2016

The Influence of Hydrothermal Plumes on Midwater Ecology, and a New Method to Assess Pelagic Biomass

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THE INFLUENCE OF HYDROTHERMAL PLUMES ON MIDWATER
ECOLOGY, AND A NEW METHOD TO ASSESS PELAGIC BIOMASS

By

BRENNAN THEODORE PHILLIPS

A DISSERTATION SUBMITTED IN PARTIAL FUFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN

OCEANOGRAPHY

UNIVERSITY OF RHODE ISLAND
2016

DOCTOR OF PHILOSOPHY DISSERTATION

OF

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2016

ABSTRACT

Submarine hydrothermal vent fields introduce buoyant plumes of chemically altered seawater to the deep-sea water column. Chemoautotrophic microbes exploit this energy source, facilitating seafloor-based primary production that may transfer to pelagic consumers. This dissertation synthesizes a growing body of scientific evidence supporting the hypothesis that hydrothermal plumes are the energetic basis of unique deep-sea pelagic food webs, including secondary and tertiary consumers. Contemporary large-scale plume events associated with periods of eruptive activity suggest that hydrothermal plumes may have influenced basin-scale ocean chemistry during periods of increased submarine volcanism. The author speculates that hydrothermal plumes are capable of driving both positive and negative impacts to pelagic communities on broad spatiotemporal scales. Increased research efforts, focused on high-resolution surveys of midwater biology relative to plume structures, are recommended to establish baseline conditions and aid in monitoring the impact of anthropogenic disturbances to the deep-sea pelagic biosphere.

Present-day plume environments constrained by seafloor bathymetry may be analogous to hypothetical large-scale plume events in geologic history. To investigate this relationship, biogeochemical exploration was conducted on Kavachi, a highly active submarine volcano in the Solomon Islands. This expedition was serendipitously timed with a rare lull in volcanic

activity, allowing for observation of the inside of Kavachi's active crater and its flanks. Our research efforts resulted in medium-resolution bathymetry of the main peak paired with benthic imagery, biological observations of multiple trophic levels inside the active crater, petrological and geochemical analysis of samples from the crater rim, measurements of water temperature and gas flux over the summit, and descriptions of the hydrothermal plume structure. Populations of gelatinous animals, small fish, and sharks were observed inside the active crater, raising new questions about the ecology of active submarine volcanoes and the extreme environments in which large marine animals can exist.

Observing pelagic biology in high spatiotemporal resolution presents unique challenges, characterized by the difficult task of measuring free-swimming animals in a three-dimensional environment. Several methods have arisen using bioluminescence as an assay for total living biomass. Building upon these efforts, a scientific complementary-metal-oxide-semiconductor (sCMOS) microscopy camera was outfitted for deep-sea imaging of marine bioluminescence. This system was deployed on multiple platforms (manned submersible, remotely operated vehicle, and towed body) in three oceanic regions (Western Tropical Pacific, Eastern Equatorial Pacific, and Northwestern Atlantic) to depths up to 2500 m. Using light stimulation, bioluminescent responses were recorded at high frame rates and in high resolution, offering unprecedented low-light imagery of deep-sea bioluminescence *in situ*. The kinematics of light production in several

zooplankton groups was observed, and luminescent responses at different depths were quantified as intensity vs. time. These initial results signify a clear advancement in the bioluminescent imaging methods available for observation and experimentation in the deep-sea. The presented method may be applicable to large-scale, high-resolution surveys of pelagic biology in the context of deep-sea hydrothermal plumes.

ACKNOWLEDGMENTS

First and foremost: I would like to thank my major advisor Dr. Chris Roman for mentoring and supporting my Ph.D research. He was an essential resource for developing ideas, supporting proposals, and presenting important opportunities for equipment development and fieldwork. Chris is also one of the best teachers I have ever met; while serving as his teaching assistant, I dramatically improved my communication skills. Above all, I am grateful for Chris's wisdom, open mind, and fair judgment.

I would like to express deep appreciation to Dr. Karen Wishner, who challenged me to improve my science on all fronts, from developing strong hypotheses to communicating in a critical and direct manner. She constrained and improved many of my ideas through constructive criticism and open conversation. Karen's lifelong experience and passion for oceanography is obvious, and I was honored to serve as her teaching assistant.

Dr. Brad Seibel is the reason I decided to return to graduate school to earn my Ph.D. His early encouragement and continued support was essential to my success, and I am grateful and honored to have him serve on my committee.

I am indebted to Dr. Steven Carey for his continual support of my ideas, his collaboration with me on several important projects related to my dissertation work, and his friendship. Steve's wisdom, positive attitude, and passion for marine geology are infectious and I consider him a great role model.

A special thank you goes to Dr. Graham Forrester for serving on my exam and defense committees, and for his rational and positive leadership in this role.

I would like to thank Dr. David Smith for advising me on several important decisions throughout my graduate studies. The URI Graduate School of Oceanography is extremely lucky to have such a generous, accessible, and engaged Academic Dean.

Dr. Robert Ballard has influenced my career many times since we first met more than 13 years ago. Bob allowed me to attain my childhood dream, and I will never forget the incredible and life-changing opportunities that he exposed me to. Along with his acknowledgement comes a special thank you to Dr. Dwight Coleman, Dr. Katy Croff Bell, Dr. Nicole Raineault, Alexandra Witten and the Ocean Exploration Trust for supporting me as I transitioned in my role from engineer to scientist.

I am deeply grateful to Dr. David Gruber, Dr. Vincent Pieribone, and Dr. John Sparks for including me in their creative, cutting-edge science and providing me with research opportunities that I never could have imagined. I am proud to consider them both friends and colleagues.

I am grateful to the following staff at the University of Rhode Island for their various forms of support throughout my graduate studies: Derek Sutcliffe, Anya Hanson, Meredith Clark, Amy Smith, April Pariseault, Bob Sands, Deb Coty, Erma Celani, and Helen Selvestri.

Most importantly, none of this would have been possible without the love, support, and patience of my family. I could not have done anything without my wife, to whom this dissertation is dedicated. My young son Murray, while he doesn't know it (yet), was the primary motivating factor for me in the final stages of my research and writing while simultaneously providing a welcome diversion. My parents, Ted and Pat Phillips, and my brother, Simon Phillips, kept me focused and grounded, and I am eternally grateful for their love and support. My extended family, in particular Sue and Ralph Bodington, Steve Smith and Maria Gooch-Smith, and Ben and Rebecca Smith aided and encouraged me throughout this endeavor.

Finally, I appreciate and acknowledge financial support from the following entities: the Ocean Exploration Trust, the National Geographic Society, the American Museum of Natural History, and the URI Graduate School of Oceanography Alumni Association.

DEDICATION

This dissertation is dedicated to my wife, Sarah Lindley Smith, and my son, Murray August Phillips. Sarah, your unwavering support and encouragement was the only way I could have ever accomplished this body of work. The expeditions that took me far away from home, the long hours and late nights when I actually was home, and my teaching schedule required a special strength from you, as my partner to make it all happen. Murray, you are the light of my life and the reason I did all of this in the first place. I learned you were coming into this world during the first year of my studies and nothing could have motivate me more to finish the job. Never give up on your dreams; you can always achieve them with hard work, determination, and passion. I love you both with all of my heart.

PREFACE

This dissertation is comprised of three chapters, each of which is presented as a journal-format manuscript. Together, they explore the influence of hydrothermal plumes on the deep-sea pelagic biosphere, using unique observations of an unexplored active submarine volcano as a case example, and introduce a new, original method to assess pelagic living biomass using light-stimulated bioluminescence.

Chapter 1 (Title: “Beyond the vent: New perspectives on hydrothermal plumes and pelagic biology”) comprehensively reviews all published literature on hydrothermal plumes and their influence on pelagic animals, with several of my own observations and data included in the discussion. I presented on this topic at the 14th Deep-Sea Biology Symposium in September 2015 in Aveiro, Portugal as part of a special session on the environmental impacts of deep-sea mining. The manuscript presented in this dissertation currently in review for a special issue of the journal *Deep-Sea Research II* (communicated February 12, 2016).

Chapter 2 (Title: “Exploring the ‘Sharkcano’: Biogeochemical observations of the Kavachi submarine volcano (Solomon Islands)”) presents novel observations of one of the most active submarine volcanoes in the world. The National Geographic Society funded this project, and I was the principal investigator for the proposal and fieldwork. While I lead research efforts and the resulting manuscript for this project, it involved collaboration

with several other scientists based at the University of Rhode Island, the University of Queensland, Queensland University of Technology, and the Bigelow Laboratory for Ocean Sciences. I presented a poster on this topic at the 2015 American Geophysical Union Fall Meeting in San Francisco in December 2015. The manuscript in this dissertation is in review for the journal *Oceanography Magazine*.

Chapter 3 (Title: “Observations of *in situ* deep-sea marine bioluminescence with a high-speed, high resolution sCMOS camera”) introduces a new method to observe bioluminescence using a state-of-the-art low-light camera and strobe-light stimulation to elicit luminescent responses from pelagic animals. The method, given further development, may be applicable to studying hydrothermal plumes and the distribution of pelagic animals in and around them using common deep-sea research platforms such as submersibles, ROV’s, and autonomous vehicles. I, and co-authors on this manuscript, have several proposals in various stages of review to continue this work. I presented on this topic at the American Geophysical Union Ocean Sciences conference in New Orleans in February 2016. The manuscript presented in this dissertation has been accepted to the journal *Deep-Sea Research I* (communicated October 14, 2015, accepted February 19, 2016). A related news article presenting a significant result from this work has also been accepted for peer-reviewed publication in the journal *Oceanography*, 29-2, 2016 (see Appendix A).

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Table 1: Reference list of studies observing bioluminescence *in situ*

Chapter 1

A manuscript in review, *Deep-Sea Research II*

Beyond the vent: new perspectives on hydrothermal plumes and pelagic biology

By

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Abstract

Submarine hydrothermal vent fields introduce buoyant plumes of chemically altered seawater to the deep-sea water column. Chemoautotrophic microbes exploit this energy source, facilitating seafloor-based primary production that evidence suggests may transfer to pelagic consumers. While most hydrothermal plumes have relatively small volumes, there are recent examples of large-scale plume events associated with periods of eruptive activity, which have had a pronounced effect on water-column biology. This correlation suggests that hydrothermal plumes may have influenced basin-scale ocean chemistry during periods of increased submarine volcanism during the Phanerozoic eon. This paper synthesizes a growing body of scientific evidence supporting the hypothesis that hydrothermal plumes are the energetic basis of unique deep-sea pelagic food webs. While many important questions remain concerning the biology of hydrothermal plumes, this discussion is not present in ongoing management efforts related to seafloor massive sulfide (SMS) mining. Increased research efforts, focused on high-resolution surveys of midwater biology relative to plume structures, are recommended to establish baseline conditions and monitor the impact of future mining-based disturbances to the pelagic biosphere.

1. Introduction

Hydrothermal vent systems exist in every ocean and are associated with a diverse range of geological and oceanographic settings (reviewed by Baker and German 2004). Since their initial discovery in 1977, an entire research community has grown around their study, with new findings reported every year. Hydrothermal fields have characteristically diverse morphology and geochemistry, spanning the extremely deep, hot, and metal-rich ‘black smoker’ vent field recently discovered in the Mid-Cayman Spreading Center (Connelly et al. 2012, Kinsey and German 2013) to the relatively shallow, lukewarm and serpentinite-hosted Lost City vent field on the Mid-Atlantic Ridge (Kelley et al. 2001, 2005). Unique fluid compositions, resulting from fluid-rock interactions occurring in the seafloor reaction zone (Amend et al. 2011), host chemosynthetic communities with distinct biogeographic provinces on a global scale (Bachraty et al. 2009, Van Dover 2011, Rogers et al. 2012). Buoyant hydrothermal plumes are just as diverse in chemistry and size, with some “megaplumes” encompassing thousands of km³ in volume (Baker et al. 1987, Murton et al. 2006). Given the known global distribution of hydrothermal vents, hydrothermal plumes are recognized as an important mechanism for thermal and chemical fluxes at the ocean basin scale (Hannington et al. 2005), as transport mechanisms for vent-endemic larvae (Tyler and Young 2003) and tracers for major deep-sea circulation patterns (Lupton 1995).

The influence of hydrothermal plumes on midwater biology is an embryonic research topic receiving increased attention by the oceanographic research community. While the population dynamics of deep-sea pelagic fauna is poorly understood, direct measurements of enriched microbiological activity inside plumes are now paralleled with observations of elevated biomass associated with higher trophic levels. Synthesizing these results endeavors to improve our understanding of hydrothermal systems on a global scale, and draws hypotheses linking the geochronology of seafloor volcanism to the evolutionary history of pelagic fauna. Furthermore, a recent surge in efforts to promote deep-sea mining of hydrothermal vent fields has prompted numerous calls for precautionary management of these environments (Halfar & Fujita 2002, Hoagland et al. 2010, Van Dover 2011).

This paper presents a comprehensive synthesis of known and inferred biological and ecological processes associated with hydrothermal plumes. This broad assessment is placed in context with geochronologic evidence of pulsed seafloor volcanism during the Phanerozoic eon (0-500 Mya), with present-day conditions likely driving minimal hydrothermal fluid output scenario on a global scale. Based on these considerations, the author advocates for increased research efforts to study the biology of hydrothermal plumes, particularly in the context of SMS mining. Such activities have the potential to dramatically alter natural plume-based ecosystems, which currently do not have an established baseline.

2. Review: hydrothermal plumes and pelagic biology

2.1 Hydrothermal plumes in space and time

The combined effects of temperature, pressure, and geologic setting drive a diverse range of fluid compositions at the approximately 600 known or inferred submarine hydrothermal vent fields in the world's oceans (Hannington et al. 2005, Beaulieu et al. 2013). Buoyant plumes originating from seafloor vents entrain background seawater as they rise, allowing them to grow in volume until they achieve a depth of neutral buoyancy where they continue to spread laterally (Baker et al. 1995, Lupton 1995). If incidence along a ridge is high enough, singular-source plumes can combine into a larger volume; this effect is further pronounced in slow-spreading ridges with deep axial valleys and other constraining bathymetry. While off-axis, diffuse flow venting may account for heat and water flux at more than an order of magnitude higher than on-axis high temperature flow (Elderfield and Schultz 1996), such fluid sources are not considered in this discussion based on the assumption that they do not rise high enough into the water column to influence the pelagic environment.

