



**COUNTY OF SACRAMENTO
DEPARTMENT OF REGIONAL PARKS**

ENCROACHMENT PERMIT NO. 21-18

AN ENCROACHMENT PERMIT IS HEREBY ISSUED BY THE DIRECTOR OF THE DEPARTMENT OF REGIONAL PARKS, PURSUANT TO SACRAMENTO COUNTY CODE SECTION §9.36.062 AS FOLLOWS:

Permittee	University of California, Berkeley Department of Integrative Biology
Permittee Address	1001 Valley Life Sciences Building #3140 Berkeley, California, 94720
Site Contact Person(s)	Forrest Freund, 415-497-9687, Lentic64@berkeley.edu Or Dr. Carl Rothfels, 919 907 8744, crothfels@berkeley.edu
Location of Encroachment	Various locations on the American River Parkway, downstream of the Hazel Bridge.
Purpose and Description of Encroachment:	To collect samples of the aquatic Azolla plant for lab testing. See attached collection protocol. Samples will be used to add to existing information on genomic diversity across California. Depending on preliminary results, we may also characterize Azolla morphology with the goal of providing end-users (conservation managers, botanical professionals, etc.) with a useable resource for identifying the Californian Azolla species in the field.
Permitted Dates:	February 23 rd , 2021 to August 30 th , 2021

PERMITTEE AGREES TO COMPLY IN ALL RESPECTS TO THE PROVISIONS AND CONDITIONS IMPOSED BY THE DIRECTOR.

EXECUTED THIS ____ DAY OF **FEBRUARY, 2021** AT SACRAMENTO, CALIFORNIA

Accepted: _____

Dr. Carl Rothfels
Department of Integrative Biology
University of California, Berkeley

County: _____

Liz Bellas, Director
County of Sacramento Department of
Regional Parks

Date: **Feb 19 2021**

Date: _____

3-2-21

**County of Sacramento, Department of Regional Parks
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APPROPRIATION OF PARK PROPERTY

Pursuant to Section § 9.36.062 of the Sacramento County Code, no person shall, without a permit from the Director of the Department of Regional Parks, deposit any earth, sand, rock, stone or other substance within any park facility, nor shall he/she dig or remove any such material from within any park facility, nor shall he/she erect or attempt to erect any building, wharf or structure of any kind by driving or setting up posts or piles, nor in any manner, appropriate or encumber any portion of the real property owned, operated, controlled or managed by

the Department of Regional Parks.

Pursuant to Section § 9.36.063 © of the Sacramento County Code regarding use of the park property, no person shall, without a permit from the Director of Regional Parks, make or place on any tree or plant, building, sign, fence, bench, structure, apparatus, equipment, or property, or any portion thereof; any rope, wire, mark, writing, printing, sign, card, display, or similar inscription or device.

THIS PERMIT IS ISSUED SUBJECT TO THE FOLLOWING PROVISIONS AND CONDITIONS:

Acceptance of Provisions and Conditions: It is understood and agreed by the Permittee that the doing of work under this Permit shall constitute an acceptance of all provisions and conditions thereof.

Notice Prior to Starting Work: Before starting work in the encroachment areas, the Permittee shall notify the Department of Regional Parks at ParksPermit@sacounty.net, at least 24 hours in advance of the date work is to begin. Message shall include your permit number, the location of your work area, and (if relevant) your vehicle information.

Keep Permit on the Work Site: This Permit shall be kept at the site of the work and must be shown to any representative of the Department of Regional Parks or any law enforcement officer upon demand (Sacramento County Code §9.36.052).

Non-Exclusive use: This Permit does not authorize exclusive rights to the property. Permittee may be required to cooperate and coordinate the work and/or encroachment activity with other interests who may require passage or encroachment. Permittee does have exclusive right to their own property (equipment and materials) and would not be required to relinquish such right to others.

Transferability: This Permit is intended for the exclusive use of the Permittee, its contractors, and those designated by the Permittee to perform work under this Permit.

Permits, Approvals, and Inspections From Other Agencies: The Permittee shall obtain and coordinate

all permits, approvals, and inspections as required by other agencies or jurisdictions.

