

Department of Land and Natural Resources

Division of Forestry and Wildlife

1151 Punchbowl St., Room 325; Honolulu, HI 96813

(808) 587-0063, (808) 587-0064 (Fax)

Application for NARS Special Use Permit



Name: **Scott R. Santos**

Title of Proposed Activity: **Microbial Biodiversity of the Hawaiian Anchialine Ecosystem**

The following activities require a Special Use Permit under HAR §13-209-5. If your work in the Natural Area Reserve (NAR) will involve one or more of the following, please indicate with an 'X' below:

- ☒ **X** remove, injure, or kill any form of plant or animal life, except game mammals and birds hunted according to department rules*
- ☐ introduce any form of plant or animal life*
- ☐ remove, damage, or disturb any geological or paleontological features or substances*
- ☐ remove, damage or disturb any historic or prehistoric remains*
- ☐ engage in any construction or improvement*
- ☐ engage in any camping activity
- ☐ establish a temporary or permanent residence
- ☐ start or maintain a fire
- ☐ litter, or to deposit refuse or any other substance
- ☐ operate any motorized or nonmotorized land vehicle or air conveyance in any area (including roads and trails) not designated for its use
- ☐ operate any motorized water vehicle of any shape or form in freshwater environments or marine waters, except as otherwise provided by DLNR's boating rules
- ☒ **X** enter into, place any vessel or material on, or otherwise disturb a lake or pond
- ☐ engage in commercial activities, defined as "the use of or activity on state lands for which compensation is received by any person for goods or services or both rendered to customers or participants in that use or activity"
- ☐ have or possess the following tools, equipments or implements: fishing gear or devices (in `Ahihi-Kina`u NAR), cutting or harvesting gear (in any NAR), and hunting gear or tools (except as permitted by the hunting rules of the department)
- ☐ hike or conduct nature study **with a group larger than 10**
- ☒ **X** presence in an area closed pursuant to HAR §13-209-4.5 or after visiting hours established by §13-209-4.6
- ☐ anchor any motorized or non-motorized water vehicle in the marine waters of `Ahihi-Kina`u NAR
- ☒ **X** other (please explain): **collection of microbial samples by bottom sampling and water filtering**

* May require additional State or Federal permits. Applicants are responsible for identifying and securing all approvals that may be required.

** The NARS rules and recent rule amendments can be viewed on-line at
<http://www.state.hi.us/dlnr/dofaw/Unofficial%20compilation%20HAR%2013.209.pdf>

***** Please allow for a minimum permit processing time of three months*****

All permits will have the following standard conditions, pursuant to HAR § 13-209-5. Additional conditions may apply.

- 1) The permittee shall adhere to the specifications given in the permit application
- 2) Disturbance of vegetation and wildlife shall be avoided as much as possible
- 3) Precautions shall be taken to prevent introductions of plants or animals not naturally present in the area. The permittee is responsible for making sure that participants' clothing, equipment, and vehicles are free of seeds or dirt to lessen the chance of introducing any non-native plants or soil animals. Should an infestation develop attributable to the permittee, the permittee is responsible for eradication by methods specified by the department
- 4) Once approved, the permit is not transferable
- 5) Once approved, the permit does not exempt the permittee from complying with any other applicable rule or statute
- 6) The State of Hawaii shall be released and held harmless from any and all liability for injuries or death, or damage or loss of property however occurring during any activity related to the permit

I certify that the information contained in this application is true and correct.



Applicant's Signature

If approved, copies of the permit will be provided to:

- Applicant
- NARS Commission Executive Secretary
- NARS Branch staff
- DLNR-DOCARE

For internal use only:

Application received on: _____

Distributed to District staff for review on: _____

Approval () recommended () not recommended by NARS Commission or authorized representative on: _____ () with the attached special conditions.

() Approved

() Not Approved

Chairperson, DLNR

Date

Applicant Contact Information

Name: **Scott R. Santos, Ph.D.**

If you are applying on behalf of an organization, the organization and your title:

Dept. Biological Sciences, Auburn University, Assistant Professor

Title of Proposed Activity: **Microbial Biodiversity of the Hawaiian Anchialine Ecosystem**

Primary contact person for this permit application: **Scott R. Santos**

Mailing Address: **101 Life Sciences Building, Auburn, AL 36849**

Phone: **(334) 844-7410**

Fax: **(334) 844-1645**

Email: **santos@auburn.edu**

Supporting Information

Please provide the following information about your proposed activity that requires a special-use permit ("proposed special-use"). Failure to provide responses to the following questions may result in your application being rejected.

