***FINAL REPORT***

**KN207-03**
***North Atlantic Virus Infection of Coccolithophore Expedition (NA-VICE)***

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## *1. BACKGROUND AND SUMMARY*

The '*North Atlantic Virus Infection of Coccolithophore Expedition* (*NA-VICE*; KN207-03; 13 June – 16 July 2012; http://www.bco-dmo.org/project/2136) consisted of a 30-day, ~2000 nautical mile **(Figure 1)** cruise aboard the *R/V Knorr* following a transect from Ponta Delgada, Azores to Reykjavik, Iceland. The primary objective of the NA-VICE cruise was to field test the cellular and molecular mechanisms, as well as the cornerstone infochemicals that regulate viral infection, PCD and cell fate in natural coccolithophore populations. We approached this through observational studies and manipulative experiments on the Northeast Atlantic (NEA) spring bloom. Processing of samples, data analysis, and development of publications are still ongoing and are supported by other funds to Bidle from other NSF-funded projects and an award from the Gordon and Betty Moore Foundation.

The NA-VICE expedition was a critical part of an NSF-funded project entitled "*Collaborative Research: Lipid lubrication of oceanic carbon and sulfur biogeochemistry by a host-virus chemical arms race*" (OCE-1061883), for which I was the lead-PI in collaboration with Co-PIs Drs. Ben Van Mooy (Woods Hole Oceanographic Institution, MA), Assaf Vardi (Weizman Institute of Science Israel; visiting faculty affiliation at WHOI), Marco Coolen (WHOI; now at Curtin University, Australia), and Jack DiTullio (College of Charleston, SC). The focus of this project was to elucidate the molecular, ecological, and biogeochemical links between GSLs (and other polar lipids) and the global cycles of carbon and sulfur. Our team aimed to extend cellular, molecular, and biochemical mechanistic markers of GSLs and viral infection to the oceans and elucidate the ecological and biogeochemical consequences of this sophisticated host-virus chemical arms race in natural blooms. Our work involved a multi-pronged approach combining a suite of lab-based, mechanistic studies using several haptophyte-virus model systems along with observational studies and manipulative field-based experiments in the Northeast Atlantic. Using a suite of GSLs and biomolecular targets as diagnostic markers, we attempted to document active viral infection of natural haptophyte populations *and* couple it with a suite of oceanographic measurements in order to quantify how viral infection (via vGSLs) influences cell fate, the dissolved organic carbon (DOC) pool, vertical export of particular organic (POC) and inorganic carbon (PIC; as calcium carbonate, $CaCO_3$) (along with associated alkenone lipid biomarkers and genetic signatures of viruses and their hosts) and the upper ocean sulfur cycle (via the cycling of dimethylsulfide [DMS] and other biogenic sulfur compounds). Furthermore, given they are unique to viruses, we posit that vGSLs can trace the flow of virally-derived carbon and provide quantitative insights into a "viral shunt" that diverts fixed carbon from higher trophic levels and the deep sea. *Our overarching hypothesis was that vGSLs are cornerstone molecules in the upper ocean, which facilitate viral infection on massive scales and thereby mechanistically 'lubricate' the biogeochemical fluxes of C and S in the ocean.*

## *2. VOYAGE INFORMATION AND SPECS*

The instruments and specifications that were used aboard the R/V Knorr for the NA-VICE were as follows:

- **Cruise No.**: KN207-03; **MOB**: 13-14June2012 (Azores); **DEPARTURE**: 15June2012 **ARRIVAL**: 14July2012 (Iceland); **DEMOB END**: 16July2016

- **General Duties of Marine Technician :**
  SSSG Technicians – David Simms (WHOI SSSG) & Anton Zafereo (WHOI SSSG)

- **Acoustics:** 12 kHz Pinger; ADCP 300 kHz; ADCP 75 kHz

- **Other:** Scientific Seawater System, Deionized Water System, Fume Hood; Scientific Team also deployed a glider and a couple of profiling floats to better characterize the in situ physical and optical conditions of blooms when they are encountered

- **Communications:** Basic Internet access via HiSeasNet

- **CTD/Rosette:** 911+ Rosette 24-position, 10-liter bottle Rosette with dual T/C sensors; Biospherical underwater PAR (1000m depth limit) with reference Surface PAR; SBE43 oxygen sensor; Seapoint STM turbidity sensor; Wet Labs C*Star transmissometer (660nm wavelength); Wet Labs ECO-AFL fluorometer

- **MET/Wx:** Air temperature, Barometric Pressure, Precipitation, Relative Humidity, Short Wave Solar Radiation, Wind speed and direction

- **Winches:** CTD Winch with .322" Electro-mechanical wire, Hydro Winch with .25" hydro wire

- **Sample Storage:** Freezer -85°C 25 cu. ft.; Refrigerator 8.6 cu. ft.; Freezer -70°C 3.2 cu. ft. ea.; Freezer -20°C

- **VANS**: Chemical Storage Van; Isotope Van; Hydrographic Van

## 3. *PERSONNEL*

The *NA-VICE* consisted of 26 science personnel and one free-lance videographer from six different institutions (listed below).

| Institution | email address |
|---|---|
| **Rutgers** | |
| Kay Bidle (lead PI/Chief Sci) | Kay Bidle <bidle@marine.rutgers.edu> |
| Chris Brown (postdoc) | Chris Brown <cbrown@marine.rutgers.edu> |
| Kim Thamatrakoln (postdoc) | thamatrakoln Kim <thamat@marine.rutgers.edu> |
| Liti Haramaty (technician) | Liti Haramaty <haramaty@marine.rutgers.edu> |
| Brittany Schieler (grad student) | Brittany Schieler <schieler@marine.rutgers.edu> |
| Christien Laber (grad student) | Christien Laber <claber@marine.rutgers.edu> |
| Benjamin Bailleul (postdoc) | Benjamin Bailleul <benjamin@marine.rutgers.edu> |
| Ana Filipa Carvalho (grad student) | Ana Filipa Carvalho <filipa@marine.rutgers.edu> |
| **Woods Hole Oceanographic Institution** | |
| Jamie Collins (grad student) | James Collins <jrcollins@whoi.edu> |
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| Justin Ossolinski (technician) | Justin E Ossolinski <jossolinski@whoi.edu> |
| Marco Coolen (co-PI) | Marco Coolen <mcoolen@whoi.edu> |
| Cherel Balkema (student) | Cherel Balkema <cherelbalkema@gmail.com> |

| **College of Charleston** | |
|---|---|
| Jack DiTullio (co-PI) | Jack DiTullio <DitullioJ@cofc.edu> |
| Peter Lee (technician) | Lee, Peter A <LeeP@cofc.edu> |
| Jacob Kendrick (grad student) | Jacob Kendrick <jacobkendrick@gmail.com> |
| Barbara (Bobbie) Lyon | Lyon, Barbara R <lyon@musc.edu> |
| Rachel Stevens | Rachel Stevens <rcsteven@g.cofc.edu> |

| **Weizmann Institute of Science** | |
|---|---|
| Assaf Vardi (co-PI) | Assaf Vardi <assaf.vardi@weizmann.ac.il> |
| Miguel Frada (postdoc) | Miguel Frada <miguel.frada@weizmann.ac.il> |
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| Daniella Schatz (technician) | Daniella Schatz <daniella.schatz@weizmann.ac.il> |
| Yoav Lehahn (postdoc) | Yoav Lehahn <yombav@gmail.com> |
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| **University of Azores** | |
|---|---|
| Ana Martins (PI) | Ana Maria Martins <anamartins@uac.pt> |
| Clara Loureiro (grad student) | Clara Loureiro <c.angela.m.loureiro@gmail.com> |

| **Broder Impact Film Crew** | |
|---|---|
| Rose Eveleth | Rose Eveleth <rose.eveleth@gmail.com> |

