## I. GENERAL INFORMATION

1. Center General Information
2. Changes in Faculty
3. Primary Contact
4. Context Statement

## II. RESEARCH

1. Overall Research Goals and Objectives
2. Research Thrust Areas
   a. Theme 1: Fluxes, Connectivity, and Energy
   b. Theme 2: Activities, Communities, and Ecosystems
   c. Theme 3: Metabolism, Survival, and Adaptation
   d. Field Projects
   e. Projects from our Grants and Fellowships Program
   f. C-DEBI Workshops
3. Performance with Respect to the Strategic Implementation Plan
4. Plans for the Next Reporting Period

## III. EDUCATION

1. Overall Education Goals and Objectives
2. Undergraduate Students
3. Graduate Students and Postdoctoral Scholars
4. K-12 and the General Public
5. Performance with Respect to the Strategic Implementation Plan
6. Plans for the Next Reporting Period

## IV. DATA MANAGEMENT AND KNOWLEDGE TRANSFER

1. Overall Data Management and Knowledge Transfer Goals and Objectives
2. Knowledge Transfer Activities and Organizations
3. Data Management and Integration Activities and Organizations
   a. Making Data Publicly Available
   b. Providing Computational Resources to C-DEBI Researchers
   c. Expanding the Impact of C-DEBI Data through External Partnerships and Collaborations
4. Performance with Respect to the Strategic Implementation Plan
5. Plans for the Next Reporting Period

## V. EXTERNAL PARTNERSHIPS

1. Overall External Partnerships Goals and Objectives
2. Activities Conducted as Part of Partnerships
3. Performance with Respect to the Strategic Implementation Plan
4. Plans for the Next Reporting Period
VI. DIVERSITY
1. Overall Diversity Goals and Objectives
2. Programs and Activities Which Enhance Diversity at the Center
3. Performance with Respect to the Strategic Implementation Plan
4. Plans for the Next Reporting Period

VII. MANAGEMENT
1. Overall Organizational Strategy
2. Management and Communications Systems
3. Performance with Respect to the Strategic Implementation Plan
4. Plans for the Next Reporting Period

VIII. CENTER-WIDE OUTPUTS AND ISSUES
1. Center Publications
2. Conference Presentations
3. Honors, Awards and Grants
4. Placement of Graduated Students and Postdoctorals
5. Outputs of Knowledge Transfer Activities
6. All Participants
7. Institutional Partners
8. Summary Table for Internal NSF Reporting Purposes
9. Media Publicity
10. Distributable Media

IX. INDIRECT/OTHER IMPACTS
1. International Activities and Other Outputs, Impacts, or Influences
I. GENERAL INFORMATION

1. Center General Information

<table>
<thead>
<tr>
<th>Date Submitted</th>
<th>2/15/2021</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reporting Period</td>
<td>1/1/2020 - 12/31/2020</td>
</tr>
<tr>
<td>Center</td>
<td>Center for Dark Energy Biosphere Investigations</td>
</tr>
<tr>
<td>Center Director</td>
<td>Jan P. Amend</td>
</tr>
<tr>
<td>Lead University</td>
<td>University of Southern California</td>
</tr>
<tr>
<td>Co-Principal Investigator</td>
<td>Steven L. D'Hondt, University of Rhode Island</td>
</tr>
<tr>
<td>Co-Principal Investigator</td>
<td>Andrew T. Fisher, University of California Santa Cruz</td>
</tr>
<tr>
<td>Co-Principal Investigator</td>
<td>Julie A. Huber, Woods Hole Oceanographic Institution</td>
</tr>
<tr>
<td>Co-Principal Investigator</td>
<td>C. Geoffrey Wheat, University of Alaska Fairbanks</td>
</tr>
</tbody>
</table>

2. Changes in Faculty

None.

3. Primary Contact

<table>
<thead>
<tr>
<th>Name of Individual</th>
<th>Jan P. Amend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center Role</td>
<td>Director</td>
</tr>
<tr>
<td>Address</td>
<td>3616 Trousdale Pkwy, Los Angeles, CA 90089-0371</td>
</tr>
<tr>
<td>Phone Number</td>
<td>213-740-0652</td>
</tr>
<tr>
<td>Fax Number</td>
<td>213-740-8801</td>
</tr>
<tr>
<td>Email Address</td>
<td><a href="mailto:janamend@usc.edu">janamend@usc.edu</a></td>
</tr>
</tbody>
</table>

4. Context Statement

C-DEBI’s stated mission is to explore life beneath the seafloor and make transformative discoveries that advance science, benefit society, and inspire people of all ages and origins. Specifically, we seek to better understand the microorganisms that inhabit the sediment, rock, and fluid in the marine subsurface. Our scientific goals are organized into three broad research themes: (1) Fluxes, Connectivity, and Energy—centering on subseafloor environmental conditions; (2) Activities, Communities, and Ecosystems—emphasizing resident microbial communities; and (3) Metabolism, Survival, and Adaptation—concentrating on the actions and traits of individual microbial species. These goals are pursued with a combination of approaches, including field investigations, laboratory experimentation,
chemical analyses, nucleic acid sequencing, and modeling studies. In addition to these research themes, C-DEBI also facilitates and encourages the synthesis and broad understanding of submarine microbiological processes within geochemical, geophysical, and hydrogeologic contexts. This is largely accomplished by funding scientific and technical activities, coordinating and hosting meetings and workshops, and supporting researchers and graduate students. These research endeavors generate a vast array of diverse datasets that need to be managed and integrated, as well as a vast array of new knowledge that needs to be disseminated. C-DEBI has established and now operates state-of-the-art programs in these arenas. Furthermore, in our education, outreach, and diversity programs, we seek to train, educate, inspire, and mentor an interdisciplinary community. Specifically, we create and operate activities aimed at three target groups: (1) undergraduate students, especially community college students; (2) graduate students and postdoctoral scholars; and (3) K-12 and the general public. The aforementioned activities in research, data integration, knowledge transfer, education, outreach, and diversity are managed by a talented and skilled team in each of these disciplines, with critical indicators and a strategic implementation plan in place to quantitatively evaluate the Center’s performance.

In 2020, C-DEBI scientists recorded numerous significant accomplishments in our three research themes; a few examples are highlighted here. Within Theme 1, Fluxes, Connectivity, and Energy, Amend et al. (2020) used a thermodynamic approach to predict a novel metabolism (sulfur comproportionation) that could support microbial communities in a variety of natural, acidic environments. Lu et al. (2020) calculated redox energetics for a shallow-sea hydrothermal system and tabulated nearly 400 energy-yielding reactions. Bradley et al. (2020) expanded on his earlier, C-DEBI-funded work to quantify power generation from organic matter degradation in global subseafloor sediments. Shalev et al. (2020) showed that low-temperature hydrothermal circulation may explain the ‘missing’ component in the Mg budget and also tackled the ‘dolomite problem’—the modest formation of dolomite in the modern. Wheat et al. (2020) used a cesium tracer to demonstrate rapid fluid flow in the shallow crust at North Pond. In two studies from the Juan de Fuca Ridge system, Lin et al. (2020) used primordial He fluxes to suggest mantle-derived sources of methane into the crust, where it would be available to support the deep biosphere, and Wheat et al. (2020) reported a 20-year dataset of temperature, salinity, and ion concentrations from hydrothermal brines, with implications for the formation of metal deposits.

Within Theme 2, Activities, Communities, and Ecosystems, C-DEBI scientists authored numerous original research and synthesis papers. Hoshino et al. (2020) synthesized much of what is known about archaeal and bacterial diversity in deep-sea sediments. Farag et al. (2020) and Buongiorno et al. (2020) interpreted sediment genomic data to describe unique heterotrophic metabolisms of important archaeal and bacterial groups. Genomic data were also used in marine crustal systems, where Seyler et al. (2020) showed that microbial groups are mostly motile heterotrophs, and Suzuki et al. (2020) used microscopy to document microbial cells in basalt veins. Isotopic approaches were used on sediment samples to investigate nitrogen fixation (Kapili et al. 2020; Metcalfe et al. 2020) and the viability of 100 My-old cells (Morono et al. 2020), and novel electrochemical techniques were employed to study extracellular electron transport in two hard rock environments (Jones et al. 2020; Rowe et al. 2020).

Investigations within Theme 3, Metabolism, Survival, and Adaptation, generated new knowledge pertaining to individual microbes. A number of studies are on-going, with key papers in preparation for submission to peer-reviewed journals. Several papers, however, were published during this reporting period. For example, Fincker et al. (2020) synthesized the acetogenic potential of Chloroflexi in anoxic subseafloor environments. The metabolic potentials of Chloroflexi, as well as Atribacteria, were also examined in abyssal clays (Vuillemin et al. 2020a and b). Lastly, genomes from a ubiquitous vent taxa, *Sulfurovum*, yielded robust evidence of natural selection in driving microbial evolution in deep-sea hydrothermal systems.

In 2020, C-DEBI’s education, outreach, and diversity (EOD) programs were impacted by the COVID-19 pandemic, but we provided on-line versions for two of our flagship programs for undergraduate students: the Genomics and Geobiology Undergraduate Research Experience (GGURE) for students from underrepresented groups at USC and the Global Environmental Microbiology (GEM) course for students across the country. A third program, the Community College Research Internship for
Scientific Engagement (CC-RISE) could not be offered, but it is slated to continue in 2021. Graduate students and postdoctoral researchers were also affected by the pandemic, but several on-line training and mentoring activities proved effective. C-DEBI scientists, heavily dominated by its junior researchers, are connected via several social media platforms. In addition, graduate students and postdoctorals continued their professional training with presentations at virtual national and international meetings. We also continued our Networked Speaker Series with 4 invited speakers (3 postdoctorals and 1 graduate student), and we held a virtual annual meeting, with plans for several similar follow-up meetings to be planned, organized, and hosted by early career scientists. Lastly, our bioinformatics leadership established the Bioinformatics Virtual Coordination Network providing tutorials on computational projects.

C-DEBI’s data management and knowledge transfer programs disseminate the most important scientific discoveries and technical advances to the scientific community and the broader population. We also ensure that the variety of data types and products are archived, shared, and accessible for the long term. C-DEBI knowledge transfer occurs via numerous mechanisms, the most effective of which for transfer to the scientific community remains to be through journal publications; during this reporting period, C-DEBI scientists authored more than 40 peer-reviewed papers. Additional avenues include worldwide access to data and new technologies, exchanges of personnel, lectures and presentations, professional development activities, conferences and workshops, and targeted courses for students and educators. The dissemination of C-DEBI products relies on our ability to reach the target audiences. In this regard, we note that C-DEBI’s mailing list counts nearly 1000 individuals in 40 countries. Of these, 150 are 'active' participants, defined as those who presented or participated at one of our workshops or conference sessions, submitted proposals to C-DEBI, served on one of our committees or panels, or otherwise engaged directly in C-DEBI science or education activities.

As we near the end of the STC, we continue to seek external funding to maintain the incredible community of deep biosphere scientists C-DEBI has developed, nurtured, and supported in the past 10 years to continue the multidisciplinary research in the subsurface biosphere only possible with center-based infrastructure. In the remaining time of our no-cost extension, we are emphasizing synthesis as well as the completion of original research projects.

II. RESEARCH

1. Overall Research Goals and Objectives

The Center’s overall research goals have not changed from the previous year. We seek to investigate the marine deep subsurface biosphere, including the physiology, phylogeny, distribution, limits, and activity in the sediments, rocks, and fluids that make up this very large biome. Our approaches combine field-based research with laboratory studies of microbial survival and propagation, and modeling studies of physico-chemical properties in the subsurface. Another way to describe our research framework is as a balance of discovery science, hypothesis testing, data integration, laboratory experimentation, and ecosystem modeling. C-DEBI continues to generate knowledge in these areas by addressing fundamental questions, which include: How metabolically active is the subseafloor biosphere? What are the physico-chemical limits of life in the subseafloor? What are the nature and extent of life in the subseafloor? What are dominant metabolic processes in the subseafloor? The central research objectives require multidisciplinary and interdisciplinary approaches, with the greatest emphasis on microbial ecology. To achieve these objectives, we directed a large portion of the research funds to the Co-Investigators (Jan Amend, USC; Julie Huber, WHOI; Steven D’Hondt, URI; Andrew Fisher, UCSC; Geoff Wheat, UAF) and Senior Scientists (Steven Finkel and John Heidelberg, USC; Beth Orcutt, Bigelow; Victoria Orphan, Caltech; Alfred Spormann, Stanford). Unlike previous years, we did not provide substantial resources to competitively awarded research grants, and graduate student and postdoctoral fellowships; this was a
necessary response to the scaled-back budget as we approach the end of our funding period. In this report, the Principal Investigator, Co-Investigators, and Senior Scientists who have equal scientific standing in C-DEBI will all be referred to as “Co-I’s”. The research themes are:

**Theme 1: Fluxes, Connectivity, and Energy**—centering on subseafloor environmental conditions.

1. (1.1) Constrain the extent, variability, and controls on fluxes and connectivity within subseafloor biomes and between the subseafloor and the overlying ocean.
2. (1.2) Map the geochemical energy sources in subseafloor ecosystems at a range of spatial scales.
3. (1.3) Develop and test the next generation of coupled geochemical-hydrological-microbial models for subseafloor ecosystems.

**Theme 2: Activities, Communities, and Ecosystems**—emphasizing resident microbial communities.

1. (2.1) Determine community composition, functional potential, and patterns of natural selection in subseafloor ecosystems.
2. (2.2) Determine metabolic activity of subseafloor microbial communities.
3. (2.3) Advance understanding of subseafloor microbe-virus interactions.

**Theme 3: Metabolism, Survival, and Adaptation**—concentrating on the actions and traits of individual microbial species.

1. (3.1) Isolate and characterize novel bacteria and archaea from diverse subseafloor habitats.
2. (3.2) Examine fundamental physiology of subseafloor microbes under conditions of low growth rates and low energy flux.
3. (3.3) Perform adaptive evolution and long-term survival experiments with subseafloor microbes to characterize molecular genetic signatures associated with particular phenotypes.

2. Research Thrust Areas

Here, we summarize the most important research accomplishments in 2020; the only noteworthy problems were limitations—in some instances substantial limitations—to field and lab work due to the COVID-19 pandemic. The first three subsections focus on the primary research themes, as described in the last section. This is followed by summaries of field programs that were active in 2020, comprised of work that cuts across the research themes. For each of these programs, we provide background/context and describe the key operational, scientific, and technical accomplishments. The last two subsections briefly highlight projects previously funded through our grants and fellowships program (2.e) and workshops supported by C-DEBI (2.f). As noted above, no new grants or fellowships were awarded in 2020.

a. **Theme 1: Fluxes, Connectivity, and Energy**

Research associated with Theme 1 addressed topics including: the energetics of distribution of microbial metabolisms, hydrothermal solute balances and flows, and development and application of new tools and methods. We highlight selected studies on these and related topics in this section.

Amend et al. (2020, C-DEBI #523) used calculations of redox reaction energetics to posit a novel microbial metabolism, sulfur comproportionation. In this putative metabolism, sulfate and sulfide react at low pH to yield elemental sulfur. Natural environments in which this reaction would be exergonic (i.e., energy yielding) could include shallow-sea hydrothermal systems. The authors note that this predictive approach, using thermodynamics to hypothesize new metabolic pathways, was successfully used previously to propose anaerobic ammonia oxidation with nitrate or nitrite (later termed anammox) and anerobic oxidation of methane with sulfate (later termed AOM).

Lu et al. (2020, #529) analyzed samples collected from a shallow-sea hydrothermal system in Greece, calculating the Gibbs energies for >700 possible redox reactions that could fuel the sedimentary biosphere. The authors found 379 reactions that were exergonic at one or more locations, generating as
much as ~160 kJ/mol of electrons from redox reactions involving oxygen, nitrate, carbon monoxide, and hydrogen sulfide. These calculations suggest that microorganisms capable of using native sulfur or hydrogen sulfide may benefit from a large energy advantage over other metabolic pathways.

Bradley et al. (2020, #534) provided global maps of marine sediments, depicting energy and power availability from several organic matter degradation processes. The primary pathways were demonstrated to be (in order of decreasing importance) sulfate reduction, methanogenesis, and aerobic respiration. The total power from these degradation reactions on a global scale is on the order of 40 GW, about the same as that generated at 80 high-temperature vent fields, or lost by conduction from 5 x 10^5 km^2 of Earth's seafloor, equal to about 0.05% of the deep sea. Much of the energy utilization associated with processing of organic matter in marine sediment occurs on continental shelves, where organic carbon contents and sedimentation rates are high. In contrast, the energetics of life in the abyssal ocean sediments are very limited, suggesting that life can perhaps survive (but not thrive) in these low energy environments.

