

Task Agreement Number P15AC00649
Under
Cooperative Agreement P14AC00921
Between
The United States Department of the Interior
National Park Service
And
Regents of the University of New Mexico
DUNS No: 868853094
Contract and Grant Administrators
Research Administration
MSC01 1247
University of New Mexico
Albuquerque, NM 87131-0001

CFDA: 15.945

Project Title: Establishing a Baseline for Bat Microbiota and Discovering Clues to Natural Defenses Against WNS in Bat Microbiota in Southeast Arizona Group (SEAZ; Chiricahua NM, Coronado NMem, Fort Bowie NHS)

Park Unit: SEAZ (CHIR, CORO, & FOBO)

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Amount of Federal Funds Obligated: \$39,048

Total Amount of Task Agreement Award: \$39,048

Funding source: NPS White-Nose Syndrome

Period of Performance: May 13th, 2015 through September 2nd, 2016

Student Involvement: Yes

Sensitive Information: No

ARTICLE I – BACKGROUND AND OBJECTIVES

Cooperative Agreement Number P14AC00921 was entered into by and between the Department of the Interior, National Park Service, (NPS), and Regents of the University of New Mexico (hereafter referred to as ‘Recipient’) for the purpose of providing research, technical assistance, and/or education, as described below. Unless otherwise specified herein, the terms and conditions as stated in the Cooperative Agreement will apply to this Task Agreement.

For performance under this task agreement, the regulations set forth in 2 CFR, Part 200, supersedes OMB Circulars A-21 (2 CFR 220), A-87 (2 CFR 225), A-110, and A-122 (2 CFR 230); Circulars A-89, A-102, and A-133; and the guidance in Circular A-50 on Single Audit Act follow-up. The recipient shall adhere to 2 CFR, Part 200 in its entirety in addition to any terms and conditions of the master agreement not superseded by 2 CFR

200, as well as the terms and conditions set forth in this agreement. In the event of a conflict between the original terms of the master agreement and 2 CFR, Part 200, relating to this task agreement, 2 CFR, Part 200 shall take precedence.

Establishing a Baseline for Bat Microbiota and Discovering Clues to Natural Defenses Against WNS in Bat Microbiota in Southeast Arizona Group (SEAZ; Chiricahua NM, Coronado NMem, Fort Bowie NHS)

Project Description:

White-Nose Syndrome (WNS), caused by the fungus *Pseudogymnoascus destructans*, threatens bat populations throughout the United States as it progresses from eastern hibernacula west. Western bat populations provide an excellent opportunity to understand bats' external bacterial and fungal inhabitants and their potential to provide natural defenses against this newly emerging pathogen. Using next generation DNA sequencing and microbial culturing techniques we propose to analyze bat microbiota across bat species, to isolate Actinobacteria for testing of anti-fungal activity against *P. destructans*. Actinobacteria are rich producers of antibiotics, including antifungals. In 2014, the Principal Investigator (PI) netted bats in caves and surface sites in five other Arizona and New Mexico sites. Thirteen target species of bats were swabbed, including species very likely and less likely susceptible to WNS. Next generation sequencing performed on DNA from swabs suggests that bats caught on the surface versus those caught in the cave have different microbial communities. Some species of bats have many Actinobacteria present, and we have identified some isolates that show significant inhibition of *P. destrucans*. We propose to extend this research to the SEAZ Group bats found in caves, mines, and surface sites. Much remains to be learned about SEAZ Group's bats, their microbiota and the potential for natural defenses against the invading newly emerging fungal pathogen, *Pseudogymnoascus destructans*, which causes WNS. This funding will allow us to expand the existing dataset to compare results from SEAZ Group bats to our other five sites.

ARTICLE II – LEGAL AUTHORITY

54 U.S.C §101702(a) Cooperative Agreements, Transfer of Service Appropriate Funds
54 U.S.C §101702(b) Cooperative Agreements, Cooperative Research and Training Programs
54 U.S.C. §100703 Cooperative Study Units

ARTICLE III – STATEMENT OF WORK

A. Statement of work:

Background:

Since the winter of 2006-2007, White-Nose Syndrome (WNS) has spread north, south and west from Albany, New York, killing hibernating bats as it continues to move westward. At present *Pseudogymnoascus destructans*, a fungus likely from Europe and causal agent of WNS, and the disease itself has been documented as far west as western Missouri and Arkansas, near the eastern Oklahoma border. Our research focus is to establish a baseline and evaluate the bats before WNS reaches the southwest.