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## 4. *PLANNING MEETINGS*

As part of the logistical cruise planning, Bidle organized and participated in several pre-cruise planning meetings. The first meetings took place with some of the aforementioned science personnel on 14 December 2011 (via Skype) and on 19-20 January 2012 (in person) at Rutgers University. Bidle also participated in an official, in-person meeting with WHOI's Marine Operations on 1 February 2012, at which time we went over the general cruise plan and discussed specs and equipment.

Bidle also organized and led a Post-Cruise Meeting for the *NA-VICE* cruise at the Carriage House on WHOI's Quissett Campus from 19-20 January 2014. The purpose of the meeting was to present our multifaceted datasets and discuss findings from the 5-week *NA-VICE* cruise. An important outcome from the meeting was also to strategize and identify possible papers coming out from this endeavor. Bidle was the Chief Scientist for the cruise/fieldwork. Personnel from the labs of co-PIs Van Mooy, Coolen, DiTullio, and Vardi were also in attendance to present their work. In total, we had 7 people from my lab at Rutgers, 5 people from Van Mooy's group at WHOI, 1 person from Coolen's lab at WHOI, 4 people from Vardi's lab at Weizmann Institute) and 1 person from DiTullio's lab at College of Charleston in attendance. We also had a few participants from MIT, University of Azores and University of Liege (Belgium).

## 5. *FINDINGS AND ACCOMPLISHMENTS*

Using satellite imagery and analyses from NASA's MODIS Aqua (PIC, Chl, Rrs 547, Rrs 555), we were able to successfully detect and locate *E. huxleyi* blooms that were associated with distinct, anticyclonic eddies **(Figure 2)**. *E. huxleyi* bloom densities ranged from ~1-4 x $10^3$ cells $l^{-1}$, which are well within reported values of other large scale *E. huxleyi* blooms in the North Atlantic and elsewhere. To our knowledge, this is the first time *E. huxleyi* has been associated with mesoscale circulation features in the open ocean. At each eddy location, we extensively sampled these blooms in lagrangian mode over a 4-5 d using traditional CTD casts, as well as deploying a variety of sediment traps (PIT and net traps) and optical profilers, which were

programmed to give vertical profiles of backscatter (700nm), Chl *a*, turbidity, CDOM, dissolved oxygen, PAR and downward irradiance (412, 440, 490 nm), and CTD at ~2.5 m 3 $h^{-1}$ resolution **(Figure 3)**.

We diagnosed the physiological state and infection stage of resident cells within these natural blooms by measuring inventories of a unique suite of structurally distinct polar lipids— glycosphingolipids (GSLs) and betaine-like lipids (BLLs)— which have emerged as powerful diagnostic biomarkers because they represent different functional aspects of the *E. huxleyi*-EhV infection process. Depth-integrated measurements of GSL and BLL species first provided a bulk inventory of resident *E. huxleyi* populations. Host-specific GSLs (hGSLs) are specific markers of *E. huxleyi* cells; virus glycosphingolipids (vGSLs) are critical to and diagnostic of successful infection; sialic acid GSLs (sGSLs) have only been detected in susceptible host strains are used to diagnose population susceptibility to EhV infection. Lastly, BLLs are a class of polar diacylglycerolipids with EhV infection triggering an exponential increase in BLL(22:6, 22:6), which becomes the major BLL species in the infected cells. We also used an independent, DNA-based, qPCR proxy to quantify the abundance of EhV associated with *E. huxleyi* cells via qPCR analysis of major capsid protein (MCP) and cytochrome oxidase (COI) genes.

Results to date indicate that each eddy-contained *E. huxleyi* bloom that we occupied was in a different phase of bloom and host-virus dynamics **(Figure 4)**. We appear to have fortuitously occupied (and sampled) *E. huxleyi* blooms that were either in early- to mid-infection (Cocco1), late termination (Process 2), and post-bloom (Cocco2) scenarios, with the former (Cocco1) showing signs of an ongoing, raging host-virus arms race and the latter having been completely decimated by EhV infection prior to our arrival. One eddy-contained station that we occupied, named *Cocco2*, appeared to have been in the midst of post-bloom demise by EhV infection **(Figures 5-6)**, based on a combination of satellite data (Chl and PIC), where we hind-casted the life stage of the bloom over a ~40 d period up through the days that we occupied and sampled it, and *in situ* water column profile data including *E. huxleyi* and EhV abundance, coccolith concentration, and EhVs $cell^{-1}$ (via qPCR). Furthermore, high-resolution data from an optical profiler sampling the upper 150-300 m over a 5 d period, indicated a dramatic reduction in turbidity and backscatter within the in the ~20 m mixed layer consistent with a pronounced vertical flux of PIC and cellular material. This shift was also concomitant with distinct increases in water column cDOM and respiration just below the mixed layer. This high-resolution optical dataset is the first of its kind to intensively sample *in situ* ecosystem and biogeochemical properties after an *E. huxleyi* bloom that was documented to have undergone virus demise. Our ongoing work aims to apply similar in situ ecosystem and biogeochemical sensors to elucidate how host-virus interactions and their associated microbe-infochemical milleu, structure and imprint ocean biogeochemistry.

A key finding from our datasets is that that that active EhV infection was not only occurring, but it was triggering intense particle sinking fluxes to 50, 150 and 300 m depth--- evidence that EhVs play an inherent role in vertical C flux. In fact, our measured POC (250 mg $m^{-2}$ $d^{-1}$) and PIC (110 mg $m^{-2}$ $d^{-1}$) fluxes are amongst the highest fluxes measured in the oceans **(Figure 7)**. The aforementioned BLL markers of infection were elevated in sinking particles, suggesting that infected cells were comprising the sinking flux; similarly, the PIC flux was correlated with the sGSL flux, suggesting that the sensitive cells were making up the bulk of the sinking material **(Figure 8)**. These findings indicated that ROS production and caspase activation in virus infected *E. huxleyi* is functionally coupled with TEP production and aggregate formation, thereby serving a mechanistic role in the vertical flux of organic matter. This is counter to the traditional paradigm invoking cell lysis, release of DOM, and stimulation of the microbial loop.

In addition to our core research efforts on *NA-VICE* to investigate the linkages of GSLs, viral infection and vertical flux, we also performed extensive measurements to characterize the aerosol particle field at our field locations and 1) relate them to prevailing conditions (e.g., wind, wave height, etc.) and the incorporation and characterization of infectious EhVs within the aerosol field as a possible infection transmission vector for other *E. huxleyi* populations in the North Atlantic. In a similar manner, the *NA-VICE* platform was used to investigate the possible interface of EhV-infected cells with macrozooplankton grazing (e.g., copepods) as an additional transmission vector to spread EhV infection through a *E. huxleyi* population. Results found that infected cells are grazed by copepods, that infectious EhVs make it through the copepod digestive cavity, and that they indeed can be used to transmit infectious EhVs throughout the bloom.

