

CHYTRID FUNGI ASSOCIATED WITH POLLEN DECOMPOSITION IN CRATER LAKE, OREGON

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ABSTRACT

We identified chytrid fungi that were attached to pine pollen on the surface of Crater Lake. Fungi were identified by large subunit (LSU) rRNA gene sequencing of lake pollen extracts and by isolation of a chytrid fungus that was present on the pollen. LSU rRNA PCR products were cloned, sequenced and identified. The majority of eukaryotic LSU rRNA sequences associated with pollen were found to be members of the chytrid order *Rhizophydiales*. A fungal isolate was characterized culturally, morphologically, and by DNA sequencing and was identified as a member of the genus *Paranamyces*, in the order *Rhizophydiales*. In addition, protist LSU rRNA sequences from the phylum *Ciliophora* were found. The concentrations of dissolved organic matter, nitrogen, and phosphate in surface water that had visible pollen rafts increased according to the concentration of pollen in the water. Each of these nutrients was detected at several fold higher levels in water with pollen rafts as compared to surface water lacking pollen rafts. These results provide evidence for the role of chytrid fungi in nutrient release from pollen deposited on Crater Lake.

INTRODUCTION

The occurrence of pollen in Crater Lake: seasonal pine pollen deposition is a source of allochthonous organic matter for Crater Lake, Oregon. Crater Lake is a high-elevation, collapsed volcanic caldera lake and the deepest lake in the United States. It has an average depth of 350 m and a maximum

depth of 594 m. It is a nitrogen limited, oligotrophic lake that supports a relatively low density of phytoplankton, heterotrophic bacteria, fungi, zooplankton, and fish (12). During spring and early summer, coniferous forests in the vicinity of Crater Lake produce massive amounts of wind-dispersed pollen,

primarily from pine trees including *Pinus contora* and *Pinus ponderosa* (35). Pine pollen grains have air bladders that allow them to float on water and form yellow aggregates known as pollen rafts. The appearance of pollen rafts on Crater Lake and on many Northern forest lakes is a yearly occurrence (7, 16, 33, 44). Terrestrial organic matter does not flow readily into Crater Lake; it is fed by rain, snowmelt, and groundwater. Other than a few small springs on the steep caldera walls, there are no stream inputs and sources of allochthonous organic matter are presumed to be scarce. Crater Lake has seasonal stratification of the water column and deep-water mixing events every 2 to 5 years. Pollen dispersal occurs during summer stratification when warmer water floats on top of colder, denser water. This effect provides stability within the photic zone and would be expected to favor growth of microbial communities associated with buoyant pollen. However, pollen grains are highly refractory nutrient sources. When not colonized by microorganisms, they eventually sink and can be preserved intact in sediments for millennia (34). Because pollen had not yet been studied as a nutrient source in Crater Lake, we set out to discover if the pollen is subject to frequent microbial colonization and what role, if any, fungi play in its decomposition.

Release of nutrients from pollen in support of aquatic food webs: pollen is rich in fatty acids and supplies organic and mineral nutrients when broken down by microorganisms (28, 32). Conifer pollen inputs have been shown to increase nutrient levels, primary

production and zooplankton biomass in oligotrophic lakes (16). Chytrid fungi are often observed attached to pollen grains that float on lake surfaces. Unlike most aquatic microbes, chytrids have the ability to break down the outer exine wall of pollen to obtain nutrients (22, 38, 41). Chytrids that grow on pollen grains can complete their life cycle attached to pollen (15) and are considered to be major pollen decomposers along with bacteria (33). Once the exine wall is breached, a variety of other organisms can participate in pollen decomposition. Organic matter released from pollen serves as a growth substrate for heterotrophic organisms. Mineralized phosphate and nitrogen from organic matter decomposition supports the growth of phytoplankton (4). The size of pollen grains and their buoyancy make them susceptible to zooplankton grazing. Partial microbial digestion of pollen greatly increases the nutrient quality of pollen for zooplankton because zooplankton lack the ability to break down the outer pollen wall (32). In addition, chytrid zoospores are consumed by zooplankton and have been shown to promote their growth (22). Thus, pollen infection by chytrid fungi initiates food webs that nourish primary producers, heterotrophic microbes and larger consumers such as zooplankton. The role of chytrid fungi in lentic food webs has received little attention, perhaps because of its seasonal pollen dependence and difficulty in fungal identification (20). Because of the crucial role that chytrid fungi play in nutrient release from pollen, we sought to identify and culture pollen-associated chytrids from Crater Lake and compare dissolved nutrient

levels in pollen rafts relative to lake water lacking pollen.