Quantifying the size of hydrothermal plumes is problematic due to the non-conservative nature and/or low concentrations of many measurable properties. Hydrographic and optical tracers (temperature, salinity, optical backscatter, light attenuation), whilst easy to measure *in situ*, dissipate quickly

and may present a low-end estimate of plume size. Suspended particles in particular are subject to precipitation and sinking mediated by both physical and biological processes (Breier et al. 2012). Chemical tracers can be used to detect hydrothermal plumes at significant distances from their source, but in most cases must also be considered non-conservative due to microbial and oxidation gradients. For example, dissolved iron is subject to rapid biogeochemical speciation in the deep-sea (Taliabue et al. 2010, Toner et al. 2012), which complicates flux estimation and makes its utility as a tracer quite subjective. In contrast, primordial helium (^3He) is a stable, non-biologically labile isotope that can be used as a conservative tracer to detect plumes thousands of km's from ridge-axis sources (e.g. Stommel 1982, Lupton 1998, Rth et al. 2000).

The relatively small size of present-day hydrothermal plumes that are measured using labile tracers compared to the total volume of the modern ocean has led to a general notion that their effect on basin-scale midwater biology is insignificant. There is, however, a line of reasoning that demonstrates this may not have always been the case. Seafloor spreading encompasses a wide range of velocities and patterns, with characteristic examples including the ultra-slow spreading Mid-Cayman Rise ($<20 \text{ mm yr}^{-1}$) and the fast-spreading East Pacific Rise ($100\text{-}150 \text{ mm yr}^{-1}$). Global average spreading rates for the observable lithosphere is now well established, limited by the maximum age of the seafloor to approximately 280 Mya (Mller et al. 2008). However, volcanism associated with mid-ocean spreading is

characteristically episodic and spreading rates vary on multiple periodicities. These eruptive cycles are ultimately driven by mantle plumes but are also sensitive to tidal patterns on the <1yr scale (Wilcock 2001) and long-term variations in sea level associated with Milankovitch cycles on the 10-100kyr scale (Lund and Asimow 2011, Tolstoy 2015, Crowley et al. 2015).

Crucially, patterns of seafloor spreading and the resulting volcanism exhibit a direct correlation with hydrothermal circulation and plume incidence (Baker et al. 1995, Baker and German 2004). Heat and mass flux calculations based on present-day observations indicate that the entire mass of the world's oceans cycles through mid-ocean ridges every ~10 My (Elderfield and Schultz 1996), but the pulsed nature of seafloor volcanism influences hydrothermal circulation on shorter time scales (e.g. Lalou et al. 1995, Chu et al. 2006, Lund and Asimow 2011). Recently observed “megaplumes” are examples of high-output plume events that can extend for 10s of kilometers laterally and beyond 1000m above the seafloor (Baker et al. 1987, Murton et al. 2006).

Asymmetric sedimentation patterns on the Western ridge flank of the East Pacific Rise align with present-day ^3He plume signals originating from the spreading axis, and may be the result of biologically enriched plume fallout persisting for >7 Mya (Hauschild et al. 2003). Given the current observed state of suppressed global volcanism due to relatively high sea level and low orbital eccentricity, it is reasonable to hypothesize that these are minimal examples of large-scale hydrothermal plumes.

Basin-scale changes in ocean chemistry, such as oceanic anoxic and euxenic events in the past 500 Mya, exhibit correlation with submarine volcanism and hydrothermal flux. The Cenomanian-Turonian boundary, a global ocean anoxic event centered at approximately 90 Mya that led to the extinction of almost a third of marine invertebrate species, is thought to be the result of a massive hydrothermal output event that introduced enough reduced inorganic material to cause basin-scale declines in dissolved oxygen (Sinton and Duncan 1997). This event may have persisted for up to 40 My based on magnetic reversal evidence in the South Pacific (Larson 1991) and has been directly connected to magmatic pulsing (Turgeon and Creaser 2008). A similar explanation has been proposed for the Jurassic oceanic anoxic event ~183 Mya (Jones and Jenkyns 2001). Global glaciation may have compounded the effect of increased hydrothermal flux driving changes to ocean chemistry (Gernon et al. 2016), and other biologically-mediated positive feedbacks may have enhanced oxygen depletion leading to euxinic conditions (Meyer and Kump 2008). The biogeochemistry of present-day hydrothermal vent plumes may offer a glimpse into how these environments functioned on much larger spatiotemporal scales.

2.2 Hydrothermal plume ecology

While thousands of publications address the biology of hydrothermal vent systems, fewer than one hundred papers focus specifically on the role of

hydrothermal plumes (Figure 1). These reports are fairly evenly distributed among the topics of larval dispersion, microbiology, and zooplankton, but the absence of a single publication addressing tertiary consumers (such as mesopelagic fishes) in relation to hydrothermal plumes is noteworthy. The following sections synthesize the findings of the publications presented in Figure 1.

Fig. 1.1: The number of primary journal articles published since 1985 that associate midwater biology and hydrothermal plumes. “Larval Dispersion” papers are restricted to studies on vent-endemic larvae. Based on a survey of Web of Science, Google Scholar and references therein.

Microbiology

Seafloor biological communities associated with vent-derived chemoautotrophy are well documented and thoroughly reviewed by Van Dover (2000). Comparatively few studies address microbial life in hydrothermal plumes, but all evidence indicates an enrichment of chemoautotrophic biomass within these unique midwater environments. These communities may originate from the subseafloor and enter the ocean via original fluid discharge, as well as derive from background seawater entrained within the plume. Early work on the Endeavour Segment of Juan de Fuca Ridge gave the first evidence of elevated bacterial biomass in hydrothermal plumes (Winn et al. 1986). Trace metal scavenging mediated by microbial activity within a plume was observed several years later (Cowen et al. 1990, Cowen and Li 1991), closely followed by evidence of an associated increase in methane oxidation (De Angelis et al. 1993). At the same site, sinking biogenic particulate matter from the surface appeared to scavenge hydrothermally-sourced Fe within the midwater plume (Bertram et al. 2002). A major eruptive event on the nearby Gorda Ridge allowed for observations of 2-3 fold increases of bacterial abundance in the resulting plumes (Juniper et al. 1998) largely associated with manganese-precipitating bacteria (Cowen et al. 1998). Observations following the 1998 eruption of Axial Seamount delivered similar results, with significantly higher counts of bacteria measured relative to background seawater (Cowen et al. 1999).

Evidence of plume-associated microbiology has now been observed in several oceanic basins. Sunamura et al. (2004) found a permanent sulfur-reducing microbial population within a plume physically constrained by an active submarine volcano's caldera in the Izu-Bonin Arc. Recently, Bennett et al. (2013) reported on three distinct zones of chemoautotrophic primary production occurring in the plume overlying the Von Damm vent field in the Cayman Spreading Center, largely driven by methanotrophic bacteria. A range of enriched chemoautotrophic processes associated with plumes in the Guaymas and Lau Basins is described by Dick et al. (2013), notably mediated by diverse communities similar to background seawater but with higher specific abundances (Dick and Tebo 2010) and largely dominated by sulfur-reducing microorganisms (Anantharaman et al. 2016). Inconclusive evidence of vent-associated pelagic microbiology over Nordic Sea vents is presented in Olsen et al. (2014). The interplay of microbial activity relative to an ephemeral hydrothermal plume was recently examined by an integrated monitoring program following the submarine eruption of El Hierro (Canary Islands). The massive discharge of acidic fluids enriched in dissolved Fe, reduced sulfur species, and CO₂ led to a completely anoxic condition in the core of the midwater plume (Santana-Casiano et al. 2013), followed by increased abundance and decreased species richness of heterotrophic bacteria throughout the water column (Ferrera et al. 2015). When the eruption ended approximately 6 months later, the water column returned to normal conditions.

Vent-associated larvae

Vent-endemic larvae and their post-larval forms present another source of biological enrichment within hydrothermal plumes (Mullineaux et al. 1995). The planktonic stages of these species can live for weeks to months, facilitating dispersal over 100s of kilometers and directly influencing the global biogeography of hydrothermal vent communities (reviewed in Tyler and Young, 2003). While estimates of larval abundance in plumes are orders of magnitude lower than microbial abundance (e.g. Mullineaux et al. 2005, Bennett et al. 2013), these energy-rich food sources may be specifically targeted by larger midwater predators (Lindsay et al. 2015). Given the distinct vent-associated biogeographic provinces now defined on a global scale for benthic communities (Bachraty et al. 2009, Van Dover 2010, Rogers et al. 2012) such ecological diversity may also be true for plume-associated biological communities and their larval constituents. The known hydrological barriers and corridors governing larval dispersion among hydrothermal vent faunas (Thomson et al. 2003, Adams et al. 2012) may offer a first-order evaluation of these pelagic provinces.

Zooplankton

Pathways of plume-mediated chemosynthetic production to higher trophic levels have not been directly observed, but there is growing evidence that such connections exist. Enriched, upward-directed lipid and POC flux

associated with secondary production was first measured at the Endeavour Segment of Juan de Fuca Ridge using moored inverted sediment traps (Cowen et al. 2001, Wakeham et al. 2001). Burd and Thomson (1995, 2012, 2015) have repeatedly observed a broad enrichment of biomass and secondary production in the water column above the Endeavour Vent Field using towed multiple-net equipment paired with backscatter measurements from acoustic Doppler current meters. Some of this research has also pointed to an increased abundance of predacious gelatinous zooplankton that may be associated with enriched microzooplankton biomass within plumes (Burd and Thomson 2000). Early work on the East Pacific Rise and within Guaymas Basin also indicate enriched benthopelagic zooplankton populations directly above and surrounding active hydrothermal vent fields (Smith 1985, Berg and Van Dover 1987, Weibe et al. 1988).

Visual-based methods to observe zooplankton distributions relative to hydrothermal plumes have produced largely inconclusive results, despite the extensive deployment of ROV's and manned submersibles to hydrothermal vent sites. Video-based quantification of zooplankton and nekton populations at Juan de Fuca Ridge revealed a complex system heavily influenced by physical circulation patterns, with distinct clustering of major organism groups relative to plume and bathymetric zones (Skebo et al. 2006). Total abundances of macroplankton relative to the Lost City and Broken Spur hydrothermal vent fields appear unrelated to these features (Vinogradov and Vereshchaka 2006), but dense aggregations of salps have been observed at

Lost City offset from their expected diel vertical migration pattern and near the maximum of their known depth range (Figure 2; author, personal observations). A diverse community of gelatinous zooplankton was observed in waters overlying a vent field in the Okinawa Trough (Lindsay et al. 2015); notably, appendicularian larvaceans were measured in high abundances relative to turbid plume waters. Similarly, larvaceans were seen in dense aggregations inside a hot ($>43^{\circ}\text{C}$), acidic ($<6.1\text{ pH}$) and turbid plume at a shallow-water volcanic vent in the Solomon Islands (Figure 2). Given that filter-feeding appendicularians have the ability to capture suspended picoplankton (Bedo et al. 1993, Gorsky et al. 1999), they are uniquely suited to exploit chemosynthetic bacteria as an energy source.

Tertiary consumers

Associations between hydrothermal plumes and tertiary consumers are hypothesized based on several noteworthy reports and observations. Coincident acoustic backscatter and deep-water trawl data give evidence of enriched fish biomass relative to the Mid-Atlantic Ridge (Sutton et al. 2008). While a recent review concluded that seafloor biological productivity likely had no influence on these observations (Priede et al. 2013), the studies were not targeting specific geographic regions of hydrothermal venting. Burd and Thomson (2015) present a strong hypothesis for an associative pattern of whale feeding and hydrothermal plume-associated zooplankton at the Endeavour Segment of the Juan de Fuca Ridge; this is best supported by

acoustic behavior studies on fin whales in the region (Soule and Wilcock 2013). In addition to swarms of larvaceans, two species of reef fish and two species of shark appeared to be living within the hydrothermal plume of the Solomon Islands submarine volcano, where catastrophic eruptions occur on a monthly basis (Figure 2; author, unpublished results). Tagged leatherback turtles have been repeatedly observed circling the same volcano in a pattern consistent with feeding behavior (Albert et al. 2014). These collective observations, while lacking definitive conclusions, inspire further questioning and highlight the potential influence of hydrothermal plumes to the pelagic biosphere.

Fig. 1.2: *Top row:* Down-looking view of a *Salpa* sp. swarm with the *HERCULES* ROV in center frame, taken from ~30m altitude above the Lost City vent field at a depth of ~750m (image courtesy of NOAA Ocean Exploration/Institute for Exploration/Inner Space Center). *Middle row:* larvaceans inside the Kavachi volcano crater circled in green (left, image copyright The National Geographic Society) and a close-up view of a similar larvacean observed in dense numbers at the Hatoma Knoll hydrothermal vent field (right, image courtesy of D. Lindsay). *Bottom row:* A hammerhead shark, *Sphyrna lewini* (left) and bluefin trevally, *Caranx melampygus* observed inside the Kavachi volcano crater (both images copyright The National Geographic Society).

3. Discussion

The spatiotemporal boundaries of hydrothermal plumes are characteristically ephemeral and span broad biogeochemical gradients. In contrast, most methodology used to observe the biology inside and around plumes do not adequately address this dynamic nature. The temporal patterns of hydrothermal venting at specific study sites, a nascent research topic of its own, must be considered to place future biological observations in proper context. With the notable exception of work presented by Burd and Thomson (2015), which includes observations made over two decades at the Juan de Fuca Ridge, most investigations into plume-associated biology have taken place on singular oceanographic cruises. In several instances published conclusions have been made based on a single submersible or ROV dive.