Shalev et al. (2020, #516) may have resolved two long-standing quandaries in marine chemistry and geology: closing the Mg budget and explaining the discrepancy between historical and modern production of dolomite. For the Mg budget, it has been recognized for decades that there appears to be a quantitative imbalance between Mg inputs into the ocean, mainly from continental weathering and riverine fluxes, and Mg sinks in the ocean. The latter are thought to comprise mainly water-mineral reactions that occur as a consequence of sedimentary diagenesis and hydrothermal circulation, but the removal of Mg as part of low-temperature hydrothermal circulation (in particular) has remained cryptic and difficult to quantify. Resolution to the "dolomite problem" (the lack of modern dolomite formation relative to the abundance of dolomite in the rock record) has depended previously in paleoenvironmental explanations (that more dolomite was formed during warmer periods when sea level was higher) and/or attribution to latent processes that create dolomite in relatively inaccessible settings. In this new study, based on analysis of Mg isotopes, the authors found evidence for a Mg sink as part of low-temperature hydrothermal circulation, which can be explained by the formation of dolomite below the seafloor. If their preferred interpretation is correct, then the dolomite problem may be a consequence of a large, heretofore unrecognized Mg reservoir in oceanic sediment and volcanic rocks.

Another long-standing conundrum involving seafloor hydrothermal systems was addressed by Wheat et al. (2020, #546), who sampled high-temperature fluids that discharged from the Cleft Segment of the Southern Juan de Fuca Ridge. These samples were the last of a long series of vent samples dating back to 1984, when venting in this area was sampled for the first time. Hydrothermal fluids are remarkably stable in temperature, salinity, and the concentrations of several major ions. For example, temperatures at one site have remained remarkably constant over ~3 to 20 years. Since 1984 hydrothermal fluids that discharge from one vent have much higher salinities than seawater and likely are a result of phase separation and segregation. On a global basis, this salt-imbalance (with fresher fluids being more common than brines) is thought to result in the cycling of solutes back into the mantle during subduction, but as these authors note, the occurrence and nature of hydrothermal brines is poorly known, with important implications for the formation of metal deposits.

Lin et al. (2020, #535) completed the first assessment of primordial helium fluxes from the mantle into the crust on ridge flanks, using fluids collected from borehole observatories in the Juan de Fuca major program area. These fluids were enriched in 3He relative to air and bottom seawater, but only half as enriched as fluids sampled from high-temperature hydrothermal fluids on ridge axes. This suggests that much of the excess helium found in ridge-axis hydrothermal fluids, equivalent to ~1 to 6% of the global flux, results from degassing from the underlying mantle and ridge-flank basalt. These results may provide a source for mantle-derived, abiotic methane production, subsequently fueling the deep biosphere.

Wheat et al. (2020, #518) analyzed a cross-hole tracer experiment completed in the North Pond major study are using ocean drilling boreholes. For this study, a cesium-rich tracer was injected into one borehole (U1383B) and the arrival of tracer was monitored in another borehole (U1383C) located ~25 m away. The rapid appearance of tracer in the monitoring hole indicates relatively rapid transport in the
shallow crust. Differences in tracer and natural solute concentrations suggest that the upper basement monitored in this area is not as well-mixed as is often supposed, likely because the upper crust is heterogeneous in permeable pathways. This finding is consistent with earlier work that investigated the presence of distinct dissolved carbon reservoirs in the volcanic crust (Walter et al. 2018).

These and additional studies highlighted the significance of new tools and methods for subseafloor research, including the use of long-term borehole observatories (CORKs) for collection of dissolved gases and characterization of organic carbon (Lin et al. 2020; #543) and collection of borehole fluid samples at high temperature (Wheat et al. 2020; #530). The development of pumping, filtration, and measurement systems for large-volume sampling of CORK fluids is especially notable, as they required nearly a decade to develop (engineer, prototype, test, validate) and resulted in a massive increase in sampled volumes and quality.

Figure 1. Examples of studies exploring connections, fluxes and energetics of coupled fluid-chemical-microbiological systems in the deep sea. A. Calculations of energy available from a novel
chemolithotrophic metabolism (Amend et al. 2020, #523). B. Calculations of cell-specific energy utilization from sulfate reduction in marine sediments (red is greatest) (Bradley et al. 2020, #534). C. Cartoon illustrating low-temperature hydrothermal flows, reactions, and potential for loss of Mg as a consequence of dolomite formation (Shalev et al. 2020, #516). D. Measuring the temperature of a briny vent discharge on the Cleft Segment of the Juan de Fuca Ridge (Wheat et al. 2020, #546). E. Schematic and photograph of novel fluid sampling system developed and deployed on long-term borehole observatories. F. Chemical records of a cross-hole tracer discharge experiment in North Pond (Wheat et al. 2020, #518).

► See References Cited in Appendix A
► See related C-DEBI Contributed Publications in Appendix G

b. Theme 2: Activities, Communities, and Ecosystems

Theme 2 emphasizes research focused on the in situ environmental microbial assemblages, their functional activities, and interactions within the deep subseafloor biosphere. Key objectives of this theme are (1) Determine community composition, functional potential, and patterns of natural selection in subseafloor ecosystems; (2) Determine metabolic activity of subseafloor microbial communities; and (3) Advance understanding of subseafloor microbe-virus interactions. In 2020, C-DEBI researchers have continued to make important contributions to our understanding in these areas. Here we highlight a few recent discoveries and publications from C-DEBI scientists including Co-I’s, postdocs and students aligned with Theme 2 objectives.

Contributing to the first objective, a global synthesis of sediment microbial diversity by Hoshino and colleagues published in PNAS (2020, C-DEBI #544), including data from several C-DEBI Major Programs and with Co-I D’Hondt as co-author, documented that oxygen and organic carbon are the key environmental factors defining microbial community composition and diversity. This work estimates a global richness of roughly $10^9 - 10^4$ unique sequence variants of both Bacteria and Archaea in marine sediment, comparable to the richness in seawater and topsoil. In addition, several studies used genomic approaches to examine the functional potential of sediment microbial taxa. For example, Vuillemin et al. (2020 a and b, #545 and #551 both involving Co-I D’Hondt) used samples from the North Atlantic to show that sediment Chloroflexi likely use homoacetogenesis to thrive in anoxic settings, and that sediment Atribacteria likely use fermentative and acetogenic pathways to fuel growth in the subsurface. These studies of Atribacteria and Chloroflexi and Atribacteria provided the first transcriptomic evidence of active cell division in subseafloor sediment. A synthesis of genomic data from several anoxic subsurface environments further documented the acetogenic potential of Chloroflexi (Fincker et al. 2020, #531, involving Co-I’s Huber, Orphan, and Spormann). Genomic datasets from organic rich sediments were leveraged by Farag et al. 2020 (#517 involving former C-DEBI postdoctoral fellow León-Zayas and C-DEBI scientist Biddle) to reveal the metabolic potential of Lokiarchaeota (i.e., degradation of hydrocarbons like benzoate) and Bathyarchaeota (i.e., unique coupling of methylotrophy to acetogenesis) and by Buongiorno et al. 2020 (#539 involving C-DEBI scientist Lloyd) to show that Woesiiales bacteria can switch from sulfur oxidation to nitrate reduction with burial. In subsurface marine crust, genomic approaches also documented that microbial groups in fluids circulating through upper basaltic crust are predominantly heterotrophic and motile (Seyler et al. 2020, #548 including Co-I Huber), and microscopic approaches documented dense microbial life in basalt veins filled with specific secondary minerals (Suzuki et al. 2020, #526 involving Co-I D’Hondt). Analysis of genomic datasets from several hydrothermal systems revealed that natural selection is a key driver of gene loss and gain in *Sulfurovum* species (Moulan et al. 2020, #525 involving Co-I Huber). Finally, C-DEBI supported postdoc Daan Speth with co-I Orphan is using comparative metagenomics to assess the community structure and physiological traits of sediment-hosted microorganisms from S. Pescadero Basin and Guaymas basin, the two major sedimented hydrothermal vent systems in the Gulf of California. These vent fields are separated by a distance of ~200 km, yet support an unexpectedly high degree of overlap at the species-
level (>95% ANI) for archaeal and bacterial MAGs, suggestive of frequent exchange of multiple microbial lineages between these two vent ecosystems. The prediction of optimal growth temperatures from genomic data was calibrated between phenotypically characterized cultured and uncultured microorganisms, providing additional insights into the distribution of thermophilic adaptation among diverse lineages from Gulf of California hydrothermal vents (Speth et al. in prep).

Contributing to the second objective, stable isotopic approaches coupled to genomics revealed new metabolic processes in marine sediment. Kapili et al. (2020, #520 involving C-DEBI scientist Dekas) documented the capacity for nitrogen fixation by diverse sediment Bacteria. Likewise, Metcalfe et al. (2020, #557 involving Co-I Orphan) documented nitrogen fixation by a newly described lineage syntrophic sulfate-reducing bacteria coupled to methane-oxidizing ANME-2b archaea in methane seeps. Long-term stable isotope incubations documented that aerobic cells can persist in 100-million-year-old sediment and are capable of rapidly consuming substrates (Morono et al. 2020, #532 including Co-I D’Hondt). Stable isotope probing experiments also offered evidence of active anaerobic methane oxidation by endolithic archaea in barites from S. Pescadero basin (Cremiere et al. in prep with C-DEBI Co-I Orphan and scientists Wu and Speth). Isotopic analysis of d13C of methoxyl groups sourced from lignin in deeply buried coalbeds (Shimokita Peninsula IODP leg 337) provide evidence for subsurface microbial methylotrophy and highlight potential sources and controls on coalbed methane generation. This work is currently in review at Science and includes C-DEBI co-I Orphan and scientist Trembath-Reichert. Novel electrochemical poised potential techniques were developed by C-DEBI postdocs to examine microbial activity and extracellular electron transport in subsurface hard rock environments, revealing the potential for aerobic iron oxidation in subsurface marine basalts (Jones et al. 2020, #503 including C-DEBI scientist Orcutt) and fluctuating oxidative and reductive interactions in the continental subsurface despite consistent microbial community structures (Rowe et al. 2020, #552 led by a former C-DEBI postdoc and involving Co-I Amend).

Contributing to objective 3, numerous genomic datasets continue to reveal the interaction of viruses with microbial hosts in the subsurface. For example, C-DEBI scientist Labonté led a study documenting prophages in diverse hydrothermal taxa (2019, #524). Unlike in the surface ocean, this environment seems dominated by lysogeny instead of lytic infections, even though highly active taxa could favor lytic infection. Lysogen prophage appear to have co-evolved with their hosts and to be involved in horizontal gene transfer for protection against toxic metals and antibacterial compounds. Recent enrichment cultivation and assembly of circularized genomes associated members of the Asgard Archaea within Pescadero Basin hydrothermal sediment (Heimdallarchaeota) using long read sequencing additionally point to an active archaea-virus ‘arms race’ in this extreme deep ocean ecosystem. Anoxic enrichment cultures harboring Heimdallarchaeota contained CRISPR/Cas viral defense systems with insert sequences matching viral-like sequences in addition to a novel genome-associated prophage that appeared to transition from passive lysogeny to active replication over the course of the incubation (led by C-DEBI supported postdoc Fabai Wu, Co-I’s Orphan and Amend).

► See References Cited in Appendix A
► See related C-DEBI Contributed Publications in Appendix G

c. Theme 3: Metabolism, Survival, and Adaptation

Theme 3 concentrates on the actions and traits of individual microbes, including the isolation and characterization of novel bacteria and archaea from diverse subseafloor habitats; examining fundamental physiology of subseafloor microbes under conditions of low growth rates and low energy flux; and carrying out adaptive evolution and long-term survival experiments with subseafloor microbes to characterize molecular genetic signatures associated with particular phenotypes. Traditionally, our understanding of the physiology of microorganisms has been historically derived from fast growing, terrestrial microorganisms. In Theme 3 (Metabolism, Survival, and Adaptation), C-DEBI seeks to investigate relevant subseafloor organisms under laboratory conditions and to study their metabolisms in
controlled environments that allow for scientific hypotheses testing. Here, we discuss several such ongoing experiments and recently published results.

Isolates from marine sediment, crustal fluids, and hydrothermal vents are being used in several projects. New laboratory studies with *E. coli* were carried out by C-DEBI researcher Dr. Alberto Robador in Co-I Finkel’s lab to identify previously unknown growth dynamics related to the temporal expression of the growth advantage in stationary phase (GASP) phenotype that allow mutants to capitalize on the decrease of energy over prolonged periods of time (Robador et al. 2019, C-DEBI #480). In addition, Ph.D. student Hans Sebastian has been comparing the growth and survival dynamics of *E. coli*, *Pseudomonas*, and *Halomonas* species under nutrient limiting conditions, including using deep subsurface sediment pore waters as the sole sources of carbon and energy. We are now combining those approaches with mass spectrometry analysis of potentially bioavailable organic molecules to bridge our understanding of how microbes access nutrients during long periods of starvation. As shown in Figure 2, we are comparing the calculated available energy of dissolved organic compounds with calculated energy needs of microbes in those environments. These analyses allow us to examine the balance between biomass production and “maintenance energy,” as well as help understand the aging of microbes found in subsurface environments.

![Figure 2. Energy distribution during the time course of growth of E. coli. The energy available in culture (solid black line) was calculated based on the elemental composition of dissolved compounds –as resolved with HRMS – using thermodynamic models. The dashed line indicates the energy consumed due to the metabolic activity of E. coli populations.](image)

The Finkel lab also continues to work with novel isolates from the crustal aquifer. Crustal fluids from the deep and shallow horizons of CORK observatory U1383C at North Pond were used to isolate 46 *Halomonas* strains on autotrophic minimal media in Co-I Huber’s lab. Co-I Finkel’s group continues to characterize the suitability of these isolates with respect to their utility as model organisms for laboratory experiments, currently focusing on the physiological properties and genetic plasticity of these organisms and their ability to grow under extremely oligotrophic conditions. We also continue to characterize the ability of both laboratory and naturally-obtained species to utilize pore water as a source of carbon and energy. Using a variety of dialysis techniques, we have demonstrated that the bioavailable carbon present in these pore waters is used differentially by two different laboratory species: *E. coli* and *Pseudomonas*.
aeruginosa. In fact, under almost all conditions *Pseudomonas* is able to grow using pore water as the sole sources of carbon and energy, while *E. coli* strains survive without proliferating. Mass spectrometry approaches are now being used to characterize which carbon compounds are accessed by which organisms. This analysis is also now being applied to a set of 8 *Halomonas* strains that have been undergoing analyses for genetic plasticity.

In Co-I Spormann’s lab, a new enrichment method is being developed for deep-sea sediment microbes. Traditional laboratory enrichments are set up as batch systems in which nutrients at high concentrations are available simultaneously to the entire community population. Such enrichment setup will select for the fastest-growing cells that can use the selected substrate in this artificial environment; however, these cells might not play important roles in their native environments. Modifying enrichments to operate as a fed-batch or chemostat, in which nutrients are added throughout the course of the incubation also does not reduce competitive bias for the fastest growing subpopulation because slower-growing microbes will always be outcompeted regardless of when a shared pool of resources is added. Therefore, in order to circumvent competition from faster-growing microbes, the Spormann lab (led by postdoc Dr. Ali McCully) are developing a novel, high throughput enrichment platform that eliminates competition by spatially segregating single cells into double oil emulsions (Figure 3). This enrichment platform selects for microbes that have higher biomass efficiency rather than faster growth, as only a finite pool of resources is available to each cell population within a single droplet. Selection occurs when the resulting grown populations are pooled and diluted, where the more efficient cells will be enriched given sufficient time to metabolize their privatized pool of nutrients. Development of this novel enrichment technique was done in collaboration with the Fordyce lab at Stanford University, who specialize in microfluidics and bioengineering technology. We have been optimizing conditions to generate stable, fully anaerobic droplets containing artificial saltwater medium that support the growth of a novel sulfate-reducing bacterium *Desulfovibrio ferrophilus* IS5 and the model methanogenic Archaea *Methanococcus maripaludis*, with plans to expand growth conditions to other model methanogens, sulfate reducers, and acetogens. For example, frozen core samples from the IODP Gulf Coast Repository will be used with the double oil emulsion enrichment method to enrich for acetogens from Guaymas Basin and Juan de Fuca Ridge.

