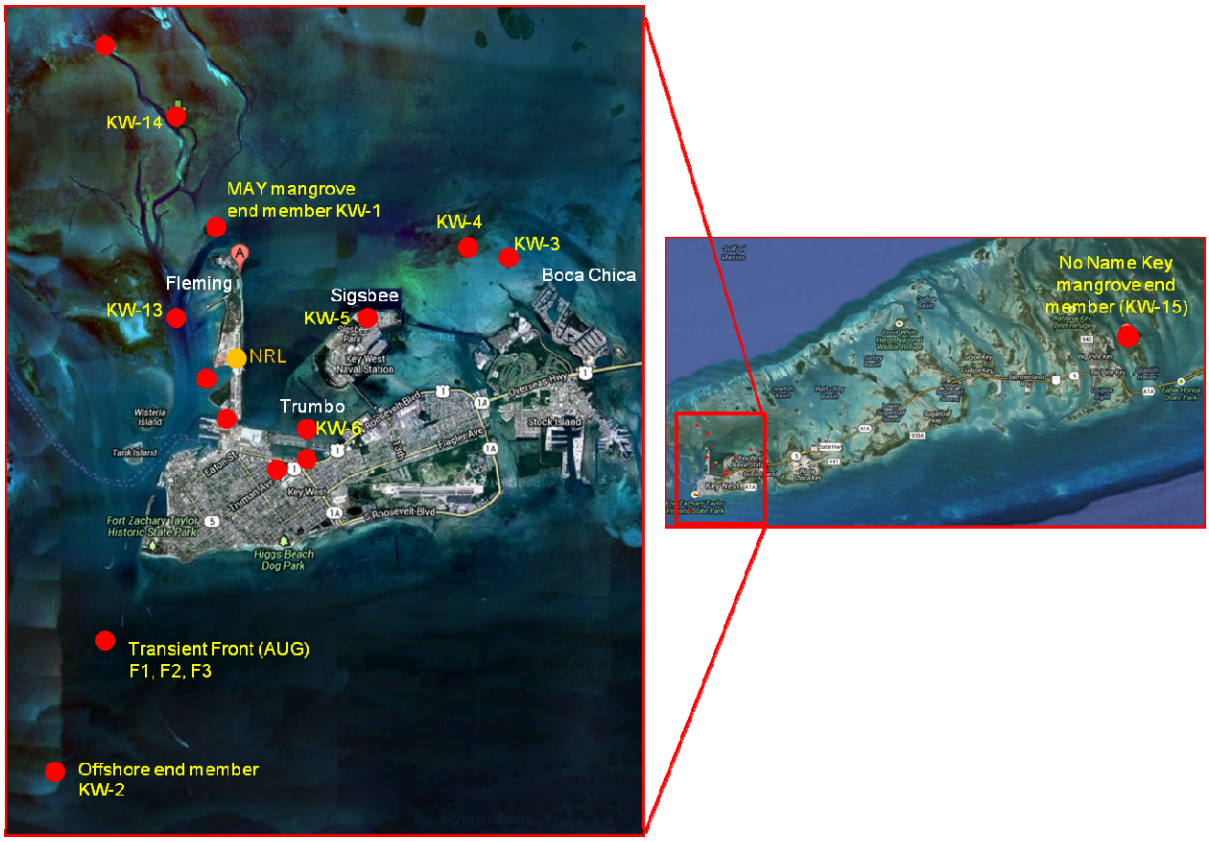


Figure 3. Sampling locations around Key West and No Name Key, Florida, USA.

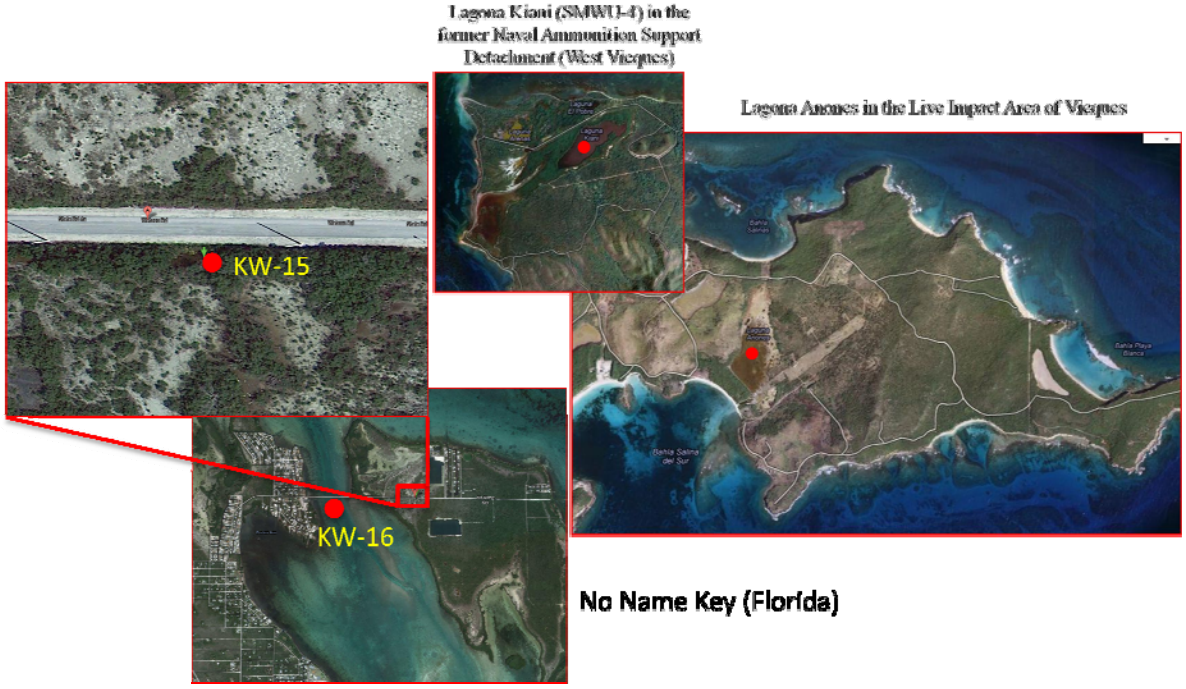


Figure 4. Potentially UXO-impacted lagoons on Vieques Island, Puerto Rico, USA. Study area at No Name Key (KW-15) was chosen to approximate less accessible areas in these other subtropical ecosystems. November mixing experiment included water from the adjacent waterway (KW-16).

During CY14, the Albemarle-Pamlico Estuarine System, North Carolina will be sampled three times with the first sampling planned for 14-18 APR. The survey of this southern, temperate lagoon estuary will include areas adjacent to 10 DoD sites in this expansive ecosystem but will not include samples from within actual site boundaries (Wood/Cat Island, Long Shoal, NAAS Edenton, Bull Bay, Drummond Point, Corolla, Duck, Southern Shores, Jockey's Ridge, and Cherry Point; Figure 5). In CY15, the northern temperate Great Bay Estuary, Portsmouth, New Hampshire will be sampled three times with months TBD. Data gathered in this survey will be relevant to three DoD sites (Fort Constitution Range Complex, Fort Stark Range Complex, and Duck Island; Figure 6). Water column (using CTD, Niskin bottles), nepheloid samples (for nepheloid sampling devices see Pohlman et al. 2002) and sediment (benthic grab, Wildco) will be collected along the salinity gradient of these coastal systems with sampling concentrated across salinity fronts between water masses and salt wedges. Standard water quality measurements of DO, salinity, temperature, pH will be made with a hand-held YSI Multiprobe™.