Observing pelagic populations in high spatial resolution is a characteristic challenge in biological oceanography. The approximate 100-meter depth intervals that most surface-controlled multiple net systems can achieve may be too coarse to adequately resolve spatial patterns associated with hydrothermal plumes. The extreme bathymetry generally associated with active spreading centers further complicates the use of towed nets to assess zooplankton distribution. New methods centered on advanced acoustic and video-based technology may address this issue; for example, AUV-based high-resolution acoustic surveys have demonstrated an approximate 5-meter critical scale necessary to resolve patchiness in deep-sea zooplankton (Benoit-Bird et al. 2013). The video transects made in Endeavour Axial Valley

approach the 10-meter scale (Skebo et al. 2006), and are a good example of a high spatial resolution survey applied to a hydrothermal plume. More studies such as these, conducted on multiple time scales, are needed; highly maneuverable autonomous platforms and long-term deployment ocean observatories may be critical to achieving success in this regard.

Despite worldwide interest from a diverse set of stakeholders, the potential impact of deep-sea seafloor massive sulfide (SMS) mining on existing plume-associated pelagic biology has essentially been ignored. An important distinction must be made: the introduction of sediment plumes from SMS mining, whether from seafloor extraction or from surface processing, is a completely different consideration than the potential disturbance of altering and/or removing existing hydrothermal fluid sources to the water column. The former topic of sediment plumes has received much attention in recent policy literature (Boschen et al. 2013) and has been the subject of at least three deep-sea experiments related to SMS and manganese nodule mining (Fukushima 1995, Borowski and Thiel 1998, Sharma et al. 2001).

Paradoxically, there appears to be no mention of hydrothermal plume-associated biology in SMS mining policy literature, and all published work on plume-associated biology (Figure 1) fails to draw connections to the long-standing threat of SMS mining. Given the observations and hypotheses presented in this work regarding secondary and tertiary consumer associations with hydrothermal plumes, and the relatively unexplored nature of

hydrothermal systems in general, it appears that an entire ecosystem is being overlooked by SMS policy stakeholders.

The International Seabed Authority (ISA) has long recognized the need to establish a proper baseline from which to judge the potential impacts of SMS mining to deep-sea ecosystems (e.g. ISA Technical Study #9 2011, ISA Technical Study #10 2011). Potential disturbance to the natural hydrothermal plume environment is missing from these high-level discussions, and existing scientific evidence is insufficient to properly define a baseline for the ISA to adopt. This presents a compounded problem: to put it simply, we do not know what is out there, and if SMS mining proceeds with the current level of oversight, we will not know if it is gone. Furthermore, efforts to create Marine Protected Areas that include deep-sea vent sites (i.e. Beaulieu et al. 2013) may also wish to include the plume environment in spatial management efforts.

4. Conclusion

This paper reviews a growing body of evidence portraying hydrothermal plumes as a distinct, biologically rich environment in the deep-sea water column. Chemoautotrophic microbial food webs have been observed in association with every plume that has been examined, and a positive relationship with secondary consumers appears to be a common occurrence. A connection between hydrothermal plumes and tertiary

consumers, such as midwater fish, sharks, and marine mammals, is hypothesized based on limited observations. The influence of plumes to ocean chemistry on large spatiotemporal scales is inferred based on the geochronology of submarine volcanism. Despite these wide-ranging considerations, the pelagic biology associated with hydrothermal plumes appears to be missing from deep-sea management efforts, specifically within the context of SMS mining. The author recommends increasing research efforts to incorporate dedicated surveys of pelagic biology relative to plume structure in high spatiotemporal resolution, and an inclusion of hydrothermal plume biology in discussions at the policy and management level for the deep-sea.

Acknowledgements

This paper is the result of a special session on deep-sea mining held at the 14th Deep Sea Biology Symposium in Aveiro, Portugal in September 2015. The author wishes to thank Richard Thomson, Karen Wishner, and two anonymous reviewers for their critical reviews of this manuscript. Dhugal Lindsay, Steven Carey, and Paraskevi Nomikou contributed helpful discussions and imagery.

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Chapter 2

A manuscript in review, *Oceanography Magazine*

Exploring the “Sharkcano”: Biogeochemical observations of the Kavachi submarine volcano (Solomon Islands)

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Ashton Flinders: Processed and interpreted bathymetric data; edited the manuscript.

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Abstract

An expedition to the Kavachi submarine volcano (Solomon Islands) in January 2015 was serendipitously timed with a rare lull in volcanic activity, permitting access to the inside of Kavachi's active crater and its flanks. The isolated location of Kavachi and its explosive behavior normally restricts scientific access to the volcano's summit, limiting previous observational efforts to surface imagery and peripheral water-column data. Here we present medium-resolution bathymetry of the main peak paired with benthic imagery, biological observations of multiple trophic levels living inside the active crater, petrological and geochemical analysis of samples from the crater rim, measurements of water temperature and gas flux over the summit, and descriptions of the hydrothermal plume structure. A second peak was identified to the southwest of the main summit and displayed evidence of diffuse-flow venting. Microbial samples collected from the summit indicate chemosynthetic populations dominated by sulfur-reducing ϵ -proteobacteria. Populations of gelatinous animals, small fish, and sharks were observed inside the active crater, raising new questions about the ecology of active submarine volcanoes and the extreme environments in which large marine animals can exist.

Introduction

The New Georgia Island Group is the result of a complex tectonic configuration, characterized by subduction of the active Woodlark Spreading Center (WSC) beneath the Solomon Islands intra-oceanic arc. This triple junction is the site of active volcanism, crustal uplift that closely matches subducted bathymetric features from the WSC, and a proposed ‘slab window’ that leaves asthenospheric mantle in direct contact with a thin overlying crust (Mann et al., 1998; Chadwick, 2009). The Kavachi submarine volcano ($8^{\circ} 59' \text{ S}$, $157^{\circ} 58' \text{ E}$) is situated $\sim 30\text{km}$ northeast of the subduction zone and rises abruptly to the surface from more than 1000 m of water depth. By far the most active volcano in the region, Kavachi is known for frequent phreatomagmatic and subaerial eruptions leading to occasional ephemeral island emergence (Johnsen and Tuni, 1987). The remote geographic location and inherent danger of frequent eruptions has prevented scientists from observing the proximal area of Kavachi’s summit crater.

Our research team took advantage of a rare lull in volcanic activity and conducted geological, biological and oceanographic observations of Kavachi using relatively simple equipment and methodologies. We report here on our initial results, which include evidence of diffuse-flow venting on a confirmed second peak, a megafaunal community thriving inside the active crater, and insights into the eruptive processes and magma source characteristics of Kavachi’s active volcanism.

Results

Of the roughly 30 active submarine volcanoes known worldwide, only a few have been observed during or closely following an active eruption, e.g. West Mata (Resing et al., 2011), El Hierro (Santana-Casiano et al., 2013), Kick'em Jenny (Devine and Sigurdsson, 1995) and Axial Seamount (Chadwick et al., 2012). Kavachi is continually erupting; reports of airborne steam and ash visible from shore are common, and islands forming and eroding multiple times in the past century indicate the summit is in a constant state of flux. The most detailed investigation of Kavachi prior to this study took place during a single day in May 2000 and was conducted from an approximate 1 km perimeter surrounding the actively erupting main peak (Baker et al., 2002). A magnitude 8.1 earthquake that shook the entire region in April 2007 prompted the reconnaissance of a conspicuous surface plume (Wunderman, 2007). The latest evidence of activity was obtained by NASA's EO-1 satellite on January 29th 2014, and appears similar in behavior to that seen in 2007. Compared with these reports, we believe our study aligns with Kavachi exhibiting its most inactive behavior. Such circumstances allowed for the first up-close look inside and around the shallow summit of the volcano.

a. Edifice Morphology

The summit of Kavachi is an oblong, pockmarked crater, measuring approximately 120 m X 75 m with a lengthwise strike of 115° and rising to an

average of 24 m depth on the rim (Figure 2.1). The deepest soundings on the peak are ~70m deep and indicate asymmetrical terrain, surrounded by almost uniform flanks with 18° slope that descend to depths greater than 1000 m. Video recorded by a SCUBA diver on the crater rim reveals widespread, vigorous gas bubbling originating from the shallowest regions surrounding orange, cloudy hydrothermal plume water inside the crater that almost completely obscures visibility (Figure 2.2A). The diver who recorded this footage reported physical discomfort from caustic water on his skin when he approached the outer line of bubbles.

We confirm the existence of a “SW Extension”, or a secondary summit located 1.3 km southwest of the main summit rising to 260 m with a narrow col between the peaks (Figure 2.1). Previously published bathymetric maps suggested the existence of this feature, but lacked the resolution to define it (Johnson and Tuni, 1987; Baker et al., 2002; Exon and Johnson, 1986). Bottom imagery was collected at 5 sites on the SW Extension, with one image presenting evidence of chemolithoautotrophic bacteria commonly associated with hydrothermal venting (Figure 2.2B).

Fig. 2.1. Bathymetric map of Kavachi and the summit crater (inset, lower right). Red circles indicate locations of water column profiles and benthic imagery; white diamonds are where baited drop cameras were deployed; the blue line delineates the path of surface drifters measuring temperature and atmospheric CO₂.

Fig. 2.2. A) Oblique view of a line of bubbling gas along the outer edge of Kavachi's crater with orange, cloudy plume fluids in the background. B) Down-looking view of a microbial mat on the summit of the SW Extension. C) Bluefin trevally, D) snapper, E) hammerhead shark and F) a silky shark observed using a baited drop camera deployed inside the crater.

b. Hydrothermal Plumes

Opportunities to sample hydrothermal plumes associated with active submarine volcanoes are often limited by safety concerns. Those that have been observed are enriched in suspended particulate matter, ^3He , and sulfur,

and exhibit depressed pH values (Walker et al., 2008). Plume-measuring instruments deployed systematically around Kavachi's summit only detected optical backscatter and oxidation-reduction-potential (ORP) signals directly over the crater. A minor ORP signal was observed to the North of the SW Extension summit, but was not large enough to warrant association with diffuse venting at that site. A surface drifter measuring atmospheric CO₂ and water temperature at 0, 2, and 5 m depths measured ΔT values of >13 °C above ambient (approximately 29 °C) over the crater, which persisted down-current from the main summit (Figure 2.3). A $>300\%$ step increase in atmospheric CO₂ concentration occurred within the surface bubble zone indicating this was the primary source of CO₂. The measured unit area flux rate was $19.5 \text{ kg m}^{-2} \text{ d}^{-1}$, and assuming a similar rate across the whole surface bubble zone the total flux rate was 153 t d^{-1} . This estimate lies within the lower range of previously reported studies (Burton et al., 2013). The increased availability of dissolved CO₂ within the bubble zone provides a labile carbon source for chemolithoautotrophic communities surrounding the venting site.

Our observations align with those made in 2000 by Baker et al. (Baker et al., 2002), who concluded from their perimeter survey that Kavachi's hydrothermal discharge disperses and/or escapes to the atmosphere almost immediately upon reaching the surface. We were unable to constrain the pH of vented fluids directly inside Kavachi's crater, but a single water sample collected within the surface plume measured a pH of 6.1. This compares well with pH measurements of two other active submarine volcanoes at 5 or lower

inside their craters (Staudigel et al., 2006; Kiliyas et al., 2013). We therefore characterize the plume inside of Kavachi's crater as a discrete, 'extreme' environment; hot, acidic, and particulate-laden.

Fig. 2.3. Autonomous drifter data collected over Kavachi's active crater. *Top:* Water temperature at three depth intervals. *Bottom:* Atmospheric CO₂ measured at the surface over roughly the same transect as the temperature drifter. Sections delineated by (A) are upcurrent of the crater, (B) are directly over the crater, and (C) are downcurrent.

c. Biology of the active crater

Seamounts serve as unique and important biological habitats that support increased biomass and biodiversity of benthic and pelagic species (Rogers, 1993; Stocks and Hart, 2008). Active seamounts, defined as those exhibiting fluid/gas venting or active eruption, are of particular interest as they offer chemosynthetic energy pathways for microbial communities to exploit. Benthic scavengers, deposit feeders, and filter feeders can utilize chemosynthetic biomass to support up to 100% of their nutritional input (MacAvoy et al., 2002). Plumes originating from hydrothermal vent fields are known to support increased microbial biomass, which may sustain higher trophic levels in the water column (Bennet et al., 2013; Dick et al., 2013; Burd and Thomson, 2015).

Extensive microbial mats in and around the active crater were visually distinct, with dense orange- and white-colored regions observed on the external flanks to approximately 100 m water depth. Orange-colored samples colonizing scoriaceous tephra were collected and used for 16s rRNA clone library analysis. In total, 12 clones were retrieved with an average length of 1050 base pairs. Phylogenetic analysis (Figure 2.4) revealed that all clones were related to *Sulfurimonas* (Epsilonproteobacteria; Campylobacteraceae; Helicobacteraceae), a genus known for growing chemolithoautotrophically using sulfur and CO₂ in low-pH hydrothermal systems (Yanagawa et al., 2013). In general, Kavachi clones were related to sequences found in other

iron/sulfur-rich hydrothermal systems, including the East Pacific Rise and Lau Basin. Kavachi clones fell into one of three distinct groups within the *Sulfurimonas*. Group 1 was related to several pure culture isolates, although the only highly similar sequence (99% identity) was an uncultured environmental clone retrieved from the Arctic Ocean in 2002. The closest relative of group 2 (95% identity) was a single clone retrieved from particulate detritus associated with the tubeworm *Ridgeia piscesae*, while the closest relative of group 3, was from iron-rich hydrothermal sediments. These *Sulfurimonas* clones may represent unique diversity within the genus, possibly restricted to Kavachi or similar systems. Given the presence of *Sulfurimonas* in clone libraries it is possible that the orange-colored microbial mats seen on the peak of the Southwest Extension indicate the presence of diffuse-flow sulfur- and CO₂-enriched hydrothermal fluids on this external feature. It is curious however that no Zetaproteobacteria were recovered during clone library analysis. Typically these bacteria are associated with orange-colored mat material in marine hydrothermal systems and indicate active iron oxidation. Organisms such as *Sulfurimonas* are typically associated with white-colored mats indicative of sulfur oxidation. More thorough geochemical and microbiological analyses are needed to elucidate the role of microbes in geochemical cycling at Kavachi.

