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Structure and Seasonal Changes of Nematode Communities from Vernal Pools (Santa Rosa Plateau)

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Abstract

Vernal pools are ephemeral wetlands, typically with a diverse and highly adapted flora and fauna. We conducted the first nematode survey on record for this ecologically important habitat. Soil samples were collected on six dates from four locations in and around each of two vernal pool basins in the Santa Rosa Plateau Ecological Reserve. Nematodes from plant roots and debris were extracted in a mist chamber separately from the rest of the soil, which was sieved. An estimated fifty-two nematode genera were isolated, including at least sixty-three species. Soils from the two pools were substantially different in the composition and dynamics of their nematode communities. Nematode abundances were analyzed using the nonparametric Friedman test. Significant differences are observed between/among exact locations, sample dates and extracted sample fractions. Differences in abundance patterns are also significant across nematode genera. Simple feeding experiments were conducted to test whether some vernal pool nematodes might feed on the cysts of fairy shrimp, and thereby potentially limit the recovery of some endangered fairy shrimp species. Our observations do not suggest that this is the case. During the dry summer phase, roots and plant debris appear to play an important role in both pools as refuges for nematodes, and by extension of microscopic organisms in general. Adults of large nematodes, in genera such as *Dorylaimus* and *Labronemella* congregate preferentially in dead plant material, perhaps not only in response to drought but also because of the fine texture of the underlying soil. "Aquatic" genera such as *Tobrilus* persist throughout the dry phase, especially in plant debris. We recommend that management of vernal pools takes care to avoid controlled burns or other forms of removal of dead plant material in dry vernal pool beds. Future surveys of nematodes and other microscopic organisms in this type of alternately inundated and desiccated habitat should include methods for extraction of roots and plant debris.

Introduction and Problem Statement

Vernal pools are ephemeral wetlands characterized by alternating seasonal phases of wetting, inundation, waterlogged soil, and drought. Exact definitions vary, but in the stricter sense vernal pools are filled only by precipitation and emptied primarily by evapotranspiration, typically occurring at lower elevations in depressions underlain by an impermeable substrate.

Vernal pools are of exceptional ecological value, as they harbor a rich diversity of plant and animal species that have adapted to extreme seasonal and daily variations in environmental conditions. Many of these species are not or rarely found in other habitats, and some are restricted to just one or a few pools. In Southern California alone, twenty-seven species of special concern were listed by the U.S. Fish and Wildlife Service.

Furthermore, vernal pools provide important feeding grounds to a wide range of highly mobile animals, such as migratory birds and pollinating insects. Aside from these immediate biological assets, vernal pools are also directly relevant to a wide range of scientific and environmental issues. Their hydrological features are as yet poorly known, but important functions include surface water storage, surface water conveyance, and subsurface water exchange.

Annual shifts in the delicate balance between precipitation and evapotranspiration in vernal pools directly influence the composition of flora and fauna, and many species have developed resting stages capable of surviving several years of inhospitable conditions before resuming development. Although this leads to short-term unpredictability in the seasonal development of vernal ecosystems, monitoring of changes in vernal populations and species could provide an interesting indicator system for climate change on a broader geographic scale.

Unfortunately, the very same properties that explain the unusual ecology of vernal pools also render them extremely sensitive to human land use. Rates of extirpation in the past century have been dramatic, and it is estimated that well over 90% of the original number of vernal pools in California are now lost to human impact. Because of their importance to ecology and conservation, the macrofauna and –flora of californian vernal pools have received considerable attention. However, no published surveys exist of the microscopic inhabitants of vernal pools and their soils. This oversight not only leaves an important gap in the knowledge needed to assess the success of conservation and mitigation efforts, but it also constitutes a scientifically interesting field of inquiry in its own right, especially because of the semi-unpredictable patterns of soil inundation and dehydration that occur in vernal areas.

In a wider context, vernal pools in Southern California are also especially interesting because they are mostly located in grasslands. Temperate grasslands have received considerable attention from soil ecologists and nematologists, especially so in Europe, North America and New Zealand. However, the same cannot be said for subtropical or mediterranean grasslands, of which nematode communities remain largely unexplored on all continents. Such an extreme sampling bias is quite likely to unduly prejudice our understanding of the composition and functioning of nematode communities in grassland soils.

Objectives

Several of the largest remaining vernal pools in California are located at 33° latitude on plateaus of the Santa Ana Mountains, which are part of the peninsular ranges of cismontane Southern California. One of these plateaus became a protected area in 1997 through the creation of the Santa Rosa Plateau Ecological Reserve (SRPER, not to be confused with the more easterly located Santa Rosa Mountains and Santa Rosa Wilderness). The vernal pools inside this reserve provide habitat for at least eleven threatened or endangered species, including two listed species of fairy shrimp and five listed plant species.

Within the constraints of strictly enforced protection measures, we have set out to produce a first survey of nematodes from two of the pools of the SRPER. Our primary purpose was to obtain a first set of baseline data, which are an essential prerequisite for follow-up studies with more directly applied purposes, such as for example:

- a) monitoring of nematode diversity as an indicator suite for assessment of the possible effects of climate change and human encroachment point of reference for more extensive;
- b) identification of the naturally occurring parasites or predators of the endangered species occurring in vernal pools, and monitoring of potentially invasive exotic pathogens;

c) investigation of co-occurring microfaunal taxa with divergent adaptations for xeric versus limnic habitats, with the aim of identifying new biological strategies and models for survival and recolonization of soil ecosystems subject to protracted inundation and desiccation.