A key part of the cruise activities were to employ two BOSS-mini vertical profiling floats to gather a suite of integrative, *in situ* data in these eddies. A total of six deployments were performed over the duration

of the cruise **(Figure 3)**, with floats collecting measurements of backscatter ($B_b$; 700nm), Chl *a* fluorescence, DO, CDOM, turbidity, downward irradiance (412, 440, 490 nm) and for photosynthetically active radiation (PAR), as well as standard CTD measurements at ~2.5 m 3 $h^{-1}$ resolution. Three of the deployments overlapped with the 4-5 d semi-lagrangian, ship-based sampling stations while the other deployments were contextually anchored by only deployment and/or recovery ship sampling. Profiles recorded data between 250-300m and the surface at roughly 2.5m and 3 h intervals. These deployments lasted between 4-6 days each. Rapid deployment and recovery paired with high temporal resolution introduces a novel use of profiling floats in contrast to the multi-month/year deployments more commonly executed.

Ship-based oceanographic approaches have a limited reach in molecular and microbial oceanography due to available ship time and course spatial resolution. *In situ* autonomous optical platforms have the potential to alleviate these problems with high sampling resolution (temporally and spatially) and long deployment times (weeks to years). Additionally, they may be deployed in advance of a study to identify areas of relevant interest, in contrast to searching blindly. Gliders and vertical profiling floats have been outfitted with bio-optical sensors and are increasingly providing detailed information on primary production and associated processes. Identifying bio-optical signatures associated with EhV infection of *E. huxleyi* (given the aforementioned optical qualities of this organism) would provide a powerful complimentary tool for exploring *in situ* infection dynamics and subsequent impacts on ecosystem and biogeochemical cycling. Once these bio-optical based proxies have been grounded with the aforementioned, diagnostic biomolecular markers of infection and cellular response mechanisms, it opens the possibility to interrogate these interactions and ecosystem response using observational platform sensors, which can be more easily and rapidly measured than molecular approaches.

The thesis work of Bidle's PhD student (Christien Laber) concentrates on the development and implementation of optical proxies to detect in situ EhV infection of *E. huxleyi* and its corresponding ecological and biogeochemical impact in the upper ocean. It expands our understanding of *E. huxleyi*-EhV interactions in different ecological settings (bloom versus non-bloom) to different oceanic regions for a more complete understanding of the extent to which EhVs regulate the fate of fixed carbon associated with *E. huxleyi* populations. *In situ* autonomous ocean optics can provide high spatial resolution of discrete water column optical properties and, when combining optical signals influenced by EhV infection, they may collectively provide accurate identification of *in situ* EhV infection in natural populations, something that has been crucially lacking in virus ecology and biological oceanography to date. These properties include scattering, fluorescence, and absorptive properties associated with infection. Laber's thesis examines the optical consequences of EhV infection by characterizing the optical signals produced in infected *E. huxleyi* blooms using a combination of optical profiling floats and ship based sampling, examining how EhV infection influences spectral absorption and fluorescence characteristics of *E. huxleyi*. Armed with this knowledge the final chapter then explores the utility of these techniques in assessing *E. huxleyi*-EhV interactions in the regions of the Pacific Ocean where *E. huxleyi* populations are often present in non-bloom abundances and where EhV infection dynamics are currently unknown.

$B_b$ and Chl *a* signatures collected during 4 day deployments of the optical profiler were distinctly different for *'Infected Bloom'*, *'Late Termination'*, and *'Post-Bloom'* locations **(Figure 9)**. Given these stations also had adjacent shipboard sampling, various biological measurements are being used to contextualize and determine the major constituents of the signals. Currently, these signals have been qualitatively compared to phytoplankton concentration and available PIC data from the cruise and suggest a promising relationship with coccolithophore abundance (Figure 8). High coccolithophore abundance is associated with a low Chl *a* and high $B_b$ signal, while the inverse is observed as abundance decreases. With PIC/POC measurements having recently been completed, $B_b$ will be compared to PIC, coccolith and coccolithophore concentration, phytoplankton and bacterial abundance.

Cellular carbon released can be remineralized and incorporated in the microbial loop, or pumped into the deep ocean via sinking aggregates. The relative contribution of EhV infection to these two diametrically opposed processes is critically dependent on infection dynamics and how infection manifests in cellular response (lysis and release of DOM versus TEP production, particle aggregation and sinking). Using the optical profiler data, Christien is exploring the extent of bacterial remineralization and vertical flux of carbon during different stages of coccolithovirus infection, using the aforementioned, three distinct eddies (and

associated documented host-virus dynamics). He has measured the $B_b$ 'spike' signal [based on: Briggs et al. *Deep Sea Research Part I: Oceanographic Research Papers*, 58, 1031-1039], which is a proxy for export flux, for four deployments and found that it is highest during infection of an active bloom **(Figure 8)**. This data has also been calibrated using concurrent sediment trap deployments to measure carbon flux ($C_{flux}$) and to extrapolate quantified $C_{flux}$ measurements from unaccompanied float deployments. The sediment trap POC and PIC flux rates suggest that these constituents have a similar influence on the total carbon (TC) flux correlation with the spike signal **(Figure 8)**. Carbon remineralization ($C_{remin}$) rates have also been determined using depth binned DO utilization rates where possible. In several cases, use of this technique is limited by mixing water bodies or float movement out of a coherent water mass. Primary production also prevents accurate measurements of DO utilization, preventing the use of this technique in the productive layer. Ship based incubations measuring $^3$H-Leucine incorporation by bacteria and DO utilization were also conducted during the cruise and will provide a comparison to the optical measurements. The ratio of $C_{flux}$ to $C_{remin}$ will also be calculated to explore the remineralization efficiency of sinking TC.

The above work resulted in several publications with several others in preparation (listed below). It has been presented as part of oral talks at the 2016 Gordon Research Conference, Marine Microbes- 'The Evolution, Nature and Function of Microbial Interactions' (Girona, Spain; 19-24 June 2016), 2014 Gordon Research Conference, Marine Microbes- 'Small Microbes, Big Data' (22-27 June 2014: Bentley University, Waltham MA), and the 2014 Ocean Sciences Meeting (23-28 February 2014; Honolulu, HI).