Aquatic food webs were initially imagined as linear food chains from phytoplankton-produced organic matter to zooplankton and animals such as fish. The current view is more complex and includes the consideration of allochthonous nutrients and the roles of heterotrophic bacteria and fungi (36, 37). Heterotrophic microbes thrive in most aquatic environments, including Crater Lake, and participate in food webs and microbial loops (9, 43). Most of the available organic matter in aquatic systems is not readily digestible by organisms other than heterotrophic bacteria and fungi (6, 8). Heterotrophic microbes effectively consume organic matter and serve as food sources for larger microbes and zooplankton. In addition, they are able to mineralize and release phosphate and nitrate that are often limiting nutrients in aquatic ecosystems (9). Phytoplankton and heterotrophic microbes may be in competition for inorganic nutrients, but on the whole, heterotrophs are considered to be essential for production of mineral nutrients that support phytoplankton (4, 38). Until now, pollen has not been considered as an allochthonous nutrient in Crater Lake. Pollen decomposition by chytrid fungi in Crater Lake would support food webs by releasing the nutrients present in pollen and by promoting chytrid zoospore production. Chytrid sporangia and zoospores serve as food for larger organisms including zooplankton and invertebrates (22, 23, 33).

Characteristics of chytrid fungi and their growth on pollen: *Chytridiomycota* is a

phylum of fungi that reproduces with flagellated spores known as zoospores. These organisms, commonly called chytrids or zoosporotic fungi, are genetically diverse and have been shown to occupy a basal position in fungal phylogeny (10). There are five taxonomic orders within *Chytridiomycota*, with *Rhizophydiales* having the majority of described genera and species (30). Two other newly recognized phyla, *Blastocladiomycota* and *Neocallimastigomycota*, are similarly comprised of zoosporotic fungi although these phyla have relatively few species represented within them (19). The morphologically distinct features of chytrids are their sporangia and their flagellated zoospores, which are produced within the sporangia. In the case of eucarpic sporangia, filamentous rhizoids are produced, which are attached to the sporangia. Zoospores develop into germlings, which are the precursors of sporangia. Zoospores and/or germlings typically attach to a nutritive substrate such as pollen or phytoplankton and develop either as epibionts or as endobionts. These morphologic and life cycle features are used to classify zoosporotic fungi (2, 41). In addition, genetic sequences, particularly large subunit ribosomal RNA sequences, are a valuable aid to identification (2, 42). Chytrids are found in aquatic and terrestrial environments and are often parasites of phytoplankton or saprophytes with the ability to break down refractory nutrient sources such as pollen, chitin, and cellulose (2, 18, 20). Lake pollen is considered to be a prime nutrient for many aquatic chytrids (15, 33). We hypothesize that Crater Lake pollen is subject to colonization and decomposition

by chytrid fungi, and that this leads to an increase in nutrient availability. Food webs at several trophic levels would be supported

by a general increase in nutrients given that Crater Lake is a nutrient-limited ecosystem.

MATERIALS AND METHODS

Sample collection and processing: A Crater Lake research and sample collection permit number CRLA-2016-SCI-0005 was obtained from the National Park Service. Surface water from Crater Lake was collected in 0.5 liter bottles from the shore at a rocky location (42°58'36.5"N, 122°5'16.8"W) where the dropoff into the water was steep. The sampling site was selected based on the presence of a thick pollen raft in early June, 2016. Approximately 0.4 liters of water (with or without pollen raft) were collected on June 3, June 20, July 27, and August 31 of 2016. Aseptic techniques included use of sterilized sample containers, careful skimming of surface water during collections to avoid contamination, and aseptic laboratory techniques. The volume of buoyant pollen raft that was collected above surface water was measured relative to total sample volume using the graduations on the sample bottles. Water was kept at ambient temperatures for up to 12 h until it could be stored in a 4°C refrigerator. Upon arrival at the laboratory, all samples were analyzed by microscopy to determine the concentration of pollen grains and observe associated microorganisms. Pollen concentrations were determined by counting the number of pollen grains in a fixed volume using a hemocytometer and microscope. In addition, 150 ml of each

sample was processed by pre-filtration through a 100-micron filter (to prevent clogging) and subsequent filtration through a 0.2 micron pore size filter to separate particulates from dissolved nutrients. The filtered water was stored frozen for later chemical analysis. Buoyant pollen raft from the June 20 water sample was separated from the water beneath it by aspiration, transferred to a 100-micron sieve, rinsed with sterile water and used for isolation of chytrid fungi and analysis of eukaryotic rRNA gene sequences.