The timeline of WNS spread through bat populations indicates the disease could be present in Arizona within the next 10 years and, based on the eastern bat species impacted by WNS, as many as 10-15 Arizona bat species could be affected. However, there is still an opportunity to address a glaring need: to identify susceptible bat communities within our region and establish baseline of microbiota present on bats and in caves prior to the arrival of WNS. By identifying the presence of *P. destructans*, or close relatives, and analyzing microbiota, we can monitor for WNS, establish baseline microbiological data about bats, and identify a potential natural defense to WNS infection.

Current research by Ms. Debbie Buecher has established the several caves in El Malpais National Monument, and other caves throughout New Mexico possess appropriate microclimate conditions for the growth of *P. destructans*. Previous work by the Northup lab has shown that there are fungi present in the soil and guano samples from some of our sites in Carlsbad Caverns National Park that test positive for *P. destructans* using Lorch primers (Lorch et al. 2010), but are negative using the more accurate real time PCR (Minnis et al. 2013). We hypothesize that these represent close relatives of *P. destructans*. Using these findings as a guide, Dr. Northup and Ms. Buecher selected sites for the investigation of bat microbiota (bacteria and fungi) on roosting bats to investigate differences in bat species with different vulnerabilities to WNS. Dr. Northup has found that naturally occurring bacteria and fungi on the surfaces of bats' wings and fur vary among bat species. In addition, some bat species have a preponderance of Actinobacteria, the bacterial phylum from which two-thirds of naturally occurring antibiotics come from (Berdy 1985; Nett et al. 2009). Drs. Northup and Porras-Alfaro are currently testing Actinobacteria cultured from twelve different bat species against *P. destructans* to determine if these bat Actinobacteria may serve as a natural defense against *P. destructans* (unpub. results). We believe that the western bats' microbiota composition may be different or greater in diversity and abundance when compared to eastern bat species that have been affected by WNS. Therefore, we propose to continue our novel research that investigates the naturally occurring microbiota of bats from SEAZ Group cave and surface roosting bats. This study will address three main objectives:

Objectives:

- Identify and describe the bacteria and fungi present within microbial communities among key bat species at SEAZ Group caves, mines, and surface sites.

- Isolate Actinobacteria that may have the potential to inhibit *P. destructans* growth from bats captured on the surface and in caves/mines at SEAZ Group.
- Test the potential of cultured Actinobacteria from bats to inhibit the growth of *P. destructans*.

Methods:

Objective 1: Identify and describe the bacteria and fungi present within microbial communities among key bat species at mines, caves, and surface sites in the SEAZ Group.

We will explore mines and caves in the SEAZ Group and will net bats at water sites within the parks and monuments. We will also work to expand the number of species from which we swab to include other species we are catching in our other five field sites. Debbie Buecher, in conjunction with Karen Krebbs, a bat biologist already working at the SEAZ Group sites, will conduct all bat cave and surface captures and handling, which will be done during a May 2015 expedition to the SEAZ Group study caves, mines, and surface sites. In total we will swab 20-30 bats (mixture of cave and surface-caught) during this year's WNS funding. Swabbing and bat handling will be done under UNM and NPS IACUC protocols issued to Northup and NM Game and Fish permits issue to Buecher. Swabs will be stored in RNA Later to preserve the DNA and transported on a combination of dry ice and a liquid nitrogen dry shipper to further ensure that the nucleic acids are preserved for extraction. The bacterial and fungal microbiota present on the bats will be identified by MR DNA who will do the DNA extraction and sequencing of the 16S rRNA gene (bacteria) and ITS genes (fungi). These sequences will be analyzed with the Qiime pipeline (Caporaso et al. 2010) and the R statistical package to provide information about what controls the diversity we observe on the bats. Our previous work is currently up on a well-known pre-print serve to obtain feedback before formal submission in about two weeks to a journal for consideration for publication. These new results will also be incorporated into additional publications.