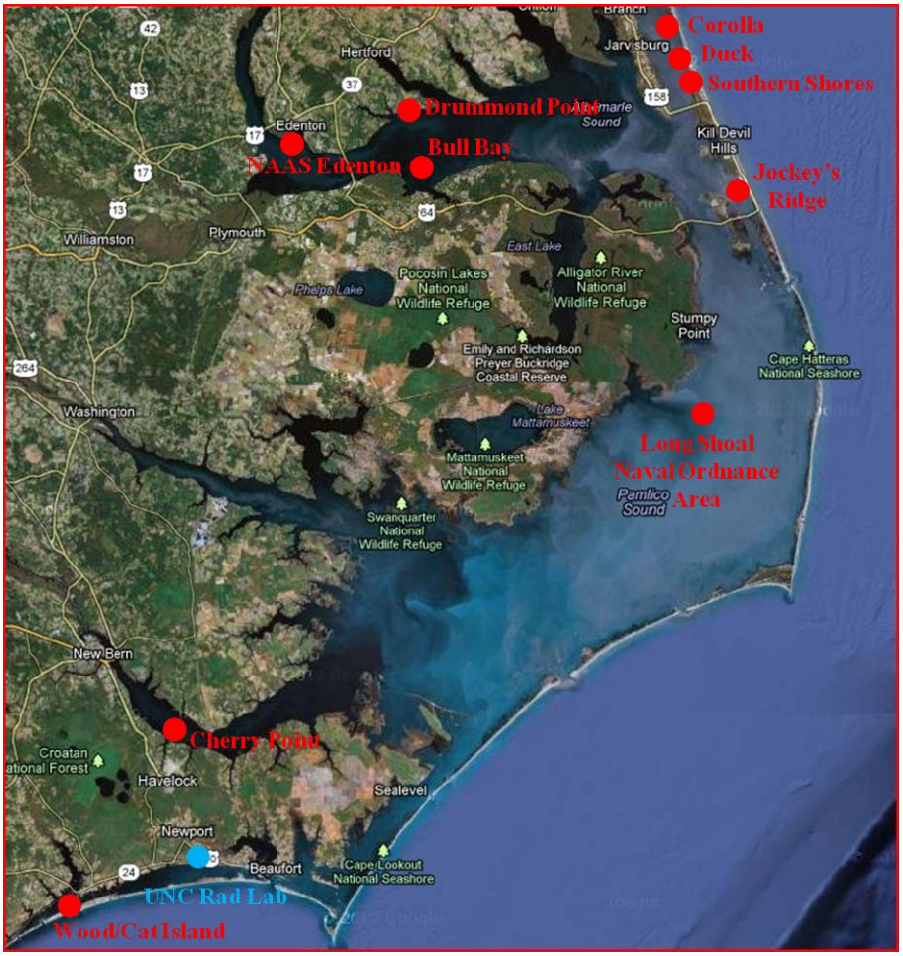


Figure 5. Potential CY14 sampling locations around Albemarle-Pamlico Estuarine System, North Carolina, USA.

TNT, RDX, HMX, and Phenanthrene Mineralization Rates of bacterial metabolism of aromatic contaminants were measured by mineralization of ^{14}C-radiolabelled substrates to $^{14}CO_2$. These assays were typically initiated within 2 h of sediment sample collection using a modification of Boyd et al. (1996) and Pohlman et al. (2002). Carbon substrates 2,4,6-TNT [ring-^{14}C(U)] (4 mCi mmol^{-1}, American Radiochemical Corporation, 99% purity), 9-^{14}C-phenanthrene (PHE; 55.7 mCi mmol^{-1}), UL-^{14}C-RDX (1.13 mCi mmol^{-1}, Defence R&D Canada), and UL-^{14}C-HMX (1.97 mCi mmol^{-1}), were added in separate incubations to 100 x 16 mm polycarbonate test tubes (ca. 0.2 µg g^{-1} or 0.04 µg mL^{-1}, depending on specific activity). For sediment, 0.5 mL of bottom water from the same station was filtered (0.22 µm

nom. pore dia., Nuclepore polycarbonate) and added to make slurries with sediment that was cored from surface of the core section using a five-mL syringe with the end cut-off. Triplicate live and one kill (2 mL of 2 N H_2SO_4) of all samples were incubated for ca. 48 h at *in situ* temperature in the dark and evolved $^{14}CO_2$ captured on NaOH-soaked filter papers. H_2SO_4 (2 mL, 2 N) was likewise added to end live incubations and to partition any remaining CO_2 into headspace of the tube and to the filter paper trap. Filter paper traps containing metabolized $^{14}CO_2$ were removed, radioassayed and subsequently used to calculate substrate mineralization. Triplicate one-mL syringed samples of wet sediment were dried (overnight; 50°C) and used to convert mineralization values sample into kg sediment dry weight. Detection limit of the assay was typically 0.01 µg C L^{-1} d^{-1} though average values that were below one standard deviation were considered non-detect (0).

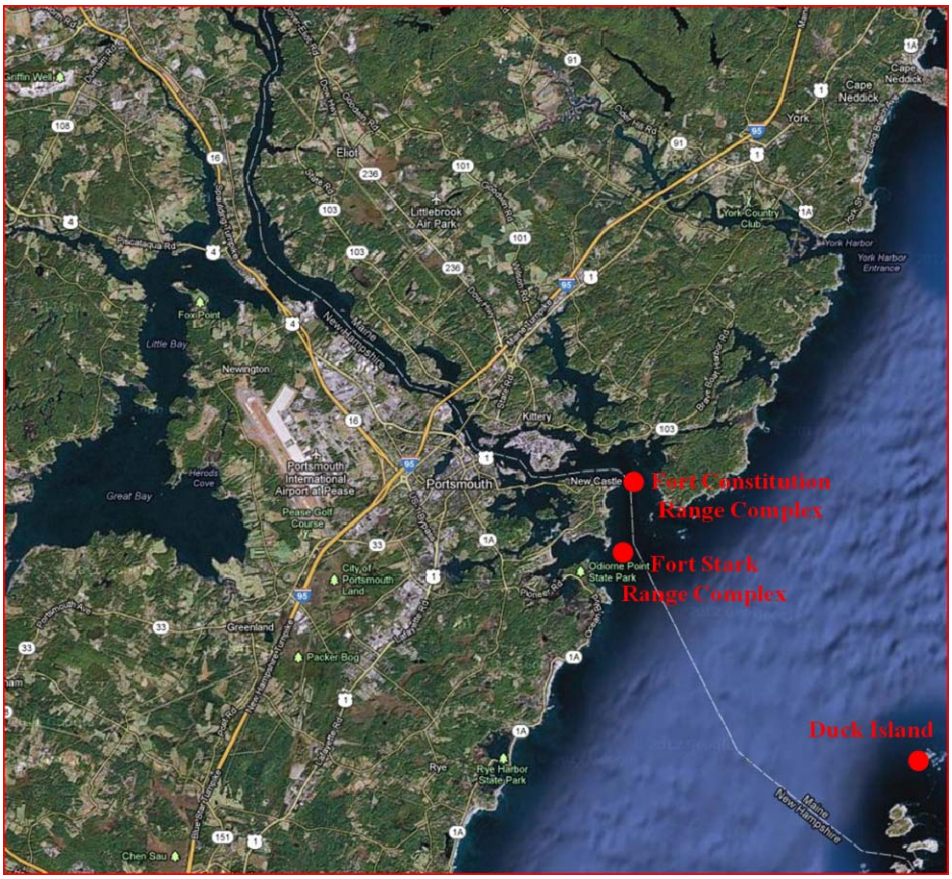


Figure 6. Potential CY15 sampling locations around Great Bay Estuary, Portsmouth, New Hampshire, USA.

Bacterial Production Growth rate of the heterotrophic bacterial assemblage (in terms of carbon) was measured by the leucine incorporation method of Smith and Azam (1992) for water or nepheloid (1.0 mL) or wet sediment (50 µL) as adapted by Montgomery et al. (2010). Environmental samples from each station were added to 2.0 mL microcentrifuge tubes (three experimental and one killed control) sealed with a cap with an O-ring that was pre-charged with [^{3}H-4,5]-L-leucine (120 mCi $mmol^{-1}$, final concn. 20 nM). Sediment was extracted from the sample using a one-mL polypropylene syringe with the end cut off and then added to the assay tube. For sediment, 1.0 mL of 0.22 µm (nom. pore dia.) filtered bottom water was then added to each tube and vortexed to form a sediment slurry. All samples were incubated at *in situ* temperature for 30 min. Incubations were ended by adding 57 µL of 100 % trichloroacetic acid (5 % final concentration; TCA, Fisher Scientific) and frozen for storage prior to processing by the method of Smith and Azam (1992). Killed controls have the TCA added prior to sample addition and these values were subtracted from those of the experimental samples. A constant isotope dilution factor of two was

used for all samples and was estimated from sediment dissolved free amino acids measurements (Burdige and Martens 1990) and saturation experiments (Tuominen 1995). Triplicate one-mL syringed samples of wet sediment were dried (overnight; 50^{o}C) and used to convert sediment production values into dry weight. Leucine incorporation rate was converted to bacterial carbon using the formula of Simon and Azam (1989). Assay detection limit was 1.0 μg C kg^{-1} d^{-1} though average values that were below one standard deviation were considered below detection.