Water chemistry: Nutrient analysis, including measurements of dissolved organic carbon (DOC), phosphate, ammonia nitrogen, and nitrite plus nitrate nitrogen was performed by the Cooperative Chemical Analytical Laboratory (CCAL, Corvallis, Oregon) operated by Oregon State University College of Forestry and the U.S. Department of Agriculture Forest Service. CCAL's methodology and quality assurance program conforms with American Public Health Association (APHA) and US Environmental Protection Agency (EPA) surface water chemistry criteria. CCAL tested random duplicate samples for each parameter to ensure that duplicate measurements of identical samples were within their quality

assurance limit of ten percent. Nitrogen concentration from ammonia was measured using APHA protocol 4500-NH₃ G (salicylate method), nitrogen concentration from nitrite plus nitrate was measured using APHA protocol 4500-NO₃ F (cadmium reduction method), ortho phosphate was measured using APHA protocol 4500-P F (ascorbic acid method), and dissolved organic carbon was measured using APHA protocol 5310 B (1).

Genetic analysis: Pollen from water collected on June 20, 2016 was retained in a 100-micron sieve and rinsed with sterile deionized H₂O. Chitinase from *Streptomyces* (Sigma Aldrich, St. Louis, Missouri) was dissolved at a concentration of 1 mg per ml in phosphate buffered saline, pH 6.0. 200 µl of pollen suspension was mixed with 200 µl of chitinase and incubated for 1h at room temperature to break down fungal cell walls. Similarly, an isolated colony from a cultured Crater Lake chytrid was digested with chitinase at pH 6.0 at room temperature for 1h. Reagents and methodology from a DNAeasy kit (Promega, Fitchburg, Wisconsin) were used to prepare DNA extracts from the chitinase digested lake pollen and fungal isolate. These extracts were used as templates during polymerase chain reaction (PCR) amplification of partial 5.8S rRNA, intragenic spacer (ITS) region, and 28S rRNA gene sequences. The primers used for PCR amplification were 5.8SR (5'-TCGATGAAGAACGCAGCG-3') and L7 (5'-TACTACCACCAAGATCT-3'). PCR reactions were catalyzed by Promega *taq* polymerase at these thermal cyler

settings: 94°C for five minutes followed by 35 cycles of denaturation at 92°C for 30 seconds, annealing at 50°C for one minute, and extension at 72°C for one minute. PCR products were purified after agarose gel electrophoresis using QiaQuick gel extraction and spin column kit reagents (Promega). Amplicons were cloned into pGEM T-Easy (Promega). Cloned DNA was sequenced using BigDye™ Terminator v3.1 Cycle Sequencing technology and a 310 Genetic analyzer (Applied Biosystems, Foster City, California). Primers for DNA sequencing were 5.8SR (5'-TCGATGAAGAACGCAGCG-3') and L7 (5'-TACTACCACCAAGATCT-3'). DNA sequences were analyzed by BLAST to determine percent sequence similarities. A phylogentic tree was prepared using a neighbor joining distance method based on pairwise alignments (39) and sequence similarity identified by BLAST analysis (<https://blast.ncbi.nlm.nih.gov/>). Pollen clone DNA sequences were submitted to GenBank under accession numbers MG132640-MG132663. Crater Lake chytrid isolate *Paranamyces* sp. CL sequence was submitted under accession number MG195571.