Laber, C.P., A.F. Carvalho, J.E. Hunter, B. Schieler, E. Boss, M. Coolen, G. DiTullio, M. Frada, A.M. Martins, L. Haramaty, A. Vardi, Y. Lehahn, K. Thamatrakoln, C. Brown, J. Ossolinski, H. Fredricks, B. Van Mooy, and Kay D. Bidle. Coccolithovirus stimulation of carbon export in the North Atlantic. *(in prep)*

Nissimov, J.I, D. Talmy, L. Haramaty, H. Fredricks, U. Zelzion, M. Eren, R. Gardella, C. Laber, K.D. More, M.J.L. Coolen, M. Follows, D. Bhattacharya, B.A.S. Van Mooy and K.D. Bidle. "Survival of the slowest": Biochemical diversity of coccolithovirus-derived serine palmitoyltransferase and its impact on host demise. *(in prep)*

Thamtrakoln, K., D. Talmy, J. Latham, L. Haramaty, K.D. Bidle. Light structuring of coccolithovirus infection. *(in prep)*

Johns, C.T., J.I. Nissimov, F. Natale, V. Knapp, A. Mui, H. Fredricks, B.A.S. Van Mooy and K.D. Bidle 'Though the looking glass': the mutual interplay between coccolithovirus infection and cellular PIC quotas in *Emiliania huxleyi*

Collins, J.R., B.R. Edwards, K. Thamatrakoln, J.R. Valdes, J.E. Ossolinski, G.R. DiTullio, K.D. Bidle, S.C. Doney, R.G. Keil, and B.A.S. Van Mooy. 2015. The multiple fates of sinking particles in the North Atlantic Ocean. *Global Biogeochem. Cycles* 29 (9): 1471–1494.

Sharoni, S., M. Trainic, D. Schatz, Y. Lehahn, J.M. Flores, K.D Bidle, S. Ben-Dor, Y. Rudich, I. Koren, A.Vardi. Infection of bloom-forming phytoplankton by aerosolized marine viruses. 2015. *Proc. Natl. Acad. Sci. USA* 112(21): 6643-6647.

Edwards, B.R., K.D. Bidle, and B.A.S. Van Mooy. 2015. Dose-dependent polyunsaturated-aldehyde regulation of microbial activity on sinking particles in the ocean *Proc. Natl. Acad. Sci. USA* 112(19): 5909-5914.

Lehahn, Y., I. Koren, D. Schatz, M. Frada, U. Sheyn, E. Boss, S. Efrati, Y. Rudich, M. Trainic, S. Sharoni, C. Laber, G.R. DiTullio, M.J.L. Coolen, A.M. Martins, B.A.S. Van Mooy, K.D. Bidle, and A. Vardi. 2014. Decoupling physical from biological processes to assess the impact of viruses on a mesoscale algal bloom. *Current Biology* 24: 2041-2046.

Lehahn, Y., I. Koren, Y. Rudich, K.D. Bidle, M. Trainic, J.M. Flores, S. Sharoni, A. Vardi. 2014. Decoupling atmospheric and oceanic factors affecting aerosol loading over a cluster of mesoscale North Atlantic eddies. *Geophys. Res. Lett.* 41(11): 4075-4081.

Frada, M. J., D. Schatz, V. Farstey, J. E. Ossolinski, H. Sabanay, S. Ben-Dor, I. Koren, and A. Vardi. 2014. Zooplankton may serve as transmission vectors for viruses infecting algal blooms in the ocean. Curr. Biol. 24:2592-2597.

## 6. *DATA MANAGEMENT*

Metadata from hydrographic and oceanographic data from CTD hydrographic casts, as well as from surface samples collected by the ships underway system, have been submitted to the Biological and Chemical

Oceanography Data Management Office (BCO-DMO) and are available at: *North Atlantic Virus of Coccolithophore Expedition* (*NA-VICE*; KN207-03; http://www.bco-dmo.org/project/2136). Several datasets of measured parameters (pigments, nutrients, GSLs, PIC/POC) have also been submitted and are available on the BCO-DMO website.

## 7. OUTREACH and BROADER IMPACT

One important aspect of the *NA-VICE* was to use it as a platform to develop novel education and outreach materials using the research being conducted in the North Atlantic on the R/V *Knorr* as a platform. Bidle worked with Janice McDonnel (Department of 4-H Youth Development, Rutgers University), along with Josh Kurz, Chris Metzler and Jeff Springer (Tilapia Film, LLC) to develop a novel video series and hands on lessons entitled *'Tools of Science'* (http://toolsofscience.org) on how science works. Initial funding for this work came from NSF (OCE-1061883) and was supplemented with funds from the Gordon and Betty Moore Foundation and Rutgers' School of Environmental and Biological Sciences. This first three videos (each ~5-7 min), on *'Collaborations'*, *'Sampling, & Proxies'*, are currently in production and highlight various scientific practices through real research investigations during the *NA-VICE*. The videos will be open access and are designed to be incorporated into both middle and high schools, as well as undergraduate classes. We are currently developing two additional videos on *'Using Mathematical Models'* and *'Asking Testable Questions'*, which are based on current NSF-funded projects (IOS-1425372).

The *'Tools of Science'* series is designed to help high school students explore the nature and process of science. The short videos are designed to introduce the science and engineering practices from the point of view of practicing scientists. They can be integrated into any STEM unit to help illustrate the non-linear, cyclical nature of science and the creative vision and skills needed to conduct scientific research. They allow students and teachers to join scientists on a virtual field trip aboard the R/V *Knorr* to experience how scientists explore, observe, question, test, and communicate their science. The videos and associated materials have been produced in close to support the implementation of the Science Framework for K-12 Science Education and in the Next Generation Science Standards (NGSS). The long-term aim of this project is to produce material to complement each of the eight NGSS practices (listed at: http://toolsofscience.org/lessons.html). Content for Practices 3 and 8 has been developed and is currently available. Material for the other practices is currently under development.