Bacterial Organotolerance Inhibition of bacterial production by the presence of naphthalene (organotolerance) was measured as a proxy for osmotic stress at salinity fronts. Naphthalene organotolerance of the bacterial assemblage was measured by adding 0, 5, 10, 15 or 25 μg of naphthalene dissolved in 5 μL of methanol to 0.50 μL of wet sediment or 1.0 mL of water and subsequently processed for bacterial production (Montgomery et al. 2010). All treatments and controls received the same addition of methanol (5 μL) though previous experiments showed that production was not affected in parallel incubations with methanol alone (Montgomery et al. 2010). The 5 μL of methanol with dissolved naphthalene was added to the microcentrifuge tube prior to sample addition. Average and standard deviation of three live incubations (with value for killed control subtracted) was regressed to the amount of naphthalene added to the leucine incorporation assay. Final concentration of naphthalene in the 25 μg addition was ca. 250 μg g^{-1} sediment (dry weight) and 25 μg mL^{-1} of water. The regression formula and r^2 value were calculated using Microsoft Excel®.

DOC Analyses DOC was quantified by wet chemical oxidation on 2 mL sample volumes, using concentrated and cleaned sodium persulfate (Osburn and St-Jean 2007). Limit of detection via this method is 12 μmol L^{-1} C and reproducibility was <5%. Potassium hydrogen phthalate was used as a calibration standard for DOC concentrations over a range of 83 to 1,666 μM. DOC stable isotope values (δ^{13}C-DOC) were measured on samples and calibrated to the international PDB scale with sucrose (δ^{13}C=-10.45‰) and caffeine (δ^{13}C=-27.77‰). δ^{13}C-DOC values indicate organic matter source with values of -27 to -25‰ indicating terrestrial (and presumably more aromatic) organic matter, while marine phytoplankton range from -22 to -19‰. Salt marsh plants such as *Spartina spp.* generally are isotopically enriched with values between -11 and -14‰.

Lignin Analyses Presence and degradation state of terrestrially-derived OM (*i.e.,* lignin) was determined by measuring lignin concentration and determining its relative degree of oxidative degradation. Lignin was measured as its component acid, aldehyde, and ketone phenols after microwave assisted CuO-oxidation (Louchouarn et al. 2000, Goñi and Montgomery 2000). Phenols were extracted into ethyl acetate, redissolved into pyridine, derivatized, and then analyzed by GC/MS on a Varian 431-220MS using a DB-5 ms column. Lignin phenols were quantified against a standard curve of each of eight phenols released during the oxidation procedure (vanillin, vanillic acid, acetovanillone, syringealdehyde, syringic acid, acetosyringone, p-courmaric acid, ferulic acid). As there is currently no accepted radiotracer method for measuring bacterial lignin metabolism, ratios of vanillic acid to vanillin ($Ac:Al_v$) content were used to indicate oxidative degradation (Hedges and Mann 1979).

Absorption and Fluorescence Spectroscopy Relative aromatic character of refractory dissolved organic carbon (DOC) was measured by its absorptive and fluorescent properties. Spectral absorption (200-800 nm) was measured on a Varian Cary 300UV spectrophotometer and excitation-emission matrix (EEM) fluorescence on a Varian Eclipse spectrofluorometer on 0.2 μm filtrates from water samples. EEM fluorescence was measured on filtrates for DOM and on 0.1 N NaOH extracts of 0.7 μm GF/F filters for particulate organic matter (POM) fluorescence (Osburn et al. 2012); the appropriate Varian Eclipse instrument corrections applied and fluorescence data were reported in Raman-normalized quinine sulfate equivalents (QSE) in ppb, corrected for sample absorption (*i.e.,* inner filter effects). All EEM data were modeled using the Parallel Factor Analysis (PARAFAC) procedure to decompose the fluorescent matrices

into fluorescent components (Stedmon and Markager 2005, Stedmon and Bro 2008). Optical data processing was performed using Matlab software.

Demarcation of Salinity Fronts, Salt Wedges and Confluence Zones We used the change in salinity over relatively narrow spatial scales to identify an area as a front, wedge or zone of confluence. Collection bottles that contain water samples whose salinity is intermediate between the surface water at the beginning of the rapid change and at the end near that of the bottom water (in the case of a salt wedge) were considered representative of the frontal water sample. Collection of samples from convergence zones that interface at the air-sea boundary (*e.g.,* horizontal gradient) is more straightforward as these areas are typically catchments for flotsam and detritus that can be clearly seen from aboard the boat deck.

Mixing Experiments Small scale mixing experiments were performed for bacterial production by mixing different water masses. Each experiment was performed once during each sampling with all treatments incubated in a 1 L polycarbonate bottle in the dark. All experiments involved 1 L of each end member from a mangrove effluent sample and an open water sample. The first two samplings involved a single 50:50 mix of the end members (MAY: KW-1, 2; AUG: KW-15,2) whereas the NOV sample involved 5 mixtures as a percentage of mangrove end member (KW-15) and a nearby open water end member (KW-16; 10%, 20%, 30%, 40% and 50%). Treatments were subsampled for bacterial production, contaminant mineralization and DOC and the start of the incubation (T_0) and for bacterial production and DOC and the end of the incubation (T_f: MAY, 65 h; AUG, 65 h; NOV, 94 h). Mineralization rates were only determined for the T_0 mix and end members because the assay incubation time was different than that for bacterial production (>48 h vs 1 h).

Data Interpretation We expected that if there is no effect of water mass interface on TNT metabolism, then the relationship between salinity and TNT metabolism (*i.e.,* mineralization) would be conservative. That is, it would not be different from a linear mixing of end members with respect to salinity of DOC. When there was enhanced or inhibited metabolism different from that which was expected from straight mixing, this was seen on a graph comparing these parameters with salinity or as a percentage mixture of the two end members. Those biogeochemical parameters that could be measured on similar spatial and temporal scales (*e.g.,* DOC fluorescence, bacterial production etc.) and that demonstrated a similar pattern of change with respect to salinity were considered candidates for further study, as they were putative factors that control TNT metabolism in nature.

RESULTS AND DISCUSSION

This limited scope project primarily focused on demonstrating that frontal boundaries have enhanced rates of TNT mineralization, bacterial metabolism (*i.e.,* heterotrophic production) and degradation of refractory OM (*e.g.,* TNT, PAH, lignin). In addition, study of biogeochemical features at the interface that control these microbial rates and what cellular changes in the microbial assemblage occur in these environments (*e.g.,* organotolerance) was initiated. If TNT metabolism rate can be related to heterotrophic production or OM transformation, then knowledge of general metabolism and carbon cycling rates may be applied to modeling TNT attenuation. *Mixing experiments* between water mass end members, a sampling of a *Sargassum front*, and three coastal *survey*s were performed to address this topic.

Mixing Experiments Variations of a mixing experiment were performed during each of three Key West sampling events (May, August, November 2013). The concept was to mimic the response of the natural bacterial assemblage to a mixing event that would occur between the relatively high organic mangrove lagoon water and lower organic open ocean water. In nature, this may occur with tidal flushing or from a rain event that overwashes the lagoon mixing in water from the adjacent coastal ecosystem. The first

mixing experiment (May) involved mangrove effluent from amongst the root system that was accessible by boat (Figure 7, KW-1) and was likely to be frequently mixed with adjacent Gulf water via wave action. The open ocean end member (Figure 7, KW-2) was collected from ca. four miles south of Key West, FL near Point of Rocks in the Gulf of Mexico. The second mixing experiment also involved this open ocean end member, KW-2, but the mangrove end member was collected from a much more isolated water body within a mangrove lagoon at No Name Key (Figure 7, KW-15). This sample appeared to have a much high concentration of colored dissolved organic matter (CDOM, Figure 8) and may be more representative of the type of mangrove lagoons impacted by unexploded ordnance (UXO) at Veiques, Puerto Rico. These types of lagoons are more likely to be flushed episodically with extreme rain events like hurricanes. The third mixing experiment also involved the No Name Key mangrove end member, KW-15, but the open ocean station (KW-2) was inaccessible so water from the adjacent channel (a few blocks away) was collected from a bridge between No Name Key and Long Key and used as the open ocean end member (Figure 7, KW-16). When mixed with the No Name Key mangrove sample, KW-15, using this latter sample (KW-16) may have better represented the response of the natural assemblage to an overwash of No Name Key lagoon than using the open ocean end member (KW-16 was a block away whereas KW-2 was ca. 20 miles away).

Figure 7. Mixing experiment stations for lagoon end members near Fleming Key (KW-1), No Name Key (KW-15) and open water end members offshore of Key West (KW-2) and between No Name and Long Keys (KW-16).

During the May 2013 survey, mixing the mangrove effluent from Fleming Key with that of the offshore sampled did little to stimulate overall bacterial growth (5% increase), TNT or RDX mineralization, however, phenanthrene mineralization was stimulated by about 58% over that predicted from the end member values (Table 2). HMX mineralization may also have been stimulated but the high standard deviation made this questionable. Note that the mangrove effluent sample from this mixing experiment was likely to be more frequently mixed with adjacent Gulf of Mexico water than those mangrove lagoon samples used in the subsequent mixing experiments.