Culture techniques and microscopy: Pollen from water collected on June 20, 2016 was transferred to a sterile sieve and rinsed with sterile deionized H₂O. The pollen was suspended in sterile water and counted. Chytrid mPmTG agar was supplemented with 120 mg/L penicillin and 200 mg/L streptomycin to inhibit bacterial growth (11). Chytrid agar plates were inoculated by spreading with approximately 50 pollen

grains in a volume of 0.5 mls of sterile water. The cultures were incubated at 15°C for three weeks until chytrid colonies appeared. Zoosporotic fungal colonies were purified by three rounds of streak plating on mPmTG agar. One of these isolates was characterized extensively by microscopy and ITS/LSU rRNA gene sequencing. Liquid cultures of the chytrid isolate were maintained in mPmTG broth or sterile distilled water with 6 mg/L penicillin, 10 mg/L streptomycin, and 2 x

10⁴ Ponderosa pine pollen grains/ml. A Leica DM1000 microscope with digital MC100 camera was used for microscopy of stained and unstained samples. Samples were observed by phase contrast microscopy and by staining with lactophenol cotton blue followed by bright field microscopy. Cooperative Chemical Analytical Laboratory performed measurements of DOC in pollen and chytrid culture supernatants after filtration through 0.2 micron membranes.

RESULTS

Chemical and microscopic analysis of water samples: Climatic conditions during late spring of 2016 resulted in the appearance of substantial rafts of aggregated pollen on Crater Lake, visible from the caldera rim. The yellow pollen rafts stood out in stark contrast to the water of this lake that is renown for its deep blue color. On June 3 and June 20, pollen rafts were observed floating in swirled patterns on the surface of the lake in various locations, especially near the shoreline. A wind-blown pollen raft accumulated as particularly thick layer near Cleetwood Cove on June 3. A sample of surface water from this location on June 3 consisted of buoyant, aggregated pollen (20% of sample volume) and optically clear water beneath the pollen raft. On June 20, a pollen raft at Cleetwood Cove constituted 5% of the surface water sample volume. Later in the summer, on July 27 and August 31, pollen rafts were absent from the lake, but pollen grains were suspended in surface

water. Pollen concentrations and chemical analysis of filtered water samples are shown in Table 1. Dissolved organic carbon (DOC), phosphate and mineralized nitrogen were present at higher concentrations in surface water associated with pollen rafts. In the month of June, pollen raft waters had 5–160 times more DOC than non-raft associated water during late July and August. Phosphate and mineralized nitrogen levels (nitrite plus nitrate) were also greatly elevated (3–330 fold) by the presence of pollen rafts. Pollen rafts on Crater Lake were concentrated at particular locations, usually near the shore. It is important to note that the pollen raft collected on June 3 was unusually thick. The vast majority of Crater Lake's surface was free of pollen rafts throughout 2016. Microscopic observations of Crater Lake water samples revealed the presence of zoosporotic fungi, bacteria and ciliates attached to and/or grazing on pollen in water samples from June 3, 20, and July 27.

In the water sample from June 3 there were an average of two zoosporotic fungal sporangia attached to each pollen grain.

A representative photograph of chytrid colonization of pollen grains is provided as Figure 1.

Table 1

Sample date	Pollen concentration (grains/ml)	DOC mg/L	Phosphate mg/L	N from ammonia mg/L	N from Nitrite/nitrate mg/L
06/03/16	9×10^6	1620	3.666	6.233	0.155
06/20/16	1×10^6	50	0.034	0.128	0.108
07/27/16	1×10^4	10	0.011	0.033	0.024
08/31/16	$>10^4$	5	0.009	0.007	0.004

Figure 1. Phase contrast micrograph of Crater Lake pollen with attached fungal sporangia and bacteria.