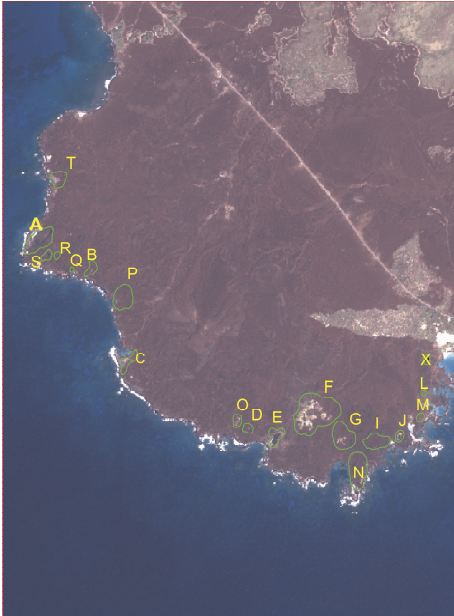
1. What is the period of time for which the permit is requested (e.g., the date of a proposed single event or an ongoing research project from when to when)?
** Please note: research permits are limited to one year in length, except where waived for permits to other governmental agencies where the board determines the waiver to be in the best interest of the State. Proposals for multi-year projects are advised of the need to apply for a new permit EACH year.*

July 1st, 2010 – July 1st, 2011 (with 2-day site visits at 3-month intervals over the one-year period.

2. List the individual Natural Area Reserve(s) involved:

Ahihi-Kinau Natural Area Reserve, Maui, Hawaii

3. Attach a map that illustrates where in the Natural Area Reserve(s) you propose to conduct your special-use. *The map should be legible and reproducible in black and white. The map should also be at the appropriate scale for the type of activity proposed and of sufficient detail to allow the Division to identify activity sites within 10 meters. For any activity off established trails, entry and exit routes should be marked.*



Visits will be to all of the anchialine pond complexes/sites indicated by letters in the above map.

4. Provide a thorough and detailed description of the proposed special use. *The description should be detailed enough so that those reviewing your application understand what you propose to do and the scope of your proposal. As part of your description, please include: a) a description of the planned method of transportation to and within the Natural Area Reserve, and b) if other people than you will participate in the proposed special-use, please note how many people, and whether they are volunteers, students, research assistants, paying customers, etc...*

For research proposals,

- a) please explain your objectives, your methods, and why the proposed special-use is necessary to your research;*
- b) if the research is part of your undergraduate or graduate studies, please include the name and affiliation of your major professor;*
- c) if you are seeking permission to remove or introduce any form of plant or animal life, please list all species involved and specifically identify which are threatened, endangered, or candidate species.*
- d) if you are seeking permission for the collection of any specimens, please note type of specimen (species and parts collected, if less than entire specimen), quantities to be collected, storage methods, and ultimate disposition.*

Failure to provide sufficient information may result in your application being returned for additional information or rejected. Please feel free to attach additional sheets as necessary.

Anthropogenic impacts such as habitat destruction and the introduction of invasive species have lead to the deterioration of numerous ecosystems around the world. This is particularly true in the Hawaiian Islands, which is known as a “hotspot” of ecosystem degradation and species extinctions. One of Hawaii’s unique ecosystems is its anchialine environments, land-locked bodies of mixohaline water that possess simultaneous subterranean connections to the ocean and groundwater aquifer. Environments fitting this definition are globally rare and Hawaii is home to the only natural representatives in the US as well as the largest concentration of them on the planet. However, it has been estimated that >90% have been degraded or lost by anthropogenic activities and it is predicted that they will likely disappear in the next two decades. Thus, we risk losing this ecosystem without knowing the nature and extent of the biodiversity contained with it. This is particularly true of their endemic cyanobacterial-bacterial communities, which degenerate rapidly following environmental perturbation. Given that the microbial constituents of anchialine environments in general have received little attention, this project’s overarching goal is to build further understanding in this area by describing and documenting the taxonomic diversity, relative abundances, distributions and dynamics of Archaea, Bacteria and micro-Eukarya populations from habitats in the Hawaii Islands using high-throughput DNA sequencing technologies. In this context, the specific objectives of the proposed work will expand our limited knowledge of anchialine microbial ecology by:

- 1) Identifying factors influencing the spatial distributions of microbial populations from anchialine habitats.**
- 2) Investigating the temporal dynamics of microbial communities inhabiting anchialine habitats.**
- 3) Quantifying changes in microbial community composition of anchialine habitats impacted by environmental perturbation.**