As a result of the May data, during the August 2013 survey, it was decided to use mangrove effluent from a more enclosed lagoon that may have more significant water quality differences than those represented

by the previous end members. No Name Key mangrove lagoon was mixed with the same offshore sampling location end member and showed little positive effect on phenanthrene mineralization (5%) and the mixing may have actually depressed mineralization of TNT and RDX (Table 3). It should be noted that the general mixing effect on bacterial production may have been a simple function of oxygen as DO in the mangrove end member was 31%. Subsequent mixing experiments using these end members focused on different (and greater) proportions of oxygenated offshore water to try and investigate this aspect of the mixing experiment. One measure of a bacterial assemblage's sensitivity to membrane destabilization as a result of osmotic or organic stress is the effect of increasing concentrations of naphthalene on bacterial production (organotolerance). Though the mangrove assemblage had much higher bacterial production than the offshore assemblage (KW-2), as expected, it turns out that the mangrove assemblage was also much more sensitive to naphthalene addition (Figure 9). This suggests that the offshore assemblage may have a disproportionate influence on assemblage functioning in the experimental mixtures.

Figure 8. Mixing experiment samples from (L to R) mangrove effluent (KW-15), 50:50 mixture (KW-15/KW-2) and the offshore end member (KW-2) during the August 2013 sampling.

Table 2. Rates of bacterial production and mineralization of TNT, phenanthrene, RDX and HMX (μg C L^{-1} d^{-1}) for a mixing experiment between mangrove effluent from No Name Key (KW-1) and offshore of Key West (KW-2; May 2013).

	Rate (AVG (SD μg C L^{-1} d^{-1})				
	Bacterial	Mineralization			
Station	Production	TNT	P	RDX	HMX
KW-1	67 (6.7)	0	0.85 (0.36)	0	0.14 (0.02)
Mix KW-1/2	42 (1.9)	0	1.46 (0.48)	0	0.65 (0.63)
KW-2	13 (2.0)	0	1.0 (0.06)	0	0

During the final sampling in November 2013, high winds prevented small craft sampling of the offshore open water station so the No Name Key mangrove end member (KW-15) was instead mixed with an open water end member sampled from the Old Wooden Bridge between No Name Key and Long Key (KW-

16). Though this sample was more likely to be impacted from the adjacent land mass than the offshore sample (4 mi south of Key West), it actually makes for a more likely scenario of what might happen during a storm-induced overwashing of the nearby No Name Key lagoon. It also turns out that bacterial production at KW-16 was similar to that of the offshore station KW-2 (3.5 (+/- 0.27) vs 13 (+/-2.0) μg C L^{-1} d^{-1}, respectively) suggesting that it might not be as impacted by the adjacent mangrove lagoons as initially suspected. Effluent from the No Name Key mangrove lagoon (KW-15) was mixed with the adjacent channel water (KW-16) with increasing percentage (*i.e.,* 10, 20, 30, 40 and 50%) of KW-16 mangrove water.

Similarly to the August incubation of No Name Key water and open ocean water, mixing these end members enhanced bacterial production above that predicted by conservative mixing. This was the case both for incubations at T_0 and T_f (ca. 94 h later; Figure 10). At T_0, the enhanced production effect increased from 10-25% (above predicted) with increasing percentage of mangrove water though this pattern was reversed to 38-31% T_f. This temporal reversal may reflect some parameter (*e.g.,* DO) that becomes limiting over incubation time in the fastest growing mixtures. Mixing had no affect on RDX mineralization but appears to have enhanced both HMX and phenanthrene mineralization (Figure 11). TNT mineralization appeared to be enhanced above either end member in 3 of the 5 mixtures, as well, though there was no clear pattern with mixture percentage.

Some changes due to water mass mixing may be a simple function of the effect of different conditions on overall heterotrophic bacterial metabolism. However, when phenanthrene mineralization rate was normalized to bacterial production, this ratio was higher than predicted for each treatment and higher than each end member for all but the 50% mixture (which was no different from the 100% mangrove end member; Figure 12). This suggests that changes resulting from mixing end members preferentially enhanced aromatic contaminant degradation rates over those of noncontaminant natural organic matter, such as mangrove leachate (e.g. by simply increasing overall heterotrophic carbon demand). This has important implications toward supporting the project hypothesis that frontal boundaries and mixing zones between water masses support aromatic contaminant degradation above that predicted from a simple survey of environmental rates along a salinity transect.

Table 3. Rates of bacterial production and mineralization of TNT, phenanthrene, RDX and HMX (μg C L^{-1} d^{-1}) for a mixing experiment between mangrove effluent from near Fleming Key (KW-1) and offshore of Key West (KW-2; August 2013).

	Rate (AVG (SD) μg C L^{-1} d^{-1})					
	Bacterial Production		Mineralization			
Station	T_0	T_f	TNT	P	RDX	HMX
KW-15	186 (5.4)	102 (5.9)	0	1.21 (0.13)	2.04 (0.31)	2.57 (0.41)
Mix KW-15/2	158 (2.5)	7.3 (0.73)	0	1.63 (0.33)	0	1.9 (1.3)
KW-2	14 (1.2)	48 (2.9)	0.31 (0.13)	1.91 (0.26)	0.82 (0.57)	0

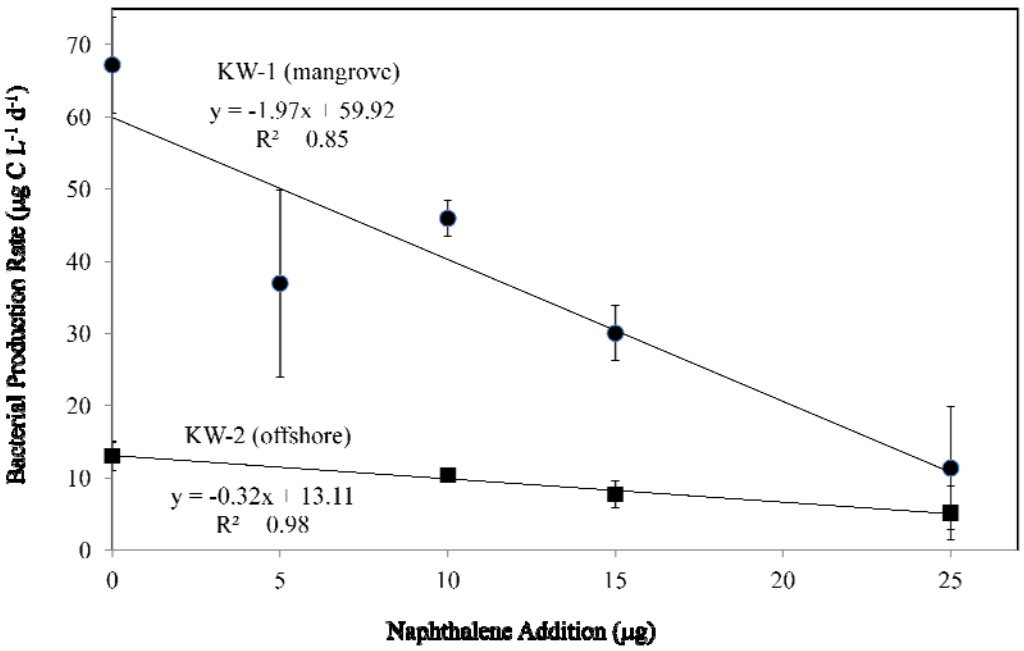


Figure 9. Organotolerance of the bacterial assemblage as bacterial production (µg C L^{-1} d^{-1}) decreases with naphthalene added (µg) to mixing experiment end members KW-1 (mangrove effluent) and KW-2 (offshore; May 2013).