Objective 2: Isolate Actinobacteria that may have the potential to inhibit *P. destructans* growth.

Bats that are captured for microbiota swabbing will also be swabbed to inoculate three media that target Actinobacteria: humic acid vitamin agar (HV), Actinobacteria Isolation Agar (AIA), and gellum gum (GG). These media are supplemented with additional chemicals that enhance the ability of the media to target just Actinobacteria. The inoculated plates will be grown in the laboratory at 20 degrees Celsius until developed enough to pick individual colonies for isolation into pure subcultures. A selection of different morphologies will be selected, their DNA will be extracted and sequences and the resulting sequences will be clustered using BioEdit. Representative isolates will be chosen from each cluster for further testing against *Pseudogymnoascus destructans* at Western Illinois University (WIU) (we don't want to bring this deadly pathogen to New

Mexico) by our colleague Dr. Andrea Porras-alfaro and her students. All isolates will be freezer stocked for long-term availability. The 20-30 swabbed bats will generate 60-90 parents plates, which will generate approximately 600-900 pure isolates for sequencing to identify the Actinobacteria present. These results will also be written up for publication.

Objective 3: Test the potential of cultured Actinobacteria from bats to inhibit the growth of *P. destructans*.

The representative isolates will be shipped to WIU for testing against *Pseudogymnoascus destructans* using a plate overlay technique developed and refined by our Azorean colleague, Dr. Lurdes Dapkevicius in her antibiotics from cave bacteria research. R2A medium is poured in the plates and the bat actinobacterium is inoculated into the center of the plate. After sufficient growth is observed, an overlay of fungal medium (richer) is poured overlap, allowed to harden, and a liquid suspension of *Pseudogymnoascus destructans* spores is poured on this. The plate is incubated for a few days and antifungal production that is effective against *Pseudogymnoascus destructans* is observed as a cleared zone of inhibition where no *P. destructans* can grow. We are currently entered into negotiations with other researchers will further explore the antifungal activity of these isolates.

Animal Care and Use:

Capture and handling of bats will follow the Fort Collins Science Center Standard Operating Procedure (SOP) SOP#: 2013-01 2001-01 (Ellison et al. 2013). Buecher has the permits from New Mexico Game and Fish that allow us to conduct these activities and Northup holds a University of New Mexico IACUC Protocol, as well as an NPS IACUC. We will follow current decontamination protocols established by the U.S. Fish and Wildlife Service to prevent any potential spread of *P. destructans* should it already be in New Mexico caves undetected.

Work Cited:

Berdy, J. 1985. *CRC Handbook of Antibiotic Compounds, Part II: Bacterial Metabolites*. Berdy, J., ed. CRC Press, Publisher.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Tumbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7(5): 335-336.

Lorch, J.M., A. Gargas, C.U. Meteyer, B.M. Berlowski-Zier, D.E. Green, V. Shearn-Bochsler, N.J. Thomas, and D.S. Blehert. 2010. Rapid polymerase chain reaction diagnosis of white-nose syndrome in bats. *Journal of Veterinary Diagnostic Investigation*. 22:224-230.

Minnis, A.M., and D.L. Lindner. 2013. Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. Nov., in bat hibernacula of eastern North America. *Fungal Biology* 117(9): 638-649.

Nett, M., Ikeda, H., Moore, B.S. 2009. Genomic basis for natural product biosynthetic diversity in the actinomycetes. *Nat. Prod. Rep.* 26(11):1362-1384.

B. Project schedule and products:

- Project start Date – May, 14th 2015
- Technical progress reports – Annually
- Investigator's Annual Report (IAR) – April 29, 2016
- Database, Collections/Specimens, Archives, and Maps provided to the NPS ATR or Technical Expert—July 29th, 2016.
- Draft Final Report – June 17th, 2016
- Final Report – July 29th, 2016
- Project End Date – September 2nd, 2016 (project reports/deliverables are due)
- Final SF424 FFR must be submitted within 90 days of project end date.