![Figure 3. Double oil emulsion generation setup. (A) Aerobic double oil emulsion setup consisting of programmable syringe pumps, a high-speed camera, stereo to visualize droplet generation, custom](image-url)
fabricated PDMS device design, and medical tubing. (B) Custom fully anaerobic double oil emulsion setup housed in an anaerobic glove box (N₂/H₂ atmosphere). (C) Diagram of fabricated device array showing inlets for medical tubing to generate double emulsions at set flow rates. “Cell” and “Inner” channels contain bacterial growth medium and BSA as a stabilizing agent. Carbon sources are added through in the “Inner” line and cells are suspended in the “Cell” line. “Outer” channel inlet contains bacterial growth medium. (D) The aqueous “Inner” and “Cell” components are surrounded by the oil shell to form single emulsions, then the water-in-oil emulsions are converted into double oil emulsions as the “Outer” aqueous layer pinches off individual droplets suspended in aqueous outer solution.

Theme 3 also uses environmental –omics to examine the metabolism of subseafloor populations. Co-I Spormann’s lab focuses on uncultured members of the Chloroflexi phylum that are highly enriched in numerous subseafloor environments. Their metabolic potential was evaluated by reconstructing 31 Chloroflexi genomes from 6 different subseafloor habitats. The near ubiquitous presence of enzymes of the Wood-Ljungdahl pathway, electron bifurcation, and ferredoxin-dependent transport-coupled phosphorylation indicated anaerobic acetogenesis was central to their catabolism (Fincker et al. 2020, #531). Most of the genomes simultaneously contained multiple degradation pathways for complex carbohydrates, detrital protein, aromatic compounds, and hydrogen, indicating the coupling of oxidation of chemically-diverse organic substrates to ubiquitous CO₂ reduction. Such pathway combinations may confer a fitness advantage in subseafloor environments by enabling these Chloroflexi to act as primary fermenters and acetogens in one microorganism without the need for syntrophic H₂ consumption (Figure 4). While evidence for catabolic oxygen respiration was limited to two phylogenetic clusters, the presence of genes encoding putative reductive dehalogenases throughout the phylum expanded the phylogenetic boundary for potential organohalide respiration past the Dehalococcoidia class. Additional work by C-DEBI community members, including Co-I D’Hondt and former C-DEBI postdoc Dr. William Orsi examined the metabolic potential and gene expression of subseafloor Chloroflexi and Atribacteria in abyssal clays (Vuillemin et al. 2020a; #545, Vuillemin et al. 2020b, #551).

Figure 4. Proposed metabolism of Chloroflexi in the subseafloor. Chloroflexi contain pathways of fermenters and acetogens integrated in one microorganism in conjunction with the CO₂-reductive WLP which enables a more efficient use of a catabolic substrate. Such efficient metabolism is independent of an obligate interspecies H₂/formate transfer to H₂/formate-consuming microorganisms, because the WLP consumes electrons from oxidative pathways internally during CO₂ reduction to acetate. Such integrated metabolism with an overall longer pathway may be more advantageous in a carbon- and mixing-limited environment.

Other work with environmental -omics in Theme 3 includes research from Co-I Huber’s lab that used genome-resolved metagenomics and metatranscriptomics to examine microbial communities in the low-temperature, ridge flank environment from North Pond crustal fluids. Our results suggest that the microbial community in the North Pond aquifer plays an important role in the oxidation of organic carbon within the crust. This community is motile and metabolically flexible, with the ability to use both autotrophic and organotrophic pathways, as well as function under low oxygen conditions by using
alternative electron acceptors such as nitrate and thiosulfate. Anaerobic processes are most abundant in subseafloor horizons deepest in the aquifer, furthest from connectivity with the deep ocean, and there was little overlap in the active microbial populations between sampling horizons. In addition, Co-I Huber and C-DEBI community member Dr. Rika Anderson and her undergraduate students evaluated the influence of selection on gene content variation in hydrothermal vent microbial populations. Metagenome-assembled genomes (MAGs) from the ubiquitous vent Epsilonbacteriaeota genus Sulfurovum were recovered from two deep-sea hydrothermal vent regions, Axial Seamount in the northeastern Pacific Ocean (13 MAGs) and the Mid-Cayman Rise in the Caribbean Sea (9 MAGs). While housekeeping functions were highly conserved, genes involved in environment-specific functions, and in particular phosphate regulation, were found mostly in Sulfurovum genomes from the Mid-Cayman Rise in the low-phosphate Atlantic Ocean environment, suggesting that nutrient limitation is an important selective pressure for these bacteria (Moulana et al. 2020, #525). These results highlight the importance of natural selection in driving the evolution of microbial populations in the dynamic hydrothermal vent habitats.

► See References Cited in Appendix A
► See related C-DEBI Contributed Publications in Appendix G

d. Field Projects

The Center continues to pursue expedition-based research. Here, we briefly describe activities that took place in 2020, identifying key C-DEBI personnel involved, and note expeditions that were postponed and rescheduled for 2021 and 2022 due to the COVID-19 pandemic.

Mid-Cayman Rise. For a month in January-February 2020, C-DEBI Postdoctoral Fellow Dr. Sarah Hu and other C-DEBI associated researchers (Drs. Rika Anderson, Tom McCollom, Susan Lang, Karyn Rogers, Kiana Frank) participated in a NSF-sponsored research cruise led by Drs. Jeff Seewald and Susan Lang to study the geochemistry and microbiology associated with hydrothermal activity at the Von Damm and Piccard vent fields, located on the Mid-Cayman Rise. Co-I Huber leveraged C-DEBI and WHOI funds to support Drs. Hu and Anderson to collect samples for microbiological analysis, with the overall goal of examining community structure and activity of eukaryotes, archaea, bacteria, and viruses inhabiting fluids in the seafloor below hydrothermal systems at both Von Damm and Piccard. Samples were collected by the ROV Jason for RNA/DNA extraction and sequencing, as well as RNA Stable Isotope Probing (SIP) analysis in order to examine the autotrophic microbial community at 55°C and 80°C. SIP incubations for single-cell NanoSIMS were conducted on behalf of Dr. Elizabeth Trembath-Reichert to examine single-cell microbial activity. In order to examine the abundance, diversity, evolution and ecology of viruses inhabiting the subseafloor near hydrothermal systems, Dr. Anderson also collected samples for viral counts as well as viral metagenomics. To further explore the ecological role of single-celled microbial eukaryotes at hydrothermal vent ecosystems, Dr. Hu conducted grazing experiments shipboard at 1 atm as well as at in situ pressure to capture the predation pressure heterotrophic microbial eukaryotes are exerting on the resident prokaryotic community. Results will quantify the rate at which microbial eukaryotes consume hydrothermal vent prokaryotic prey.

Future expeditions. C-DEBI Co-I’s are also involved in other upcoming drilling/coring/sampling expeditions and in numerous deep biosphere proposals (submitted and in-development) to the IODP for future drilling.

Atlantis Massif and Iceland Basin. Co-I Wheat and Co-I Orcutt’s lab (including postdoctoral and research associates) will be participating in upcoming IODP Expeditions that were rescheduled due to the COVID-19 pandemic, to lead the collection of novel deep biosphere samples. On IODP Expedition 395E (April 6 – June 6, 2021), fluid samples will be collected from a legacy borehole in gabbric oceanic crust across a thermal gradient that spans the known temperature limit for life. Although this is an engineering expedition, scientific objectives from IODP Proposal 937-Full2 (co-proponents Orcutt and Wheat) are being incorporated into the operational plan as a value-added benefit of the expedition. On the expedition, Wheat will deploy the new Multi-Temperature Fluid Sampler technology designed for
borehole applications (Wheat et al. 2020, Scientific Drilling). The MTFS can collect fluids at temperatures up to 190°C. During the expedition the MTFS will be lowered into the IODP Hole U1309D, which has a maximum temperature of 145°C at its base. Fluid samples will be collected for a variety of microbial and geochemical interests. Orcutt’s laboratory will also participate in the collection and analyses of these borehole fluids. In addition, subsurface rock samples from the serpentinizing Atlantis Massif will be collected, allowing comparison to shallow subsurface samples from this environment that were collected in 2015 on IODP Expedition 357. As IODP Expedition 395E is a primarily an engineering expedition, with a very minimal science party on board, Wheat and the Orcutt lab team will be responsible for collecting and preparing samples for a dozen shore-based participants from the C-DEBI community. In June-August 2021, postdoctoral scientist Melody Lindsay from the Orcutt lab will participate in IODP Expedition 395, leading the collection of deep sediment and upper basement samples from the western flank of the Mid-Atlantic Ridge offshore Iceland.

**Pescadero Basin.** Co-I Orphan is the co-chief scientist for a 2022 Schmidt Ocean Institute expedition to the South Pescadero Basin hydrothermal vent field in the Gulf of California. This system represents the deepest known hydrothermal vent field in the Pacific at 3800 m water depth and is distinctive in its carbonate-dominated mineralogy and near seabed oil generation. Two legs of the expedition are planned. The first leg will focus on high resolution AUV mapping of the vent field (led by MBARI scientist Dave Caress) followed by a biology/microbiology/geochemistry leg where AUV data will be used to guide strategic sampling and analysis of seafloor habitats to create a 3D perspective of the microbial assemblages, geochemical gradients, and energy availability within hydrothermal sediments.

**Mariana Trench.** During IODP Expedition 366 (December 8, 2016 - February 8, 2017) to the Mariana trench, three cased boreholes were deployed into the summit of three different serpentinite mud volcanoes. These mud volcanoes represent a continuum along the subduction channel in which fluid compositions differ with increasing pressure and temperature as the Pacific Plate subducts under the Philippine Plate. Each cased borehole included a screened section at depth and each is actively discharging fluids that ascend from the subduction channel. Co-I Wheat is funded by NSF to deploy CORK-Lites into each of the cased boreholes and to sample the discharging fluids from them. Such data will provide a measure of reactions within the subduction channel and provide a foundation for in situ experimentation and “taking the pulse of subduction”. Scheduled operations have been postponed from 2021 to potentially early 2022.

**Southern Mid-Atlantic Ridge.** C-DEBI scientists are involved in the upcoming drilling expeditions to the south Atlantic. A transect of six sites has been rescheduled for drilling in 2022 (IODP Expeditions 390 and 393 originally scheduled for 2020 and 2021). This two-part expedition will drill crust on the western flank of the Mid-Atlantic Ridge at crustal ages of 13 to 54 Ma, complementing the transect of boreholes (ODP Expedition 168) on the eastern flank of the Juan de Fuca that served as the foundation for one of the three primary C-DEBI sites. C-DEBI scientist Sylvan will be one of the Co-Chief Scientists and Co-I Wheat will participate as an inorganic geochemist.

**Blake Nose and the Central Pacific.** Co-I D’Hondt is the lead proponent of IODP Proposal 929 (Blake Nose Drilling: Effects of Subseafloor Life of a Major Lithologic Unconformity and Past Oceanic Events). This drilling expedition is currently being considered for scheduling in 2022 or 2023. D’Hondt is also a co-proponent of IODP Proposal 951-Full2 (Drilling Middle Aged Oceanic Crust on North Arch off Hawaii, lead proponents are Susumu Umino and Gregory Moore). 951-Full2 proposes the first stage in a multi-stage program to drill from the seafloor to the mantle.

► See more at the Field Sites webpage
► See related C-DEBI Contributed Publications in Appendix G

e. Projects from our Grants and Fellowships Program

C-DEBI has an extensive grants and fellowships program that supports small research projects, research and travel exchanges, and graduate student and postdoctoral fellowships. The funded projects
cut across all three Research Themes. A list of the 5 funded projects that were active in 2020 is provided in Appendix B. The breakdown of active grants and fellowships is as follows: 1 small research project (up to $80k), 1 graduate student fellowship (1-2 years), and 3 postdoctoral fellowships (1-2 years). Nine different individuals, comprised of 4 graduate students and postdocs representing 5 institutions, received financial support for these projects.

The small research grants and fellowships support a wide variety of field projects, experimental and analytical investigations, and modeling efforts relevant to C-DEBI. These include analyses of samples and data from deep subseafloor sites, laboratory studies of microbial activity, and investigations of analog environments. These small research grants and fellowships are the final awards of the program, however the research and travel exchange program continues with an open call for proposals. No new proposal submissions were received in 2020 for the research exchanges presumably due to the COVID-19 pandemic’s limitation on travel.

► See more at the Funded Projects webpage
► See related C-DEBI Contributed Publications in Appendix G

C-DEBI Workshops

In 2020, C-DEBI supported students and other junior researchers to attend the Geobiology Gordon Research Conference (January 12-17) in Galveston, Texas. In this conference “The Interaction of Microbial and Geological Processes Through Time” the scientific sessions were centered around nine unanswered questions within the broad remit of Geobiology, each of which are key to understanding the evolution of a living planet. The interdisciplinary conference explored the limits of life at the surface of the planet, the role that microbial populations play in key processes including mineral formation, the carbon cycle, silicate weathering, what drove key changes in the redox state of Earth's surface environment, and what are the new tools that exist to explore these ideas further.

One C-DEBI supported workshop initially planned for 2020 was postponed due to the COVID-19 pandemic, first to 2021 and then to 2022. This workshop, entitled Microbial Life Under Extreme Energy Limitation, will be held in Denmark, with Co-I’s Amend and Orphan among the organizers.

► See more at the Workshops webpage

3. Performance with Respect to the Strategic Implementation Plan

Our primary research goal is to enable, produce, and communicate transformative, synergistic research through an inclusive and collaborative culture that crosses disciplinary and institutional boundaries and is embedded throughout the Center’s activities. In Phase 1 (2010-2015), C-DEBI focused primarily on the exploration and discovery of subseafloor ecosystems, with most studies concentrated at four major sites: Juan de Fuca Ridge flank, South Pacific Gyre, North Pond, and the Dorado Outcrop. In Phase 2 (2015-2020), C-DEBI is developing an integrated understanding of microbial subseafloor life, covering and connecting the molecular, cellular, and ecosystem scales. Maintaining highly multidisciplinary and interdisciplinary approaches, C-DEBI is emphasizing microbial ecology while ensuring that essential context is provided through studies and advances in geochemistry, hydrology, oceanography, and related disciplines. The three overarching research themes are (1) fluxes, connectivity, and energy; (2) activities, communities, and ecosystems; and (3) metabolism, survival, and adaptation. C-DEBI research projects target two distinct subseafloor biosphere environments—the igneous ocean crust and overlying sediments—that have historically been studied independently; field investigations of these environments are complemented by coordinated laboratory studies and modeling. C-DEBI is led by five Co-PIs and five senior scientists from eight U.S. universities and research labs, but seeks to build and leverage scientific, educational, and technological partnerships with numerous other U.S. and
international institutions (educational, research, outreach, engineering, not-for-profit). In addition, C-DEBI seeks to develop a diverse community of multidisciplinary collaborators, to identify promising topics, and to develop new projects that will help to advance the Center's objectives.