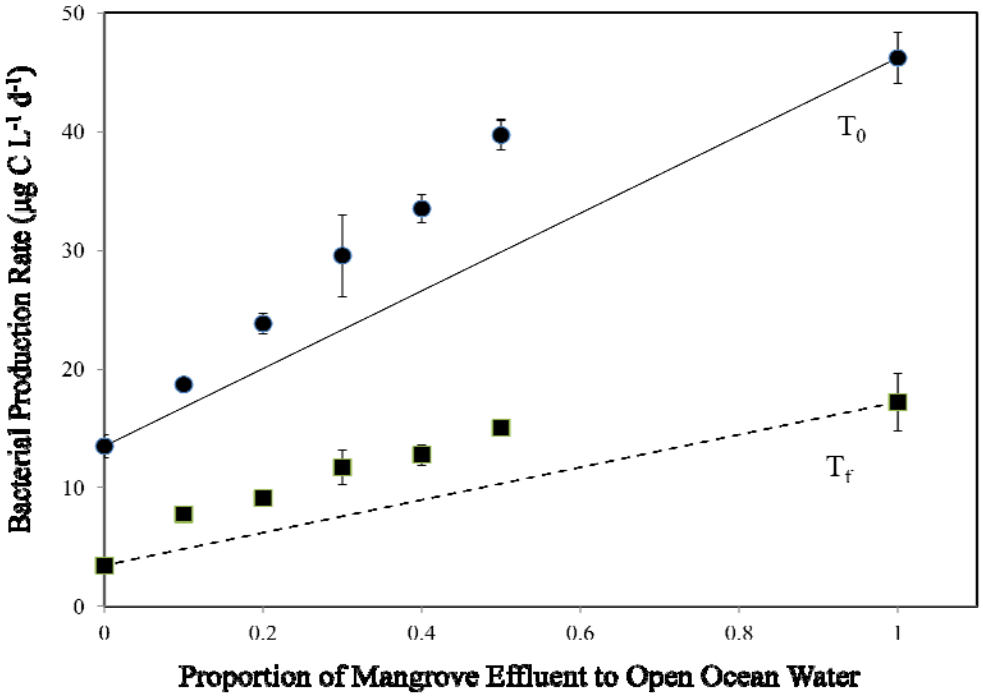


Figure 10. Bacterial production rate (µg C L^{-1} d^{-1}) in mixing experiment with different proportions of No Name Key mangrove effluent (KW-15) and open ocean water from the adjacent waterway (KW-16; November 2013) at the incubation start (T_0) and after 95 h (T_f).

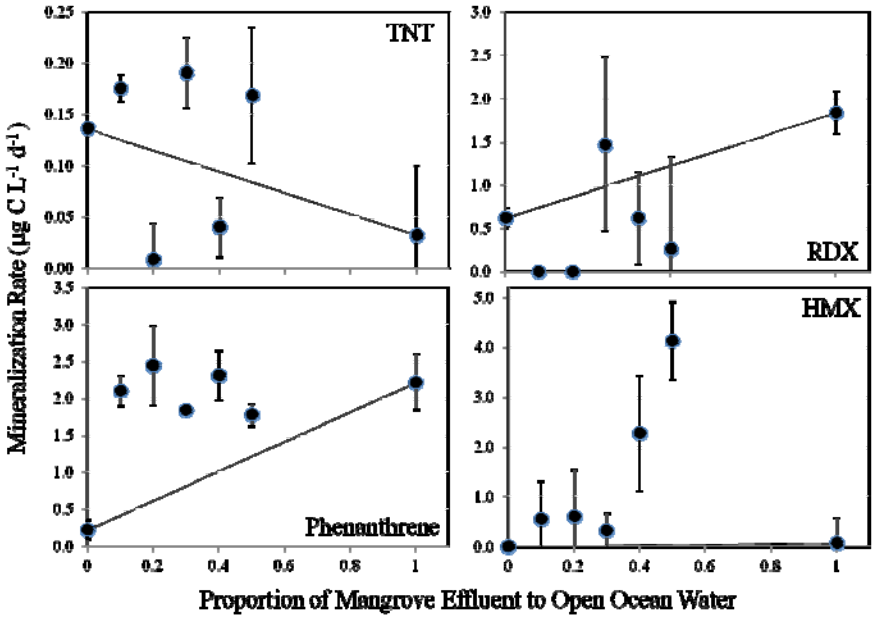


Figure 11. Mineralization of TNT, Phenanthrene, RDX and HMX (µg C L^{-1} d^{-1}) in mixing experiment with different proportions of No Name Key mangrove effluent (KW-15) and open ocean water (KW-16; November 2013).

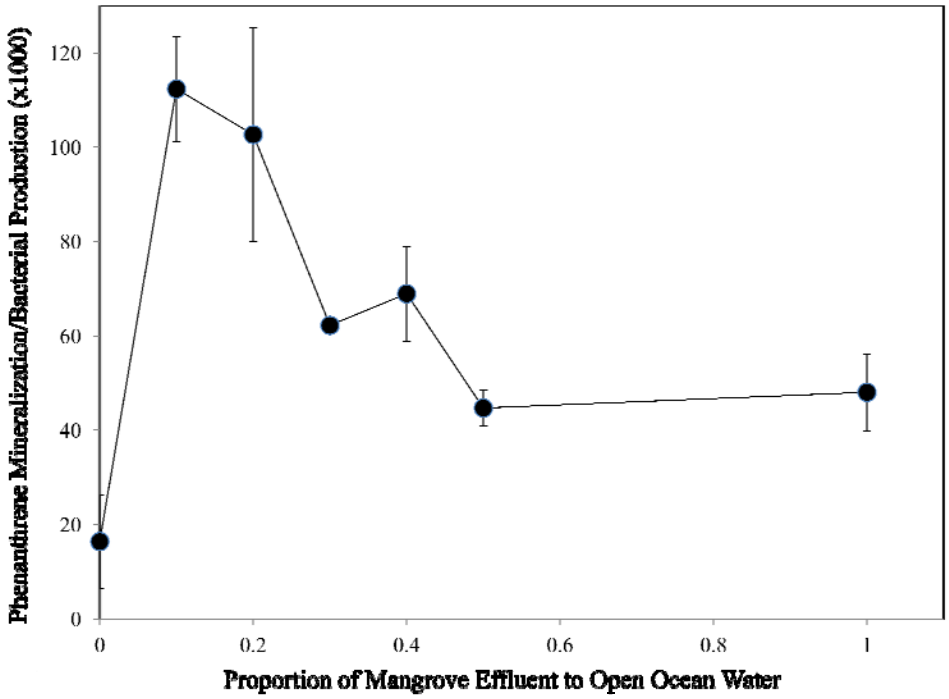


Figure 12. Phenanthrene mineralization/bacterial production rate (x1000) in mixing experiment with different proportions of No Name Key mangrove effluent (KW-15) and open ocean water (KW-16; November 2013).

Frontal Boundary Mixing experiments are performed because locating and sampling water mass boundaries in nature can be difficult as these areas can be spatially narrow, ill defined and transient. In addition, although a sample may have the salinity or other geochemical parameter that is intermediate between the two water mass end members, determining the time from mixing (or residence time) in the boundary area can require expensive and cumbersome isotopic analyses of earth metals. However, during the August 2013 sampling, we came across a frontal boundary between two water masses (here designated west (KW-F1) and east (KW-F3)) that could be delineated by a Sargassum collected at the sea surface (Figure 13).

Figure 13. Sargassum collecting at frontal boundary between two water masses just south of Key West, FL. Surface samples were collected just west of the front (KW-F1), at the front (KW-F2) and just east of the front (KW-F3; August 2013).

Bacterial production was only slightly higher at the interfacial sample of a Sargassum front found about a mile offshore south of Key West and there were little differences in mineralization of TNT, phenanthrene and RDX (Table 4). However, HMX mineralization appears to be stimulated from non-detect on each side of the front (F1 and F3) to 0.43 (+/-0.13) µg L^{-1} d^{-1} at the interfacial sample (KW-F2). Sargassum was collected and incubated separately from this survey and was found to enhance bacterial production by about an order of magnitude. Future experiments will investigate the effect of the Sargassum on aromatic mineralization in the surrounding water.

Table 4. Frontal boundary rates of bacterial production and mineralization of TNT, phenanthrene, RDX and HMX (µg C L^{-1} d^{-1}; August 2013).

		Rate (AVG (SD) µg C L^{-1} d^{-1})				
	Bacterial	Mineralization				
Station	Production	TNT	P	RDX	HMX	Notes
KW-F1	13 (3.7)	7.8 (7.3)	2.25 (0.37)	0.78 (0.28)	0	Front -- West/ocean side
KW-F2	14 (1.0)	0.38 (0.35)	2.06 (0.59)	0	0.43 (0.13)	Front -- interface
Sargassum	134 (3.6)	---	---	---	---	Front -- Sargassum
KW-F3	13 (0.16)	0.59 (0.40)	1.91 (0.57)	0	0	Front -- East/bay side

DOC Analyses DOC concentrations for sites around Key West ranged from 93 to 251 µM and had a weak positive correlation with salinity ($R^2 = 0.23$; $P<0.05$). This appeared to be an effect of evaporation of shallow open water on the shelf rather than dilution of seawater with terrestrial-rich freshwater because corresponding salinities ranged only from 36.01 (KW-13/14) to 36.74 (KW-5). DOC was correlated to absorption at 254 nm (a_{254}; $R^2 = 0.76$, $P<0.05$). Specific ultraviolet absorption (SUVA) which is correlated to aromatic ring content in organic matter ranged from 0.51 to 2.13. These values are characteristic of marine or planktonic dominated systems with some terrestrial inputs (Weishaar et al. 2003).