C. Recipient agrees to:

- Apply for NPS Research permit through Research Permit Reporting System ([Irma.nps.gov](http://irma.nps.gov))
- Complete objectives utilizing methods outlined above
- Provide reports as requested above (Project Schedule and Reports).
- Assist in capturing WNS research science-in-action photos with appropriate text, which can be used by NPS to create exhibits and other interpretive materials.

D. NPS agrees to:

- Assist with scheduling and conducting WNS fieldwork.
- Encourage staff to assist with WNS photography and fieldwork.
- Assist with coordination of fieldwork with Visitor and Resource Protection.
- Be involved in the planning of what WNS research science-in-action photos will be taken.

- Utilize results and photos in providing interpretation to the public concerning WNS and the potential threat it poses to southwestern bats.
- Assist in capturing WNS research science-in-action photos with appropriate text, which can be used by NPS to create exhibits and other interpretive materials.
- May co-author publications or assist with review of materials published regarding project work.

ARTICLE IV – TERM OF AGREEMENT

This Task Agreement will become effective on the date of final signature and extend through September 2nd, 2016.

ARTICLE V – KEY OFFICIALS

A. Key officials are essential to ensure maximum coordination and communication between the parties and the work being performed. They are:

1. **For the NPS:**

Agreement Technical Representative:

Jason Mateljak
Chief of Resource Management
Southeast Arizona Group
National Park Service
Intermountain Region
12856 E Rhyolite Creek Road
Willcox, AZ 85643
Phone: 520.824.3560 ext 204
Fax: 505-285-5661
jason_mateljak@nps.gov

Awarding Officer:

Kelly Adams
Financial Assistance Officer
National Park Service
Intermountain Region
12795 W. Alameda Parkway
Lakewood, CO 80228
Phone: 303-969-2303

Fax: 303-969-2786
Email: Kelly_adams@nps.gov

CPCESU Research Coordinator:

Todd Chaudhry, Ph.D.
Research Coordinator
Colorado Plateau Cooperative Ecosystem Studies Unit
National Park Service
Northern Arizona University
PO Box 15018
Flagstaff, AZ 86011
Phone: 928-523-6638
Email: todd_chaudhry@nps.gov

2. **For Recipient:**

Principal Investigator:

Diana E. Northup, Ph.D.
Visiting Associate Professor; Professor Emerita
Regents of the University of New Mexico
Biology
MSC03 2020; 1 University of New Mexico
Albuquerque, NM 87131-0001
Phone: 505-277-5232
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Administrative Contact:

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ldsalas@unm.edu

B. **Communications** - Recipient will address any communication regarding this

Agreement to the Awarding Officer with a copy also sent to the ATR. Communications that relate solely to routine operational matters described in the current work plan may be sent only to the ATR

- C. **Changes in Key Officials**-Neither the NPS nor Recipient may make any permanent change in a key official without written notice to the other party reasonably in advance of the proposed change. The notice will include a justification with sufficient detail to permit evaluation of the impact of such a change on the scope of work specified within this Agreement. Any permanent change in key officials will be made only by modification to this Agreement.

ARTICLE VI – AWARD AND PAYMENT

- A. Financial Assistance: NPS will provide funding to Recipient in an amount not to exceed \$39,048.00 for the work described in Article III and in accordance with the approved budget (Attachment A). Any award beyond the current fiscal year is subject to availability of funds.
- B. Recipient shall request payment in accordance with the following:
1. **Method of Payment.** Payment will be made by advance and/or reimbursement through the Department of Treasury's ASAP system.
 2. **Requesting Advances.** Requests for advances must be submitted via the ASAP system. Requests may be submitted as frequently as required to meet the needs of the FA Recipient to disburse funds for the Federal share of project costs. If feasible, each request should be timed so that payment is received on the same day that the funds are dispersed for direct project costs and/or the proportionate share of any allowable indirect costs. If same-day transfers are not feasible, advance payments must be as close to actual disbursements as administratively feasible.
 3. **Requesting Reimbursement.** Requests for reimbursements must be submitted via the ASAP system. Requests for reimbursement should coincide with normal billing patterns. Each request must be limited to the amount of disbursements made for the Federal share of direct project costs and the proportionate share of allowable indirect costs incurred during that billing period.
 4. **Adjusting payment requests for available cash.** Funds that are available from repayments to, and interest earned on, a revolving fund, program income, rebates, refunds, contract settlements, audit recoveries, credits, discounts, and interest earned on any of those funds must be

disbursed before requesting additional cash payments.