Codes and Standards: This Permit does not in any way allow for deviations from governing codes and laws in the implementation of this temporary project.

Indemnification: Permittee shall indemnify, hold harmless, and defend COUNTY, its officers, agents and employees against any and all claims, demands, damages, costs, expenses or liability costs arising out of the development, construction, operation or maintenance of the encroached property described herein resulting from permittee's negligence or willful misconduct during the dates of permitted encroachment. In the event COUNTY is named as co-defendant, PERMITTEE shall notify the Director of such fact and shall represent COUNTY in such legal action unless COUNTY takes action to be otherwise represented as co-defendant in such legal action, in which event COUNTY shall bear its own litigation costs, expenses and attorney's fees. In the event judgment is entered against COUNTY and PERMITTEE, agents, or others, an apportionment of liability to pay such judgment shall be made by a court of competent jurisdiction setting without a jury.

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SPECIFIC CONDITIONS:

Containment of Work Area: The project sites and permitted encroachments shall be located on the American River Parkway, downstream of the Hazel Bridge.

Hazards/Liability: Permittee shall take all reasonable actions to insure that Permittee's activities on the encroached areas do not present a safety hazard or attractive nuisance to park users. All trenches, holes, or such, shall be safely protected during construction and overnight. Equipment or fuel shall be safely operated and stored to prevent fire from occurring. Paved trails, including bicycle trail and all paved Parkway access points, shall be kept clear of dirt, mud, rock, gravel, debris and any other navigational hazards.

Minimum Interference with Park Use: All work shall accommodate safe use of the Parkway by park users and shall be planned and carried out so that there will be the least possible inconvenience to the general public using the park

Parking: Permittee shall not allow employee vehicles or construction equipment to fill public parking lots or displace public users unless the daily parking fee has been paid and the receipt is prominently displayed. Parking fees will not be waived for Permittee's daily employee parking.

Biological Resources: All trees, shrubs, and woody vines in the project area and vicinity and not part of the project shall be protected and preserved. No tree or shrub taller than 24 inches in height shall be trimmed, pruned, damaged or removed, and no excavation or placement of fill shall be allowed within the canopy dripline of any native tree, unless specifically so stated in this Permit under "Description of Encroachment."

Elderberry and Longhorn Beetle Habitat: Elderberry shrubs or trees are known to exist in the project vicinity. Elderberry shrubs are host plants for the Valley Elderberry Longhorn Beetle (VELB), listed as Threatened under the federal Endangered Species Act. Therefore, no elderberry shrub or tree shall be disturbed without an appropriate permit from the United States Fish and Wildlife Service.

Cultural Resources: If any cultural resources, such as structural features, unusual amounts of bone or shell, artifacts, human remains, or architectural remains be encountered during any activities, work shall be suspended and the Sacramento County Department of Planning and Environmental Review (PER) shall be immediately notified at (916) 874-6141. At that time, PER will coordinate any necessary investigation of the find with appropriate specialists as needed. The project applicant shall be required to implement any mitigation deemed necessary for the protection of the cultural resources. In addition, pursuant to Section 5097.97 of the State Public Resources Code and Section 7050.5 of the State Health and Safety Code, in the event of the discovery of human remains, all work is to stop and the Sacramento County Coroner shall be immediately notified. If the remains are determined to be Native American, guidelines of the Native American Heritage Commission shall be adhered to in the treatment and disposition of the remains.

Termination: County may withdraw or cancel this Encroachment Permit upon written notice.

Permit Type: It has been determined that this encroachment request constitutes a Type II Permit: modification to facilities or grounds. In lieu of the permit processing fee, the Permittee shall submit a written report of work completed and study results to the Department of Regional Parks.

Azolla collection protocol.

Field

Supplies for field collections:

- Rectangular storage containers (6"x9"x3"?) ([Amazon link to 12pk](#))
- GPS
- Camera, Color Scale, Scale Bar and Tripod
- Label tape and sharpie
- Write in rain notebook, mechanical pencil
- Paper towels
- Large coolers for samples
- Physical printout of Azolla key & hand lens
- Physical printout of permits, etc
- Nalgene bottles with FPA
 - Vials for FPA-ed samples? Extra pencils/paper for labels
- Plant press, silica
- Waders? Dip net?
- Spraybottle (for cleaning the FPA ones, etc.)
- [Surface sanitizer (?)]
- [LN₂ transport (?)]
- [15ml plastic tubes for flash freezing samples (?)]