Carbon stable isotope (δ^{13}C-DOC) values indicated largely phytoplankton-derived, marine DOM in the coastal waters of Key West, with some notable terrestrial input. The offshore values typically ranged from -19 to -22‰, typical for coastal seawater DOC. Closer inshore samples (e.g., KW-4, Channel Key mangrove) had relatively depleted values of -25.16‰ reflecting terrestrial input.

DOM excitation-emission (EEM) fluorescence supported the DOC findings (Figure 14). The Offshore sample was measured in the frontal zone (KW-F1) encountered in Aug 2013. That pattern was very similar to the Mangrove water sampled at No Name Key. By contrast, fresh Sargassum DOM, leached overnight into seawater, produced a very different EEM pattern, unlike that measured on the majority of our samples and indicated by the Offshore frontal zone station KW-F1. Also of note, the magnitude of fluorescence for No Name Key mangrove water was nearly 40-fold greater than the Offshore sample. The latter also had stronger protein fluorescence (centered on Ex/Em 280/340), which reflected the marine phytoplankton signature in the coastal seawater DOM surrounding Key West.

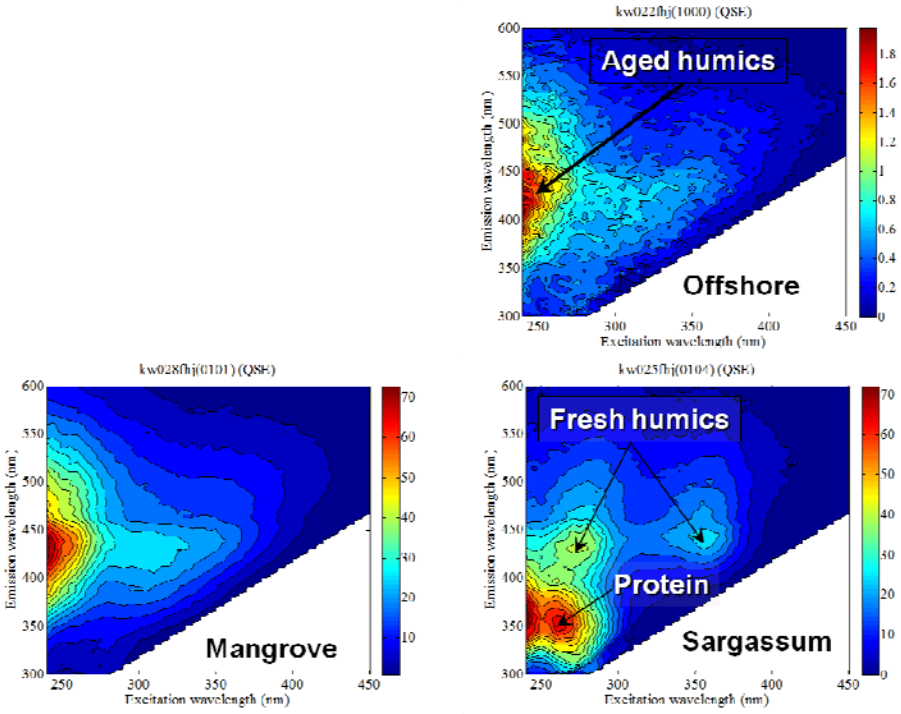


Figure 14. EEM fluorescence analyses of potential OM sources.

Rate Survey of NAVFAC and Reference Sites Several stations were repeatedly sampled during the three surveys (*e.g.,* KW-1, 2, 5, 6, 15). Bacterial production was generally similar amongst the different samplings at the same station with the exception of No Name Key (KW-15) at the August and November samplings, the former of which was immediately preceded by a substantial rain event. Phenanthrene mineralization was most frequently measured in water samples with detects in 15 of 18 samples though it

was not detected in any of the four sediment samples (Table 5). Most rapid rates of TNT (KW-3), RDX (KW-1) and HMX (KW-1) were all measured in surface sediment. RDX mineralization was most commonly measured in the sediment (4 of 4 samples) and the rates were among the most rapid we have reported for natural assemblages (previously 46 μg kg^{-1} d^{-1} for RDX and 23 μg kg^{-1} d^{-1} for HMX; Montgomery et al. 2012).

Table 5. Survey of bacterial production and mineralization rates of TNT, phenanthrene, RDX and HMX for water (μg C L^{-1} d^{-1}) and sediment (μg C kg^{-1} d^{-1}).

		Rate (AVG (SD), μg C kg^{-1} d^{-1} or μg C L^{-1} d^{-1})					
			Mineralization				
Station	Sampling	Bacterial Production	TNT	P	RDX	HMX	Notes
KW-1	MAY	67 (6.7)	0	0.85 (0.36)	0	0.14 (0.02)	Fleming Key mangrove
	AUG	58 (4.9)	9.5 (8.4)	1.82 (0.45)	0	0	
KW-2	MAY	13 (2.0)	0	1.0 (0.06)	0	0	Offshore
	AUG	14 (1.2)	0.31 (0.13)	1.91 (0.26)	0.82 (0.57)	0	
KW-F1	AUG	13 (3.7)	7.8 (7.3)	2.25 (0.37)	0.78 (0.28)	0	
KW-F2	AUG	14 (1.0)	0.38 (0.35)	2.06 (0.59)	0	0.43 (0.13)	Sargassum front offshore
KW-F3	AUG	13 (0.16)	0.59 (0.40)	1.91 (0.57)	0	0	
KW-3	MAY	26 (1.9)	0.59 (1.04)	0	0.77 (0.23)	0	Boca Chica shallows
KW-4	MAY	46 (1.6)	0	1.22 (0.32)	0.76 (0.54)	0	Channel Key mangrove
KW-5	MAY	47 (2.8)	0	0	0	0.62 (0.19)	Sigsbee Park
	AUG	44 (3.4)	0	1.90 (0.54)	0	0.51 (0.23)	
KW-6	MAY	33 (1.5)	0.98 (0.75)	0	0.25 (0.16)	0.82 (0.38)	Trumbo Point
	AUG	34 (1.7)	0	2.07 (0.13)	0	0	
KW-13	AUG	37 (3.5)	0	1.85 (0.39)	0.25 (0.23)	0.51 (0.37)	channel W of Fleming Key
KW-14	AUG	15 (2.6)	1.47 (1.16)	1.94 (0.49)	0	1.17 (0.32)	
KW-15	AUG	186 (5.4)	0	1.21 (0.13)	2.04 (0.31)	2.57 (0.41)	No Name Key mangrove
	NOV	46 (2.1)	0	2.22 (0.37)	1.84 (0.25)	0	
KW-16	NOV	3.5 (0.27)	0	0.22 (0.13)	0.62 (0.11)	0	Open water off Long Key
KW-1	AUG	335 (85)	2.12 (0.99)	0	690 (100)	50 (18)	Fleming Key mangrove
KW-3	MAY	279 (83)	8.7 (6.5)	0	76 (58)	0	Boca Chica shallows
KW-5	MAY	273 (37)	0	0	134 (68)	0	Sigsbee Park
	AUG	93 (48)	0	0	290 (102)	0	

To account for vagaries in mineralization rates that are a simple function of bacterial growth rate differences, mineralization rates were normalized to bacterial production and then multiplied by 1000 to obtain whole number ratios. Highest ratios are indicative of bacterial assemblages that are more adapted to preferentially biodegrading that particular carbon substrate. During the May 2013 sampling, TNT mineralization to production ratios were highest in the sediment at Boca Chica shallows (KW-3) followed by water at Trumbo Point (KW-6) and the overlying water at Boca Chica (Table 6). Ratio for phenanthrene mineralization was highest in the offshore sample (KW-2). Both RDX and HMX mineralization to production ratios were highest for the sediment at Sigsbee Park (KW-5). It should be noted that these comparisons are probably most valid when comparing same station rates at different times or amongst different mixing treatments at the same time.

During the August 2013 sampling, highest ratio of mineralization to bacterial production for both phenanthrene and HMX was found in open ocean water at the offshore station (KW-2) and the sargassum front (KW-F1, -F2, -F3; Table 7). Highest ratios for RDX were amongst sediment samples while the highest HMX ratio was also in sediment (off Fleming Key, KW-1). Ratio of TNT mineralization to production showed little pattern across sites but ranged from 0.02 in the mangrove lagoon to 602 offshore. During the November 2013 sampling, phenanthrene appeared to be mineralized preferentially

by mangrove lagoon assemblages and especially when there were large dilutions of mangrove water with adjacent open ocean water. HMX was preferentially mineralized in less diluted (*e.g.*, 40, 50%) mangrove water (Table 8). For HMX, it's possible that the assemblage in the mangrove effluent was skewed towards aromatic degradation but that DO was too low for efficient mineralization in the unmixed mangrove lagoon.