To achieve the above, 2-day site visits at 3-month intervals over the one-year period will be done to the Ahihi-Kinau NAR by a 3-4-person (myself and graduate students under my direction) team. All movement within the boundaries of the NAR will be by foot. Between 6-12 anchialine pools will be visited and sampled (to encompass the range of habitat types [i.e., orange crusts, green crusts, sediments, etc] within the NAR as identified by Matt Ramsey, Ahihi-Kinau NAR ranger) at each visit. From each anchialine habitat, two (2) samples of water (~5 l) and surface (~ 1.0 cm depth) benthic material (~10 g) will be collected during the daytime high tide (i.e., when these habitats’ basins are at or near maximum (< 1.0 m) depth) using a hierarchical randomized sampling regime to evaluate within-site variation. Surface and benthic levels of photosynthetically active radiation (PAR) will be also be measured using a LI-190SA Quantum Sensor (LI-COR). Collections will be done into sterile containers and transported on ice from the NAR for processing on the same day. A total of up to 48 samples (i.e., 2 water * 2 benthic * 12 sites) will be collected at each visit over the course of the year.

Processing of the samples will result in the isolation and purification of microbial DNA for laboratory analyses (i.e., DNA sequencing). Portions of the samples will also be 1) preserved in 95% EtOH and cryopreservation with DMSO and glycerol and 2) creation of a formalin voucher for morphological identifications/cell enumerations via microscopy. Filtered water from each site will be sent to the University of Hawaii-Hilo Analytical Laboratory to determine water chemistry of each anchialine habitat at the time of sampling. Replicate samples will be shipped on ice within 24 h of their collection and nutrients (e.g., DOC, NO₂⁻ + NO₃⁻, PO₄³⁻), physical properties (e.g., pH, alkalinity) and levels of SiO₂, Mg⁺², Ca⁺², HCO₃⁻¹, and SO₄⁻² quantified. Temperature and salinity measurements will be made in the field with a thermometer and refractometer, respectively.

Given the rate at which Hawaii's anchialine environments are being lost, we will soon miss the opportunity of studying their microbial communities in their natural context. Thus, there is a need to maintain future access to these communities since they could potentially contain taxa of basic research and/or applied scientific value. Thus, materials that are in cryostorage (for future isolation and characterization of cells via culturing), EtOH preservation (for genomic DNA) and formalin vouchers (for morphological identifications/cell enumerations) will be archived with The Ocean Genome Legacy (OGL; <http://www.oglf.org/>), a non-profit "biobank" dedicated to preserving threatened marine biological diversity. The cost of this service will be covered under the current project and it will serve as a resource to future generations interested in Hawaii's anchialine ecosystem as well as environmental microbiology in general (subsamples will be freely available upon request). Access to information on this repository will be made publically available via links from OGL to additions at the current project's website (<http://www.auburn.edu/~santosr/anchialine.htm>). All information will also be prepared as a specific report to the State of Hawaii Department of Land and Natural Resources for their records.

5. Please answer the following questions about your proposed special use:
 - a. Can your proposed special use be conducted elsewhere? If not, why not?

No. This project is funded by the National Science Foundation (NSF; <http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=0949855>) to survey the microbial diversity present within the anchialine environments across the State of Hawaii. Since 1) a large number of Hawaii's anchialine habitats occur in the Ahihi-Kinau NAR and 2) are unique relative to those elsewhere on Maui (such as Waianapanapa Cave) or Oahu and the Big Island, it is important to sample those within the NAR.

- b. Is your proposed special-use consistent with the purpose and objectives of the Natural Area Reserves System (the purpose and objective of the NARS is to protect in perpetuity specific land and water areas which support communities, as relatively unmodified as possible, of the natural flora and fauna of Hawai'i)? If so, how?

Yes, one of the major overarching goals of proposed research is to provide State of Hawaii, Federal and conservation agencies with information that has applied utility in the

development of management strategies for anchialine habitats across the islands. Specifically, this project will provide direct knowledge of the natural microbial flora and fauna of the anchialine habitats in the NAR, which has been little studied to date.

- c. Is your proposed special-use consistent with the management plan developed for the individual Reserve(s) (*Management plans are available for review at www.dofaw.net/nars or by contacting the NARS office*)?