**Target 1:** Transfers of fluid, heat, solutes, carbon, and microbes are quantified within and between subseafloor biomes, and between the subseafloor and the overlying ocean; the nature of energy sources available to microbes in these ecosystems is determined; and the next generation of coupled fluid-energy-biochemical-microbial models is developed.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantify transfers of fluid, heat, solutes, carbon, and microbes within and between subseafloor biomes, and between the subseafloor and the overlying ocean</td>
<td>Pending</td>
</tr>
<tr>
<td>i. Continue time-series observations and sampling at selected sites and analyze data and samples from earlier studies to resolve the extent of natural variability (within and between biomes), determine environmental controls on crustal microbial community composition, and assess how this variability impacts flows and connections</td>
<td></td>
</tr>
<tr>
<td>ii. Develop studies, including some at new field sites, as needed to test and extend understanding of coupled fluid-rock-geochemical-microbial systems</td>
<td></td>
</tr>
<tr>
<td>Determine the nature of energy sources available to microbes in subseafloor ecosystems</td>
<td>Pending</td>
</tr>
<tr>
<td>i. Map the distributions of electron acceptors and electron donors regionally and globally as a function of depth at a range of spatial scales</td>
<td></td>
</tr>
<tr>
<td>ii. Quantify metabolic reaction energetics as well as fluxes of electron acceptors and donors by combining internally consistent thermodynamic data, available kinetic parameters, and reactive transport modeling</td>
<td></td>
</tr>
<tr>
<td>Develop the next generation of coupled fluid-energy-biochemical-microbial models</td>
<td>Pending</td>
</tr>
<tr>
<td>i. Combine existing physical and thermal models with rate constants for primary reactions and transport (advection and diffusion) that involve solutes in basement fluids; then attempt to couple these with microbial processes to increase the model complexity</td>
<td></td>
</tr>
<tr>
<td>ii. Test, calibrate, and apply coupled geochemical-microbiological models to a variety of seafloor and subseafloor environments</td>
<td></td>
</tr>
<tr>
<td>Publish 25 (in aggregate) papers in this research theme</td>
<td>Met</td>
</tr>
<tr>
<td>Publish 5 (in aggregate) method/instrument papers demonstrating new techniques and tools developed and/or applied in this research theme</td>
<td>Met</td>
</tr>
</tbody>
</table>
**Target 2:** The composition of subseafloor microbial communities and the functional potential of these communities are illuminated, based on the diversity of metabolic activities and interactions with the physicochemical aspects of the system.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
</table>
| Determine community composition, functional potential, and patterns of natural selection in subseafloor ecosystems  
  i. Quantify the number, diversity, and relative abundances of microbes at multiple taxonomic levels—from domain to “species-level” operational taxonomic units (OTUs), ecotypes, and oligotypes  
  ii. Determine the functional potential embodied in these communities  
  iii. Integrate data on community composition and microbial activities to identify how sources of energy and microbial interactions drive natural selection in subseafloor ecosystems | Pending |
| Determine metabolic activity of subseafloor microbial communities  
  i. Document actual rates of *in situ* activities using gene expression in sediment and rock samples  
  ii. Identify potential activities in laboratory experiments using subseafloor samples incubated with isotope-labeled substrates  
  iii. Closely examine microbe-mineral interactions in conjunction with activity measurements in *in situ* incubations and laboratory microcosms | Pending |
| Advance understanding of subseafloor microbe-virus interactions  
  i. Integrate correlation network techniques using subseafloor archaeal, bacterial, microeukaryote, and viral diversity datasets combined with microbial activity measurements  
  ii. Incorporate the isotopic and diversity datasets collected as part of 2.a. and 2.b. to develop a food web model in combination with statistical diversity-based networks | Pending |
| Publish 25 (in aggregate) papers in this research theme | Met |
| Publish 5 (in aggregate) method/instrument papers demonstrating new techniques and tools developed and/or applied in this research theme | Met |
**Target 3:** A ‘portfolio’ of selected model subseafloor organisms is established, and their physiological and genetic traits are characterized; in addition, these microorganisms are used to investigate energy and carbon use for growth and maintenance under kinetically limiting conditions and to determine rates of metabolism under specific conditions.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
</table>
| Isolate and characterize novel bacteria and archaea from diverse subseafloor habitats  
  i. Enrich subseafloor bacteria and archaea from sediment, crustal fluids, and rock samples, using, among others, plugged flow, chemostat, and hanging sponge reactors  
  ii. Fully characterize novel organisms, including their genomes  
  iii. Interrogate their abundance and activity in the original sample to help infer their ecological roles                                                                 | Pending |
| Examine fundamental physiology of subseafloor microbes under conditions of low growth rates and low energy flux  
  i. Use long-term chemostat-like culturing systems to study the coupling of catabolism and growth in the Chloroflexi  
  ii. Use down-flow hanging sponge bioreactors to explore the molecular and physiological underpinnings of the hypothesis that archaea may be low-energy specialists and well adapted to the energetic extremes that define many subseafloor environments                                                                 | Pending |
| Perform adaptive evolution and long-term survival experiments with subseafloor microbes to characterize molecular genetic signatures associated with particular phenotypes  
  i. Use subseafloor isolates to determine the genotypic, phenotypic, and biochemical and physiological bases for metabolic traits  
  ii. Develop genetic markers for model organisms to be used in competition experiments                                                                 | Pending |
| Publish 25 (in aggregate) papers in this research theme                                                                 | Met |
| Publish 5 (in aggregate) method/instrument papers demonstrating new techniques and tools developed and/or applied in this research theme                                                                 | Pending |
Target 4: Field investigations at the four ‘major sites’ identified in C-DEBI Phase 1 are largely completed (i.e. at Juan de Fuca, South Pacific Gyre, North Pond, and Dorado Outcrop with the latter two potentially continuing beyond C-DEBI Phase 2). Environmental data and samples from these sites are compiled and analyzed along with laboratory experiments and modeling to address questions across the three Phase 2 research themes.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
</table>
| C-DEBI researchers lead and participate in expeditions to these and other sites of interest  
   i. Collect samples for laboratory analyses and experiments  
   ii. Collect environmental data for use in experiments and ecosystem modeling | Met |
| Convene workshops and conference sessions  
   i. Develop approaches to integrate results from field, lab, and modeling studies  
   ii. Synthesize results and methods from multiple sites | Met |

Target 5: The C-DEBI senior scientists are integrated in all aspects of the Center, and cross-disciplinary and cross-institutional research training is thriving through our grants programs, thereby expanding the community of deep biosphere researchers, technologists, and educators.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provide substantial research funds to the 5 Co-Investigator labs as well as to the 5 new senior scientist labs</td>
<td>Met</td>
</tr>
<tr>
<td>Award $1M in research grants/fellowships annually (for the first 3.5 years) to predominantly graduate students, postdoctoral scholars, and other junior researchers through annual RFPs</td>
<td>Met</td>
</tr>
</tbody>
</table>

4. Plans for the Next Reporting Period

The research plans for 2021 remain as stated in our Phase 2 proposal, incorporating field, laboratory, and modeling approaches. As outlined in Theme 1, we will constrain the extent, variability, and controls on fluxes and connectivity within subseafloor biomes and between the subseafloor and the overlying ocean; map geochemical energy sources in subseafloor ecosystems at a range of spatial scales; and develop and test the next generation of coupled geochemical-hydrological-microbial models for subseafloor ecosystems. In Theme 2, we will determine community composition, functional potential, and patterns of natural selection in subseafloor ecosystems; determine metabolic activity of subseafloor microbial communities; and advance understanding of subseafloor microbe-virus interactions. In Theme 3, we will isolate and characterize novel bacteria and archaea from diverse subseafloor habitats; examine fundamental physiology of subseafloor microbes under conditions of low growth rates and low energy flux; and perform adaptive evolution and long-term survival experiments with subseafloor microbes to characterize molecular genetic signatures associated with particular phenotypes. However, our primary focus for the final reporting period will be on finishing long-term investigations and generating synthesis products in addition to a chapter in press led by Co-I Orcutt on microbial life in the ocean crust and a PNAS article with Co-I D’Hondt on the global diversity of microbial communities in marine sediments that appeared in 2020. Lastly, PI Amend and Managing Director Sylvan are generating an hour-long
multi-media presentation that summarizes and highlights the major scientific and educational accomplishments of 10 years of C-DEBI; Amend intends to deliver this ‘story’ at multiple occasions and venues in 2021 and 2022.

III. EDUCATION

1. Overall Education Goals and Objectives

The main goal of C-DEBI’s education program is to generate distinctive and targeted activities in and around ocean sciences, in general, and the marine subsurface biosphere, specifically. To achieve this goal, we focus our efforts on three target groups: undergraduate students, graduate students and postdoctoral scholars, and the general public. Central to our mission is the integration of our education programs with our expansive and diverse research effort—we do not see these as independent pursuits. To engage and retain young people in STEM fields and to develop the next generation of specialists, C-DEBI provides training, mentoring, and professional development opportunities, but we also leverage numerous educational partnerships nationally to work with K-12 students and the public to ensure engagement at all levels.

As the Center sunsets, our goals have not changed. However, as planned the programs to support our education objectives have been scaled back. Despite the COVID-19 pandemic, we were able to make successful adjustments to most of our activities.

2. Undergraduate Students

As part of our undergraduate education program, we focus heavily on hands-on research activities for community college students, including the Community College Research Internship for Scientific Engagement (CC-RISE). For undergraduate students from underrepresented minority (URM) groups at USC, we again supported the Genomics and Geobiology Undergraduate Research Experience (GGURE), and for undergraduate students across the country who are interested in microbial ecology and biological oceanography, we developed our Global Environmental Microbiology (GEM) course.

CC-RISE is a non-residential REU-style program at our partner institution, Woods Hole Oceanographic Institution (WHOI) in Massachusetts, led by Co-I Dr. Julie Huber. In a typical year, four academically competitive students spend eight weeks during the summer in state-of-the-art research labs. For 40+ hours a week, they carry out experiments and analyses, and participate in professional development seminars that range from how to read and write a scientific paper to how to apply to graduate school. This year, however, WHOI has restricted undergraduate access in research labs due to the pandemic, and the program is planned instead for an in-person or virtual experience in the summer of 2021.

GGURE, a research internship program that targets URMs, continues a 17-year effort led by Co-I Dr. Steven Finkel to recruit and maintain USC undergraduate students in STEM fields. Coordinated by Education and Diversity Director Gwen Noda, the program is part-time during the academic year and full-time over the summer, with students carrying out research in a wide range of USC labs. All students were able to participate in some research with their respective lab groups from their homes during Los Angeles County shelter-in-place orders and USC research laboratory restrictions in 2020. Regular meetings provide opportunities for students to delve deeper into research topics while forming a tight cohort. They also include professional development sessions with topics ranging from ‘Demystifying Graduate School’ to ‘Career Paths’. Students present their research at conferences as well as co-author peer-reviewed papers. As reported in the external evaluations (Appendix C/Appendix D), these research experiences
made students more likely to include research in their career goals. A 17-year retrospective survey is currently being conducted.

Another flagship component of our undergraduate education program is the GEM course, which targets URMs early in their academic careers. Now in its tenth year, this 3-week course led by USC faculty Co-I Dr. John Heidelberg and Dr. Eric Webb with directional support from Education and Diversity Director Gwen Noda was designed to be field-based and hands-on. This year, in-person USC summer programs were cancelled due to the pandemic, so a 3-week virtual program was developed. The Summer OnLine Interactive/Discussion Global Environmental Microbiology (SOLID-GEM) met synchronously 3 hours each weekday with additional virtual office hours. The program included lectures and discussions about microbiology, oceanography, and molecular biology; labs to help develop students’ skills working with data and understanding phylogenetic trees; opportunities to practice creating and delivering science presentations; graduate student, graduate school, and early career scientist panels for students to meet and talk with graduate students and faculty about their science career pathways; and assistance in finding and applying to future REU opportunities. In lieu of students doing field and lab work themselves, we provided virtual experiences via short talks from graduate student, postdoctoral, and faculty researchers about their own field and lab work (e.g., Dr. Karla Heidelberg (USC faculty/NSF) on Antarctica, Heidi Aronson (USC graduate student) on caves, and Dr. Sarah Hu (WHOI postdoctoral researcher) on oceanography). Fourteen students participated with all but one from community colleges.

We remain in close contact with all graduates of the course through email and social media, and we maintain a community of young researchers with this common experience. This year’s online version of the course was well received with all of the students reporting that it was a worthwhile experience and would recommend it to others (Appendix E). All students rated the fieldwork talks “8” or higher on a 10-point scale and most gave them a perfect “10.” All students also felt that the program impacted their educational goals and they continue to plan a career in science. Almost half (43%) said the program has given them a new target, helping them to see graduate school as an attainable goal and/or having exposed them to a wider breadth of science careers including policy, teaching, and fieldwork.

Undergraduate students were also engaged by C-DEBI researchers, senior scientists, graduate students, and postdoctoral scholars through mentoring (e.g., Dr. Steven Finkel (USC), Dr. Andrew Fisher (UCSC), Dr. Beth Orcutt (Bigelow), Dr. Geoff Wheat (UAF), Dr. Benjamin Tully (USC)), workshops and training events (e.g., Dr. Benjamin Tully (USC)), and lectures (e.g., Dr. Julie Huber (WHOI), Dr. Steven D’Hondt (URI)).

### Activity Summary: Undergraduate and Community College Programs

<table>
<thead>
<tr>
<th>Led by</th>
<th>Co-I Dr. Julie Huber, Co-I Dr. Steven D’Hondt, Co-I Dr. Andrew Fisher, Co-I Dr. Geoff Wheat, Co-I Dr. Steven Finkel, Co-I Dr. John Heidelberg, Co-I Dr. Beth Orcutt, Dr. Eric Webb, Dr. Benjamin Tully</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intended Audience</td>
<td>Undergraduates</td>
</tr>
<tr>
<td>Approximate Number of Attendees</td>
<td>100+</td>
</tr>
</tbody>
</table>

► See more at the [CC-RISE webpage](#)
► See more at the [GEM webpage](#)
► See more at the [GGURE webpage](#)
3. Graduate Students and Postdoctoral Scholars

The close integration of education and research is particularly evident in the activities of our graduate students and postdoctoral scholars. C-DEBI provides both formal and informal training to these early career scientists. First and foremost, graduate students and postdoctoral scholars make up the bulk of the research personnel in the Co-I labs. In addition, C-DEBI has awarded numerous 2-year research fellowships, hosts an online seminar series, and supports a range of professional development opportunities.

At any time, approximately 30-40 graduate students and postdoctoral scholars are working on C-DEBI research in the 10 Co-I labs. In 2020, these graduate students and postdoctoral scholars received a wide variety of professional training that intertwined research with education and outreach, including oral and poster presentations at virtual national and international meetings, opportunities to deliver virtual classroom and public lectures, inclusion in and leadership of grant proposals, and mentoring of undergraduate and graduate student research.

C-DEBI also invests in the next generation of subseafloor researchers via its graduate student and postdoctoral scholar fellowship programs. These fellows have always integrated education and outreach with their research activities, and since 2016 have been required to include a formal broader impacts statement in the proposal process. The cohort of C-DEBI fellows (together with the C-DEBI graduate students and postdoctoral scholars in Co-I labs) are connected by a listserv to discuss research problems, professional development, and future employment opportunities. Gwen Noda also coordinates regular updates with information on a variety of topics from organizations including, but not limited to, the AGU, IODP, National Postdoc Association, Council of Graduate Schools, and National Association of Geoscience Teachers.

The C-DEBI Networked Speaker Series (NSS) is another opportunity for early career scientists to interact with the larger community. Speakers can be nominated by anyone, with selections made by ExCom. The speakers give live, 30-minute web seminars, followed by a Q&A session. The seminars are recorded for those unable to attend, and C-DEBI hosts 4 per year. In 2020, the speakers were Dr. Rose Jones (postdoctoral researcher, University of Minnesota) and Dr. Julia McGonigle (postdoctoral researcher, Bigelow Laboratory for Ocean Sciences), and in early 2021, the remaining speakers in the current series will be Dr. Jesse Colangelo-Lillis (postdoctoral researcher, University of Colorado Boulder) and Megan Mullis (graduate student, Texas A&M Corpus Christi).

With the pandemic still impacting travel and in-person meetings, we held our 2020 annual meeting virtually on December 2-3, composed of 2 sessions on current research activities including 14 early career lightning talks, and 1 session for a professional development diversity training. Jerika Loren Heinze, a cultural anthropologist and the founder of the Fieldwork Initiative, presented the Fieldwork Initiative’s FISST Training that provides a practical safety tool kit based on survivors’ collective knowledge and empowers researchers to prioritize their own safety and wellness in the field. In contrast to professional development workshops at previous annual meetings, participation was open to all meeting participants. The spread of participation across career stage of the 43 C-DEBI students, postdocs and faculty was appreciated in the post-meeting survey, and most survey respondents found the session “very useful” (69%) or “somewhat useful” (24%; Appendix F).

Individual C-DEBI graduate students and postdoctoral scholars also participated in a range of other professional development activities, from mentoring undergraduates to organizing workshops. For example, many C-DEBI postdocs mentored C-DEBI graduate students; Dr. Jeanine Ash (postdoctoral fellow, Rice University) compiled polar science and policy jobs for the minority-serving non-profit Polar Impact’s website; Megan Mullis (graduate student, Texas A&M Corpus Christi) led a workshop about microbiology, conservation, and sustainability activities camp directors could include in Camp Aranzazu, which serves children and adults with special needs and chronic illnesses by providing camping experiences; and Dr. Sarah Hu (postdoctoral scholar, WHOI) gave a talk about her lab and fieldwork to the SOLID-GEM undergraduate students.
Building on the success of previous bioinformatics workshops, C-DEBI Bioinformatics Specialist Dr. Benjamin Tully established the Bioinformatics Virtual Coordination Network (BVCN), a digital community led by bioinformaticians and computational biologists providing bioinformatic lessons and tutorials to assist wet-lab biologists in learning computational skills and begin computational projects during the pandemic. Thus far, this effort has attracted 590 undergraduates, graduate students, postdoctoral researchers, and faculty to the online Slack community and culminated in 1,300+ hours and 12,300+ views on the community YouTube page.