Table 6. Mineralization to production ratios (x1000) for Key West water and sediment samples collected during May 2013.

		Mineralization/Production (x 10^3)				
Station		**TNT**	**P**	**RDX**	**HMX**	**Notes**
KW-1	mixing experiment		13		2.1	Fleming Key mangrove
Mix KW-1/2			35		15	Mixing experiment
KW-2			78			Offshore end member
KW-3	surface water	23	5	30		Boca Chica shallows
KW-4		0.49	27	17	3.3	Channel Key mangrove
KW-5			14		13	Sigsbee Park
KW-6		30	19	7.5	25	Trumbo Point
KW-3	sediment	31		272		Boca Chica shallows
KW-5				491	26	Sigsbee Park

Table 7. Mineralization to production ratios (x1000) for Key West water and sediment samples collected during August 2013.

		Bacterial	Mineralization/Production (x 10^3)				
Station		**Production**	**TNT**	**P**	**RDX**	**HMX**	**Notes**
KW-1	surface water	58 (4.9)	163	31	20	29	Fleming Key mangrove
KW-5		44 (3.4)	1.0	43	0	12	Sigsbee Park
KW-6		34 (1.7)	1.6	61	6.3	15	Trumbo Point
KW-13		37 (3.5)	88	50	6.8	14	channel W of Fleming Key
KW-14		15 (2.6)	98	129	31	78	channel W of Fleming Key
KW-1	sediment	335 (85)	6.3	0	2059	148	Fleming Key mangrove
KW-5		93 (48)	0	8.7	3120	0	Sigsbee Park
KW-F1	front	13 (3.7)	602	173	60	116	Front -- West/ocean side
KW-F2		14 (1.0)	27	147	0	30	Front -- interface
sargassum		134 (3.6)					Front -- sargassum
KW-F3		13 (0.16)	45	147	178	3.4	Front -- East/bay side
KW-15	mixing experiment	186 (5.4)	0.02	6.5	11	14	No Name Key mangrove
Mix KW-15/2		158 (2.5)	0	10	0.15	12	Mixing experiment
KW-2		14 (1.2)	22	137	59	35	Offshore end member

These rates of contaminant degradation can be a compelling line of evidence in support or against a typical site conceptual model which assumes that surface soil or sediment contaminants can run off with rainfall and negatively impact adjacent waterway sediment. Several NAVFAC Key West sites within the survey area have maximum reported PAH concentrations of 20-26 ppm in soil and sediment. If a rain event were to transport similar PAH concentrations to surface water and sediment in the adjacent waterway, degradation rates measured during these surveys suggest that this amount would be metabolized in ca. 1-21 days. Likewise, nitrogenous energetic (nitroglycerine, NG) concentrations of

0.11-0.25 ppm would be mineralized in <16 hours by surface water and sedimentary assemblages at these NEC mineralization rates. Although these time estimates can be affected by other environmental factors (*e.g.,* weather), the magnitude of these turnover times (*i.e.,* hours to weeks) is a line of evidence that contradicts the site conceptual model that these contaminants have existed in the surface soil and sediment for the past 60 years. A more likely model would involve the ambient PAH concentrations resulting from a balance of current day flux to the site (*e.g.,* atmospheric deposition) minus degradation by microbiota and abiotic processes (*e.g.,* photodegradation). Though NG mineralization rates were not specifically measured in this survey, NG biodegradation rates are reported to be more rapid than those of TNT used in this analysis. Based on our previous experience and given the transient nature of NG in the environment, it is more likely that these are NG values are actually false detects due to lower cost chemical analyses (i.e. shouldering with glycerine and FID detection rather than using GC/MS).

Table 8. Mineralization to production ratios (x1000) for Key West water samples collected during November 2013.

Station		Bacterial Production	Mineralization/Production (x 10^3) TNT	P	RDX	HMX	Notes
KW-15		46 (2.2)	0.69	48	40	2	No Name Key mangrove
KW-A		40 (1.3)	4.25	45	7	104	50% KW-15
KW-B		34 (1.2)	1.19	69	18	68	40% KW-15
KW-C	mixing experiment	30 (3.5)	6.45	62	50	10	30% KW-15
KW-D		24 (0.85)	0.34	103	0	25	20% KW-15
KW-E		19 (0.44)	9.38	112	0	29	10% KW-15
KW-16		14 (0.98)	10.09	16	46	0	Open water (off Long Key)

CONCLUSIONS

During the first year of this three year project, we found that mixing experiments between tropical mangrove lagoon water and open ocean Gulf of Mexico water resulted in more rapid rates of bacterial growth aromatic contaminant mineralization than would have been predicted by interpolation using unmixed samples. This line of evidence supports the hypothesis that coastal mixing zones may stimulate more rapid energetic and PAH degradation than would be expected using standard measures and techniques. Surveys of energetic and PAH mineralization rates in areas adjacent to DoD sites in the Key West area suggest that contaminants in surface runoff from shoreside area would be rapidly metabolized (hours to weeks) in the adjacent seawater and surface sediment potentially mitigating ecological risk. In addition, the most rapid RDX and HMX mineralization rates associated with any natural assemblage to date were found in the tropical sediment at some stations around Key West. Taken together, this work supports site conceptual models where PAH and energetics are rapidly biodegraded by natural microbial assemblages as they migrate from mangrove dominated lagoon systems to adjacent coastal waterways in tropical ecosystems.

LITERATURE CITED

Borsheim, K.Y. 1990. Bacterial biomass and production rates in the Gulf Stream front regions. Deep-Sea Res. 37(8):1297-1309.

Boyd, T.J., B.J. Spargo and M.T. Montgomery. 1996. Improved method for measuring biodegradation rates of hydrocarbons in natural water samples. In B.J. Spargo (eds.) *In Situ bioremediation and efficacy monitoring.* US Naval Research Laboratory. NRL/PU/6115--96-317:113-121, Washington, DC.

Burdige, D.J., and C.S. Martens. 1990. Biogeochemical cycling in an organic-rich marine basin – 11. The sedimentary cycling of dissolved free amino acids. Geochim. Cosmochim. Acta 54:3033-3052.

Caron, D.A., J.C. Goldman, and M.R. Dennett. 1988. Experimental demonstration of the roles of bacteria and bacterivorous protozoa in plankton nutrient cycles. Hydrobiologia 159:27-40.

CH2M HILL. 2006. Response to comments on Time Critical Removal Action (TCRA) of unexploded munitions in the former Vieques Naval Trainnig Range (VNTR). Memorandum, 28 March.

Clark, B., and R. Boopathy. 2007. Evaluation of bioremediation methods for the treatment of soil contaminated with explosives in Louisiana Army Ammunition Plant, Minden, Louisiana. J. Hazard. Mater. 143:643–648.

Claus, H. 2014. Microbial degradation of 2,4,6-Trinitrotoluene in vitro and in natural environments. In S.N. Singh (ed.), Biological Remediation of Explosive Residues, Environmental Science and Engineering, DOI: 10.1007/978-3-319-01083-0: 15-38, Springer International Publishing, Switzerland.

Chappell, M.A., B.E. Porter, C.L. Price, B.A. Pettway, and R.D. George. 2011. Differential kinetics and temperature dependence of abiotic and biotic processes controlling the environmental fate of TNT in simulated marine systems, Mar. Poll. Bull. 62(8):1736-1743.

Conder, J. 2002. Disappearing Doses: Fate and toxicity of TNT in sediment toxicity tests over time. Presentation at the 23rd Annual Meeting of the Society of Environmental Toxicology and Chemistry, Salt Lake City, UT, November 16-20.

Douglas, T.A., L. Johnson, M. Walsh, and C. Collins. 2009. A time series investigation of the stability of nitramine and nitroaromatic explosives in surface water samples at ambient temperature. Chemosphere 76:1–8.

Fahrenfeld, N., J. Zoeckler, , M.A. Widdowson, and A. Pruden. 2012. Effect of biostimulants on 2,4,6-trinitrotoluene (TNT) degradation and bacterial community composition in contaminated aquifer sediment enrichments. Biodegradation 24(2):179-190.

Floodgate, G.D., G.E. Fogg, D.A. Jones, K. Lochte, and C.M. Turley. 1981. Microbiological and zooplankton activity at a front in Liverpool Bay. Nature 290:133-136.