## 8. *FIGURES*

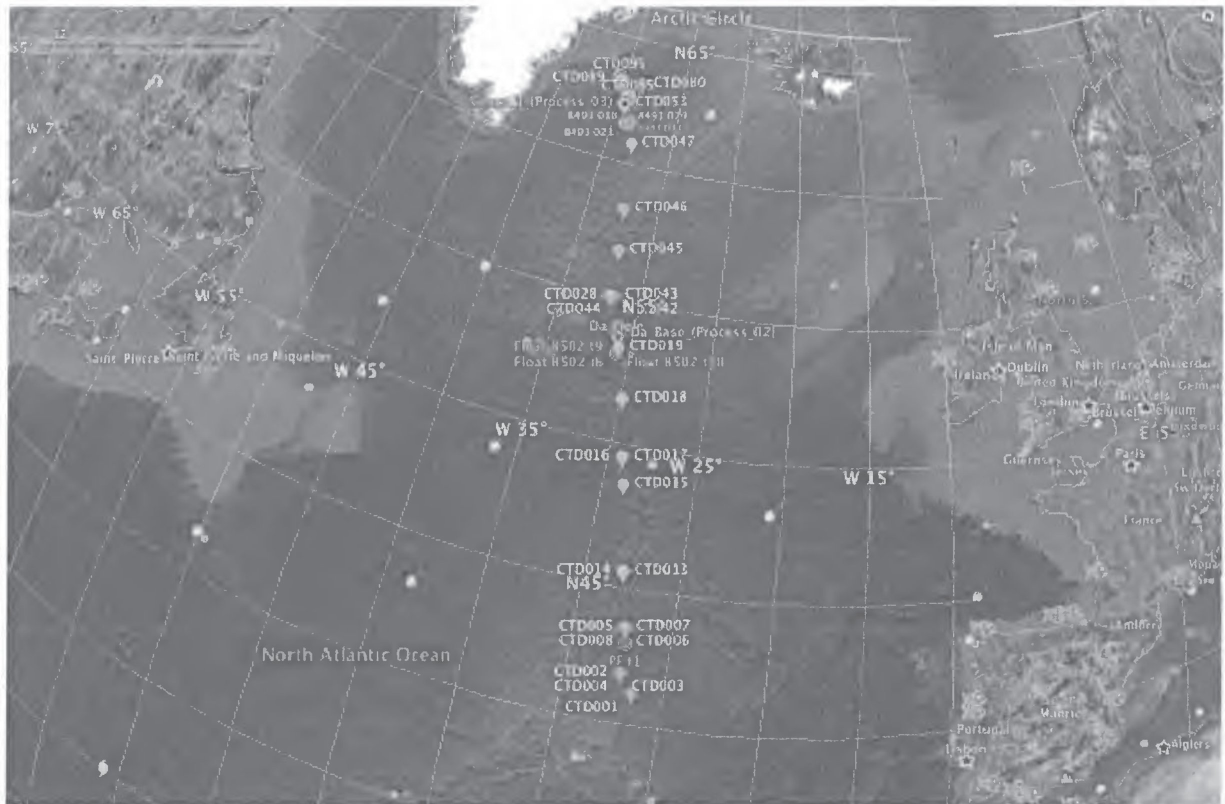


**Figure 1.** Cruise track and station locations of KN207-03 between Ponta Delgada, Azores to Reykjavik, Iceland (green line & pins). The R/V Knorr departed Ponta Delgada on 15 June 2012 and arrived Reykjavik on 14 July 2012. Locations of CTD casts are indicated (in white) and 'process' stations, where the R/V Knorr held it's location and operated in a lagrangian mode for 4-10 days and where various instruments were deployed for in situ measurements, are indicated in blue, orange, and yellow.

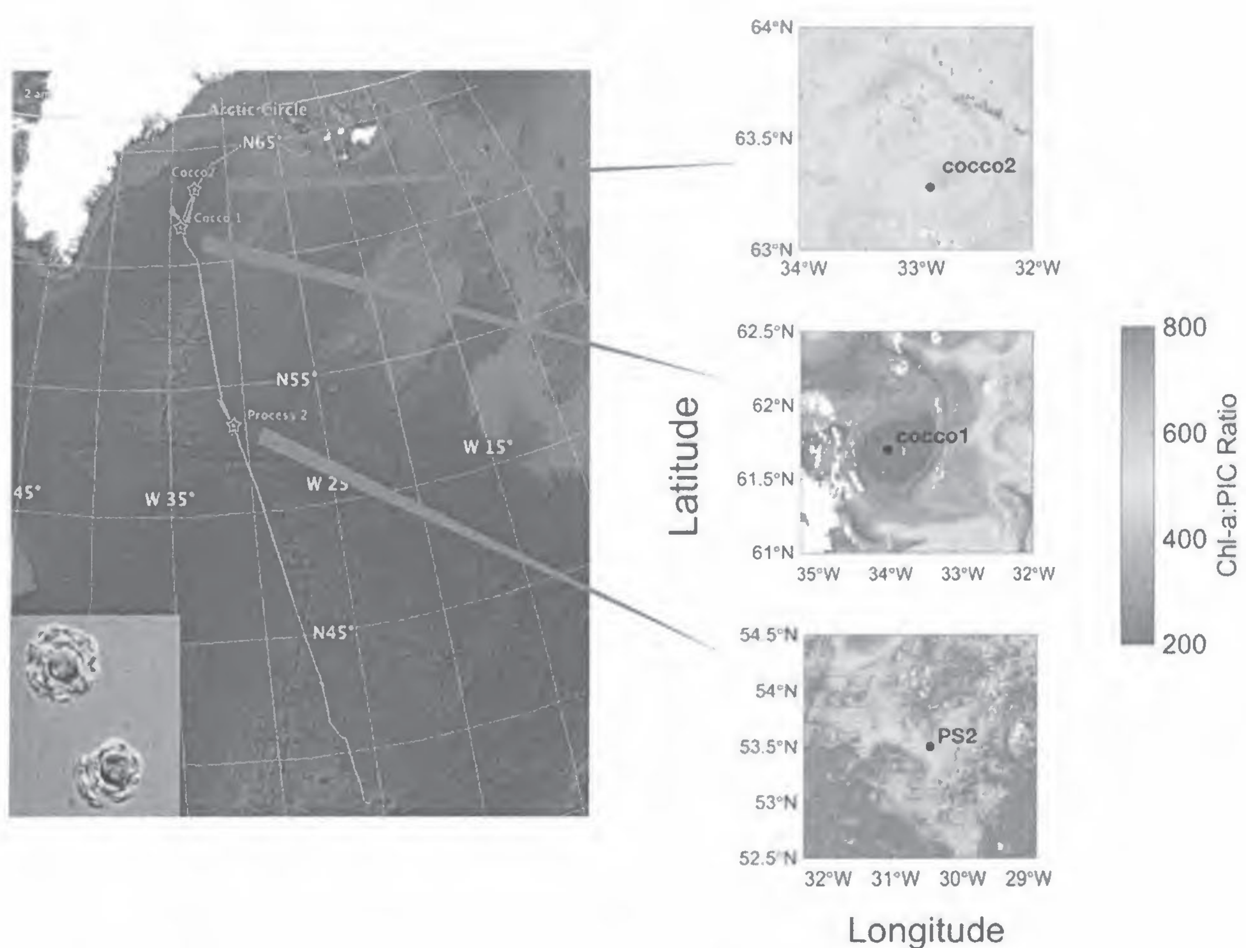


**Figure 2. Detection of EhV-infected *E. huxleyi* blooms in the North Atlantic.** (Left panel) Cruise track of KN207-03 (R/V *Knorr*; 15 June – 14 July 2012; between Ponta Delgada, Azores to Reykjavik, Iceland; green line) highlighting three locations (mesoscale eddies) whereby *E. huxleyi* bloom populations were found to present and at different stages of EhV infection. Using satellite imagery and analyses from NASA's MODIS Aqua (high PIC, low Chl, high Rrs 547, high Rrs 555), we were able to successfully and consistently detect and locate *E. huxleyi* blooms that were generally associated with four distinct, anticyclonic eddies. *E. huxleyi* bloom densities ranged from ~1-4 x $10^3$ cells $l^{-1}$, which are well within reported values of other large scale *E. huxleyi* blooms in the North Atlantic and elsewhere. (Inset) microscopic image of *E. huxleyi* cells collected at Cocco1. To our knowledge, this is the first time *E. huxleyi* has been associated with mesoscale circulation features in the open ocean and this finding will be an important component of subsequent papers. Process 2 and Cocco 1 were 'process' stations, where the R/V *Knorr* held it's location and operated in a Lagrangian mode for 4-10 days and where various instruments were deployed for in situ measurements (see Figure 3). Cocco2 was found to be at 'post bloom' situation whereby a large *E. huxleyi* bloom had been terminated prior to our occupation of the eddy (see Figure 5-6). Consequently, it was sampled extensively via CTD casts and an optical profiler (see Figures 3,9), which allowed us to assess the ecosystem and biogeochemical impact of EhV-infection.