Baited drop-camera deployments inside Kavachi's crater at 50 m depth revealed multiple species of fish and zooplankton, apparently associated with active venting processes (Figure 2.2C-F). Two species of shark, the scalloped

hammerhead *Sphyrna lewini* and the silky shark *Carcharhinus falciformis*, approached the baited camera multiple times in an aggressive pattern; in some cases, sharks appeared to be swimming from greater depths inside the crater. Bluefin trevally, *Caranx melampygus*, and a *Lutjanus* sp. snapper were also seen; notably, these groups of fish were impacted the least on reefs following a subaerial volcanic eruption in the Mariana Archipelago (Vroom and Zglicynski, 2011). Appendicularians (pelagic tunicates) drift in abundance throughout the recordings, possibly correlating with similar observations of an *Oikopleura* sp. seen in great densities inside a hydrothermal plume at Okinawa Trough (Lindsay et al., 2015). In two other active submarine volcanoes, Vailulu'u Seamount (American Samoa) and Kolumbo (Greece), the craters are dominated by microbial mats and "kill zones" where carcasses of larger animals such as fish are found on the seafloor. It is likely that the high crater walls at these sites cause physical entrainment and concentration of vent fluids, while Kavachi's crater is relatively shallow and subjected to high surface currents allowing for rapid mixing to occur.

Fig 2.4: Phylogenetic tree of 16S rRNA clones retrieved from orange-colored microbial mats collected from scoriaceous tephra at the crater rim. All 12 clones fell into three distinct clades within the genus *Sulfurimonas*.

Baited drop-camera deployments inside Kavachi's crater revealed multiple species of fish and zooplankton, apparently associated with active venting processes (Figure 2.3 C-F). Two species of shark, the scalloped hammerhead *Sphyrna lewini* and the silky shark *Carcharhinus falciformis*, approached the baited camera multiple times in an aggressive pattern; in some cases, sharks appeared to be swimming from greater depths inside the crater. Bluefin trevally, *Caranx melampygus*, and a *Lutjanus* sp. snapper were also seen; notably, these groups of fish were impacted the least on reefs following a subaerial volcanic eruption in the Mariana Archipelago (Vroom &

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d. Petrology/geologic samples

Three volcanic samples collected from the shallow rim of Kavachi's crater (~25 m depth) encompass a broad range of eruptive textures, from vesicular scoria to dense lava flow, to scoriaceous tephra. These are the only samples collected directly from the summit area during this recent stage of activity. Visual analysis of thin-section slides indicates that Kavachi's explosive eruptions are driven by a combination of primary volatile degassing and phreatomagmatic interactions (Figure 2.5 A&B). Major element analysis bulk samples indicates they are basaltic and relatively mafic at ~6 wt.% MgO. Trace elements (Figure 2.5C) show patterns typical of arc lavas, although Ba/La and Ba/Nb ratios of Kavachi are among the highest of the entire

Solomon Arc, suggesting an extreme composition for the subducted slab-derived fluid. Ratios of moderately- to highly-incompatible high field strength elements, which are thought to reflect the mantle source composition independent of slab-derived influences, are also among the highest in the Solomon Islands (e.g. $Zr/Nb = 93-95$) suggesting that the mantle source of Kavachi is highly depleted. Given Kavachi's position between the trench and the main Solomon volcanic front, the volcano likely taps a fore-arc mantle that is highly depleted due to prior melting beneath the main arc front volcanoes.

Fig. 2.5: A) Photomicrographs of basaltic lava from Kavachi summit crater with phenocrysts of plagioclase-clinopyroxene-olivine set in a groundmass of microlite-rich glass. Left image is transmitted light, right image is crossed polars, and each image is 2.5 mm in width. B) Photomicrograph of basaltic scoria from Kavachi summit crater with phenocrysts (conspicuous crystals) of plagioclase-clinopyroxene-olivine and abundant vesicles (left image is transmitted light, right image is crossed polars) C) NMORB-normalized trace element diagram after Hofmann (1988). Thick, solid lines are three whole-rock Kavachi lava/tephra samples from this study, and the thin solid line is a highly depleted NMORB for reference. Compositions are normalized to the NMORB of Sun and McDonough (1989).

Discussion

Our observations of primary, secondary and tertiary consumers inside Kavachi's hydrothermal plume contributes to ongoing research into the physiological and behavioral resiliency of marine animals to increased temperature and acidity due to climate change, as well as increased water turbidity due to sediment loading. Appendicularians demonstrate a positive response to decreased pH values (Troedsson et al., 2013), which may explain the apparent plume-associative behavior seen among *Oikopleura* sp. Glass tunicates, gelatinous chordates in the same subphylum as appendicularians, were the only observed metazoan living inside the Kolumbo submarine caldera 'kill zone' (Sigurdsson et al., 2006; Carey et al., 2013). Given their apparent resiliency to hydrothermal fluids and their unique ability to filter-feed on picoplankton (Bedo et al., 1993; Gorsky et al., 1999; Ribes et al., 2003; Lesser and Slattery, 2015), animals such as *Oikopleura* sp. and glass tunicates could mediate an energy pathway for suspended chemolithoautotroph production into higher trophic levels. While no study has yet to directly investigate oceanic climate change effects on bluefin trevally or snapper, cardinalfish and damselfish exhibit decreased metabolic performance and significant mortality when exposed to temperatures above 32 °C (Munday et al., 2009; Johansen and Jones, 2011) and depressed antipredator responses when exposed to increased CO₂ concentrations (Ferrari et al., 2011; Munday et al., 2014). Elevations in water turbidity are a well-established stressor to reef fish (Wenger et al., 2011; Wenger et al., 2013;

Hess et al., 2015). Elasmobranchs demonstrate behavioral thermoregulation with an affinity for warmer temperatures (Schlaff et al., 2014), and while there is evidence suggesting that ocean acidification may have a negative effect on odor tracking in sharks (Dixon et al., 2015), there are also indications of physiological and behavioral resiliency (Heinrich et al., 2014; Heinrich et al., 2015).

Importantly, Kavachi's hydrothermal plume is comparatively extreme relative to climate change-driven ocean temperature and acidification effects. Projections to the year 2100 agree on a maximum Δ -0.4 pH and Δ 4 °C for surface seawater on a global scale (Pörter et al., 2014), compared to our estimated Δ -1.9 pH and Δ 13 °C between Kavachi's plume and the surrounding surface waters. These extreme conditions, however, may be representative of ocean basin-scale 'megaplume' events caused by periods of increased volcanism in Earth's geologic history (e.g. Sinton and Duncan, 1997). A further understanding of the physiology and behavior of organisms that thrive in these environments could offer new insights into the evolutionary history of marine animals.

This study offers unique observations of one of the most active submarine volcanoes in the world. Kavachi's ephemerally active summit area consists of a shallow oblong crater and a small secondary peak to the southwest that exhibits signs of diffuse-flow hydrothermal venting. Mafic basaltic lavas collected from the summit are among the most extreme compositions in the Solomon arc, likely reflecting an unusual tectonic position

between the trench and the main line of arc volcanoes. Inside the crater, vigorous fluxing of CO₂-rich gas and warm turbid water convect towards the surface and are rapidly diluted and dispersed by the prevailing currents. Bacteria collected from Kavachi's summit indicate these fluids are enriched in sulfur, coupled with high-temperature and low-pH measurements made inside the surface plume. Surprisingly, this hostile environment hosts a vibrant ecosystem including gelatinous zooplankton, reef fish, and sharks. The appearance of these animals apparently thriving in such extreme conditions poses new questions centered on the resiliency of marine animals to rapid changes in their environment. If larvaceans, elasmobranchs and other fish species have a particular tolerance for hot and acidic water, do these groups have a greater chance of surviving human-induced changes to ocean chemistry and periods of increased submarine volcanism on a global scale? What happens to the crater community before, during and after an eruption, and how often do these cycles occur? Kavachi represents a fascinating natural laboratory to investigate these questions and remains full of mysteries to explore.

Conclusion

This study offers unique observations into the crater of one of the most active submarine volcano systems in the world. Kavachi's ephemerally active

summit area consists of a shallow oblong crater and a small secondary peak to the southwest that exhibits signs of diffuse-flow hydrothermal venting. Mafic basaltic lavas are among the most extreme compositions in the Solomon arc, likely reflecting an unusual tectonic position between the trench and the main line of arc volcanoes. Inside the crater, vigorous fluxing of CO₂-rich gas and warm turbid water convect towards the surface and are rapidly diluted and dispersed by the prevailing currents. Bacteria collected from Kavachi's summit indicate these fluids are enriched in sulfur, coupled with high-temperature and low-pH measurements made inside the surface plume. Surprisingly, this apparently toxic environment hosts a vibrant ecosystem including gelatinous zooplankton, reef fish, and sharks. The appearance of these animals apparently thriving in such extreme conditions poses new questions centered on the resiliency of marine animals to rapid changes in their environment. If larvaceans, elasmobranchs and other fish species have a particular tolerance for hot and acidic water, do these groups have a greater chance of surviving human-induced changes to ocean chemistry and periods of increased submarine volcanism on a global scale? What happens to the crater community before, during and after an eruption, and how often do these cycles occur? Kavachi represents a fascinating natural laboratory to investigate these questions and remains full of mysteries to explore.

Methods

This work was conducted in January 2015 with operations based out of Gatokae Island, Western Province, Solomon Islands. All equipment used was lightweight, relatively low-cost, and deployed using small boats. Down-looking images of the seafloor paired with water-column measurements were made with a GoPro camera in a deep-rated housing, battery-powered lights, and a NOAA/PMEL Miniature Autonomous Plume Recorder (Walker et al., 2007) mounted on an instrument frame that was raised and lowered with an electric fishing reel. Baited drop-camera recordings were achieved using an autonomous National Geographic Remote Imaging DropCam following methods described by Friedlander et al. (2014). Surface drifters measuring water temperature, light transmission and atmospheric $p\text{CO}_2$ were deployed as described by Dunbabin et al. (2016). The bubble zone surface area was calculated using a downward looking underwater camera on the hull of an autonomous surface vessel. This vessel logged its position and after multiple runs through the bubble zone it was possible to estimate surface area by noting the appearance and disappearance of bubbles on camera footage. Bathymetry was measured using a Lowrance HDS-5 echosounder (50/200 kHz). IVS Fledermaus software was used to manually remove soundings that qualitatively deviated away from a general trend depicting the volcano, similar to swath-editing in multibeam sonar processing. This “cleaned” point-cloud (~85,000 soundings) was then used to create three individual continuous curvature spline surfaces, each with a unique spatial extent and resolution.

This multi-surface approach was necessary in order to accurately represent varying degrees of data density over the study area, with the most soundings concentrated at the summit caldera, and the least at the foot of the volcano (Flinders et al., 2014). The three surfaces (100 m study-wide, 35 m volcano focused, and 7 m summit focused) were then blended to create a single individual surface for visualization. Blending constraints ensured that within each specific area, only the highest resolution surface would be used.

Rock and bacterial samples were collected by hand using SCUBA divers. Major and trace element analyses were conducted using a Perkin Elmer Optima 3100 XL ICP-AES and a Thermo X-Series 2 quadrupole ICP-MS (Kelley et al., 2003). Sample slides were prepared by the Marine Geological Sample Laboratory at the University of Rhode Island. Bacterial DNA was extracted using PowerSoil (MO BIO Laboratories, Carlsbad CA), PCR amplified using universal 16S primers (27F: AGR GTT YGA TYM TGG CTC AG, 1100R: GGG TTN CGN TCG TTG) and cloned with pGEM-T Easy (Promega). After removal of vector and primer sequences all clones were imported into mothur to screen for putative chimeras and generate an initial alignment against a SILVA-based reference set (SEED version 119). Quality screened and aligned 16S rRNA clones were then imported into the ARB environment, manually curated, and added to the master ARB tree (tree_SSURefNR99_1200_slv_123). Based on the location of Kavachi clones within the tree, we chose an additional 32 related sequences from the ARB database for further phylogenetic analysis, including representatives from

Campylobacter (Campylobacteraceae) and *Sulfuricurvum* (Helicobacteraceae) as outgroups. Where possible 16S sequences from pure cultures were included. Phylogenetic analysis was conducted using rapid bootstrapping (1000 inferences) and subsequent maximum likelihood search within RAxML (v. 8.2.4) under a GAMMA model of rate heterogeneity and GTR substitution matrix. Sequence data is deposited in GenBank under the accession numbers KU761314-KU761325.

Acknowledgements

The authors wish to thank the Solomon Islands government and the Peava Village community for permitting and assisting in our research activities. We thank Dr. Fabio Estaban Amador, Eric Berkenpas, and the National Geographic Society for making this project possible. The NOAA/PMEL VENTS program provided plume-sensing instrumentation. This work was supported by National Geographic/Waitt Grants Program grants #W324-14 and W364-14 to B.T. Phillips.

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Chapter 3

A manuscript published in *Deep-Sea Research II*, 111: 102-109, March 2016

Observations of *in situ* deep-sea marine bioluminescence with a high-speed, high-resolution sCMOS camera

By

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Abstract

Observing and measuring marine bioluminescence *in situ* presents unique challenges, characterized by the difficult task of approaching and imaging weakly illuminated bodies in a three-dimensional environment. To address this problem, a scientific complementary-metal-oxide-semiconductor (sCMOS) microscopy camera was outfitted for deep-sea imaging of marine bioluminescence. This system was deployed on multiple platforms (manned submersible, remotely operated vehicle, and towed body) in three oceanic regions (Western Tropical Pacific, Eastern Equatorial Pacific, and Northwestern Atlantic) to depths up to 2500 m. Using light stimulation, bioluminescent responses were recorded at high frame rates and in high resolution, offering unprecedented low-light imagery of deep-sea bioluminescence *in situ*. The kinematics of light production in several zooplankton groups was observed, and luminescent responses at different depths were quantified as intensity vs. time. These initial results signify a clear advancement in the bioluminescent imaging methods available for observation and experimentation in the deep-sea.