<table>
<thead>
<tr>
<th>Activity Summary: Professional Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Led by</td>
</tr>
<tr>
<td>Co-I Dr. Julie Huber, Dr. Benjamin Tully, Dr. Jesse Colangelo-Lillis, Dr. Jeanine Ash, Megan Mullis, Dr. Rose Jones, Dr. Julia McGonigle, Dr. Sarah Hu</td>
</tr>
<tr>
<td>Intended Audience</td>
</tr>
<tr>
<td>Graduate students, postdoctoral researchers</td>
</tr>
<tr>
<td>Approximate Number of Attendees</td>
</tr>
<tr>
<td>1000+</td>
</tr>
</tbody>
</table>

► See more at the [Networked Speaker Seminar Series webpage](#)
► See more at the [Bioinformatics Virtual Coordination Network webpage](#)

4. K-12 and the General Public

C-DEBI’s outreach activities engaged audiences from kindergarten through adults, including students, educators, and many others. Engagement efforts range from short, personal interactions at event tables to longer, more in-depth interactions at workshops and lectures to broadly distributed communications such as interviews for print media and podcasts, many virtual events, and social media efforts that have the potential to reach thousands of people. Here are just a few examples of our efforts in 2020: Christopher Petrone of Delaware Sea Grant, funded through a C-DEBI E&O Small Grant, reports over 55,000 views of Project VIDEO content since 2016 which includes 15 Second Science and Dive Deeper videos; In Search of Earth’s Secrets: A Pop-up Science Encounter, a traveling exhibit about the JOIDES Resolution and the deep biosphere, continues its tour of the US next year (postponed due to the pandemic; Associate Director Co-I Dr. Julie Huber participated in a webinar for MASS/Cape Cod STEM Week, a state-wide STEM promotion event, was featured in WHOI’s Ocean Encounters webinar series, and spoke virtually with high school students about deep sea research and middle school students about oceans beyond earth; Co-I Dr. Andrew Fisher was interviewed for Scientists Rescue Historical Data Taken on Floating Ice Island for Eos (Science News by AGU); Co-I Dr. C. Geoffrey Wheat hosted an outdoor display for Whalefest in Monterey, CA; Dr. James Bradley participated in Skype a Scientist with students ages 10-16 and was interviewed by Mark Fallows for The Impossible Network podcast; Co-I Dr. Victoria Orphan was interviewed for the Finding Genius podcast about deep sea extremophiles and methane; Co-I Dr. Steven D’Hondt presented the deep biosphere in virtual talks with the Princeton Club of Chicago, Astronomy News with The Cosmic Companion, and the IODP School of Rock for educators. Additional stories appeared in media outlets such as Forbes, Vice, Nautilus Oceans, Quanta Magazine, Smithsonian Magazine, Oceanus, Daily Mail, Eos, National Geographic, New Scientist, Science Daily, Wired, New York Times, and Science Daily which are listed in [Center-wide Outputs and Issues Section VIII](#) and Appendix G.

C-DEBI, in partnership with institutions across the country, engages K-12 students in a variety of activities to increase their knowledge about ocean science and subseafloor biosphere research. Due to the pandemic, Co-I Dr. Geoffrey Wheat canceled the long-running in-person Seafloor Science and ROV
Summer Camp for 6th-8th graders this year which emphasizes crucial technology to conduct subsea floor research, and instead provided a virtual view of the Monterey Bay through a series of live ROV dives via the SS-ROV Camp Facebook page. C-DEBI ROVs were also not able to be used in education programs this year at the USC Wrigley Institute’s Marine Science Center due to the pandemic.

<table>
<thead>
<tr>
<th>Activity Summary: K-12 Programs and General Outreach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Led by</td>
</tr>
<tr>
<td>Co-I Dr. Julie Huber, Co-I Dr. Geoffrey Wheat, Co-I Dr. Victoria Orphan, Co-I Dr. Andrew Fisher, Co-I Dr. Steve D’Hondt, Christopher Petrone, Sharon Cooper, Dr. James Bradley</td>
</tr>
<tr>
<td>Intended Audience</td>
</tr>
<tr>
<td>K-12 students, general audience</td>
</tr>
<tr>
<td>Approximate Number of Attendees</td>
</tr>
<tr>
<td>1000+</td>
</tr>
</tbody>
</table>

5. Performance with Respect to the Strategic Implementation Plan

Our strategic implementation plan seeks to bring C-DEBI research and the role of subsea floor microbes to the forefront by increasing microbiology literacy in the general public, engaging and retaining students in STEM fields, and training the next generation of subsea floor researchers. As noted above, we focus our efforts on undergraduate students, graduate students and postdoctoral scholars, and the general public. To engage these groups and retain young people in STEM fields, C-DEBI provides training, mentoring, and professional development opportunities, but we also leverage numerous educational partnerships nationally to work with K-12 students to ensure engagement at all levels. At the undergraduate level, we focus heavily on community college students and students from marginalized groups (underrepresented minorities, first generation, low income, etc.) because of the huge potential for educational impact. In addition to providing training in state-of-the-art technologies and instrumentation for graduate students and beyond, we emphasize professional development training targeting skills needed both in and out of academia.

Target 1: The general public is engaged in discovery science through public seminars, outreach activities, and social media.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present at 3 informal science events or national education conferences</td>
<td>Met</td>
</tr>
<tr>
<td>Communicate the deep biosphere in 3 general audience, non-scientific publications</td>
<td>Met</td>
</tr>
<tr>
<td>Communicate C-DEBI and related science content to a science-interested public audience through 2-3 weekly social media posts</td>
<td>Met</td>
</tr>
</tbody>
</table>
**Target 2:** Cutting-edge university research opportunities are provided to undergraduates, especially for community college students and members of underrepresented minorities, attracting early and potentially undecided undergraduate students into STEM majors and strengthening their interest and passion for science and research.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incorporate deep biosphere content into 3 C-DEBI and partner post-secondary programs</td>
<td>Partially met due to limited activities during the COVID-19 pandemic (CC-RISE postponed)</td>
</tr>
<tr>
<td>Assess program effectiveness using formative and summative evaluations with 70% of respondents rating programs as “very good” or “excellent” (4 or 5 out of 5)</td>
<td>Met (SOLID-GEM and GGURE)</td>
</tr>
<tr>
<td>Follow the pathway of former program participants using long-term tracking with 50% of former community college participants successfully transitioning to 4-year institutions in STEM majors, and 25% of all former undergraduate participants earning a degree in a STEM major and continuing on to graduate school and/or STEM careers</td>
<td>Met</td>
</tr>
<tr>
<td>Sustain long-term engagement of C-DEBI and STEM opportunities with &gt;75% of former program participants</td>
<td>Met</td>
</tr>
</tbody>
</table>

**Target 3:** The next generation of subseafloor researchers (graduate students and postdoctorals) are trained in state-of-the-art laboratories across the country and nurtured through professional development opportunities provided to expand their transferable skills.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support 30-40 individuals from varied institutions through C-DEBI awards (fellowships, exchanges, research and education grants) and in C-DEBI leadership laboratories</td>
<td>Met</td>
</tr>
<tr>
<td>Develop and conduct 2-3 regular and varied professional development activities for graduate students and postdoctoral researchers</td>
<td>Met</td>
</tr>
<tr>
<td>Assess professional development workshop effectiveness using formative and summative evaluations with 70% of respondents rating workshops as “very good” or “excellent” (4 or 5 out of 5)</td>
<td>Met</td>
</tr>
<tr>
<td>Follow the pathway of former program participants using long-term tracking with 50% of former graduate student and postdoctoral participants successfully transitioning to postdocs or STEM careers</td>
<td>Met</td>
</tr>
</tbody>
</table>
6. Plans for the Next Reporting Period

C-DEBI is committed to the continued development of broad-based, targeted education programs that train and foster the next generation of deep subseafloor biosphere researchers and on a broader scale, engage and retain STEM researchers. Our future objectives as C-DEBI sunsets are to: 1) maintain partnerships with community colleges by promoting undergraduate courses and summer research internship programs; 2) enhance the website summaries of education program success, lessons learned, and evaluations; 3) use networking, existing organizations, social networking tools and local contacts to increase the scope of C-DEBI’s impact; 4) promote calls for community workshops and travel grants to nurture the next generation of innovative scientists, and 5) continue to seek funding from various sources to continue the undergraduate programs GEM, GGURE, and CC-RISE which USC and WHOI are externally supporting in 2021.

IV. DATA MANAGEMENT AND KNOWLEDGE TRANSFER

1. Overall Data Management and Knowledge Transfer Goals and Objectives

C-DEBI facilitates the exchange of knowledge, expertise, intellectual and physical resources, experimental methods, and application of new technologies within its diverse community and between the STC and the community at large. This commitment is demonstrated through open access of all new discoveries, sensors, samplers, data, methods, and platforms. This access has many avenues including, but not limited to, the distribution of information through teleconferences, our website, workshops, meetings, newsletters, presentations, technical documents, peer-reviewed publications, educational activities, and outreach events. C-DEBI is also committed to mentoring students and scientists of all ages, including the exchange of personnel among laboratories and professional development.

The overarching objective of Data Management and Integration (DMI) and Knowledge Transfer (KT) is to disseminate C-DEBI scientific discoveries and technical advances both to the scientific community and broader population. This objective has not changed during the transitions from growth in Phase 1 to nurturing in Phase 2. As such, our DMI and KT goals include (1) implementing effective mechanisms to facilitate intellectual exchanges between institutions of various types, (2) maintaining worldwide access to C-DEBI data and information, (3) nurturing a new generation of C-DEBI researchers, (4) developing and making available targeted education, public outreach, and community interactions, and (5) promoting economic growth through technology development. Significant accomplishments and focused objectives of Center activities during the period of performance have been the implementation of an effective data portal, a concentrated effort that continues to archive C-DEBI produced data within the construct of BCO-DMO, the development of an internet-based system for distributing laboratory and analytical protocols, and continued development and implementation of a center-wide bioinformatics program.

During this near final year of C-DEBI and in the fog of the COVID-19 pandemic, we continue to communicate exciting results to the C-DEBI community, educators, managers, and to the general population through a variety of media types. In appreciation of the tenure of the C-DEBI STC, we have published several synthesis manuscripts and have additional manuscripts that will be submitted next year.

2. Knowledge Transfer Activities and Organizations

C-DEBI knowledge transfer (KT) occurs on a near-constant basis with numerous organizations, most significantly with those highlighted in External Partnerships Section V. Here, we specifically call
out KT activities that occurred during the reporting period and focused on overall Goals 1, 2, and 5 above (implementing effective mechanisms to facilitate intellectual exchanges, maintaining worldwide access to C-DEBI data and information, and economic growth and technical development). Other forms of KT (e.g., those involving Goals 3 and 4) are covered in other sections of this report, such as classroom lectures, public presentations, the Networked Speaker Series, fellowship and travel grants, professional development (online and in person), workshops, conferences, field trips, GEM, CC-RISE, GGURE and teachers-at-sea.

One of the most effective tools for transferring knowledge to the scientific community is the publication of peer-reviewed papers. During the reporting period, the C-DEBI community published 42 peer-reviewed journal articles and one book chapter. Of the 42 articles, 10 included graduate students, postdoctoral fellows, or both and 3 of the first authors were either graduate students or postdoctoral fellows. Each of these contributions was posted on our web page and introduced to the community in a monthly newsletter that reaches ~1000 individuals globally. In addition, during the performance period, 32 reported presentations/posters were given at numerous special sessions and workshops of large national and international meetings hosted by scientific organizations and partners even with the challenges caused by the COVID-19 pandemic forcing some meetings to be canceled and others to be presented in a virtual format (e.g., AGU, ISME, ASM, ISSM). Other, smaller C-DEBI leadership-hosted workshops and meetings also contributed to knowledge transfer. These meetings included the C-DEBI Annual Meeting (~80 virtual people this year) that provides opportunities for experienced and new C-DEBI members to report and discuss recent results, and plan for ongoing and future work. Also, C-DEBI and the work that was funded by C-DEBI were included multiple media bursts, including Facebook, Instagram and Twitter.

Two new technological advances were made during the reporting period. Last year, Wheat and others developed a high-temperature, borehole fluid sampler with a trigger that uses shape memory alloys. During 2020, several new trigger mechanisms were tested including new titanium bolts. These tests were conducted to see if we could achieve a smaller deviation in the temperature at which a sampler is triggered. We also tested some lower temperature springs in preparation for use on IODP Expedition 395E. After IODP Expedition 395E, the sampler will be transferred to IODP as a standard shipboard sampler. The other new technology includes the design and fabrication of a 2 to 5-m-long heat flow probe for geothermal surveys in oceanic sediment. Existing probes have out-of-date control systems with parts and software that are difficult to replace. The new system will use state-of-the-art technology and increase functionality by including tilt and accelerometer sensors, protocols for complete in situ data communication to the ship, and more power.

3. Data Management and Integration Activities and Organizations

The C-DEBI Data Management and Integration (DMI) team is in place to ensure that C-DEBI data and products are archived, shared, and accessible for the long term. The data types and products covered by C-DEBI include a wide variety of geophysical, geological, geochemical, and biological information, in addition to education and outreach materials, technical documents, and samples. The overall DMI goal is to make sure that all data and information generated by C-DEBI-supported researchers as part of their C-DEBI projects are made publicly available either following publication or within two years of data generation (see details in our Data Management Plan). A second goal is to make certain that no C-DEBI researcher is limited by computational resources (e.g., computers or tools). As part of Phase 2, the DMI team also takes the responsibility to make sure the C-DEBI data are integrated in ways to allow larger, more comprehensive analysis. Finally, we have begun to mandate all future small grant awardees make their protocols available on the protocols.io site. We have further suggested previous awardees also deposit protocols at the site and have made C-DEBI staff available to help researchers in depositing their protocols.
a. Making Data Publicly Available

C-DEBI produces many kinds of products that need long term archiving. These include diverse data sets (biological, chemical, physical, and geological), samples, peer-reviewed publications, technological advances with associated engineering drawings and software, educational/outreach materials (such as K-12 and community college lesson plans relating to subseaﬂoor science), and model parameters (e.g., inputs, grids, reaction rates). Biological products include, but are not limited to, molecular data, activity data (isotope abundance, community enzymatic, etc.), frozen samples, living microbial strains, and post-processed molecular data (e.g., 16S rDNA and 16S rRNA sequences, single-cell genome, metagenome and metatranscriptome sequences). Non-biological data include multi-beam maps, seismic reflection proﬁles, and thermal, chemical, and physical data from recovered samples of ﬂuids, sediment, rocks, and experiments. Measurements are also made in situ using borehole observatories, drilling platforms, cabled observatories, and coring facilities.

In 2020, we continued our efforts to ensure that all post-embargo data were deposited in appropriate internationally accessible data repositories. The principal repository is the Biological and Chemical Oceanography Data Management Office (BCO-DMO). The C-DEBI DMI team has worked with BCO-DMO to make this location either the primary host of C-DEBI data or to have them provide stable links to data housed in other repositories (e.g., NCBI, IODPdb, PRIDE, etc.). Products for which a suitable national repository does not exist, such as educational materials, outreach materials, and technical advances, have been posted directly on the C-DEBI main page under the headers ‘For Teachers’, ‘For Everyone’, etc. To the extent possible, all such products have also been described in the peer-reviewed literature to ensure public dissemination and long-term accessibility beyond C-DEBI. All C-DEBI intellectual products (publications, technical advances, software, education and outreach materials) are directly linkable by searching the main C-DEBI page. This provides direct electronic access to the data repository, publication, and protocols.

We have made major progress in our mandate to deposit C-DEBI data in public repositories. There are currently 77 “Projects” currently on the C-DEBI BCO-DMO webpage (https://www.bco-dmo.org/program/554979). Additionally, several others are queued in the BCO-DMO quality check phase. The majority of the Small Grants programs that ended more than two years ago have deposited their required data in public repositories.

We have also made progress depositing protocols used into public databases. In 2020, we continue our effort to encourage C-DEBI researchers to voluntarily deposit in the C-DEBI group in protocols.io (https://www.protocols.io/groups/center-for-dark-energy-biosphere-investigations). With the final set of grants, we have made this a mandatory compulsion, and have 12 protocols shared thus far.

b. Providing Computational Resources to C-DEBI Researchers

In 2020, C-DEBI continued to maintain a computing resource to accommodate data analysis on scales too large for laboratory computational resources, but too small (or poorly designed) for high powered computing centers. Currently, there are 11 C-DEBI researchers with access, provided on a rotating basis. These users are members of the larger C-DEBI community, and include graduate students, postdoctoral researchers and faculty from multiple labs and grants, not just the Co-I’s. This resource will undergo a sundown period in the coming year with expected end of support in September 2021.