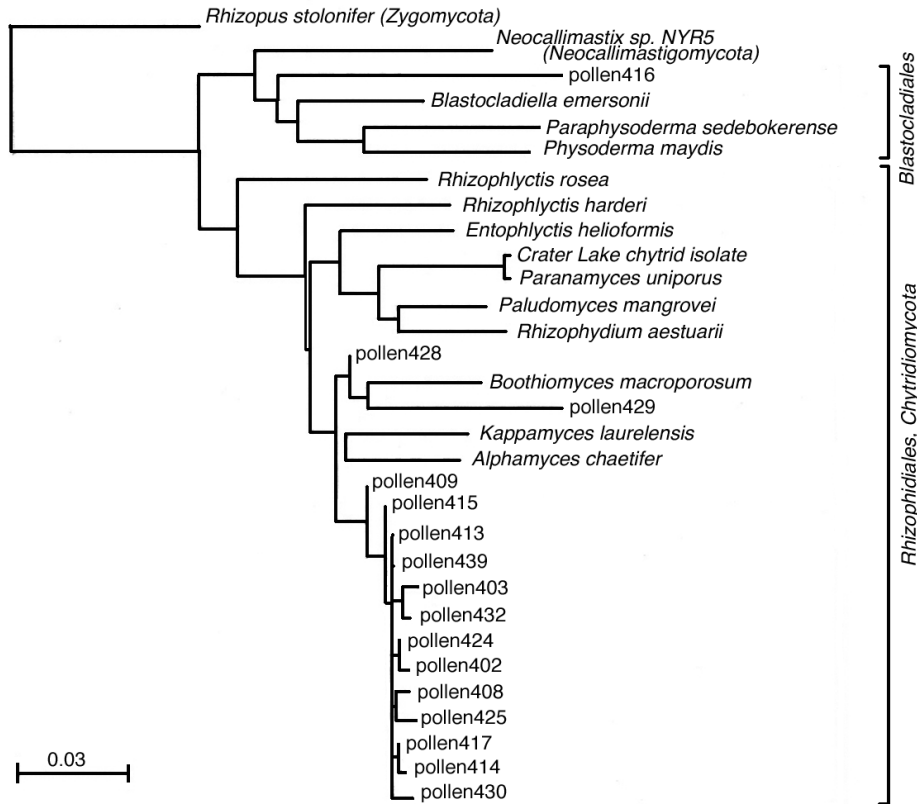
Genetic analysis of pollen associated organisms: DNA was extracted from pollen water collected on June 20, 2016 and LSU rRNA sequences were amplified by PCR and cloned. Twenty-four LSU rRNA clones were sequenced and identified by BLAST and by phylogenetic analysis of fungal sequences (Table 2 and Figure 2). Fourteen LSU clones were identified as members of the chytrid order *Rhizophydiales*. Among these, 12 clones were 98–100% identical to each other, forming a distinct clade most closely related to chytrid genera *Alphamyces* and *Kappamyces*. The two other *Rhizophydiales* clones, pollen428 and pollen429, did not fall within the tight clade of clones but clustered with known

Rhizophydiales genera in the neighbor joining tree shown in Figure 2. The closest BLAST matches to clones pollen428 and pollen429 were *Rhizophylictis* (96% sequence similarity) and *Entophylictis* (91% sequence similarity), respectively. Clone pollen416 had a relatively novel LSU rRNA sequence that was most similar to species in the chytrid order *Blastocladiiales* and phylum *Blastocladiomycota*. Nine of the LSU clones were identified as members of the protist phylum *Ciliophora* including three clones that were 99%–100% similar to *Sterkiella histriomuscorum*, and one clone that was 99% similar to *Oxytricha longa*. Five of the protist clones had LSU rRNA sequences that could not be definitively identified

Table 2. Classification of cloned pollen LSU rRNA gene sequences based on BLAST analysis and neighbor joining analysis. The range of percent DNA sequence similarity to the closest matches in GenBank are shown.

Number of clones	Phylum	Order (percent similarity)	Closely related genera
14	<i>Chytridiomycota</i>	<i>Rhizophydiales</i> (91–96%)	<i>Rhizophylictis</i> <i>Alphamyces</i> <i>Kappamyces</i>
1	<i>Blastocladiomycota</i>	<i>Blastocladiiales</i> (87%)	indeterminant
4	<i>Ciliophora</i>	<i>Oxytrichida</i> (99%)	<i>Sterkiella</i> <i>Oxytricha</i>
5	<i>Ciliophora</i>	<i>Sessilida</i> (91%) <i>Haptorida</i> (92%) <i>Endogenida</i> (85%)	indeterminant

Figure 2. Neighbor joining phylogenetic analysis of cloned pollen LSU rRNA gene sequences identified as fungal, Crater Lake chytrid isolate sequence, and closely related sequences from GenBank. Scale bar indicates percent DNA sequence difference.



beyond the phylum Ciliophora, but were most likely members of the orders *Sessilida*, *Haptorida*, and *Endogenida*. DNA sequence results were consistent with microscopic observations of zoosporotic fungi attached to Crater Lake pollen and protozoa grazing on pollen.