1. Introduction

Marine bioluminescence has generated increasing interest among the scientific community in the past several decades, spanning the disciplines of comparative biology (e.g. Haddock and Case 1999), biochemistry (e.g. Prasher et al. 1992), physiology (e.g. Contag and Bachmann 2002), neuroscience (e.g. Martin 2008), population dynamics (e.g. Moline et al. 2009), and naval applications (e.g. Fucile 2002). This research began with terrestrial fireflies (Green and McElroy 1956) and later expanded to readily accessible marine organisms, most notably bacteria (Bassler et al. 1993; Surette et al 1999), copepods (Campbell and Herring 1990), and cnidarians (Prasher et al. 1985). Whereas almost all major phyla of deep-sea zooplankton exhibit bioluminescence (Haddock et al. 2010), with new discoveries reported annually, only a handful of studies have been published on *in situ* marine bioluminescence. As a result, the deep-sea offers vast potential for future multidisciplinary discoveries related to bioluminescence.

The greatest limitation to observing marine bioluminescence *in situ* is technology. As early as 1955, photomultiplier tubes (PMTs) were employed to quantitatively measure light in the ocean from a single point (Boden and Kampa 1957), a method still used today (Adrián-Martinez et al. 2014, Johnsen et al. 2014, Craig et al. 2015). In the past three decades, a number of studies utilizing Intensified Silicon Intensifier Target (ISIT) cameras were published (Widder et al. 1989, Robison 1992, Priede et al. 2006). ISIT cameras are very

sensitive, but are limited in resolution and retain residual images between frames, offering visually-pleasing images but making them less than ideal quantitative imaging platforms. More recently, image-intensified and electron-multiplied CCD cameras have been employed to measure extremely low light sources in high resolution (e.g. Johnson et al. 2012, Craig et al. 2015).

Scientific complementary-metal-oxide-semiconductor (sCMOS) cameras offer high sensitivity, low noise, wide dynamic range, and high-resolution, high-speed capabilities without image amplification (Coates et al. 2009, Fowler et al. 2009).

We hypothesize that applying sCMOS technology *in situ* offers the ability to study the kinematics of luminescent organisms at an unprecedented resolution, and by pairing this camera with light-stimulation methods, it may be possible to quantify the luminescence of the midwater community in both spatial and temporal scales. Here we present the results of cross-platform deployment of a sCMOS camera *in situ* to depths up to 2500 m, utilizing light stimulation to elicit bioluminescent responses in an exploratory fashion.

2. Methods

A Hamamatsu Photonics ORCA-Flash4.0 V2 sCMOS camera was chosen for its high dynamic range (16-bit), high quantum efficiency (72%), 2048 x 2048 resolution, and high speed (up to 100 fps). All recordings in this study were conducted at 30fps using a global shutter. The camera was fitted

with a Navitar 17 mm $f0.95$ lens and integrated into a 2500 m-rated housing (Prevco) with a flat acrylic viewport (Figure 3.1). The entire system was run on 24 VDC provided from surface power, with a nominal power draw of 100 W. Due to the high data output of the camera, a solid-state computer (Copperhead from VersaLogic, Tualatin, OR) with a 1 TB RAID0 SSD drive array was included in the housing along with a water-cooling pump and focus/aperture control. All internal components producing light, such as LEDs, were blacked out inside the housing. The recorded 16-bit intensity images were processed with Matlab v. R2012a. The camera power and focus was controlled by an RS232 serial connection. Remote access via Ethernet was used to control the embedded computer. Images were collected using software either from Stanford Photonics (Palo Alto, CA) or using custom software LabView GUIs. Raw data was uploaded from the embedded SSDs via Ethernet upon recovery of the instrument.

Image processing to count lit ‘blobs’ and calculate Intensity Units (IU) in each frame is described in the following pseudo-code:

for each sequential image in a recorded movie
 Subtract minimum image intensity calculated from entire
 movie (dark field; average value, 472 AU);
 Calculate maximum image intensity;
 Subtract reference image frame from current image (composite
 mean of 100 dark field images recorded with no
 bioluminescent sources);
 High image pixel intensity thresholding to discount strobe-lit
 frames;
 Further thresholding of low-light image, with threshold defined
 by the standard deviation of the image added to mean image
 intensity;

Morphological opening of thresholded image to isolate and count regions larger than 2 pixels in diameter;
end

Fig. 3.1. (Top) Block diagram and internal assembly of subsea sCMOS camera system: RC = RS232 power/focus control; FO = fiber optic to Ethernet mux; PC = control computer; WP = cooling water pump; HD = RAID hard drive array; CA = camera head; VP = viewport. (Bottom) Internal view of camera system. The assembled subsea housing measures 75 cm long, 20 cm diameter, and has an approximate in-water weight of 18 kg.

Field sites for this study were chosen based on collaborative opportunity with several research expeditions. All recordings were made at night to eliminate the incidence of weak ambient light from the surface. Bioluminescent responses were induced using strobe light stimulation following methods first described by Neshyba (1967). Limited mechanical stimulation was induced when bioluminescence animals randomly came into contact with the viewport.

Initial deployment of the camera system was aboard a Triton 3k3 submersible as part of a biodiversity exploration expedition to the Solomon Islands in September 2013 (Figure 3.2C). The camera was mounted on a forward-facing instrumentation frame so that recorded video matched what observers inside the submersible sphere viewed in real-time. All lights inside and outside the submersible were either turned off or blacked out with opaque tape. Light stimulation was done using a handheld Nikon Speedlight SB-700 strobe, set to produce a sequence of 5 flashes at 2 Hz. Several dives were conducted at night to depths up to 973 m, with two notable dives taking place approximately 22 km SE of Gizo Island and 6 km N of Mborokua Island, respectively. At Mborokua Island, a vertical transect through the water column was conducted via submersible with a depth station every 100 m down to the seafloor at 900 m.

A second research cruise, to the New England shelf break, was conducted in November 2014 (Figure 3.2B). The camera system was attached to a 1-ton towed body equipped with power switching and DSL internet to the

surface, run through a standard 0.322" coaxial oceanographic wire. The viewport was oriented sideways to limit transverse water movement against it. Light stimulation was accomplished using a Vivitar Thyristor strobe placed inside a borosilicate instrument sphere, set to produce a sequence of 5 flashes at 2 Hz. Two deployments were conducted at night to 900 m at Veatch Canyon and Atlantis Canyon.

A third deployment took place onboard the ROV HERCULES in the Galapagos Islands region in June/July 2015 (Figure 3.2A). The camera was mounted vertically pointing downwards into open water. Light stimulation was conducted using a pair of Ocean Imaging Systems 3831 strobes mounted 3 m forward, pointed at an oblique 45° angle towards the camera, and set to produce a sequence of 2 flashes spaced by a system cycle time of 2 s. Recordings were made opportunistically in the water column and on the seafloor, including at a 'black smoker' hydrothermal vent and a lower-temperature vent community dominated by *Riftia* sp. tubeworms.

Fig. 3.2: Deployment sites (yellow stars) for bioluminescent imaging in the Galapagos Islands (A), the New England shelf break (B) and the Solomon Islands (C). Bathymetry contours are spaced at 500 m.

3. Results

Over 200 15-30 second-length recordings were made at depths ranging from 30 m to 2500 m. Observed bioluminescence responses were most pronounced using repetitive flash stimuli; this was investigated on a submersible dive, when different flash characteristics were compared visually. At depths exhibiting a high bioluminescence response, human observers witnessed an omnidirectional display that momentarily resembled a bright night sky (i.e. the “Milky Way”). In many cases, major zooplankton groups

were recognized based on the shape and pattern of the emitted bioluminescence. Siphonophores, ctenophores, and fishes were among the organisms discernable by their light response in otherwise complete blackness. Notably, light-stimulated bioluminescence responses on the seafloor were almost non-existent, and therefore layers of dense zooplankton populations in the water column were targeted for subsequent recordings.

Recordings of bioluminescence along a vertical cast in the water column illustrate the system's potential to measure light-stimulated bioluminescence as an assay of zooplankton population density (Figure 3.3). Spatially, total bioluminescence exhibited an increasing trend with depth to a maximum at 400 m, followed by a steady decrease towards the seafloor. Typical bioluminescent responses at each depth were characterized by a sharp increase following the flash stimulation, rising to a maximum within 1 s followed by a complete decline within ~ 4 s (Figure 3.4). Figure 3.4A demonstrates a relatively short-lived bioluminescent response event of approximately 0.5 s that occurred after the fourth strobe event, while the response in Figure 3.4B shows a discrete response after each strobe with increasing intensity. Figure 3.4C is characteristic of many light-stimulated responses observed in this study, with a delay in luminescence of ~ 1 s following an initial strobe event and a gradual decline thereafter. In some cases, the decline in luminescence follows an exponential pattern (Figure 3.4B) and in others a more linear regression was observed (Figure 3.4A and

C). Low levels of light measured before stimulus (<50 AU) reflect a background signal on the image sensor itself.

Fig. 3.3. A) Maximum counts of bioluminescent sources, or ‘blobs’ following light stimulation at 100 m-spaced depth levels at a station located 6 km N of Mborokua Island, Solomon Islands at approx. 22:00-23:30 local time. B) Representative images of maximum bioluminescent response at three depth stations shown in (A).

Fig. 3.4. Maximum image intensities, reported in arbitrary units (AU) vs. time, for whole image light-stimulated responses at a) 100 m, b) 200 m, and c) 400 m depths recorded from a manned submersible in the Solomon Islands in September 2013. Vertical red dashed lines indicate strobe events.

Full-frame image sequences recorded at 30fps demonstrate the system's ability to track light production on free-swimming pelagic animals (Figure 3.5), offering the potential to gain new insight into the pathways that mediate bioluminescence in multicellular organisms. Two recordings of medusa show a swift response from swimming bell to tentacles, indicating the use of eyespots to sense light and a sequential reaction that dissipates quickly. In a spectacular video of a *Tomopteris* sp. pelagic polychaete, the organism displayed an elongated, >2.5 s luminescent reaction initiating in the head and traveling outward to the parapodia. Several recordings of bioluminescence in *Cestum veneris*, the 'venus girdle' ctenophore, illustrate light traveling through the animal in a single wave (not shown). Many other patterns were observed, but are not presented due to the absence of positive identification of the organism.

Fig. 3.5: Sample image sequences of *in situ* bioluminescence responses recorded at 30 fps. Time between frames is indicated in milliseconds in the lower left of each image. Top and middle row: tentacles from a hydromedusa show a distinct wave of bioluminescence traveling from the bell outwards and dissipating completely within 0.2 s, recorded at 700 m and 900 m depth (respectively) off the New England shelf. Bottom row: a *Tomopteris* sp. pelagic worm at 850 m in the Galapagos Islands, which exhibited a bright response for a total time of ~2.5 s.

4. Discussion

The three different deployment setups described in this paper allow for a comparison of methodology and recommendations for future research. The manned submersible was by far the most stable platform for observing midwater bioluminescence, and offered the unique opportunity to witness bioluminescent responses with the human eye. Phylum-level identification of

several bioluminescent animals was possible, an effort that may be improved upon through repetitive application of this method. The use of a towed body with low-bandwidth telemetry produced poor results that were largely unusable, mostly due to the unstable nature of this setup. The use of a heave-compensated winch would likely resolve this issue, at least in moderate seas. ROV-based observations worked very well due to the stable nature of the decoupled platform and the high-bandwidth telemetry offered by the system. However, in both the manned submersible and ROV, the need to switch off all internal and external lighting hindered all other concurrent observations. While all of the deployment methods used in this study are viable for further development efforts, the authors envision using an autonomous vehicle to conduct light-stimulated bioluminescence surveys in high spatial and temporal resolution.

The video-based vertical profile of bioluminescence presented in Figure 3.3 matches previous measurements of deep-sea zooplankton distribution (Banse 1964, Weikert 1982, Sameoto 1986, Kosobokova and Hirche 2000), roughly characterized by a maximum at 300-500 m followed by an exponential decline beyond depths >1000 m. Our observed maxima of bioluminescence at 400m may also represent a vertically migrating population of zooplankton as this dive was conducted in the early evening, just after sunset. Vertically migrating populations of major planktonic groups are known to rise from depths exceeding 700m in the daytime to near-surface waters at night (Ringelberg 2009); repeated vertical casts using this method

over a 24-h period might produce results similar to that shown using net tows (Nishikawa and Tsuda 2001), video plankton recorders (Ashjian et al. 2001), and acoustic analysis (Kringel et al. 2003). One other study has observed the vertical migration of plankton using bioluminescence as an indicator (Widder et al. 1992), and Gillibrand et al. (2007) report an extensive bioluminescent layer at ~1500 m occurring seasonally in the NE Atlantic Ocean. Depressed bioluminescence responses approaching the seafloor corroborate similar observations in the Bahamas (Johnsen et al. 2012) and on the Mid-Atlantic Ridge (Craig et al. 2015).

In strict terms, these data represent the light-responsive bioluminescent community that exists in the water column. Because of the widespread prevalence of bioluminescence in deep-sea midwater species (with the exception of one major phylum, Tunicata), we therefore assert that the number of bioluminescent sources may be a proxy of total living biomass, at least among animals that are large enough to be imaged by the camera. The same line of comparison has been drawn with instruments using physical stimulation to elicit a bioluminescence response (e.g. Widder 2002, Herren et al. 2005, Heger et al. 2008, Craig et al. 2015).

Varying patterns of response to individual stimulus events may represent the diversity of the bioluminescent community itself, a corollary also drawn by Dominjon et al. (2012) and Craig et al. (2015). We envision developing a library of bioluminescent response profiles based on intensity vs. time and shape, paired with animal identification, which could allow for

automated classification based solely on light production measured passively using a camera system. Applying such a method spatially and temporally would address a major technical challenge in deep-sea biological oceanography that has resulted in a dearth of zooplankton distribution data (Wiebe and Benfield 2003, Webb et al. 2010, Sutton 2013). It may also produce the resolution needed to resolve zooplankton population patchiness at critical time and space scales (Benoit-Bird et al. 2013, Benoit-Bird and McManus 2014).