Beyond basic access to a maintained computer resource, several initiatives have been implemented to make sure C-DEBI researchers are not limited by any step in the bioinformatics process. One important aspect of this is the training of researchers on available tools. To this end, we have successfully hosted/co-led 3 beginner bioinformatics workshops and, because the people interested at the beginner level seem to have been reached, we are in the planning stages for hosting an intermediate bioinformatics workshop. Dr. Benjamin Tully created a new bioinformatics learning initiative in response to the COVID-19 pandemic. The Bioinformatics Virtual Coordination Network (BVCN) was initiated as a resource to provide training and hands-on experience for scientists who were restricted from accessing
their laboratories during early lockdowns (https://biovcnet.github.io/). From April through June, the BVCN created 99 lessons and hands-on tutorials for various aspects of computational biology. To date, these videos have accumulated 12,300+ views and 1,300+ hours watched. The BVCN continues to function as a platform for hosting future educational content and for ongoing discussions about specific questions that can be outsourced by the community.

c. Expanding the Impact of C-DEBI Data through External Partnerships and Collaborations

We continue to collaborate with Drs. DeLong, Hurwitz, and Wood-Charlson to integrate C-DEBI data into their EarthCube Building Block, “Planet Microbe.” C-DEBI believes collaborations are the soundest way to meet our goals for data discovery, integration, synthesis, and open sharing, and we strive to leverage available infrastructure and to partner with excellent groups like “Planet Microbe.” We are excited to collaborate at all stages of Planet Microbe, from development and validation to implementation and sustainability. Co-I Dr. John Heidelberg, Professor of Biology at USC and the C-DEBI Data Management and Integration manager, and Dr. Benjamin Tully, Bioinformatics Specialist for C-DEBI, have committed their expertise and support needed to validate current C-DEBI data. In addition, they will be instrumental in the development of standardized workflows to ensure that future C-DEBI data will also be contributed to and validated in Planet Microbe. While Planet Microbe has only begun (http://www.hurwitzlab.org/projects/planet-microbe/), we collaborated to make the Juan de Fuca Ridge Flank data currently available on the project. All the C-DEBI data, being deposited in BCO-DMO is available to Planet Microbe moving forward.

4. Performance with Respect to the Strategic Implementation Plan

Our data management and knowledge transfer goal is to implement effective mechanisms and pathways to facilitate the exchange and application of knowledge, expertise, physical resources, and novel methods and technologies within the STC and between the STC and the broader community. The overall data management plan is in place to 1) assure all data generated from the STC are deposited in publically accessible data repositories, 2) efficiently allow STC researchers tools and computational resources that allow them to efficiently perform data analysis, and 3) develop and maintain a data portal for visualization and hypothesis generation from the STC data.

**Target 1:** Innovations are imported/exported/shared and partnerships are developed with other fields, research institutions, industry and government.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Publish and promote scholarly activity via 10 publications</td>
<td>Met</td>
</tr>
<tr>
<td>Continue to develop research collaborations through networking at 2-3 interdisciplinary meetings and talks/posters/exhibition at 2-3 conferences</td>
<td>Partially met due to limited meeting activities during the COVID-19 pandemic</td>
</tr>
<tr>
<td>Lead 3 C-DEBI-focused meetings or special sessions at national or international meetings</td>
<td>Partially met due to limited meeting activities during the COVID-19 pandemic</td>
</tr>
<tr>
<td>Enhance, develop, or commercialize 2 tools, analytical capabilities, software products, sensors and platforms</td>
<td>Met</td>
</tr>
</tbody>
</table>
**Target 2:** New innovation in the field is communicated through web tools, publications, media, presentations, and educating the next generation of researchers and ocean stewards.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continue to ensure all data generated through the STC are in stable public data repositories within 2 years of generation (or for graduate students upon defense of the dissertation or thesis)</td>
<td>Met</td>
</tr>
<tr>
<td>Continue to develop web-based data portal bringing data together from various repositories for synthesis efforts</td>
<td>Met</td>
</tr>
<tr>
<td>Train researchers in new tools for data analysis by producing 3 webinars on data analysis tools and 2 small workshops for data analysis</td>
<td>Met</td>
</tr>
<tr>
<td>Engage 20 new undergraduates to the fields of microbiology and oceanography and mentor 10 graduate students in C-DEBI fields</td>
<td>Met</td>
</tr>
<tr>
<td>Communicate with the public through non-scientific journals via social and journalistic media (5 significant contributions)</td>
<td>Met</td>
</tr>
</tbody>
</table>

5. Plans for the Next Reporting Period

C-DEBI will continue its long-term data management and knowledge transfer activities, consistent with the current practices and those outlined in the renewal proposal and in response to feedback from previous NSF Site Review committee members and NSF personnel. The management team has and will continue to focus on synthesis products from high level contributions to regional-, theme-, and program-based summaries. Such products will focus on a range of media outlets from scientific and educational journals to more broad-based community resources (e.g., newspapers, magazines, and social media).

V. EXTERNAL PARTNERSHIPS

1. Overall External Partnerships Goals and Objectives

C-DEBI supports cross-disciplinary and cross-institutional partnerships that facilitate, augment, and expand the education, training, and research opportunities of Center participants.

2. Activities Conducted as Part of Partnerships

C-DEBI continues to strongly support long-standing relationships with high-profile external partnerships, and these efforts cover both research and education. Of particular note on the research side are partnerships with the International Ocean Discovery Program (IODP), University-National Oceanographic Laboratory System (UNOLS), National Deep Submergence Facility (NDSF), Japan
Agency for Marine-Earth Science and Technology (JAMSTEC), Schmidt Ocean Institute (SOI), NASA, Los Alamos National Laboratory (LANL), the International Center for Deep Life Investigation (ICDLI), and the International Earth 4D program through the Canadian Institute for Advanced Research (CIFAR). On the education side, we continue to leverage our partnerships with a variety of institutions (e.g., the USC Wrigley Institute for Environmental Studies) and through training programs (e.g., the International GeoBiology) to foster the next generation of deep subseafloor biosphere researchers.

IODP

Since its inception, C-DEBI science has been firmly connected to IODP's focus on exploring and documenting the deep biosphere. During Phase 1, C-DEBI scientists led several expeditions (327, 329, 336) and were integral science party members of many others (323, 330, 331, 337, 347, 353). During Phase 2, C-DEBI scientists have led (357, 385) and been involved in several other expeditions (360, 366 and 376). Research continues from those expeditions in the current reporting year. Due to the COVID-19 pandemic, no deep biosphere sampling occurred on IODP Expeditions in the current reporting year. C-DEBI scientist Jason Sylvan will co-lead expedition 390, which was postponed from 2020 until 2022. In the next reporting period, C-DEBI scientists will participate in several upcoming expeditions to collect new deep biosphere samples (i.e. Co-I Wheat and members of Co-I Orcutt’s lab on IODP Expeditions 395E and 395). IODP Expedition 395E will have operational objectives taken from an approved proposal to return to the Atlantis Massif, of which Wheat and Orcutt were proponents. C-DEBI scientists are also leading other proposal efforts under consideration by the IODP Science Evaluation Panel: (1) Co-I D’Hondt is lead proponent of IODP Proposal 929, which is presently under consideration to schedule for an expedition; (2) Co-I Huber submitted an invited full proposal to IODP to drill Axial Seamount, involving many C-DEBI scientists; and (3) former C-DEBI postdoc Elizabeth Trembath-Reichert has taken leadership roles in several proposal efforts under consideration. C-DEBI scientists also continue to engage in service to the IODP community. Co-I Orcutt currently serves on the IODP Science Evaluation Panel, and Orcutt and Co-I D’Hondt served on the subcommittee of the US Science Support Program for IODP focused on devising a plan for scientific ocean drilling after the current phase ends in 2023 (Koppers and Coggon, 2020). Co-I Wheat and C-DEBI early career scientist Stephanie Carr serve on the science advisory committee to the U.S. Science Support Program, which facilitates involvement of the U.S. scientific community in IODP.

UNOLS and NDSF

C-DEBI science relies heavily on the UNOLS fleet of research vessels for expeditions, including the NDSF fleet of remotely operated vehicles (e.g., Jason), autonomous underwater vehicles (e.g., Sentry), and the human occupied vehicle Alvin, particularly for CORK servicing activities and coring expeditions. Co-I Huber and other C-DEBI scientists sailed to the Mid-Cayman Rise to examine vent geochemistry and microbiology at the beginning of 2020, but due to the COVID-19 pandemic, very little deep biosphere focused sampling occurred with NDSF assets. In June-July 2020, the Deep Submergence Science Committee of UNOLS worked with the NDSF to host the online “Abyss” workshop focused on the scientific and societal opportunities and needs of expanding Alvin’s depth capability to 6,500 m (https://ndsf.whoi.edu/alvin/workshop-alvin-in-the-abyss/). C-DEBI scientists were invited to provide recorded presentations for this workshop (i.e. Co-I D’Hondt in the Abyssal Plains & Seamounts session, Co-I Orcutt in the Abyssal Technology & Societal Relevance session) and co-lead discussion groups, and many from the C-DEBI community participated in these fora.

JAMSTEC

C-DEBI collaborates closely with the Geomicrobiology Group at the JAMSTEC-Kochi Institute for Core Sample Research. Members of C-DEBI and JAMSTEC partner on international expeditions, and on proposals for new expeditions. Members of JAMSTEC and C-DEBI also collaborate closely on studies of life in marine sediment and in the igneous crust beneath the sediment. Multiple C-DEBI students have conducted research at the state-of-the-art subsurface life facilities at the Core Sample Research Center in
Kochi. Co-I Orphan and JAMSTEC scientist (Yuki Morono) are collaborating on methods development for imaging subseafloor life in sediments.

**SOI**

SOI is a private foundation that serves as an oceanographic operator for the seagoing community by providing ship and vehicle time via community solicited, peer-reviewed proposals. During C-DEBI, a number of Co-I’s and other C-DEBI scientists have participated in and led cruises aboard the SOI research vessel *Falkor*. Several C-DEBI members will be leading expeditions with the *Falkor* in the coming year. Co-I’s Orcutt, Wheat, Huber, and Fisher will lead two *Falkor* expeditions to the Dorado Outcrop offshore Costa Rica (a C-DEBI Major Program) in late 2021 and early 2022. These expeditions will focus on examining the connection of hydrogeology with animal and microbial distributions and ecosystem services in seamount systems. This work will connect to ecosystem conservation policy goals of Costa Rica in collaboration with scientists and students from Costa Rica, and also contribute to goals of the *Challenger 150* program of the UN Ocean Decade. In 2022, Co-I Orphan will also lead an expedition to S. Pescadero Basin, Gulf of California.

**NASA**

C-DEBI continues its numerous collaborations with NASA. The *Life Underground* node of the Astrobiology Institute (NAI), led by PI Amend at USC, ended in 2019. However, several C-DEBI scientists were involved in developing, testing, and ground-truthing the deep UV resonance Raman and fluorescence technology. This technology defines the Mars 2020 flight instrument SHERLOC on the Perseverance rover that just landed on the red planet. In addition, PI Amend is an investigator on a funded NASA PSTAR project called In-situ Vent Analysis Divebot for Exobiology Research (InVADER) that will use *in situ* observation, real-time data gathering, and sample collection and analysis to study a deep-sea hydrothermal vent system at Axial Seamount. The planned expedition was rescheduled to 2021 due to COVID-19, but during 2020, progress was made on the design and build of the divebot, laser system, and underwater drill. Co-I Huber is part of a different NASA PSTAR project termed Systematic Underwater Biogeochemical Science and Exploration Analog (SUBSEA) that conducted a real telerobotic expedition to Gorda Ridge to understand the habitability potential of Ocean Worlds in our solar system. Former C-DEBI Graduate Student Fellow Amy Smith (now a postdoc with Julie Huber) and current C-DEBI Postdoctoral Fellow Sarah Hu participated in the research cruise, while Huber ran operations from the Inner Space Center at the University of Rhode Island. This project also included access to infrastructure enabled through in-kind support from the NOAA Office of Ocean Exploration and Research and the Ocean Exploration Trust. Further, Co-I’s Huber and Fisher are part of a new NASA project termed Exploring Ocean Worlds (ExOW) that will address a central question in astrobiology research today: "On which ocean worlds, and using which measurements, do we have the highest probability of finding life beyond Earth within the next human generation?" This project is led out of WHOI. Lastly, building on a 2019 NASA Exobiology grant to Co-I Orcutt, Dr. Julia McGonigle was awarded a NASA Postdoctoral Program Fellowship to work with Orcutt on bioinformatic-based investigation of metabolic strategies used by uncultivated taxa in the marine subsurface. McGonigle also teamed up with current Orcutt-lab postdoc Melody Lindsay and former C-DEBI postdocs Jacqueline Goordial and Elizabeth Trembath-Reichert to propose a session on "Life Detection in Deep Biosphere Earth Analog Environments” at the 2021 Astrobiology Science Conference (delayed until 2022 due to COVID-19).

**LANL**

C-DEBI (through the UCSC Hydrogeology group) continues to collaborate with researchers in the Earth and Environmental Sciences Group at LANL, to develop complex simulations of seafloor hydrothermal circulation. Co-I Fisher and current members of his group (Price and Dickerson) were not able to visit with LANL colleagues, as in past years, but have continued to work together from a distance. Price is finalizing a paper describing North Pond simulations, and Fisher is preparing a manuscript on the Dorado Outcrop area and is extending earlier studies to assess how conditions on Ocean Worlds could
influence hydrothermal convection and the maintenance of microbial ecosystems. Former postdoc Tess Weathers is advancing simulations started in earlier work periods, but has been busy as a professor at a community college, so progress has been slowed. LANL researchers have been engaged in all of these studies.

ICDLI

The ICDLI was established at Shanghai Jiao Tong University in 2018 to provide a platform for continued international collaborations that seek to address key scientific issues in deep life research. Several C-DEBI members, including Co-I’s Amend, Orcutt, and D’Hondt, joined and continue to serve on ICDLI’s Advisory Board. In 2020, this board compiled a list of the top publications in deep life science and voted on the top 5 to be recognized as the most important/breakthrough papers. A large percentage of the nominated studies were authored or co-authored by C-DEBI scientists.

CIFAR Earth 4D

The CIFAR-supported ‘Earth 4D’ program, founded in 2019, is an international research group focused on 3 broad interdisciplinary themes of life, water, and space, with specific interests in the feedbacks and co-evolution between deep microbial life and the physical and chemical environment over time. C-DEBI Co-I Orphan is one of the funded fellows, and C-DEBI PI Amend is the chair of the international advisory committee. In 2020, Earth 4D convened two virtual multi-day meetings and hosted several guest speakers for special (virtual) seminars, including C-DEBI scientist Karen Lloyd. Lastly, applications for the Azrieli Global Scholars Program were received and reviewed, and several of the finalists are former C-DEBI postdocs (final selections pending).

Education

The interdisciplinary nature of C-DEBI research lends itself magnificently to a diverse array of external education partnerships as well. One of our primary education goals is to train the next generation of deep subseafloor biosphere researchers, and to do so, we partner with one of the top training courses for graduate students and postdoctoral scholars, the Agouron Institute-funded International GeoBiology summer course. In 2020, this course was postponed due to the COVID-19 pandemic but in 2021 is planned to again co-directed by Co-I Victoria Orphan and feature C-DEBI members as guest lecturers. Just as C-DEBI and the International GeoBiology course share key personnel (administrative and instructional), so do C-DEBI and the USC Wrigley Institute. This facilitates our training of undergraduates through programs such as the Global Environmental Microbiology course (based heavily on the successful GeoBiology program) at the Institute’s marine lab on Catalina Island. To further provide opportunities for community college students, we also began a partnership with a NSF SAGE/2YC (Supporting and Advancing Geoscience Education at Two-Year Colleges) network of local community college and four-year institution faculty to provide educational resources and facilitate tours of the Wrigley research station for students.

3. Performance with Respect to the Strategic Implementation Plan

Our overarching goal is to support cross-disciplinary and cross-institutional partnerships that facilitate, augment and expand the education, training and research opportunities of Center participants. Partnerships among individuals, institutes, organizations, and programs are the core of C-DEBI research and educational efforts. One of the strengths of these partnerships is the quality and broad appeal of publications. C-DEBI has a range of cross-disciplinary and cross-institutional collaborations that have transformed our view of subsurface microbial conditions, activity, and mechanisms within the hydrologic and geochemical context of fluid flow within the oceanic crust. Another strength of C-DEBI partnerships is the web of interaction of the C-DEBI community within other organizations and programs and the joint efforts of these organizations and programs in collaboration with C-DEBI to promote and facilitate
synergetic research objectives. These partners also extend to education and diversity efforts, leading to a community of junior scientists that are engaged in cross-disciplinary and cross-institutional training activities and exchanges.