Procedures

During the past three years soil samples were collected on six dates from four locations in and around each of two vernal pool basins in the Santa Rosa Plateau Ecological Reserve. This includes four sample series collected during dry conditions, and two sample series collected during full inundation. After initial troubleshooting, we settled on the following sampling and processing protocols:

Each sample consisted of five 200 ml cores taken randomly with a 6x2 inch auger in between plant stems (to make sure no plants were removed), within a 1 m² square. The four samples in each series were taken to the nematology greenhouse at UCR for cyst and nematode extractions. Each sample was mixed with one gallon of water in a bucket, stirred for one minute, allowed to settle for one minute, poured into a second bucket over a coarse sieve (20 US standard mesh number or 850 µm pore size) to separate stems, stalks, roots and larger seeds from the remainder.

Next, the second bucket was poured into a third bucket over a finer sieve (120 mesh number or 125 µm pore size) to separate cysts and smaller seeds and plant fragments from the remainder. Lastly, the third bucket was poured through a fine sieve (325 mesh number or 45 µm pore size) to retain nematodes, which were then rinsed off into a small vial. In the following days and weeks, nematodes were processed for microscopy and PCR using standard methods for nematodes.

Each sample extraction lasted no more than 10 minutes, and all water used was at room temperature. No live fairy shrimp or cyst hatching was observed at any stage of the process. Material collected on the intermediate sieve was immediately spread out on paper towels and allowed to dry, while undergoing inspection with a stereo microscope to count numbers of cysts and to check for evidence of nematode predation or parasitism.

The coarse root and debris fraction of the samples was first inspected visually to confirm the absence of any remaining cysts, and then subjected overnight to extraction by mist chamber (essentially the material is sprayed intermittently with a fine mist, and all runoff water is collected by a funnel below the sample fraction). In 2004 and the first half of 2005, all remainders were returned to the respective sampling sites and transferred into the core holes. After approval of changes to our US Fish and Wildlife Service permit, dry cysts were instead deposited with Dr. Marie Simovich at the University of San Diego, and dry plant seeds were deposited with the Natural Resources Manager of SRPER.

Five of the six sample series were processed quantitatively for statistical analysis, one sample series (collected during inundation in March 2005) was aimed instead at maximal recovery of live adults of predatory and omnivorous nematodes, to be used in a simple experiment to test for their potential feeding behavior in the presence of fairy shrimp cysts. Nematodes were transferred individually to multiwell plates with water and debris from their sample of origin, and 10-20 cysts

were added to each well. Observations were made daily for a week. Cysts of cultured, unlisted fairy shrimp cysts were kindly provided for this experiment by Dr. Clay Sassaman (UCR) .

Results

At the time of writing we have completed identifications of all nematodes from the five quantitative sample series. An estimated fifty-one nematode genera were isolated, including at least sixty-two species. An estimated fourteen species are thought to be new to science, two manuscripts with descriptions of three among these are currently in preparation and will be submitted before the end of the year. Additional taxonomic manuscripts will be submitted in 2007. A first detailed statistical analysis of the 2003-04 sample series (collected during dry conditions) revealed that the two sampled pools differ substantially in the composition and dynamics of their respective nematode communities. Significant differences were observed between and among the four locations, three sample dates and two extracted sample fractions (soil fraction versus root and plant debris fraction). Differences in abundance patterns were also significant across nematode genera. An ecological/analytical manuscript is currently in preparation and will also be submitted before the end of 2006.

We have also conducted some simple experiments with predatory nematodes from the vernal pools to test whether they feed on cysts of laboratory cultured fairy shrimp. No predation was observed, although it is possible our experiments need to be modified in order to reflect natural conditions more closely.

Conclusions

We have conducted the first vernal pool nematode survey worldwide. Our results show that the microscopic soil fauna of the two studied vernal pools is healthy and diverse, and that adjacent pools can have quite different communities. There is no indication that nematode parasitism or predation poses a threat to the endangered plants and fairy shrimp in either pool. During the dry summer phase, roots and dry plant debris on the soil surface of the pool beds play an important role as refuges for omnivorous, bacterivorous and phytoparasitic nematodes. Adults of large nematodes in genera such as *Dorylaimus* and *Labronemella* congregate preferentially in dead plant material, perhaps not only in response to drought but also because of the fine texture of the underlying soil. Surprisingly, “aquatic” genera such as *Tobrilus* persist throughout the dry phase, especially in plant debris.

Our research has the following practical implications:

- We recommend that management of vernal pools takes care to avoid controlled burns or any other form of large-scale removal of dead plant material in dry vernal pool beds. This plant material plays an important role as a refuge for microscopic organisms during dry conditions.

- Adjacent vernal pools do *not* necessarily harbor interchangeable faunas of microscopic organisms. It is not appropriate to transfer large amounts of soil or plant material from one pool to another for mitigation or restoration efforts, without first surveying the local micro-organisms in each pool.
- Based on present evidence, nematodes do not appear to feed on (and thereby reduce numbers of) the survival stages of fairy shrimp. Many plant parasites occur in the sediment, but none of these was found in numbers that suggest a severe parasite load on the endemic plant species.
- We recommend periodic follow-up studies to monitor nematode diversity in both pools, for example by sampling during one wet and one dry year every five years. Such studies will be important to monitor water and soil quality, as well as possible occurrence and effects of local climatic changes.
- Future surveys of nematodes and other microscopic organisms in this type of alternately inundated and desiccated habitat should include methods for extraction of roots and plant debris.

Publications

None submitted yet (we plan to submit at least five papers during the current and following year).

Thesis, Dissertations

None completed yet (this project contributes to the research of two graduate students, neither of whom have as yet completed their qualifying exams).