Yes. According to the management plans available from the website: “Preserving the integrity of the anchialine pools is a major management focus.” for the Ahihi-Kinau NAR. This project will contribute directly to that effort by 1) inventorying the diversity of microbes present in these habitats and 2) tracking how these communities change in composition over the course of a year. Such knowledge should have a significant impact on understanding the ecology of these anchialine pools since the microbes that will be studied are the foundation of the food web in the habitat.

- d. Does your proposed special-use provide a benefit (direct or indirect) to the Natural Area Reserves System or to the individual Reserve(s) or both? (*For research, please note whether any studies have previously been made similar to the one proposed and how you will convey your research findings to the Department*).

The proposed special-use permit will provide direct benefits to the Ahihi-Kinau NAR. The last surveys of the microbial populations of the Ahihi-Kinau NAR anchialine pools were conducted by Wong (1975) for her Master’s thesis and remain unpublished in the scientific literature to this day. The work to be conducted here will include the Ahihi-Kinau NAR anchialine pools as one site in a statewide survey, thus leading to a better understanding of how those within the NAR relate (and are unique) to those from across the State of Hawaii. Research findings from this work will be prepared for peer-review and publication in the scientific literature and copies of all publications will be supplied to DLNR for its records.

- e. Will the proposed special-use damage or threaten to damage the integrity or condition of the natural, geological, or cultural resources in the individual Natural Area Reserve(s) and adjacent area or region? If so, how? If not, why not?

No, the proposed special-use will not damage, or threaten to damage, the integrity or condition of the natural, geological, or cultural resources within the Ahihi-Kinau NAR. Sampling will be relatively non-intrusive (i.e., filtering of water and/or small (~10 mm diameter) fragments of the orange cyanobacterial/bacterial crusts found in the anchialine pools) along the edge of a given pond, so no visual or long-term impact/damage is anticipated.

- f. Does the proposed special-use comply with the provisions and guidelines contained in HRS Chapter 205A, entitled ‘Coastal Zone Management,’ where applicable? *HRS Chapter 205A can be accessed at: http://www.capitol.hawaii.gov/hrscurrent/Vol04_Ch0201-0257/HRS0205A/*

Yes, the proposed special-use will have no impact on how coastal zones are managed.

- g. Have you (the applicant) previously received a NARS Special Use Permit? If so, did you comply with the conditions of any previously approved permit (including providing a final report as requested)?

No NARS Special Use Permit has been previously received from DLNR.

- h. Do you (the applicant) have any other current NARS special-use permits? If so, please list and state whether you are currently in compliance with the conditions of those permits.

No other NARS Special Use Permits are currently issued by DLNR to the applicant.

6. Is the proposed special-use expected to have an environmental impact on the Natural Area Reserve(s) or the surrounding area? If, so please elaborate. If not, why not? *Please include discussion of any off-trail work, such as mist-netting, setting of traps, removal of vegetation, etc. and any measures planned to mitigate any short and long-term damage.*

No, the proposed special-use should have no measureable impact on the Ahihi-Kinau NAR and/or the surrounding area. All travel to and from anchialine pools for sampling within the NAR will be done on foot. Furthermore (and as stated previously), sampling will be relatively non-intrusive (i.e., filtering of surface water and/or small (~10 mm diameter) fragments of the orange cyanobacterial/bacterial crusts found in the anchialine pools) along the edge of a given pond.

Since this survey is specifically targeting microbial organisms, sterile technique will be utilized to preserve the integrity of the samples. This has the added benefit of negating the risk of introducing non-native organisms into the Ahihi-Kinau NAR. To further prevent the introduction of non-native organisms into the NAR, quarantine protocols will be utilized where sampling equipment will be 1) sterilized before being brought into the NAR and 2) will not be used to sample between different ponds (to prevent cross-contamination of sites). Thus, no visual or long-term impact/damage is anticipated.

7. There is an application fee of \$50 to cover the cost of processing; please attach a check made out to: *Department of Land and Natural Resources*. Fee waivers are rarely granted; if you would like to be considered for a fee waiver, please check here, and explain in the space below why the proposed special-use is in the public interest, benefits the State, or is for traditional and customary practices. If the waiver is granted, the \$50 check will be returned.

Check for \$50 fee has been attached.

8. For research proposals, please list any local collaborators or contacts (if any).

Robert A. Kinzie III, Ph.D. (University Hawaii Manoa)
Annette Tagawa (State of Hawaii Division of Aquatic Resources)
Lorena Wada (US Fish and Wildlife Service)
Matt Ramsey (DLNR - Division of Forestry and Wildlife)