**Target 1:** Strong cross-disciplinary research projects and strong cross-institutional programs are demonstrated in all aspects of Center activities, including publications, presentations, proposals, educational exchanges, and educational programs.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Publish 5 (in aggregate) cross-disciplinary papers per each of the three research themes with support from calls for small research and travel grants to facilitate the interaction of dispersed Center researchers</td>
<td>Met</td>
</tr>
<tr>
<td>Submit 2 cross-disciplinary and cross-institutional proposals</td>
<td>Met</td>
</tr>
<tr>
<td>Support 2 interdisciplinary workshops or meetings in concert with other national programs</td>
<td>Partially met due to limited meeting activities during the COVID-19 pandemic</td>
</tr>
<tr>
<td>Fund 10 graduate students, postdoctoral fellows and C-DEBI community scientists in their pursuit of generating data or developing cross-discipline techniques and tools to further Center objectives</td>
<td>Partially met due to limited activities during the COVID-19 pandemic</td>
</tr>
<tr>
<td>Provide the funds that allow 3 student/researchers the opportunity to participate in research expeditions or travel to another institution to expand the scope of their education/research in the use of novel techniques and tools</td>
<td>Partially met due to limited activities during the COVID-19 pandemic</td>
</tr>
</tbody>
</table>

**Target 2:** Partnerships are developed with other fields, research organizations, industry, government, and foundations.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Build 10 (total) partnerships by networking at interdisciplinary meetings, developing industrial and governmental partners, and targeting partnerships and interactions for new applications of existing or budding technologies</td>
<td>Partially met due to limited meeting activities during the COVID-19 pandemic</td>
</tr>
<tr>
<td>Develop documents and materials that highlight significant results through C-DEBI research, education, and diversity programs suited to specific organizations, foundations, and programs to form the basis of a dialogue between C-DEBI and that organization to further fiscal and research needs</td>
<td>Pending</td>
</tr>
</tbody>
</table>
4. Plans for the Next Reporting Period

C-DEBI has had long-standing partnerships with most of these external partners—others have come on board more recently—and the close collaborations are likely to continue at similar levels of commitment for the foreseeable future.

VI. DIVERSITY

1. Overall Diversity Goals and Objectives

C-DEBI seeks to train a new, diverse generation of K-12, undergraduate, graduate and postdoctoral researchers within an integrated and collaborative multidisciplinary community. We are committed to improving access and support for members of underrepresented and marginalized groups to be able to succeed in STEM fields. It should be noted, however, that the activities intended to enhance diversity do not happen in a vacuum—they are purposefully integrated with C-DEBI’s fundamental research, education, and outreach missions. Through its academic programs, C-DEBI promotes inclusion and retention among underrepresented groups, including women and first-generation college students. With a strong base established to increase STEM diversity at all levels, both academically and nationally, we have developed and are exporting distinctive, in-depth education and research experiences that encourage historically underserved students. These initiatives will be targeted toward three primary sectors: 1) pre-college; 2) undergraduate (including community college) populations; and 3) early-career and established scientists.

Our specific targets are to: a) increase the diversity of C-DEBI participants to reflect the diversity of the United States; b) emphasize diversity awareness with the active C-DEBI community through an diversity and inclusion training workshop at every other C-DEBI annual meeting, and among individual student cohorts of each C-DEBI summer education program; and c) promote C-DEBI research opportunities to diverse audiences through several different partners that primarily serve underrepresented groups.

As the Center sunsets, our goals have not changed, however, the programs to support our diversity objectives have been scaled back as planned. Despite the COVID-19 pandemic, we were able to make successful adaptations to most of our activities.

2. Programs and Activities Which Enhance Diversity at the Center

C-DEBI continues to build on program successes of the past years by evaluating distinct programs targeting underrepresented minorities, women, and first generation and low-income students: the Community College Research Internship for Scientific Engagement (CC-RISE), the Global Environmental Microbiology (GEM) course, and the Genomics and Geobiology Undergraduate Research Experience (GGURE; see Education section for details). These programs enrich the scientific skills of students through a combination of field-based and lab-based research with professional development activities, however have required adjustments this year due to the pandemic’s limitation of in-person undergraduate access to research laboratories (CC-RISE postponed until 2021, GEM redesigned as the online SOLID-GEM course, GGURE transitioned to remote research and meetings). We continue to have success in recruiting a diverse group of participants for each of these programs. We advertise widely for all except the USC-based GGURE which, through word-of-mouth via participants and mentors/PIs, already receives more interest than we can support. We leverage our existing contact lists (applicants and participants of each of our programs, writers of recommendation letters, etc.) by sending
information to them about other C-DEBI programs for which we think they are qualified or would know someone who is qualified. In addition to utilizing our undergraduate list, we advertise undergraduate opportunities through the SACNAS opportunity board, and the Institute for Broadening Participation Pathways to Science, among other partners. Similarly, to recruit participants at higher levels, we advertise graduate student openings and postdoctoral positions through social media and our listserv, as well as with our partners listed above.

<table>
<thead>
<tr>
<th>Program</th>
<th>Number of Participants</th>
<th>Diversity Objective</th>
<th>Measurement of Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community College Research Internship for Scientific Engagement (CC-RISE)</td>
<td>0, postponed to 2021 (56 in 8 years)</td>
<td>Summer research internship for community college students at WHOI (previously also at UCSC and USC)</td>
<td>External evaluation, Longitudinal tracking</td>
</tr>
<tr>
<td>Genomics and Geology Undergraduate Research Experience (GGURE)</td>
<td>9 (225 positions from Fall 2014 to present)</td>
<td>Academic year and summer research internship program for underrepresented undergraduate students at USC</td>
<td>External evaluation, Longitudinal tracking</td>
</tr>
<tr>
<td>Summer OnLine Interactive/Discussion-Global Environmental Microbiology (SOLID-GEM) Course</td>
<td>14 (156 in 10 years including in-person GEM)</td>
<td>Hands-on experience for 2- and 4-year undergraduate students in environmental microbiology</td>
<td>External evaluation, Retrospective survey, Longitudinal tracking</td>
</tr>
</tbody>
</table>

This year, we have again successfully incorporated diversity discussions into our primary undergraduate courses. From the SOLID-GEM post-survey, we learned that students valued the program’s focus on diversity and inclusion and felt SOLID-GEM was very respectful of a variety of kinds of diversity with 93% of students rating the program an 8, 9 or 10 out of 10 (mean of 9.5) for being respectful of diversity and inclusion (Appendix E). In the 2019-2020 GGURE academic year post-survey, 75% of students rated the program a perfect “10” on diversity and inclusion (a mean of 8.8 out of 10; Appendix C).

As part of our virtual Center-wide Annual Meeting, Jerika Loren Heinze (founder of the Fieldwork Initiative) presented the Fieldwork Initiative to Stop Sexualized Trauma (FISST) Training in a 2 hour session to students, postdocs, and faculty of the C-DEBI community. The training covered misconceptions about fieldwork, problems that researchers encounter, problems reporting violence and seeking help while in the field, and a self-advocacy toolkit. The training was well-received as timely and important in a post-meeting survey, and most survey respondents (93%) found the session useful (Appendix F).

A number of activities are designed to build and maintain a community and to keep participants active in STEM. To keep in touch with participants and share STEM opportunities, we run an email listserv for undergraduates and one for graduate students and postdoctoral researchers as well as invite everyone to sign up for our email newsletter and engage with us through our social media accounts. To strengthen the C-DEBI network, we create connections between participants in different programs as well as bring back alumni to engage with current students. For example, members of our C-DEBI graduate student and postdoctoral community presented their field work to and participated in career-path panels for the virtual SOLID-GEM summer undergraduate students.

We continue to work toward increasing underrepresented minorities in C-DEBI by promoting deep subsurface research through Minority Professional Organizations and national networks. This year,
we disseminated program and graduate training opportunities with partners such as the Institute for Broadening Participation (IBP), Society for Advancing Chicanos and Native Americans in Science (SACNAS), American Indian Science and Engineering Society (AISES), and the broader STC Education and Diversity network. In addition, we attended SACNAS USC Chapter meetings and InterTribal Education Collaboration (ITEC) meetings to share opportunities and resources directly with students and their mentors. To connect with community college faculty and students, we again participated in the SAGE/2YC (Supporting and Advancing Geoscience Education at Two-Year Colleges) workshop for faculty of 2- and 4-year institutions and are an active member of their listserv.

We also continue to leverage our diversity initiatives through the diversity infrastructure at USC. For example, we maintain our connection with Dr. Kimberly Freeman, hired in 2018 as the Chief Diversity Officer for the USC Dornsife College, and this summer discussed adapting the SOLID-GEM model for a USC audience. In February 2020, the ITEC College Exploration Day was held at USC: a one day program of college-focused workshops (e.g., on financial aid, scholarships, how to apply to college, what college is like, and an opportunity to ask questions of a panel of professors) to increase awareness of opportunities in higher education to Native American students and their families. We coordinated with SACNAS USC and USC SeaGrant among many other organizations at USC to help host the in-person event.

Of the 150 participants active in C-DEBI this year, 65 are faculty and research scientists, 41 are postdoctoral researchers and graduate students, and 29 are undergraduate students indicating the breadth of participation across career stage. Participant gender distribution (Table 1) is nearly balanced with women representing ~58% and men ~41%; ~1% reported as non-binary.

Ethnicity (Hispanic/Latinx or not) and race (White, Native Hawaiian or other Pacific Islander, Asian, Black or African American, American Indian or Alaska Native, multiracial) were reported by ~83% of participants (Table 2). Out of all individuals, ~14% identified as Hispanic/Latinx. Regarding race, ~64% of participants identified as White, ~13% Asian, ~2% Black or African American, ~0.7% American Indian or Alaska Native, and ~3% multiracial. Multiracial individuals identified various combinations of White, Asian, and/or American Indian or Alaska native.

Of all participants, 115 (~77%) identified as U.S. Citizens, 6 (4%) identified as Permanent Residents, and 14 (~9%) identified as other non-U.S. Citizens. Fifteen individuals (10%) did not provide citizenship information. One individual (~1%) identified one or more disabilities, 129 individuals (86%) reported none, and 20 individuals (~13%) did not provide disability information.

<table>
<thead>
<tr>
<th>Table 1. Gender Distribution of C-DEBI Participants (Total Number)</th>
<th>Women (Percent)</th>
<th>Men (Percent)</th>
<th>Non-binary (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faculty (58)</td>
<td>52</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>Other Research Scientist (7)</td>
<td>29</td>
<td>71</td>
<td>0</td>
</tr>
<tr>
<td>Postdoctoral (19)</td>
<td>68</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Graduate Student (22)</td>
<td>68</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Undergraduate (29)</td>
<td>62</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td>Other Participant (11; e.g., evaluator, lab manager, educator)</td>
<td>64</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>Staff (4)</td>
<td>75</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total (150; all individuals reported gender)</strong></td>
<td><strong>58</strong></td>
<td><strong>41</strong></td>
<td><strong>1</strong></td>
</tr>
</tbody>
</table>
Comparing the 2020 C-DEBI community to the July 1, 2019 U.S. Census population (figure below; race is separate from the concept of Hispanic origin in the U.S. Census), we have broadly captured the racial/ethnic diversity of society through our active participants and continue to strive towards that distribution across all career levels of our participants.

Diversity, in the broadest sense and at all levels, is an active goal for C-DEBI—and always has been. We remind the reader that in the natural sciences (vs. the social sciences), women remain vastly underrepresented, and C-DEBI has a strong record of appointing women to leadership positions, selecting women in our grants and fellowship program, and featuring women in numerous public forums. We also note that improving diversity at ‘higher levels’ is only possible when existing positions become vacant or new positions are created. At C-DEBI, diversity was considered in the few vacant and new positions, and of the 13 current leadership positions at C-DEBI (consisting of the Directorship, Co-Investigators, Data
Management, Knowledge Transfer, and Senior Scientists) nearly half are filled by women, including the Associate Director (Huber) and Managing Director (Sylvan). Lastly, we want to reiterate that our approach to building ‘diversity in leadership’ has always been to train the next generation of leaders. Note that to date, nearly 70 C-DEBI-funded graduate students and postdoctoral scholars, 46 of whom are women and 11 of whom are from underrepresented minorities, have transitioned (or are in the process of transitioning) to permanent career appointments with opportunities for leadership in their chosen professions. Those women and underrepresented minorities include a Nature editor, a number of research scientists, and 45 faculty members at universities in the USA and elsewhere.

3. Performance with Respect to the Strategic Implementation Plan

**Target 1:** The diversity, especially underrepresented minority representation, of participants in all levels of C-DEBI from undergraduate, graduate students, and postdoctoral scholars to participating researchers and staff, has increased in recent years to more appropriately reflect our diverse society.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase the diversity of C-DEBI participants to reflect the diversity of the United States</td>
<td>Met</td>
</tr>
<tr>
<td>Emphasize diversity awareness with the active C-DEBI community through 1 diversity and inclusion training workshop at every other C-DEBI annual meeting, and among individual student cohorts of each C-DEBI summer education program</td>
<td>Met</td>
</tr>
<tr>
<td>Promote C-DEBI research opportunities to diverse audiences through 4 different partners that primarily serve underrepresented groups</td>
<td>Met</td>
</tr>
</tbody>
</table>

**Target 2:** Pathways to careers in STEM fields are developed for minority undergraduate students who are interested in STEM majors.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conduct 3 programs attracting underrepresented students into STEM fields</td>
<td>Partially met due to limited activities during the COVID-19 pandemic (CC-RISE was postponed)</td>
</tr>
<tr>
<td>Assess program effectiveness using formative and summative evaluations with 70% of respondents rating programs as “very good” or “excellent” (4 or 5 out of 5)</td>
<td>Met</td>
</tr>
<tr>
<td>Follow the pathway of former program participants using long-term tracking with 50% of former community college participants successfully transitioning to 4-year institutions in STEM majors, and 25% of all former undergraduate participants earning a degree in a STEM major and continuing on to graduate school and/or STEM careers</td>
<td>Met</td>
</tr>
<tr>
<td>Sustain long-term engagement with &gt;75% of former undergraduate program participants with C-DEBI and STEM opportunities</td>
<td>Met</td>
</tr>
</tbody>
</table>
**Target 3:** Partnerships are initiated with other organizations, institutions, programs, or informal science centers that target underrepresented students and engage them in STEM fields of study.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduce C-DEBI science with appropriate resources and training to 10 institutions and/or educators that primarily serve underrepresented groups</td>
<td>Met</td>
</tr>
</tbody>
</table>

**4. Plans for the Next Reporting Period**

Our future goals are to: 1) actively encourage undergraduates to progress to graduate school in areas of deep subsurface research by promoting summer research or intensive programs being led at C-DEBI networked institutions; 2) continue to leverage support services and potential connections, organizations and institutional resources within partnering universities to promote diversity; 3) actively promote all opportunities throughout the Center to underrepresented groups and recruit at all levels of Center activity; 4) inform and encourage the C-DEBI community to participate in conferences and outreach that engages them with underrepresented students to promote recruitment into C-DEBI fields; 5) continue to document and codify components of the GGURE program such as ‘Demystifying graduate school’ and ‘Applying to Graduate School’ to create a more robust resource website for students who cannot attend meetings or are not directly in the GGURE program; and 6) continue to reconnect with and track past C-DEBI participants to see how their C-DEBI experiences influenced their career paths.

**VII. MANAGEMENT**

**1. Overall Organizational Strategy**

Our management plan facilitates the achievement of the principal scientific, education, and diversity goals of C-DEBI. C-DEBI management is composed of these major leadership groups: Directorship; Executive Committee; Administration; Research; Knowledge Transfer, Data Management and Integration; and Education, Outreach, and Diversity. The major advisory groups for C-DEBI are the Ethics Panel and External Evaluator; as C-DEBI sunsets, we no longer rely on our External Advisory Board and the Education & Outreach Steering Committee. We maintain a simple hierarchy in the management structure (rectangles below) with several advisory groups (ovals below) to encourage communication and collaboration, as well as provide transparency in decision-making. Their roles and interactions are described below and further detailed in our Operations Manual.
Directorship
The Center is led by the Director, PI Jan Amend (USC), the Associate Director, Julie Huber (WHOI), and the Managing Director, Rosalynn Sylvan. The Director is responsible for overall C-DEBI coordination and performance. He provides leadership in C-DEBI scientific, education, diversity, outreach, and administrative activities; he represents C-DEBI in interactions with USC administration and funding agencies; and he promotes the Center worldwide.

The Associate Director is the ‘right hand’ of the Director; she assumes all responsibilities and powers of the Director should he, for any reason, be unable to carry out his duties. Together with the Managing Director, she coordinates the grants program and communicates with grant recipients about outcomes, products, and dissemination of results.

The Managing Director manages fiscal matters and grants administration and oversees the administrative staff.

Executive Committee
The Executive Committee (ExCom) manages, supports and leads the direction of the Center’s science initiatives. ExCom also provides guidance to integrate research, education, and data across the Center. ExCom coordinates with the Senior Scientists (see Research management section below) on C-DEBI research directions.