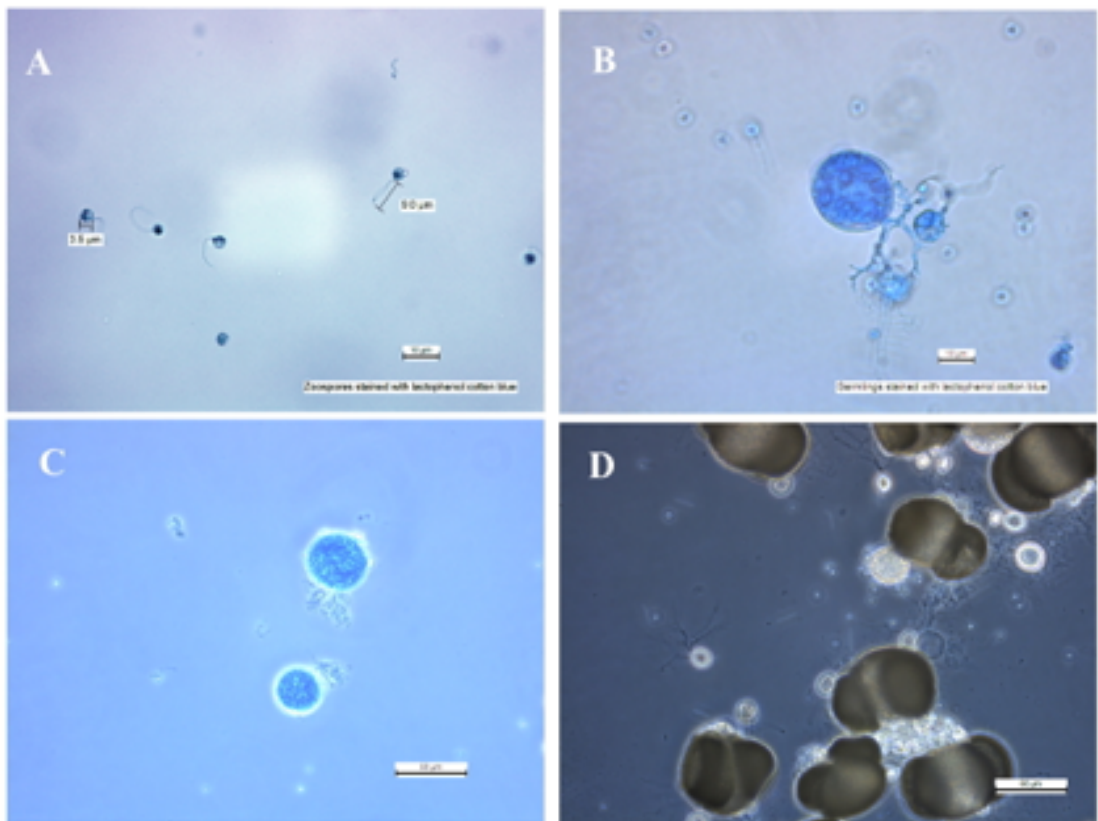
Cultivation and characterization of a fungus from Crater Lake pollen: A chytrid isolate was cultured from Crater Lake pollen and identified by ITS and LSU rRNA gene sequencing, morphology and cultural characteristics. The DNA sequences had

99.8% sequence identity to *Paranamyces uniporus* (812/814 nucleotides), a chytrid in the family Halomycetaceae and order *Rhizophydiales* (31). DNA sequence similarity between *Paranamyces uniporus*, the Crater Lake pollen isolate, and other *Rhizophydiales* is shown in Figure 2. The Crater Lake chytrid isolate grew in mPmTG broth and on pollen grains suspended in distilled water, and completed its life cycle in six days at 15°C. It generated zoospores that were 3–4 microns in diameter, spherical to slightly oval, with a single flagellum 8–15 microns long. Zoospores developed into spherical

germlings with branched rhizoids on single axis. Mature thalli were spherical sporangia ranging from 20 to 80 microns in diameter. Sporangia were monocentric and eucarpic, inoperculate with fine rhizoids tightly aggregated on a single axis. Exit pores were not apparent. The morphologic features of the Crater Lake isolate were consistent with its DNA sequence identification as *Paranamyces* (31). Photographs of its various developmental stages are shown in Figure 3. In an experiment designed to determine if organic carbon was released during chytrid

growth on pollen, the Crater Lake isolate was mixed with Ponderosa pine pollen in distilled water, penicillin and streptomycin. After four days at 15°C, the pollen grains were heavily colonized with developing sporangia. The culture supernatant contained 469 mg/L DOC, whereas control samples of incubated pollen alone or fungi alone had 178 mg/L and 84 mg/L DOC, respectively. The growth experiment provides further evidence that chytrid fungi are able to thrive on pollen and participate in nutrient release.