5. **Bank Accounts.** All payments are made through electronic funds transfer to the bank account identified in the U.S Treasury ASAP system by the FA Recipient.
6. **Supporting Documents and Agency Approval of Payments.** Additional supporting documentation and prior Agency (NPS) approval of payments may be required when/if a FA Recipient is determined to be “high risk” or has performance issues. If prior Agency payment approval is in effect for an award, the ASAP system will notify the FA Recipient when they submit a request for payment. The Recipient must then notify the NPS Awarding Officer identified on the Assistance Agreement that a payment request has been submitted. The NPS Awarding Officer may request additional information from the Recipient to support the payment request prior to approving the release of funds, as deemed necessary. The FA Recipient is required to comply with these requests. Supporting documents may include invoices, copies of contracts, vendor quotes, and other expenditure explanations that justify the reimbursement requests.

ARTICLE VII – REPORTS AND/OR DELIVERABLES

- A. Specific projects or activities within this agreement will be tracked and reported by quarterly submission of a SF-425 Federal Financial Report (FFR) and quarterly submission of a Performance Report. A final SF-425 and Performance Report shall be submitted at the completion of the Agreement. The following reporting period end dates shall be used for interim reports: 3/31, 6/30, 9/30, 12/31. For final the SF-425 and Performance Report, the reporting period end date shall be the end date of the agreement. Interim reports shall be submitted no later than 30 days after the end of each reporting period. Annual and final reports shall be submitted no later than 90 days after the end period date. All reports shall be submitted via email to the NPS Awarding Officer with a copy to the NPS Agreements Technical Representative via email.
- B. An electronic version of the final report and separate abstract suitable for public distribution will be submitted by the Recipient to the ATR. The ATR will send the final report electronically to NPS’s Technical Information Center and carbon-copy the CESU Research Coordinator. Please send Catherine Kisluk at Technical Information Center (TIC) one hard copy and one digital copy of the final report and abstract. Mail the hard copy to:

NPS Technical Information Center (TIC) 12795 West Alameda Parkway,
Lakewood, Colorado 80228 Attn: Catherine Kisluk and email the digital version to catherine_kisluk@nps.gov and cc the CESU Research Coordinator.

If the report does not contain sensitive material, the Research Coordinator will send it to the host university for posting on the CESU website. If it does contain sensitive material, then a brief abstract must be supplied that will be posted on the CESU website.

ARTICLE VIII – MODIFICATION AND TERMINATION

This Task Agreement may be modified at any time, prior to the expiration date, by the mutual concurrence of the Recipient and the NPS. Modifications will be in writing, approved and signed by the NPS Awarding Officer and the Recipient's signatory official.

ARTICLE IX – GENERAL PROVISIONS

1. **OMB Circulars and Other Regulations.** The following Federal regulations are incorporated by reference into this Agreement (full text can be found at <http://www.ecfr.gov>):

- a) **Administrative Requirements:**

2 CFR, Part 200 – Uniform Administrative Requirements, Cost Principles, and Audit Requirements for Federal Awards, in its entirety;

- b) **Determination of Allowable Costs:**

2 CFR, Part 200 – Uniform Administrative Requirements, Cost Principles, and Audit Requirements for Federal Awards, Subpart E;

and

- c) **Audit Requirements:**

2 CFR, Part 200 – Uniform Administrative Requirements, Cost Principles, and Audit Requirements for Federal Awards, Subpart F.

ARTICLE IX – ATTACHMENTS

The following documents are attached and made a part of this Task Agreement:

- A. Detailed Budget
- B. SF-424s
- C. Other Attachments

ARTICLE X - SIGNATURES

IN WITNESS WHEREOF, the parties hereto have executed this Task Agreement on the date(s) set forth below.

FOR RECIPIENT

Name
Title

Date

FOR THE NATIONAL PARK SERVICE



Kelly Adams
Awarding Officer

6/1/15

Date