ExCom consists of six permanent members and two rotating members. The permanent members are Director and PI Jan Amend (USC), Associate Director and co-PI Julie Huber (WHOI), co-PI Steven D’Hondt (URI), co-PI Andrew Fisher (UCSC), co-PI C. Geoffrey Wheat (U Alaska-Fairbanks), and Data Management Director and Senior Scientist John Heidelberg (USC). The rotating members consist of Senior Scientists (see Research management section below) added to complement the research expertise on ExCom and serving 15-month terms. Victoria Orphan (Caltech) and Alfred Spormann (Stanford) served as rotators in 2020.

ExCom maintains communication via biweekly videoconference meetings and ad hoc meetings at selected C-DEBI, national and international meetings, with participation by the Managing Director and as needed by members of the Administration; Research; Knowledge Transfer, Data Management and Integration; and Education, Outreach and Diversity Teams. ExCom typically also meets at two annual face-to-face meetings, but met virtually instead in 2020 due to the COVID-19 pandemic’s limitation on travel.

Administration
The administrative staff, led by Managing Director Rosalynn Sylvan, is based at USC, where they manage the Center’s day-to-day activities. They link to C-DEBI activities at the partner institutions and communicate with all participants worldwide. The Managing Director manages fiscal matters and grants administration and oversees the administrative staff. The Managing Director attends the biweekly ExCom videoconference meeting and any other face-to-face ExCom meetings as the administrative liaison. Since 2015, the Managing Director has been operating remotely at Texas A&M University as a Visiting Scholar at the International Ocean Discovery Program (IODP) with regular email and telecommunications in addition to the biweekly administrative and ExCom meetings via videoconferencing.

The Data Manager, Matthew Janicak, is responsible for supporting the database infrastructure (see Data Management and Integration below) and development and maintenance of the website and other community communications. The Administrative Assistant, Nerissa Rivera-Laux, implements day-to-day activities of the Center and is responsible for meeting coordination. As the Center sunsets and scales back activities, the Administrative Assistant position was to be eliminated at the end of 2020 with the Managing Director resuming her duties; in early December, Rivera-Laux resigned.

Research
C-DEBI research is organized in three cross-cutting themes: Fluxes, Connectivity, and Energy (Theme 1); Activities, Communities, and Ecosystems (Theme 2); and Metabolism, Survival, and
Adaptation (Theme 3). They are led by the PI Amend; co-PIs D’Hondt, Fisher, Huber, and Wheat; and Senior Scientists Finkel, Heidelberg, Orphan (California Institute of Technology), Orcutt, and Spormann.

Knowledge Transfer, Data Management and Integration

Knowledge Transfer (KT) is central to all of C-DEBI’s research, education, and outreach programs, and hence, it is the responsibility of all our senior personnel. As KT Director, Wheat coordinates and tracks the various knowledge transfer activities, with a special focus on dissemination of scientific and technical knowledge, increasing public awareness of the subseafloor biosphere, and promoting development and application of novel technologies through commercialization and entrepreneurial use of C-DEBI products.

The Data Management and Integration (DMI) team has the primary objective to make C-DEBI data and products accessible to the world. The products include C-DEBI publications, data generated by C-DEBI projects, documentation of technological advances, and products for education and outreach. Heidelberg leads the DMI effort, with support from personnel at USC (Data Manager Matthew Janicak and Bioinformatics Specialist Benjamin Tully) and URI (Data Portal Lead Robert Pockalny). The DMI Director is also responsible for ensuring that C-DEBI participants have access to the Center’s computational resources and/or bioinformatics expertise, as well as making certain C-DEBI generated data are properly deposited in public archives and databases, including future EarthCube initiatives.

Education, Outreach, and Diversity Administration

The Education, Outreach, and Diversity (EOD) team is based at USC and develops, implements, and coordinates EOD programs and activities. The Education and Diversity Director Gwen Noda provides oversight, leadership, and commitment to the integration of C-DEBI research with our EOD efforts at all levels and reports to the Managing Director. The Education and Diversity Director specifically leads the professional development and mentoring efforts for undergraduate and graduate students, postdoctoral scholars, and K-12 teachers; leads programs to entrain members of underrepresented groups into STEM fields with a special focus on microbiology, geochemistry, and oceanography; and expands the reach of C-DEBI through social media communication. The Administrative Assistant, Nerissa Rivera-Laux, also supports the administrative activities of the EOD programs.

Ethics Panel

The Ethics Panel advises ExCom on any issue pertaining to ethics, including concerns regarding administration, funding, and scientific conduct. This Panel handles all C-DEBI ethics complaints and convenes (electronically or in person) on an ‘as needed’ basis or on request of ExCom. The panel also makes recommendations to ExCom with respect to ethics training programs for C-DEBI members. The Ethics Panel consists of Chair Karen Lloyd (Associate Professor at U Tennessee), Frederick Colwell (Professor at Oregon State), Andrew Fisher (ExCom), Sharon Cooper (Education Officer of the IODP US Science Support Program at Lamont-Doherty Earth Observatory), and William Orsi (Associate Professor at LMU Munich), representing several groups within C-DEBI. To date, the committee has not received any ethics complaints.

In addition, we continue to emphasize a comprehensive ethics policy for C-DEBI participants based on existing models starting with NSF and integrating with specific IODP and other institution policies. This sets forth a community standard to minimize and resolve conflicts effectively. The online ethics training is mandatory for all C-DEBI participants and completion by deadline is enforced. See more at our Ethics Policies webpage.

External Evaluator

The External Evaluator, Beth Rabin, assesses and evaluates the effectiveness of C-DEBI management, research, and education, outreach, and diversity programs and provides thorough, rigorous, independent, and results-based assessments to ExCom.
2. Management and Communications Systems

C-DEBI is a geographically distributed center, with members and participants around the world. Our Center and its participants have ample experience in long-distance collaboration and communication. There are biweekly administrative and monthly ExCom meetings via videoconferencing, a biweekly newsletter (sent to nearly 1000 e-mail addresses), an active social media presence, and regular updates to our website. C-DEBI’s annual meeting includes leadership groups, graduate and postdoctoral fellows, and invited guests. We typically organize several targeted workshops annually and encourage members (especially postdoctoral scholars and early career scientists) to organize sessions at national and international meetings; however this has been somewhat impacted by the COVID-19 pandemic’s cancelation and rescheduling of many of those events. Coordination of these communication activities is under the purview of the Administrative team.

C-DEBI’s social media platforms include Facebook (cdebi, ccrise, CdebiGlobalEnvironmentalMicrobiologyCourse), Twitter (@deepbiosphere), YouTube, and LinkedIn. Education and Diversity Director Noda manages the Center’s social media presence amplifying Center news, opportunities, resources, and more to stay engaged with our deep sea scientific community as well as engage members of the science-interested public. Social media also serves as an outlet to share related science news, opportunities, and professional development from organizations such as DCO, IODP, Schmidt Ocean, MBARI, and the NSF/NSF Geosciences as well as articles on diversity in STEM/academia, work/life balance, resume writing, applying for graduate school, and more.

The C-DEBI website plays several important roles in Center communications. As the website is often the first point of contact, numerous cosmetic and performance enhancements have been implemented to improve user experience and first impressions. The website also serves as the definitive source for Center goals, policies and programs. As a principal source of community news and activities, the website lists time-sensitive items on the front page and archives them for search. Lastly, the website is intended to serve as a working resource for active research and education participants, and we have improved the website’s search interface and metadata to crosslink subawards, participants, publications and other outputs. For dataset entities, we have adopted BCO-DMO’s ontology-focused metadata system. As the Center comes to an end, we are taking steps to archive a "static" version of the website for future reference.

3. Performance with Respect to the Strategic Implementation Plan

Our leadership and management goals are to envision and enable the Center’s mission through inclusive and transparent decision-making; inspire Center members; and facilitate collaborative effort and guide all participants in the Center via a cross-disciplinary and multi-institutional ethics program to instruct them on ethical and responsible conduct of scientific research. A fundamental challenge for C-DEBI leadership is to maintain trust and support among a diverse and interdisciplinary community of scientists, educators, and technologists. Maintaining confidence in STC leadership, throughout the existence of the Center, is essential if busy STC participants are to retain a willingness to allocate some of their limited time for advisory, review, and collaborative activities. STC leaders will also need to assure that there are robust opportunities for inter-institutional and cross-disciplinary exchanges and training, and help to secure external resources in support of ongoing and future STC activities. In addition, the Center will maintain a rigorous ethics training system for all C-DEBI participants and an Ethics Panel overseeing policies and procedures. Finally, STC leadership needs to remain focused on the critical goal that motivated formation of C-DEBI in the first place: creating a vibrant, innovative, and focused community, who will work together to achieve what cannot be accomplished by individuals working alone, to transform the nature of deep biosphere research.
**Target 1:** The decision-making process is defined, transparent and effective leading to a high degree of confidence, ownership, and engagement by STC participants in the Center.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hold biweekly administration meetings as well as monthly ExCom meetings and an annual ExCom face-to-face retreat to enable clear and effective management of the Center</td>
<td>Partially met due to limited meeting activities during the COVID-19 pandemic</td>
</tr>
<tr>
<td>Update the C-DEBI Operations Manual to elucidate the functions of key individuals and groups and main research, education, outreach, and administration activities, programs, operations and procedures and post on the website with the Annual Report and Strategic Implementation Plan</td>
<td>Met</td>
</tr>
</tbody>
</table>

**Target 2:** Communication is effective in facilitating the exchange of science, education of students, and promotion of other C-DEBI activities and opportunities.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regularly update the comprehensive website at <a href="http://www.darkenergybiosphere.org">www.darkenergybiosphere.org</a> with research and education portals and resources</td>
<td>Met</td>
</tr>
<tr>
<td>Distribute biweekly newsletters to the C-DEBI community to highlight recent and upcoming C-DEBI research and education programs and events and other relevant/partner activities and opportunities</td>
<td>Met</td>
</tr>
<tr>
<td>Continue to improve the private login site for internal documents and community reporting</td>
<td>Met</td>
</tr>
<tr>
<td>Solicit 3 nominations for the next season of the videoconferenced Networked Speaker seminar series to present early career scientist research to the C-DEBI community</td>
<td>Met</td>
</tr>
<tr>
<td>Maintain protocol/procedure for issuance and usage of C-DEBI contributed publication numbers and of logo and branding information</td>
<td>Met</td>
</tr>
</tbody>
</table>

**Target 3:** STC participants are engaged in cross-Center training and collaboration.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organize 5-7 C-DEBI-specific opportunities for collaboration and training and entrain new membership (e.g., Center-wide Annual Meetings, Research Workshops, and Exchange Grants)</td>
<td>Partially met due to limited meeting activities during the COVID-19 pandemic</td>
</tr>
<tr>
<td>Support 4-6 research and professional development opportunities specifically for graduate students and postdoctorals (e.g., workshops at Annual Meetings, professional development webinars, and fellowships and networking activities in Research and Education sections above)</td>
<td>Partially met due to limited meeting activities during the COVID-19 pandemic</td>
</tr>
</tbody>
</table>
**Target 4:** Community commitment to an environment promoting high ethical standards in the conduct of research is maintained.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Require 100% of participants complete ethics training within these standards</td>
<td>Met</td>
</tr>
<tr>
<td>Ethics Panel composed of Research, Education, ExCom and Early Career representatives resolves complaints regarding C-DEBI administration, funding and scientific conduct in a timely manner (within 6 months of being presented to C-DEBI)</td>
<td>Met</td>
</tr>
</tbody>
</table>

**Target 5:** Strategies, tools, and resources are developed for sustainability of C-DEBI activities.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Status/Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secure $3M in aggregate (beyond initial STC funding) in support of C-DEBI activities</td>
<td>Met</td>
</tr>
</tbody>
</table>

*4. Plans for the Next Reporting Period*

To further enhance C-DEBI’s culture of collaboration and cross-disciplinary thinking, we will continue to develop cyber-infrastructure for our website enabling public access and data sharing among the C-DEBI research community. The architecture to aid our online communities in collaboration and learning has two principal objectives: 1) to support the connection among scientists and others in the C-DEBI project research community, and 2) to foster the connections between C-DEBI scientists and educators. See also Data Management and Knowledge Transfer Section IV.

**VIII. CENTER-WIDE OUTPUTS AND ISSUES**

**1. Center Publications**

In the current reporting period, the C-DEBI community produced 43 publications, including 42 peer-reviewed journal articles (Appendix G). Details in the appendix include graduate and postdoctoral authors, contributing C-DEBI funding, expedition, site, habitat and theme association.

**2. Conference Presentations**

Center participants reported 34 oral or poster presentations at venues including the 2020 AGU Ocean Sciences Meeting and the Goldschmidt Virtual Conference (Appendix G). C-DEBI’s two-day Annual Meeting was moved online in December and featured multiple plenaries, lightning talks, and randomized breakouts.
3. Honors, Awards and Grants

C-DEBI participants reported receiving 17 (with another 4 pending) honors, awards and grants during the reporting year related to their C-DEBI funding (Appendix G). Senior Scientist Victoria Orphan was elected to the American Academy of Arts and Sciences and C-DEBI Research Exchange Grantee recipient Megan Mullis to the Texas Branch of the American Society of Microbiology. Additionally, C-DEBI Director Jan Amend was named Divisional Dean for the Life Sciences at the University of Southern California.

4. Placement of Graduated Students and Postdoctorals

Eighteen C-DEBI undergraduates, graduate students, postdoctoral scholars and early-career scientists obtained degrees or placement during the current reporting year (Appendix G). C-DEBI funding contributing to the degrees or placement is identified.

5. Outputs of Knowledge Transfer Activities

The C-DEBI community reported developing two technologies in the current year, including a platform and field measurement system. See also Education Section III and Data Management & Knowledge Transfer Section IV.

6. All Participants

Of the 150 individuals reported as being involved with Center activities, 69 are classified as “participants” (per NSF: individuals who have spent over 160 hours on Center activities), while 81 are “affiliates,” reported spending under 160 hours. Affiliates are included where they were reported as personnel on a C-DEBI grant or other budgeted item, attended a C-DEBI event, or have a titular role in the Center. Sources of Center support and reported event attendance are included per participant to provide further differentiation of engagement level. See Appendix G for details.

7. Institutional Partners

C-DEBI has identified 69 participating institutions categorized per NSF reporting requirements. “Partner Types” are determined based on the activities of affiliated participants as follows:

- Graduate student: education, research
- Postdoctoral or researcher: research
- Outreach or professional development: education
- Program targets diverse groups: diversity, education
- Participant worked on new tools, software, methods or products: knowledge transfer
- Participant plays an advisory or managerial role in the Center: all types

Whether the institution has “participated” less or more than 160 hours is likewise determined by its affiliated participants. See Appendix G for details. See also External Partnerships Section V.
8. Summary Table for Internal NSF Reporting Purposes

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participating institutions (all academic institutions that participate in activities at the Center)</td>
<td>54</td>
</tr>
<tr>
<td>Number of institutional partners (total number of non-academic participants, including industry, states, and other federal agencies, at the Center)</td>
<td>15</td>
</tr>
<tr>
<td>Total leveraged support (funding for the Center from all sources other than NSF-STC)</td>
<td>$82,729</td>
</tr>
<tr>
<td>Number of participants excluding affiliates (total number of people who utilize center facilities; not just persons directly supported by NSF)</td>
<td>69</td>
</tr>
</tbody>
</table>

9. Media Publicity

C-DEBI research was featured in over 300 articles this year, with papers in Nature Communications, Nature and PNAS being picked up broadly among news outlets (Appendix G). Additionally, C-DEBI has an active presence on Facebook (886 page follows), Twitter (1,634 followers), and produces a bi-weekly newsletter (959 subscribers).

10. Distributable Media

Our current brochure provides an overview of the deep biosphere, C-DEBI research programs and themes, C-DEBI education and outreach programs, and how to get involved.

IX. INDIRECT/OTHER IMPACTS

1. International Activities and Other Outputs, Impacts, or Influences

C-DEBI regularly and consistently engages the international community in many of its activities, with individual and institutional partnerships as described throughout this report. Of particular note are research expeditions, where multi-national participation is common and often mandated (e.g., IODP), and C-DEBI-led conference sessions and workshops, where scientists from Germany, Japan, China, Great Britain, France, and other countries are commonly invited. Several C-DEBI Co-I’s serve in leadership roles (e.g., science panels, steering committees, advisory boards) for international programs, including the IODP, ICDLI, and Earth 4D. All other outputs, impacts, or influences related to C-DEBI’s progress and achievement in 2020 have been captured in other sections of this report.