The utilization of light stimulation to produce a bioluminescent response offers a unique advantage over mechanical, electrical and chemical stimulation techniques. In dinoflagellates, mechanical stimulation results in the deformation of cell membranes, initiating a cascade of reactions within the unicellular organism (Latz and Rohr 1999, Cussatlegras and Le Gal 2005). Larger animals, such as gelatinous zooplankton and fishes, may also exhibit a bioluminescent response based on the location and magnitude of the physical and/or electrical disturbance (e.g. Barnes and Case 1974, Herring and Widder 2004). Chemical stimulation is typically achieved by injecting a solution into laboratory aquaria, resulting in a widespread stimulus unlikely to be encountered in a natural setting. As such, luminescent responses in relation to an organism's neural/sensory network are poorly understood. Light stimulation induces bioluminescence with no mechanical or chemical invasion, and may be the least intrusive method for eliciting a 'natural' response from animals *in situ*.

The high-speed global shutter capability of the sCMOS camera offers a new window into the kinematics of bioluminescence. Our observation of the *Tomopteris* sp. polychaete supports the conclusions of Gouveneaux and Mallefet (2013) who conducted laboratory experiments on *T. helgolandica* and witnessed neural control of bioluminescence under contrived conditions. In several recordings of siphonophores, most of the organism becomes luminescent at the same time, which correlates with previous work on a siphonophore species that exhibited no coordinated response to mechanical stimulation (Freeman 1987). Without a size scale, velocities cannot be strictly calculated, but the image sequences presented here illustrate the potential of the system for future research in this area. Several methods could be employed to resolve scale such as utilizing stereo cameras (e.g., Rife and Rock 2001), scaling lasers, or simply constraining the volume being imaged to minimize the depth of field. The stereo method is particularly attractive, since it would allow volume-pixel regions or ‘voxels’ to be constrained and characterized.

A comprehensive review of published literature presenting *in situ* observations of bioluminescence is given in Table 1. While the human eye can still outperform the best digital cameras in terms of sensitivity, dynamic range and resolution, and PMTs have long been able to sense single photons, the inability to record imagery is an obvious drawback to using these methods. The electron-bombarded CMOS camera used by Dominjon et al. (2012), while likely the most sensitive instrument ever used to record

bioluminescence *in situ*, does not collect focused imagery and is therefore categorized as similar to a PMT device. The defining feature of sCMOS technology is achieving high quantum efficiency (QE) and low readout noise without electron multiplication or image intensification. This distinction makes direct comparison of sCMOS cameras to electron multiplier CCD cameras (EMCCD) and image intensified CCD cameras (I²CCD) not straightforward, and best quantified by empirical studies (e.g. Fullerton et al. 2012, Beier and Ibey 2014). While contemporary EMCCD cameras still report higher quantum efficiencies (e.g. Andor iXon 2015 series at >90% QE) compared to the most advanced sCMOS cameras (e.g. Hamamatsu ORCA-Flash4.0 2015 model at >82% QE), the electron multiplication process can dramatically reduce the effective QE of EMCCD cameras (Long et al. 2012). Given the upward trajectory in sCMOS QE performance combined with their characteristic high speed and resolution capability, sCMOS cameras have the potential to become the benchmark of bioluminescence imaging technology.

Regardless of the imaging sensor employed, most *in situ* bioluminescence research has relied exclusively on mechanical stimulation to elicit a response. For example, Herren et al. (2005), Moline et al. (2009), and Johnsen et al. (2014) used essentially the same excitation chamber/PMT instrument; Widder (1989), Widder and Johnsen (2000), Widder et al. (2005), and Johnsen et al. (2012) used similar ‘splat-screen’ approaches; and Priede et al. (2006), Heger et al. (2008), Craig et al. (2010), Craig et al. (2011), and Craig et al. (2015) use the ‘splat-screen’ method for their vertical profiling

work. By far the most comprehensive investigation into light-stimulated bioluminescence was conducted by Neshyba (1967), who measured responses using PMTs. Notably, Buskey and Swift (1985) performed a series of light-stimulation experiments on copepods and euphausiids, but these were conducted in a laboratory environment. Lapota et al. (1986) conducted a vertical dive in a manned submersible similar to that in this study, but bioluminescence was not recorded beyond written observation. Therefore, we believe these data presented herein represent the only published imagery of the phenomenon *in situ*.

Table 1: Reference list of studies observing bioluminescence *in situ*

	Sensor	Citation	Depth (m)	Observation platform
Mechanical stimulation	Human observer	Beebe 1934*	0-660	Bathyscape
		Galt et al. 1985	Surface water	SCUBA diver
	Photomultiplier tube	Boden & Kampa 1957	0-300	Vertical profiler
		Hardy & Kay 1964	62	Vertical profiler
		Bradner et al. 1987	4300	Vertical profiler
		Webster et al. 1991	4500	Vertical profiler
		Amram et al. 1999	~2350	Moored array
		Widder et al. 1999	0-250	Vertical profiler
		Herren et al. 2005	0-30	Muiltplatform
		Moline et al. 2009	0-40	AUV
		Tamburini et al. 2013*	~2350	Moored array
		Johnsen et al. 2014	20	Vertical profiler
	ISIT camera	Widder et al. 1989	0-600	Submersible
		Robison 1992	n/a	Submersible
		Widder & Johnsen 2000	0-200	Submersible
		Widder et al. 2005	600	ROV, benthic lander
		Priede et al. 2006	0-4000	Vertical profiler
		Gillibrand et al. 2007	0-4800	Vertical profiler
		Heger et al. 2008	0-3000	Vertical profiler
		Craig et al. 2010	0-5000	Vertical profiler
	I ² CCD (image-intensified CCD)	Craig et al. 2011	1500-2700	Benthic lander, ROV
		Craig et al. 2015	0-2500	Vertical profiler
	ebCMOS camera (electro-bombarded CMOS)	Johnsen et al. 2012*	1000	Submersible
		Dominjon et al. 2012	2500	Moored array
Light stimulation	Human observer	Lapota et al. 1986	0-600	Submersible
	Photomultiplier tube	Neshyba 1967	700	Profiler
	sCMOS camera	This study	0-2500	Submersible, ROV & vertical profiler

* These studies did not actively stimulate bioluminescence. Their results may result from naturally occurring mechanical stimulation (i.e. currents causing collisions) or may be from animals actively producing bioluminescence with no external stimulation.

5. Conclusion

Marine bioluminescence research has resulted in transformative applications to a wide range of medical, military and industrial disciplines (e.g. Shimomura et al. 1962), and the potential for important new discoveries is high. The diversity of light-producing organisms is remarkable, and observed in every ocean from shallow to deep seas. Despite the ubiquity of bioluminescence among marine taxa, the phenomenon is categorically underexplored and limited by the technology available to observe it *in situ*. To address this, an sCMOS camera was deployed on multiple oceanographic platforms in three different oceanic regions to explore bioluminescence *in situ*. Using light stimulation to elicit responses, counts of bioluminescent sources were compared against water depth, demonstrating a potential new method to assess midwater zooplankton populations. High-resolution, quantitative measurements of light intensity responses were observed to vary greatly among depth zones and individual organisms. The kinematics of bioluminescence was imaged among several animals at a high frame rate and resolution, presenting new visualizations of how marine animals produce light. While this study demonstrates the utility of light stimulation for eliciting bioluminescent responses, it also highlights the current lack of scientific knowledge on the subject; further work is needed to identify which organisms respond to light stimulation and varying light intensities, patterns, and wavelengths. Future work should focus on pairing spatial and temporal observations of light-stimulated bioluminescence with traditional-method

biomass assessments, in a true effort to estimate total living biomass.

Advanced control software will lead towards support onboard autonomous vehicles and other self-powered platforms, and may allow for bioluminescent surveys to be conducted in high spatial and temporal resolution. We conclude that sCMOS cameras paired with light stimulation can offer a wide range of new applications in bioluminescence research.

Acknowledgements

The authors wish to acknowledge the crew and technical team of the M/Y *Alucia*, the E/V *Nautilus* (leg NA064), and the R/V *Endeavor* (leg EN564). We are grateful for the engineering and fabrication support of The John B. Pierce Laboratory and the generous technical support provided by David Casagrande, Donald Dansereau, John Buckley, Tom Delessandro, Andrew Wilkens and Ron Goodman. This work was funded in part by the Ray and Barbara Dalio Family Foundation, the Rhode Island Endeavor Program, the Ocean Exploration Trust, and NSF grant DEB-1257555 to JSS. We thank Igor Belkin and 6 anonymous reviewers who contributed greatly to this manuscript with critical and constructive commentary.

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Appendix A: News Article

“First evidence of bioluminescence on a ‘black smoker’ hydrothermal vent”

By

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Oceanography, 29-2, June 2016 (peer-reviewed news article)

Bioluminescence in the deep-sea is omnipresent, yet imagery of the phenomenon is scarce. While this dearth in observations can be largely explained by sampling effort, the camera technology available for *in situ* low-light imagery is also a limiting factor. Recent work by Phillips et al. (2016) describes the application of a Hamamatsu ORCA Flash2.0 V2 sCMOS camera to detect light-stimulated luminescence in the water column. This system was recently employed to make an observation of bioluminescence on a deep-sea ‘black smoker’ hydrothermal vent.

As part of a multidisciplinary expedition aimed at exploring the Galapagos Spreading Center, the *HERCULES* ROV system conducted dive #H1439 on the “Iguanas” hydrothermal vent field on July 1 2015 (Bell et al. 2016). This vent system was initially discovered in 2006 (Haymon et al. 2008). Outfitted with the low-light camera and two high-power strobes oriented vertically towards the seafloor, the ROV was positioned almost directly over the vent orifice of one of the larger active chimneys at 1643 m (approx. 1 m altitude, chimney height 11 m). All lights on the ROV system were turned off (including external LED indicators), and closed-loop inertial navigation control was used to maintain vehicle position within <10 cm on all axes. The vehicle hovered over the chimney for ~20 minutes while light-stimulation techniques, as performed in Phillips et al. (2016), were used to elicit bioluminescent responses. No light-stimulated bioluminescence was observed, but two recordings made as a ‘control’ without any strobes detected a weak, mobile luminescent source within 1 m of the vent orifice (Figure

1, <https://youtu.be/YoVTCG2AJv0>). The source appears to be moving quickly in one recording, indicating a mobile organism. Review of footage obtained 5 minutes prior to the low-light recordings revealed a relatively low abundance of macrobiology on and around the chimney, with a few scattered Bythograeid crabs, squat lobsters, and numerous Alvinocarid shrimp. Based on these observations and the behavior of the luminescence, it is likely biologically sourced and could be a free-swimming shrimp. Whether such an organism is a vent-associate or a member of the surrounding midwater community cannot be determined by these observations alone.

Blackbody radiation and visible light has been measured using various methods at several hydrothermal vents (Van Dover et al. 1996, White & Chave 2002). While the low-light camera that was used in this study can detect wavelengths up into the near infra-red spectrum, no patterns were visible in our recordings. This may be attributed to the relatively low venting temperatures measured at the vent orifice (maximum reading 154.7° C) and the diminished quantum efficiency of the camera in the low infrared wavelength spectrum (800-1000nm). Given the absence of any solar radiation from the surface and negative observations of bioluminescence on vents by human observers, it has been hypothesized that blackbody radiation from deep-sea vents may be a signal for vent shrimp to locate and/or avoid heat sources through the use of primitive photoreceptor organs (Pelli & Chamberlain 1989, Van Dover et al. 1989, O'Neill et al. 1995, Nuckley et al. 1996). Given our observations of naturally occurring bioluminescence

directly adjacent to an active vent, it may be that such photosensitive capabilities serve more than to simply locate and avoid extreme heat sources. For example, Johnsen et al. (1995) suggest that the measured peak sensitivity of *Rimicaris* sp. shrimp at ~500 nm is more attuned to visible-spectrum light than blackbody radiation, which may be an artifact of their proposed evolution from surface-dwelling bresiliid shrimp (Chamberlain 2000).

It is our hope that these crude observations will serve to stimulate further interest regarding the role of bioluminescence within and around hydrothermal vent systems, with a possible focus on vent shrimp as potential light-producing animals. The continual improvement of low-light scientific cameras used for *in situ* imagery, paired with reliable and precise vehicle navigation, make such endeavors possible.

Figure 1: Left) Strobe-lit, down-looking view of the active black smoker chimney. Right) Same field of view as the strobe-lit image, with a single bioluminescent source visible in the lower-right corner. The location of the bioluminescent source is circled in red in both images.

Acknowledgements

This research used data provided by the *Nautilus* Exploration program, Expedition #NA064. The authors wish to thank the E/V *Nautilus* crew and the ROV team for their expertise and technical support, the Inner Space Center at the University of Rhode Island, and Chuck Fisher for facilitating this research. Sheri White, Cindy Van Dover, and one anonymous reviewer contributed constructive reviews that greatly enhanced this article. This work was funded in part by the Ray and Barbara Dalio Family Foundation and NSF grant DEB-1257555 to JSS.

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Appendix B: Speculative Discussion

As outlined in the first two manuscripts of this dissertation, hydrothermal plumes constitute a discrete, dynamic pelagic environment. Chapter 1 synthesizes a growing body of scientific evidence supporting the hypothesis that hydrothermal plumes host endemic communities of chemosynthetic primary producers along with secondary and tertiary consumers. A charismatic example is presented in Chapter 2, where a relatively accessible submarine volcano exhibiting hydrothermal venting was investigated using relatively simple and economical methods. The unexpected results of this work support the original hypothesis, while prompting further questioning. Chapter 3 describes a novel method that may be applied towards observing deep-sea pelagic communities relative to hydrothermal plume structure. Due to a number of financial and logistical constraints, it is unfeasible to comprehensively address the original hypothesis presented in Chapter 1 during a short research project such as this. Thus, I hope to use this concluding section to consider the results of the dissertation as a whole and speculate upon the practical and theoretical implications of this work.

The role of oxygen in hydrothermal plumes

End-member fluids of hydrothermal vents are characteristically anoxic. The rapid entrainment of background seawater re-introduces oxygen in the mixing zone, where it is readily consumed by reduced Fe, S,

Mn and other compounds. These oxidation gradients host a diverse phylogeny of microbes that can take advantage of both aerobic and anaerobic conditions (Nakagawa and Takai 2008, Anantharaman et al. 2013); plume- associated microbial communities show strikingly similar characteristics to those found in oxygen minimum zones (Dick et al. 2013). While genomic and metabolic plasticity of plume-associated microbes may stabilize their existence amongst the various geochemical gradients that they experience, higher consumers dependent on respiration are directly influenced by dissolved oxygen concentrations (Maas et al. 2014).