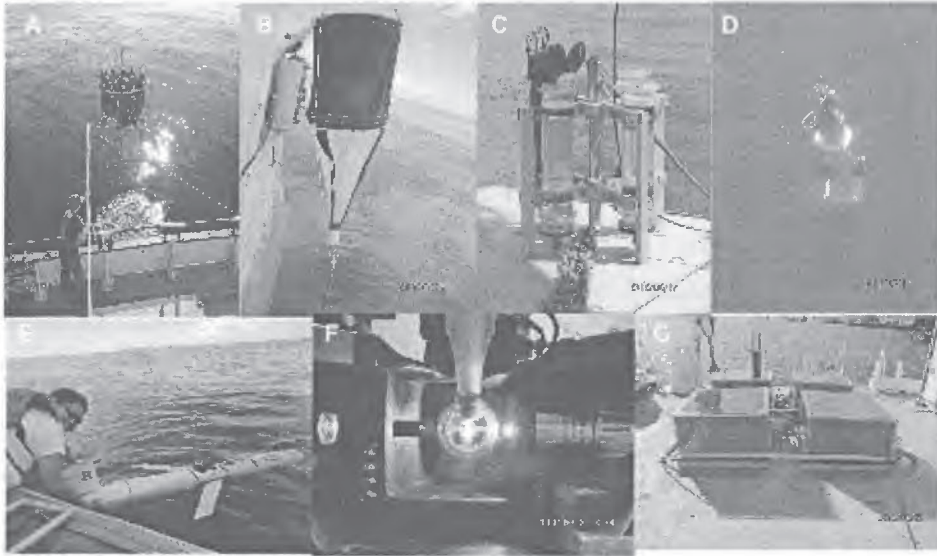


**Figure 3. Array of measurements performed during KN207-03 at eddy 'process' stations while in Lagrangian mode for 4 d.** Aside from standard CTD casts colleting water from 6 depths (mostly within the upper mixed layer and extending down to 150 m; Panel A), neutrally buoyant net (B) and PIT (C) sediment traps were deployed, along with a Webb optical profiler (D) and an autonomous glider (E). Analytical flow cytometry and cell sorting was used to count *E. huxleyi* cells, EhVs and perform diagnostic staining analyses. Lastly, on-deck incubations (G) were performed with *in situ* communities under a variety of conditions to test for critical controls on EhV infection.

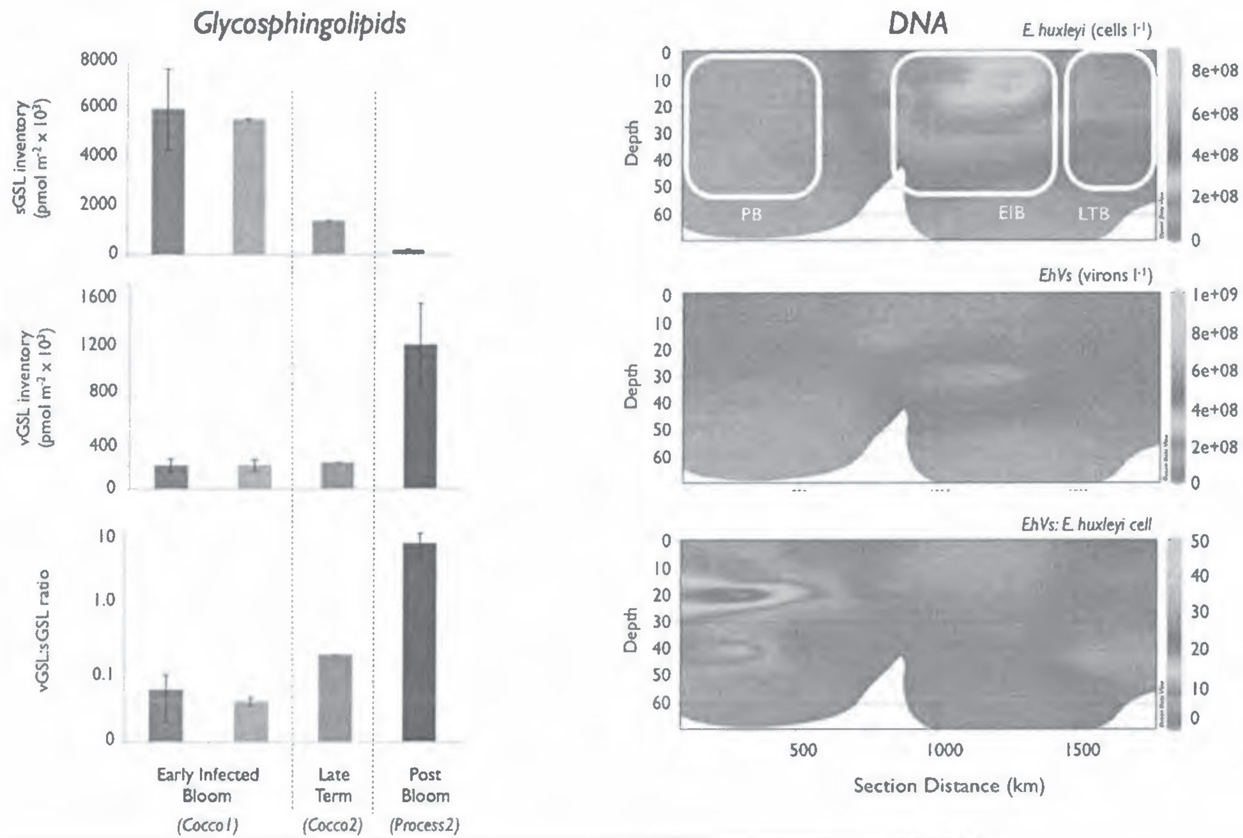


**Figure 4. Diagnosis of virus infection using lipid- and DNA-based proxies.** Average (A) sGSL and (B) vGSL inventories, down to 150m at Cocco 1 (blue), Cocco1$_R$ (green), Cocco2 (red), and PS2 (grey) stations. The average vGSL:sGSL (C) and vGSL:hGSL inventory ratios at each station revealed distinct stages of infection. (E-G) Ocean Data View contour plots of *E. huxleyi* and EhV abundance across the cruise transect (south to north) as assessed by qPCR of COI and MCP genes, respectively. (E) COI gene copy number representing *E. huxleyi* concentration; (F) MCP gene copy number representing EhV abundance, and (G) EhVs per E. huxleyi cell, based on the ratio of COI:MCP gene copy numbers. Note that analyses of gene copy numbers derives from nucleic acids extracted from biomass collected onto 0.8 μm pore-size filters; hence, the data presented in (B,F) represent EhVs associated with (and replicating within) *E. huxleyi* cells. Taken together, these *E. huxleyi* blooms were at distinct stages of progressive EhV infection and defined as: Cocco1, Early Infected Bloom (EIB); Cocco1$_{R,}$, Early Infected Bloom-Redux (EIBR); Cocco2, Late Termination Bloom (LTB); and PS2, Post Bloom (PB).