Field collections - on site

- Upon arrival at the site
 - Search for fertile ones, as priority,
 - Survey for morphological variation, make multiple collections as warranted. [[I think morphotypes should be given different numbers, rather than "If two morphotypes are present (such as in terrestrial forms), both kinds should be preserved in separate containers but with the same collection number". Because you never know when different morphologies might end up being different taxa with Azolla]]
- Photograph the landscape and habitat.
- Photograph the plants in situ with the color palette and scale bar, preferably using the macro.
 - Include root photos (trial run, see if it's easier in the field or if this should be done in lab)
- Record the collection number, photo numbers and take a GPS waypoint.
- Collect ca. 5" x 6" of Azolla (for herb. voucher, etc)
 - For terrestrial forms, use a spade or hori-hori knife to gently lift patches from the soil and transfer to the container. Place wet paper towel into the container to keep Azolla moist.
 - Rinse as best as possible

- For aquatic forms, use the container lid to gently skim the surface of the water and push the Azolla into the larger container. Keep a small amount of water in the container.
- For three individual plants:
 - Place single plant into a separate container (small ziplock with paper towel?), for freezing and subsequent DNA extraction. Aim for as large a plant as possible (while still keeping morphotypes separate, etc.), ideally have it be fertile.
- Place tupperwares into cooler, keep on ice, recharge ice as necessary, check on and potentially ventilate containers ~daily.
 - For this, might want to bring a cold-bag into the field, if the site is a ways from the car (have a freezer pack in one of those insulated bags?). If it's hot, at least, and there's a risk of the plants cooking before they get back to the car.

Field collections - at camp

- Make a preliminary ID of the samples using the key.
- Take several pieces of Azolla and preserve in FPA. In each bottle, include a small label, written in pencil, that includes the date and collection number.
 - Include whole-plant (roots, sporangia)
- [[** Clean a small subsample of the plants with the surface sanitizer and flash freeze in LN₂. (May not be possible/advisable)]]

Back at Lab

Supplies for lab work

- Stereoscope
- Scale and color palette
- Digital camera and tripod
- Silica archive packets and silica bin
- Surface sterilizer
- 15ml plastic tubes for -80 storage.

Dry lab

- For trial run:
 - Surface sterilize two of the for-extraction samples. And then compare the DNA yield (qubit) of the two surface-sterilized and two unsterilized samples to see whether surface-sterilization is ok, and how much material needs to be included in a sample.
- For main runs (contingent on results of the trial run):
- Remove roots and clean each for-extraction individual, and one of the for-silica-archive individuals with surface sterilizer. Henriette's paper -- soap and bleach (New Phyt paper).
 - [[Deluxe solution, put wash water on LB and see if anything grows. If nothing grows you've done a great job.]]

- Place into a 5ml tube and freeze at -80°C.
- Place one surface-sterilized, one non-sterilized, and one population sample (a few individuals) into their own coin envelope and dry on silica for archival purposes. (labeled was collection number.1, collection number.2, and collection number.pop).
- Take a ca. 1cm piece of the sample used for macromorphology and photograph it under the stereoscope at “low” and “high” magnification with a scale and color palette. Photograph both the adaxial and abaxial faces of the central axis.
- Press remaining materials as the herbarium voucher.
- Note preservation date

Wet lab - Azolla DNA extraction

- Extract DNA of specimens in a 96 well plate using standard CTAB extractions.
- Extant from the surface-sterilized individual plants (one individual per extraction), contingent on the trial runs (does one individual give enough DNA)

Trial run – test the tupperwares for a couple days, check impact of surface sterilization on the DNA yield. See if one thumbnail is sufficient (need to check with UCLA for how much DNA they want). FW – 100ng to 1ug should suffice (100ng is low). By qubit.