The midwater effects of anoxic conditions due to a major pulse in hydrothermal output was somewhat demonstrated during the eruption of the submarine volcano El Hierro (Canary Islands) in 2011-12. Large pelagic fish were killed immediately, followed by a shoaling of the deep scattering layer (Fraile-Nuez et al. 2012, Ariza et al. 2014). A number of other variables involved in the El Hierro submarine eruption, such as pH and turbidity, obscure the specific role of oxygen in the observed changes to the midwater community; but the extreme nature of the deviation brings it to the forefront of the discussion.

The short-lived nature of El Hierro's 2011 eruption and its effect on the water-column are predictable given the plume's relative size and exposed location (Coca et al. 2014). In contrast, active sites that pair continuous hydrothermal output with constrained bathymetry

demonstrate persistent changes to the demersal and pelagic community. Several case examples display how dissolved O₂ may play a role in these deviations. Exploration of the summit crater of Vailulu'u Seamount (maximum depth 940m, sill depth ~780m) revealed marked variability in dissolved O₂ ranging from 218-150 µM, which may have contributed to the discrete biological gradients and 'dead zones' observed within (Staudigel et al. 2006). The semi-enclosed crater of the active Kick'em Jenny submarine volcano (maximum depth ~260m, sill depth 180-230m) has dense bacterial mats on the seafloor, midwater shrimp 'dead zones' (Wishner et al. 2005) and apparently unaffected populations of pelagic and reef fish (Bell et al. 2014). Measurements of dissolved O₂ in the water column over the crater have moderately depressed values compared to open ocean sites nearby, possibly coupled with plume-associated redox measurements (Figure 1). The tightly constrained volcanic caldera of Kolumbo (maximum depth ~500m, sill depth 20-220m) contains a well-mixed volume of acidic water moderately depleted in oxygen (Figure 2, Carey et al. 2013); the entire volume of the basin below the caldera's sill is a 'dead zone' with the exception of extremely dense bacterial mats and glass tunicates (Sigurdsson et al. 2006). Certainly dissolved O₂ is not the only variable driving biological differences between these constrained-volume hydrothermal zones. Oxygen, however, specifically affects respiring metazoans and may partially explain why hydrothermal plumes host vibrant microbial communities but inconsistent observations of

higher consumers. The resiliency of certain respiring organisms to hypoxic conditions, such as teleost fishes (Randall et al. 2014), may relate their evolutionary success to hypothetical basin-scale plume events in the past.

Figure 1: *Upper left:* Bathymetric map of the Kick'em Jenny submarine volcano, Lesser Antilles. *Lower left:* Image of the seafloor inside the active crater showing extensive bacterial mats with a large grouper in the foreground. Other pelagic and reef fish, such as jacks, snapper and stingrays were also observed within close proximity of the vent field. *Right:* Water-column profiles of dissolved oxygen, temperature and redox potential (ORP) at a site directly overlying the vent field inside the crater ("KEJ", marked with a red star in the bathymetric figure) and at a non-active site ("Open sea"). The "Open sea" location was approx. 15km SW of the island of Dominica with a water depth ~2300m. Water-column profiles of temperature, salinity, and dissolved oxygen were collected using the research vessel E/V *Nautilus* and ROV *HERCULES* using vehicle descent data in November 2013. Temperature and salinity were measured using a SeaBird FastCat49 CTD mounted to the forward light-bar of the ROV, and dissolved oxygen was measured with an Aanderaa 3830 optode mounted in the center of the ROV. ORP (redox) values were

measured using a NOAA/PMEL MAPR instrument (Walker et al. 2007) mounted to the ROV frame. Map reproduced with permission from the Ocean Exploration Trust, image copyright Ocean Exploration Trust..

Figure 2: Upper left: Bathymetric map of the Kolumbo submarine volcano, Aegean Sea. Lower left: Image of the seafloor inside the active caldera showing thick bacterial mats and an inactive hydrothermal chimney. Similar adjacent structures were densely populated with glass tunicates, but no pelagic metazoans were observed within 200m altitude of the caldera's basement. Right: Water-column profiles of dissolved oxygen and temperature at a site directly overlying the vent field inside the caldera ("Kolumbo", marked with a red star in the bathymetric figure) and at a relatively non-active site inside the nearby Santorini caldera ("Santorini"). The "Santorini" location was approx. 12km SW of the "Kolumbo" site with a water depth of ~500m. Water-column profiles of temperature, salinity, and dissolved oxygen were collected using the research vessel E/V *Nautilus* and ROV *HERCULES* using vehicle descent data in July/August 2010. Temperature and salinity were measured using a SeaBird FastCat49 CTD mounted to the forward light-bar of the ROV, and dissolved oxygen was measured with an Aanderaa 3830 optode mounted in the center of the ROV. Map reproduced with permission from Nomikou et al. (2012), image copyright Ocean Exploration Trust.

Hydrothermal plumes: an understudied pelagic environment

Submarine hydrothermal venting occurs worldwide in correlation with volcanically active regions. The vast majority of hydrothermal vent fields exist below 1000m, and while fast-spreading divergent margins account for a higher incidence of activity, hydrothermal plumes are found in a diverse range of geologic settings (Baker and German 2004). Traditionally, plumes have been used as detectors for seafloor activity; sensing their existence is a step along the way towards discovering benthic features that are then explored and studied in great detail (Baker et al. 1995). To this end, most plume-oriented research has focused on measuring the geochemical properties of natural systems and modeling plumes under various conditions. A relatively small body of research, usually conducted as part of larger studies focused on the seafloor, has addressed the influence of hydrothermal plumes on pelagic biology. To the author's knowledge Chapter 1 is the first synthesis of such literature, which constitutes a scientific contribution in its own right.

Choosing an appropriate geochemical or physical tracer is a fundamental problem when observing pelagic biology relative to a hydrothermal plume. Many properties are non-conservative (e.g. oxidation-reduction-potential based on the presence of electron donors such as Fe and H₂S, suspended particulate matter), some quickly dilute to immeasurable amounts (e.g. temperature), and others have no apparent influence on biology in general (e.g. primordial helium, ³He). To further complicate the problem, plume-associated microbes exhibit metabolic plasticity and can exploit a wide

range of geochemical gradients (Anantharaman et al. 2013), while larger animals are more susceptible to diversions from their ‘normal’ environment. Thus, defining what a plume *is* in terms of size, structure, and concentration relative to biological activity remains an open question.

Based on the work presented in Chapter 1, I am prepared to speculate upon several conclusions. First, it is clear that hydrothermal plumes support chemosynthetic microbial biomass that is elevated relative to background seawater. These observations have been made at plumes around the world and are likely the result of relatively accessible methods to collect such data (e.g. only a small volume of seawater is needed to sample microbiology at specific points inside and outside the plume). As I will elaborate upon in further detail in the final section of this appendix, measuring macrobiology (zooplankton, fish, etc.) relative to plume structure is less straightforward and subject to the achievable spatiotemporal resolution of existing methodology. Some data, in particular a multi-decadal synthesis of acoustic observations made over the Juan de Fuca Ridge (Burd and Thomson 2015) indicates an associative behavior of macrobiology to a persistent midwater plume. Furthermore, several observations of filter-feeding tunicates possibly associated with hydrothermal plumes, paired with their known ability to capture and consume picoplankton (such as chemolithoautotrophic bacteria) offers the hypothesis that certain secondary consumers may be suited to exploit seafloor-based chemosynthetic primary production. If this hypothesis is supported, it is not a stretch to consider the association of tertiary

consumers with this enriched food source, particularly in the energy-starved deep-sea water-column.

Applying existing hydrothermal plume observations to large spatiotemporal scales

Through the course of compiling data based on present-day observations of hydrothermal plumes I became aware of a growing body of literature investigating patterns of seafloor volcanism and plume incidence relative to changes in hydrostatic forcing (water pressure). Starting with the first paper to bring these ideas to light (Lund and Asimow 2011), these cycles have now been shown to occur concurrent with tidal forcing and Milankovitch cycles (Burley and Katz 2015, Crowley et al. 2015, Tolstoy 2015). Delving further into geologic history, I learned that several major ocean extinction events in the past 500 Mya have been associated with increases in global volcanism. These same events are also correlated with reduced dissolved oxygen levels, the signatures of which are recorded in deep-sea sediment cores. Knowing the current Earth state of reduced volcanic activity, I am led to ask the question: could hydrothermal plumes have been much larger, on average, than we currently observe them to be? Could they have basin-scale effects on ocean anoxia and euxinia? The answer has important implications for future research into the biology of hydrothermal plumes, a topic that is

largely ignored based on the assumption that plumes are a negligible component of the pelagic biosphere.

Since most plumes that overly mid-ocean ridges are subject to rapid mixing with background seawater, their structure is dynamic and tracing gradients quickly becomes a difficult endeavor. Furthermore, the proposed connection between plumes and anoxic conditions is not apparent given the well-oxygenated nature of our current deep-sea basins. Specific hydrothermal environments; either massive ‘megaplume’ events, or persistent venting that occurs within confined bathymetry, may be analogous to hypothetical basin-scale plume events. This topic is explored in detail in Chapter 1, and aligns with the crater observations made at the Kavachi volcano described in Chapter 2. Since major ocean extinction events in the past 500 Mya are correlated with ocean anoxic events supposedly initiated by volcanic forcing, I assert that future investigations into the biology of hydrothermal plumes should focus on using dissolved O₂ as a plume tracer along with other geochemical properties.

Another idea worth mentioning in this speculative discussion is the possible effect of anthropogenically-derived sea level rise on the incidence and structure of existing hydrothermal plumes. While coastal tidal cycles can vary up to 10 m in height, in regions overlying mid-ocean ridges the tidal signal can be less than 1 m (Tolstoy 2015). We have already observed a global rise in sea level nearing 0.5 m, with predictions for the year 2100 nearing 1m (Field et al., IPCC AR5 Technical Summary 2014), on the same order of magnitude as many offshore tidal ranges. If this constant increase in

hydrostatic pressure correlates with a decrease in plume incidence and size, our window of opportunity to observe large-scale hydrothermal plumes may be diminishing.

Using novel technology to observe the macrobiology of hydrothermal plumes

Separate from the challenge of choosing and measuring an appropriate plume tracer to correlate with biological observations, simply observing zooplankton and fish at depths beyond 200m (where most known hydrothermal vents exists) in high spatiotemporal resolution is difficult. Critical time (<20 minutes) and space (5 m) scales have been demonstrated by recent acoustic-based surveys (Benoit-Bird et al. 2013, Benoit-Bird and McManus 2014) above which meso- and micro-scale distribution patterns may be masked. While multi-net systems such as the MOCNESS are able to conduct layered surveys separated by <10m, mobile animals such as mesopelagic fish can avoid nets altogether and towing such apparatus over the characteristically rough terrain of mid-ocean ridges is a risky affair. Video-based survey platforms such as video plankton recorders (reviewed in Benfield et al. 2007) offer high spatiotemporal resolution, but are generally limited in sample volume and generate massive datasets. Acoustic survey methods can pair large sample volumes with high spatiotemporal resolution, but lack the ability to differentiate among even the coarsest phylogenetic classification levels. Thus, there is a continued need to improve upon the methods available for observing the distribution of pelagic macrobiology.

The bioluminescence-based method presented in Chapter 3 builds upon previous work using light production as a representation of zooplankton distribution patterns (e.g. Widder 2002, Craig et al. 2015). These ‘splat-screen’ systems use mechanical stimulation to elicit bioluminescent responses. Diverging from this approach, I propose that light-stimulation offers several advantages; it has the potential to assess a larger, more discrete sample volume, animals are not touched or flattened out against a screen (which they may also avoid, similar to net-tows), and both stimulation and observation can be done from a single plane. An obvious drawback to this new method is the lack of scientific knowledge on light-stimulated bioluminescence in general, making conclusive results difficult to produce. Future efforts to observe light-stimulated bioluminescence *in situ*, correlated with observations of pelagic biology using established methods (such as net-tows), will aid in developing the necessary background research required to advance the application of this method. In the process, new questions and discoveries related to the light sensing capabilities and kinematics of bioluminescence will result.

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Appendix C:

Detail of methods and data presented in Chapter 1

Figure 1 data: List of primary journal articles published since 1985 that associate midwater biology and hydrothermal plumes. Based on a survey of Web of Science, Google Scholar and references therein.