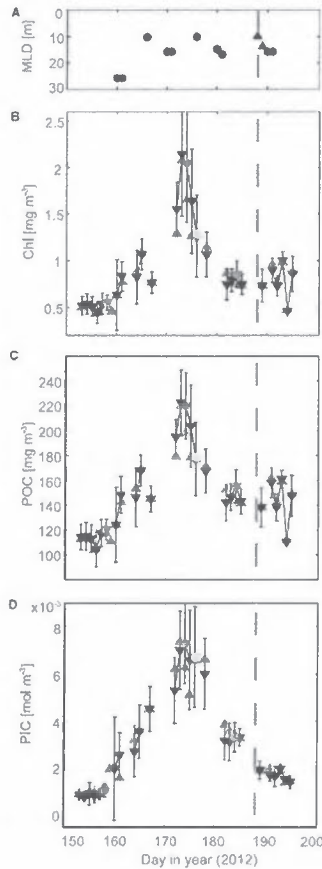


**Figure 5. Temporal changes in physical and biological variables associated with *E. huxleyi* bloom and bust dynamics of the Cocco2 station during NA-VICE.** (A) Changes in mixed layer depth (MLD) at Cocco2 eddy patch as calculated from temperature profiles obtained from Argo floats (solid circles) and shipboard CTC casts (triangles). (B–D) Changes in concentrations of surface chlorophyll (Chl; B), particulate organic carbon (POC; C), and particulate inorganic carbon (PIC; D) at Cocco2, derived from the MODIS instrument on board the Aqua satellite. Time series are extracted by averaging all available data over a disc (r = 30 km) around the phytoplankton patch centroid (black data points) and over the area associated with the core of the eddy (blue data points). Error bars show SD of the data sampled over the disc. Red data points mark the benchmark time steps for which the patch is fully exposed. Yellow data points mark the end of the 3-day period during which Chl and POC decrease rapidly while PIC remains almost constant. Green dashed line marks the timing of the in situ sampling aboard the R/V Knorr. Figure taken from: Lehahn et al. (2014) *Curr. Biol.* 24:2041-2046.

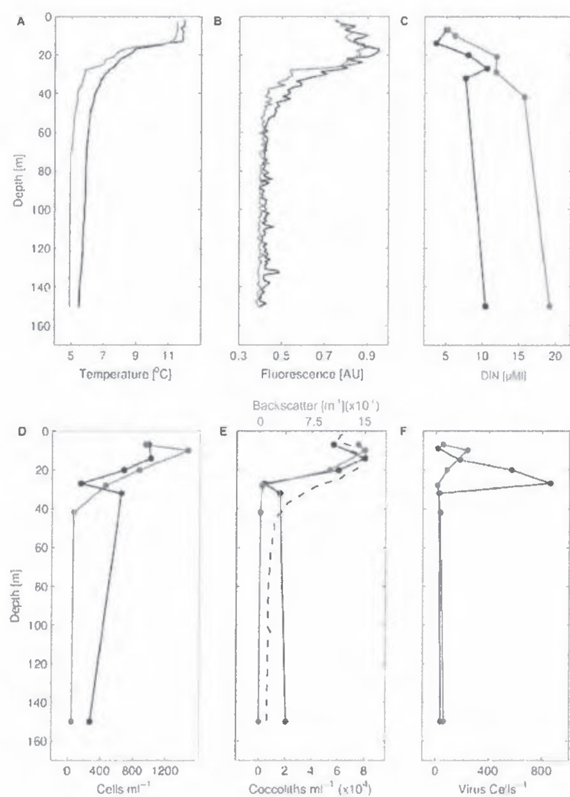


**Figure 6. Vertical profiles of physical and biological variables during 'post-bloom' *E. huxleyi*-EhV dynamics bloom at the Cocco2 station during NA-VICE.** Measurements of (A) temperature and (B) fluorescence, dissolved inorganic nitrogen (DIN) concentrations (C), abundance of (C) *E. huxleyi* cells (D) abundance of coccoliths (solid line) and backscatter (E; dashed line; as measured via the Webb optical profiler), and concentration of coccolithoviruses in coccolithophore cells (F) measured by qPCR analysis during coccolithophore bloom demise in the Cocco2 eddy. Profiles were measured in situ on July 6, 2012 at 09:12 local time at 63°110' N/32° 47' W (cast 1, black lines) and at 19:07 local time at 63°20' N/32°49' W (cast 2, blue lines).Figure taken from: Lehahn et al. (2014) *Curr. Biol.* 24:2041-2046.

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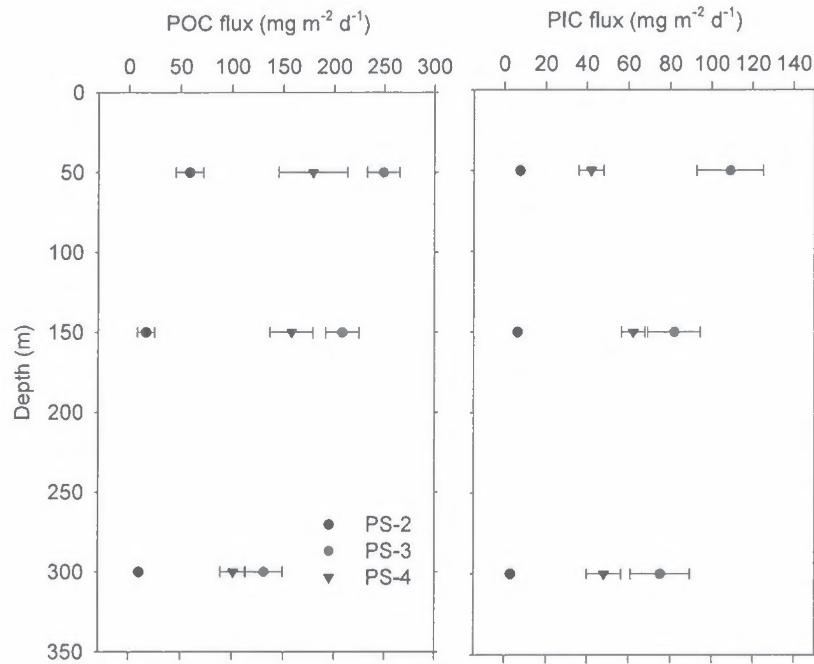


**Figure 7. Vertical fluxes of POC and PIC associated with different stages of EhV infection of E. huxleyi populations. Enhanced POC and PIC fluxes, as measured from neutrally-buoyand PIT traps, were associated with** *E. huxleyi* blooms that were in early- to mid-infection (Cocco1: PS-3 and PS-4) and undergaoing active infection, as opposed to late termination scenario (Process 2: PS-2), whereby many of the sensitive sGSL containing hosts had presumably already been removed to depth. The measured POC (250 mg $m^{-2}$ $d^{-1}$) and PIC (110 mg $m^{-2}$ $d^{-1}$) fluxes are amongst the highest fluxes measured to date in the oceans. [Figure adapted from Collins et al. *Global Biogeochem. Cycles* 29 (9): 1471–1494].