Gallagher, E.M., L.Y. Young, L.M. McGuinness, and L.J. Kerkhof. 2010. Detection of 2,4,6-Trinitrotoluene-Utilizing Anaerobic Bacteria by ^{15}N and ^{13}C Incorporation. Appl. Environ. Microbiol. 76(5):1-5.

Goñi, M.A., and S. Montgomery. 2000. Alkaline CuO oxidation with a microwave digestion system: lignin analyses of geochemical samples. Anal. Chem. 72:3116-3121.

Hawari, J., S. Beaudet, A. Halasz, S. Thiboutot, and G. Ampleman. 2000. Microbial degradation of explosives: biotransformation versus mineralization. Appl. Microbiol. Biotech. 54:605-618.

Hedges, J.I., and D.C. Mann. 1979. Characterization of plant tissues by their lignin-oxidation products. Geochim. Cosmochim. Acta 43:1803-1807.

Josefson, A.B., and D.J. Conley. 1997. Benthic response to a pelagic front. Mar. Ecol. Prog. Ser. 147:49-62.

Kirchman, D.L. 1994. The uptake of inorganic nutrients by heterotrophic bacteria in microbial ecology, 28(2) The Microbial Loop (Sep. - Oct., 1994), pp. 255-271.

Kirchman, D.L., E. K'nees, and R. Hodson. 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Env. Microbiol. 49:599-607.

Kubota, A., T. Maeda, N. Nagafuchi, K. Kadokami, and H.I. Ogawa. 2008. TNT biodegradation and production of dihydroxylaminonitrotoluene by aerobic TNT degrader *Pseudomonas* sp. strain TM15 in an anoxic environment. Biodegradation 19:795–805.

Louchouarn, P., S.Opsah, and R. Benner. 2000. Isolation and quantification of dissolved lignin from natural waters using solid-phase extraction and GC-MS. Anal. Chem. 72:2780–2787.

Montgomery, M.T., T.J. Boyd, C.L. Osburn, and D.C. Smith. 2010. PAH mineralization and bacterial organotolerance in surface sediments of the Charleston Harbor estuary. Biodegrad. 21:257–266. DOI 10.1007/s10532-009-9298-3

Montgomery, M.T., and C.L. Osburn. 2004. Bacterial metabolism, aromatic biodegradation, and lignin biogeochemistry in sediment cores from Pearl Harbor, HI. U.S. Naval Research Laboratory, NRL/FR/6114--04-10,077, Washington, D.C.

Montgomery, M.T., T.J. Boyd, J.P. Smith, S.E. Walker, and C.L. Osburn. 2011a. "2,4,6-Trinitrotoluene mineralization and incorporation by natural bacterial assemblages in coastal ecosystems", in, (Eds. M.A. Chappell, C.L. Price, R.D. George) Environmental Chemistry of Explosives and Propellant Compounds in Soils and Marine Systems: Distributed Source Characterization and Remedial Technologies, ACS Symposium Series, Vol. 1069, ACS Publications, Washington, DC, pp. 171-184. DOI:10.1021/bk-2011-1069.ch009.

Montgomery, M.T., Coffin, R.B., Boyd, T.J., Smith, J.P., Plummer, R.E., Walker, S.E., and C.L. Osburn. 2011b. Mineralization rates of 2,4,6-Trinitrotolene and bacterial production amongst natural microbial assemblages in coastal sediments. Environ. Poll. 159:3673-3680. DOI:10.1016/j.envpol.2011.07.018

Montgomery, M.T., R.B. Coffin, T.J. Boyd, and C.L. Osburn. 2012. TNT biodegradation by natural microbial assemblages at estuarine frontal boundaries. Naval Research Laboratory Memorandum Report, NRL/MR/6110—12-9390.

Montgomery, M.T., Coffin, R.B., Boyd, T.J., and C.L. Osburn. 2013. Incorporation and mineralization of TNT and other anthropogenic organics by natural microbial assemblages from a small, tropical estuary. Environmental Pollution 174: 257-264.

Montgomery, M.T., S.W. Walker, C.L. Osburn, L.J. Hamdan, T.J. Boyd, Y. Furukawa, J. Hawari, F. Monteil-Rivera, D.W. O'Sullivan, D. Luning-Prak, H.W. Paerl, and Q.X. Li. 2008. Biotic and Abiotic Attenuation of Nitrogenous Energetic Compounds (NEC) in Coastal Waters and Sediments: final report (ER-1431). Strategic Environmental Research and Development Program, www.serdp.org.

Myers, T.E., J.M. Brannon, J.C. Pennington, D.M. Townsend, M.K. Ochman, and C.A. Hayes. 1998. Laboratory Studies of Soil Sorption/Transformation of TNT, RDX, and HMX. Technical Report IRRP-98-8, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

NOAA. 2006. Final data report for the Vieques Island Biota Sampling Project.

Osburn, C.L., L.T. Handsel, M.P. Mikan, H.W. Paerl, and M.T. Montgomery. 2012. Fluorescence tracking of dissolved and particulate organic matter quality in a river-dominated estuary. Environ. Sci. Technol. 46(16):8628-8636.

Osburn, C.L., and G. St-Jean. 2007. The use of wet chemical oxidation with high-amplification isotope ratio mass spectrometry (WCO-IRMS) to measure stable isotope values of dissolved organic carbon in seawater. Limnol. Oceanogr.-Meth. 5:296-308.

Paerl, H.W., and M.F. Piehler. 2008. Nitrogen and Marine Eutrophication. pp 529-567, in, D. G, Capone, M. Mulholland and E. Carpenter (Eds.), *Nitrogen in the Marine Environment*, Vol. 2. Academic Press, Orlando.

Pohlman, J.W., R.B. Coffin, C.S. Mitchell, M.T. Montgomery, B.J. Spargo, J.K. Steele, and T.J. Boyd. 2002. Transport, deposition and biodegradation of particle bound polycyclic aromatic hydrocarbons in a tidal basin of an industrial watershed. Environ. Monit. Assess. 75:155-167.

Pomeroy, L.R. 1970. The strategy of mineral cycling. Annu. Rev. Ecol. Syst. 1:171–190.

Rappé, M.S., and S.J. Giovannoni. 2001. The uncultured microbial majority. Ann. Rev. Microbiol. 57:369-394.

Rocha, C. 2008. Sandy sediments as active biogeochemical reactors: compound cycling in the fast lane. Aquat. Microb. Ecol. 53:119–127.

Simmons, C.C., K.M. Carvalho-Knighton, and A.J. Pyrtle. 2007. Small scale characterization of the presence of the explosive octahydro-1,3,5,7-tetranitro-1,3,5,7 tetrazocine (HMX) near former naval sites on Vieques Island, Puerto Rico. American Geophysical Union, Fall Meeting, abstract #OS31B-0409.

Simon, M., and F. Azam. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol. Prog. Ser. 51:201-213.

Smith, D.C., and F. Azam. 1992. A simple, economical method for measuring bacterial protein synthesis rate in seawater using ^{3}H-leucine. Mar. Microbial Food Webs. 6:107-114.

Spain, J.C., J.B. Hughes, and H.J. Knackmuss (eds). 2000. *Biodegradation of Nitroaromatic Compounds and Explosives*. Lewis Publishers, New York, 434 pp.

Stedmon, C.A., and S. Markager. 2005. Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARFAC analysis. Limnol. Oceanogr. 50:686-697.

Stedmon, C.A., and R. Bro. 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. Limnol. Oceanogr.: Meth. 6:572-579.

Travis, E.R., N.C. Bruce, and S.J. Rosser. 2008. Microbial and plant ecology of a long-term TNT-contaminated site. Environ. Poll. 153:119-126.

Tuominen, L. 1995. Comparison of leucine uptake methods and a thymidine incorporation method for measuring bacterial activity in sediment. J. Microbiol. Meth. 24:125-134.

Wang P.-F., Q. Liao, R. George, and W. Wild. 2009. (ER-1453) Defining Munition Constituent (MC) Source Terms in Aquatic Environments on DoD Ranges (Phase II) Draft Technical Report Provided for Strategic Environmental Research and Development Program.

Weishaar, J.L., G.R. Aiken, B.A. Bergamaschi, M.S. Fram, R. Fujii, and K. Mopper. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environmental Science & Technology, 37(20):4702-4708.

Weston, N.B., M.A. Vile, S.C. Neubauer, and D.J. Velinsky. 2011. Accelerated microbial organic matter mineralization following salt-water intrusion into tidal freshwater marsh soils. Biogeochem. 102:135–151.

Zheng, W., J. Lichwa, M. D'Alessio, and C. Ray. 2009. Fate and transport of TNT, RDX, and HMX in streambed sediments: Implications for riverbank filtration. Chemosphere 76(9):1167-1177.