	Citation	Year	Larvae	Microbiology	Zooplankton
1	Smith 1985	1985			1
2	Winn et al. 1986	1986		1	
3	Berg & Van Dover, 1987	1987			1
4	Wiebe et al. 1988	1988			1
5	Roth & Dymond 1989	1989		1	
6	Thomson, Gordon & Dymond 1989	1989			1
7	Mullineaux et al. 1991	1991	1		
8	Thomson, Gordon & Dolling 1991	1991			1
9	Thomson, Burd & Dolling 1992	1992			1
10	Burd, Thomson & Jamieson 1992	1992			1
11	de Angelis et al. 1993	1993		1	
12	Burd & Thomson 1994	1994			1
13	Kim et al. 1994	1994	1		
14	Burd & Thomson 1995	1995			1
15	Mullineaux, Wiebe & Baker 1995	1995	1		
16	Herring and Dixon 1998	1998	1		
17	Kim & Mullineaux 1998	1998	1		
18	Cowen et al. 1999	1999		1	
19	Burd & Thomson 2000	2000			1
20	McCollum 2000	2000		1	
21	Khripounoff et al. 2001	2001	1		
22	Marsh et al. 2001	2001	1		
23	Wakeham et al. 2001	2001			1
24	Bertram et al. 2002	2002			
25	Burd, Thomson & Calvert 2002	2002			1
26	Mullineaux et al. 2002	2002	1		

27	Hauschild et al. 2003	2003			
28	Thomson et al. 2003	2003	1		
29	Tyler & Young 2003	2003	1		
30	Vinogradov et al. 2003	2003			1
31	Lam et al. 2004	2004		1	
32	Sunamura et al. 2004	2004		1	
33	Mullineaux et al. 2005	2005	1		
34	Berdeal et al. 2006	2006	1		
35	Dick et al. 2006	2006		1	
36	Herring et al. 2006	2006	1		
37	Skebo et al. 2006	2006			1
38	Vinogradov & Vereshchaka 2006	2006			1
39	Khripounoff et al. 2008	2008	1		
40	Lam et al. 2008	2008		1	
41	Bailly-Bechet et al. 2009	2009	1		
42	Brooke et al. 2009	2009	1		
43	Dick & Tebo 2010	2010		1	
44	Jackson et al. 2010	2010	1		
45	McGillicuddy et al. 2010	2010	1		
46	Adams et al. 2011	2011	1		
47	Bennett et al. 2011	2011		1	
48	Bennett et al. 2011	2011		1	
49	Baker et al. 2012	2012		1	
50	Breier et al. 2012	2012		1	
51	Adams et al. 2012	2012	1		
52	Burd & Thomson 2012	2012			1
53	Lesniewski et al. 2012	2012		1	
54	Sylvan et al. 2012	2012		1	
55	Anantharaman et al. 2013	2013		1	
56	Bennett et al. 2013	2013		1	
57	Dick et al. 2013	2013		1	
58	Mullineaux et al. 2013	2013	1		
59	Ariza et al. 2014	2014			1
60	Olsen et al. 2014	2014		1	
61	Burd & Thomson 2015	2015			1
62	Ferrera et al. 2015	2015		1	
63	Lindsay et al. 2015	2015			1
64	Reed et al. 2015	2015		1	
65	Anantharaman et al. 2016	2016		1	

66	Cao et al. 2016	2016	1
67	Millard et al. 2016	2016	1
68	Mitarai et al. 2016	2016	1

Title

- 1 Macrozooplankton of a deep sea hydrothermal vent: In situ rates of oxygen consumption
- 2 Microorganisms in deep-sea hydrothermal plumes
- 3 Benthopelagic macrozooplankton communities at and near deep-sea hydrothermal vents in the eastern Pacific Ocean and the Gulf of California
- 4 Deep-water zooplankton of the Guaymas Basin hydrothermal vent field
- 5 Transport and settling of organic material in a deep-sea hydrothermal plume: evidence from particle flux measurements
- 6 Acoustic Doppler current profiler observations of a mid-ocean ridge hydrothermal plume
- 7 Hydrothermal vent plumes- larval highways in the deep-sea
- 8 An intense acoustic scattering layer at the top of a mid-ocean ridge hydrothermal plume
- 9 The deep scattering layer associated with the Endeavour Ridge hydrothermal plume
- 10 Composition of a deep scattering layer over-lying a mid-ocean ridge hydrothermal plume
- 11 Methane oxidation in deep-sea hydrothermal plumes of the Endeavour segment of the Juan de Fuca Ridge
- 12 Hydrothermal venting at Endeavour Ridge: Effect on zooplankton biomass throughout the water column
- 13 Larval dispersal via entrainment into hydrothermal vent plumes
- 14 Distribution of zooplankton associated with the Endeavour Ridge hydrothermal plume
- 15 Larvae of benthic invertebrates in hydrothermal vent plumes over Juan de Fuca Ridge
- 16 Extensive deep-sea dispersal of postlarval shrimp from a hydrothermal vent
- 17 Distribution and near-bottom transport of larvae and other plankton at hydrothermal vents
- 18 Microbial biomass in the hydrothermal plumes associated with the 1998 Axial Volcano eruption
- 19 Distribution and relative importance of jellyfish in a region of hydrothermal venting
- 20 Geochemical constraints on primary productivity in submarine hydrothermal vent plumes
- 21 Particle flux in the Rainbow hydrothermal vent field (Mid-Atlantic Ridge): Dynamics, mineral and biological composition
- 22 Larval dispersal potential of the tubeworm *Riftia pachyptila* at deep-sea hydrothermal vents
- 23 Lipid-rich ascending particles from the hydrothermal plume at Endeavour Segment, Juan de Fuca Ridge
- 24 Compositional variability in the ascending fluxes from a hydrothermal plume

- 25 Isotopic composition of hydrothermal epiplume zooplankton: evidence of enhanced carbon recycling in the water column
- 26 Implications of cross-axis flow for larval dispersal along mid-ocean ridges
- 27 Asymmetric sedimentation on young ocean floor at the East Pacific Rise, 15 degrees S
- 28 Constrained circulation at Endeavour ridge facilitates colonization by vent larvae
- 29 Dispersal at hydrothermal vents: a summary of recent progress
- 30 Zooplankton distribution over hydrothermal fields of the Mid-Atlantic Ridge
- 31 Autotrophic ammonia oxidation in a deep-sea hydrothermal plume
- 32 Two Bacteria Phylotypes Are Predominant in the Suiyo Seamount
- 33 Vertical, lateral and temporal structure in larval distributions at hydrothermal vents
- 34 Vertical structure of time-dependent currents in a mid-ocean ridge axial valley
- 35 Manganese(II)-oxidizing bacillus spores in Guaymas Basin hydrothermal sediments and plumes
- 36 Presence of postlarval alvinocaridid shrimps over south-west Indian Ocean hydrothermal vents, with comparisons of the pelagic biomass at different vent sites
- 37 Spatial patterns of zooplankton and nekton in a hydrothermally active axial valley on Juan de Fuca Ridge
- 38 Zooplankton distribution above the Lost City (Atlantis Massif) and Broken Spur hydrothermal fields in the North Atlantic according to the data of visual observations
- 39 Temporal variation of currents, particulate flux and organism supply at two deep-sea hydrothermal fields of the Azores Triple Junction
- 40 Microbial ammonia oxidation and enhanced nitrogen cycling in the Endeavour hydrothermal plume
- 41 A modeling approach of the influence of local hydrodynamic conditions on larval dispersal at hydrothermal vents
- 42 Where do the embryos of Riftia pachyptila develop? Pressure tolerances, temperature tolerances, and buoyancy during prolonged embryonic dispersal
- 43 Microbial diversity and biogeochemistry of the Guaymas Basin deep-sea hydrothermal plume
- 44 Dispersion of a tracer on the East Pacific Rise (9 degrees N to 10 degrees N), including the influence of hydrothermal plumes
- 45 Larval dispersion along an axially symmetric mid-ocean ridge
- 46 Surface-generated mesoscale eddies transport deep-sea products from hydrothermal vents
- 47 Tracing iron-fueled microbial carbon production within the hydrothermal plume at the Loihi seamount
- 48 Dissolved and particulate organic carbon in hydrothermal plumes from the East Pacific Rise, 9 50' N
- 49 Genome-enabled transcriptomics reveals archaeal populations that drive nitrification in a deep-sea hydrothermal plume
- 50 Sulfur, sulfides oxides and organic matter aggregated in submarine hydrothermal plumes at 9 50'N East Pacific Rise
- 51 Larval dispersal: Vent life in the water column

- 52 Estimating zooplankton biomass distribution in the water column near the Endeavour Segment of Juan de Fuca Ridge using acoustic backscatter and concurrently towed nets
- 53 The metatranscriptome of a deep-sea hydrothermal plume is dominated by water column methanotrophs and lithotrophs
- 54 Time-series analysis of two hydrothermal plumes at 9°50'N East Pacific Rise reveals distinct, heterogeneous bacterial populations
- 55 Evidence for hydrogen oxidation and metabolic plasticity in widespread deep-sea sulfur-oxidizing bacteria
- 56 Trophic regions of a hydrothermal plume dispersing away from an ultramafic-hosted vent-system: Von Damm vent-site, Mid-Cayman rise
- 57 The microbiology of deep-sea hydrothermal vent plumes: ecological and biogeographic linkages to seafloor and water column habitats
- 58 Active positioning of vent larvae at a mid-ocean ridge
- 59 The submarine volcano eruption off El Hierro Island: effects on the scattering migrant biota and the evolution of the pelagic communities
- 60 The influence of vent systems on pelagic eukaryotic micro-organism composition in the Nordic Seas
- 61 The importance of hydrothermal venting to water-column secondary production in the north east pacific
- 62 Transient changes in bacterioplankton communities induced by the submarine volcanic eruption of El Hierro (Canary Islands)
- 63 The Gelatinous Macroplankton Community at the Hatoma Knoll Hydrothermal Vent
- 64 Predicting the response of the deep-ocean microbiome to geochemical perturbations by hydrothermal vents
- 65 Metagenomic resolution of microbial functions in deep-sea hydrothermal plumes across the Eastern Lau Spreading Center
- 66 Delta-proteobacterial SAR324 group in hydrothermal plumes on the South Mid-Atlantic Ridge
- 67 Biogeography of bacteriophages at four hydrothermal vent sites in the Antarctic based on g23 sequence diversity
- 68 Quantifying dispersal from hydrothermal vent fields in the western Pacific Ocean

Figure 3 and Figure 4 Data: Please download file “MS1_Fig3_Fig4_Data.xlsx”, available on URI’s ProQuest ETD site under “Supplementary Material” associated with this dissertation.

Appendix D:

Detail of methods and data presented in Chapter 2

Figure 1 data: Please download file “MS2_Fig1_Data.xyz”, available on URI’s ProQuest ETD site under “Supplementary Material” associated with this dissertation.

Figure 2 data: Please download file “MS2_Fig2_Data.xlsx”, available on URI’s ProQuest ETD site under “Supplementary Material” associated with this dissertation.

Figure 4 data: Sequence data is deposited in GenBank under the accession numbers KU761314-KU761325 (<http://www.ncbi.nlm.nih.gov/genbank/>)

Figure 5 data:

Sample Name		KAV-001	KAV-002	KAV-003
SiO ₂	wt. %	50.59	50.51	51.43
TiO ₂	wt. %	0.565	0.608	0.503
Al ₂ O ₃	wt. %	17.19	19.19	18.46
Fe ₂ O ₃	wt. %	9.93	9.83	9.48
MnO	wt. %	0.17	0.16	0.16
MgO	wt. %	6.77	5.21	6.33
CaO	wt. %	11.74	10.54	11.71
Na ₂ O	wt. %	1.98	2.31	2.21
K ₂ O	wt. %	0.75	0.77	0.73
P ₂ O ₅	wt. %	0.09	0.10	0.08
Total		99.78	99.23	101.10
H ₂ O-	wt. %	0.00	0.00	0.00
LOI	wt. %	0.00	0.00	0.00
Li	ppm	5.91	4.33	5.59
Be	ppm	0.239	0.259	0.222
Sc	ppm	42.8	27.9	33.1
TiO ₂	wt. %	0.586	0.599	0.512
V	ppm	306	283	256
Cr	ppm	65.4	18.4	43.8

Co	<i>ppm</i>	36.3	30.7	32.8
Ni	<i>ppm</i>	33.6	24.0	30.9
Cu	<i>ppm</i>	144	151	131
Zn	<i>ppm</i>	86.1	70.2	63.7
Ga	<i>ppm</i>	15.4	16.4	15.8
As	<i>ppm</i>	0.512	0.505	0.471
Se	<i>ppm</i>	0.0205	0.0393	0.0190
Rb	<i>ppm</i>	12.7	13.9	11.3
Sr	<i>ppm</i>	310	340	338
Y	<i>ppm</i>	15.2	14.9	12.8
Zr	<i>ppm</i>	25.2	27.6	22.1
Nb	<i>ppm</i>	0.265	0.297	0.237
Sb	<i>ppm</i>	0.0131	0.0184	0.0085
Cs	<i>ppm</i>	0.120	0.132	0.106
Ba	<i>ppm</i>	83.8	94.2	76.5
La	<i>ppm</i>	1.77	1.96	1.58
Ce	<i>ppm</i>	4.36	4.78	3.87
Pr	<i>ppm</i>	0.752	0.806	0.661
Nd	<i>ppm</i>	4.06	4.29	3.53
Sm	<i>ppm</i>	1.45	1.48	1.25
Eu	<i>ppm</i>	0.572	0.589	0.513
Gd	<i>ppm</i>	2.10	2.08	1.78
Tb	<i>ppm</i>	0.392	0.386	0.333
Dy	<i>ppm</i>	2.54	2.49	2.14
Ho	<i>ppm</i>	0.558	0.543	0.470
Er	<i>ppm</i>	1.59	1.57	1.36
Yb	<i>ppm</i>	1.61	1.60	1.36
Lu	<i>ppm</i>	0.253	0.254	0.217
Hf	<i>ppm</i>	0.900	0.952	0.782
Ta	<i>ppm</i>	0.0228	0.0248	0.0194
Pb	<i>ppm</i>	1.46	1.57	1.30
Th	<i>ppm</i>	0.231	0.252	0.197
U	<i>ppm</i>	0.121	0.127	0.106

Appendix E:

Detail of methods and data presented in Chapter 3

Figure 2: Coordinates of deployment sites identified with yellow stars in map figure.

Region	ID	Date	Latitude	Longitude	Depth (max)
Solomon Islands	D130	9/21/13	-8.9619° S	158.7408° E	908 m
	D133	9/26/13	-8.3675° S	156.9874° E	942 m
New England Shelf Break	n/a	11/4/14	39.8406° N	-69.6076° W	1000m
	n/a	11/5/14	39.8936° N	-70.2395° W	900m
Galapagos Islands	H1434	6/21/15	0.7697° N	-85.912° W	2530 m
	H1435	6/26/15	1.2131° N	-91.0864° W	1105 m
	H1436	6/27/15	1.6584° N	-91.6732° W	2087 m
	H1439	6/30/15	2.1045° N	-91.9253° W	1643 m
	H1443	7/5/15	-0.3798° S	-90.8108° W	638 m

Figure 3: Please download file “MS3_Fig3_Data.xlsx”, available on URI’s ProQuest ETD site under “Supplementary Material” associated with this dissertation.

Figure 3: Please download file “MS3_Fig4_Data.xlsx”, available on URI’s ProQuest ETD site under “Supplementary Material” associated with this dissertation.