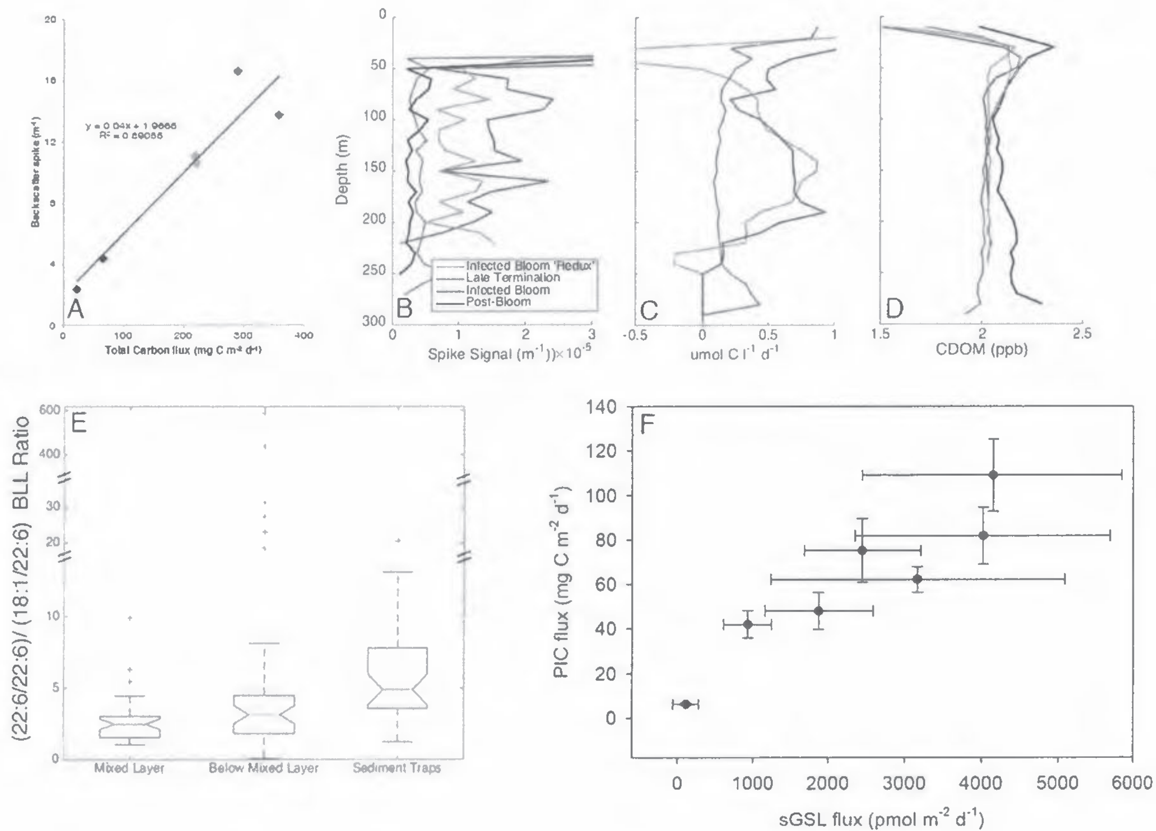


**Figure 8. Use of profiler-based backscatter spike signals to approximate particle aggregation and fluxes during *E. huxleyi* infection.** (A) Bb (700 nm) spike signal transformed (reference) from data measured with the optical profiling floats deployed at three different locations during the NA-VICE cruise, all of which contained *E. huxleyi* at different stages of EhV infection. Bb spike signal has been binned (over 10 m and 12 h) and each bin averaged over the time of each deployment. Infected Bloom (blue), Infected Bloom 'Redux' (green), and Post-Bloom (grey) had corresponding total carbon (TC) flux measurements using neutrally buoyant PIT traps that had been simultaneously deployed at 50 and 150m. The 'Late Termination' location was unaccompanied by the PIT traps and did not have corresponding flux data. Using regression analysis between the $\log_{10}$ of the Bb spike signal and corresponding PIC and POC fluxes (B), the TC flux at 150 m for the 'Late Termination' location was calculated at ~240 mg TC m-2 d-1. This data supports our hypothesis that vertical fluxes of carbon are maximal during active EhV infection and tapers off as termination progresses.

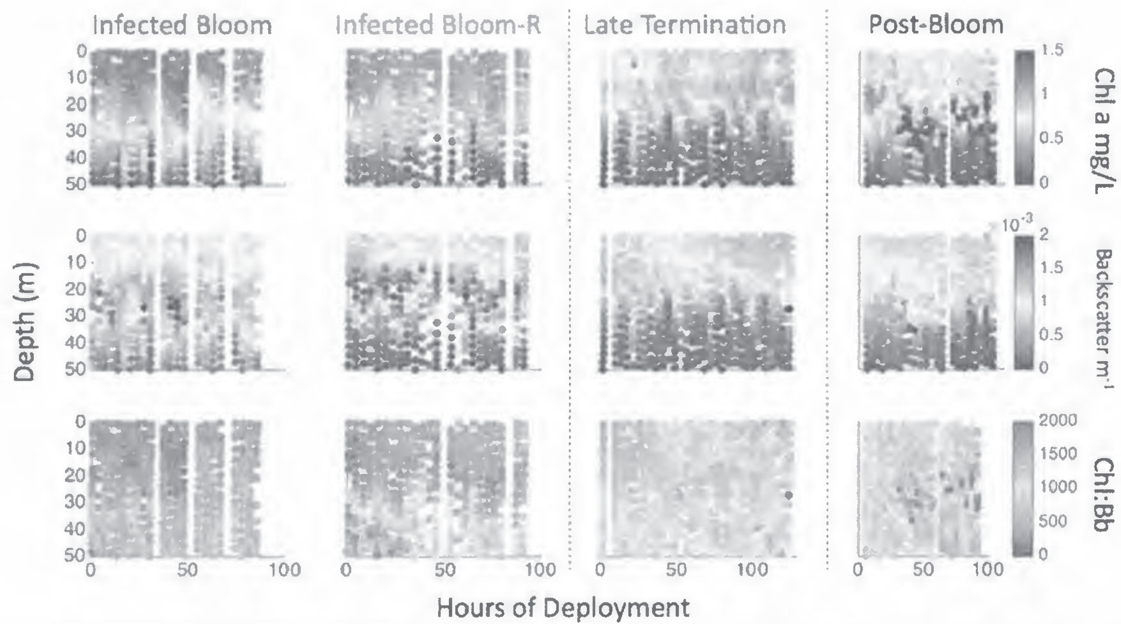


**Figure 9. Dynamics of in situ biooptical and community properties measured for different phases of the E. huxleyi-EhV interactions in the North Atlantic.** (A) Backscatter at 700 nm and (B) chlorophyll fluorescence measured with the optical profiling floats deployed at three different locations during the NA-VICE cruise, all of which contained E. huxleyi at different stages of EhV infection. (C) Cell abundance of E. huxleyi (green symbols and lines) and all other phytoplankton (black symbols and lines; includes eukaryotic and cyanobacteria) as determine by analytical flow cytometry (via chlorophyll fluorescence @ 692 nm and side scatter to distinguish coccolithophores). (D) Vertical profiles of backscatter (measure with optical profiling float) and PIC (measured via the difference between TPC minus the POC on an elemental analyzer). Note: symbols above the figure denote samples collected during different CTD casts, relative to float profile data. Chlorophyll measurements have been corrected for non-photosynthetic quenching.