Figure 3. Crater Lake chytrid developmental stages. A) zoospores stained with lactophenol cotton blue. B) Germlings stained with lactophenol cotton blue. C) sporangia stained with lactophenol cotton blue. D) unstained Lodgepole pine pollen after four



DISCUSSION

We used microscopy, rRNA gene sequence analysis, and isolation of a chytrid fungus to demonstrate the presence and growth of chytrids on Crater Lake pollen. Similar to other published results (21, 29, 45), we found that the majority of eukaryotic rRNA gene sequences associated with lake pollen were *Chytridiomycota* in the order *Rhizophydiales*. The abundant presence of chytrid fungi on pollen in Crater Lake undoubtedly contributes to the release of nutrients for consumption by other aquatic microorganisms and improves the digestibility of pollen for zooplankton (32). Zoospores produced during chytrid growth on pollen can also nourish zooplankton directly (22). We showed that lake water associated with pollen rafts had increased concentrations of DOC, phosphate, and mineralized nitrogen. We measured nutrients in surface water near shore, whereas previous researchers reported dissolved nutrient levels in Crater Lake surface water miles from shore, over the deepest part of the lake. For example, Larson et al (26) reported finding 0.012 mg/L phosphate, 0.003 mg/L nitrogen from ammonia, and 0.001mg/L nitrogen from nitrite plus nitrate. These values are similar to our late summer water sample that lacked pollen. In 1999, Hargreaves et al detected DOC values of 0.05 to 0.15 mg/L in Crater Lake surface water over the deepest part of the lake (17). We found higher levels of DOC, perhaps because pollen and other

allochthonous macronutrient inputs are likely to be higher near shore. Our results are consistent with results from other lake systems where the addition of pollen increased dissolved nutrients (16). Isolation of a *Paranamyces* chytrid from Crater Lake pollen adds to the list of zoosporotic fungi available for laboratory studies. We anticipate that this isolate will be useful for studies of pollen decomposition and metabolic activities of chytrid fungi.

Fungi have been identified as important decomposers in aquatic and terrestrial ecosystems but the dominant research focus has been on woody and leafy detritus found in streams, soils, shallow lakes and wetlands (23, 24). Filamentous, hyphomycete fungi are commonly observed in these systems. However, hyphomycete growth is limited by their non-motile spores and their reliance on hyphal networks (45). In a deep-water, oligotrophic lake such as Crater Lake, leafy and woody detritus are rare, whereas the seasonal pulse of pollen is predictable. Chytrid fungi produce motile zoospores that are able to chemotax toward nutritive substrates such as pollen (14) and chytrids are well adapted to nutrient extraction from pollen. This study detected a diversity of chytrids in association with pollen from Crater Lake, with representatives from *Chytridiomycota* and *Blastocladiomycota*. Our results align well with previous reports of chytrid diversity within aquatic

ecosystems (21, 29, 40, 45).

High trophic transfer efficiencies have been reported from pollen to chytrid zoospores as well as from chytrid zoospores to daphnid zooplankton establishing a direct connection between pollen and higher trophic levels within aquatic ecosystems (13, 22). *Daphnia* and 11 other species of zooplankton with similar feeding patterns are present in Crater Lake (4, 12, 27). Previous research on zooplankton within Crater Lake found significant seasonal and annual variability in zooplankton density but could not identify a mechanism for that variability (27). Because pollen may represent a large fraction of the macronutrients that enter Crater Lake, its degradation and nutrient uptake by chytrid fungi likely contributes to zooplankton abundance. We speculate that variation in pollen abundance contributes to variations in zooplankton abundance in Crater Lake. *Daphnia* are present within Crater Lake and are a species of interest for the Crater Lake Long-term Limnological Monitoring Program because of their important relationship with fish species (3, 12). Based on analysis of stomach contents, the diet of Crater Lake's Kokanee salmon consists almost entirely of *Daphnia* (3). Increases in fish populations were correlated with increases in *Daphnia* abundance over a period of 28 years (12). Allochthonous nutrients such as pollen that support *Daphnia* would therefore be predicted to support salmon in Crater Lake.

Crater Lake is oligotrophic with low concentrations of minerals and DOC.

Thermal stratification and nutrient upwelling from water below 200m to productive water above 120m are important Crater Lake processes that regulate ecosystem dynamics (5). The depth and frequency of vertical water column mixing control the extent that deeper, nutrient-rich water mixes with relatively nutrient-poor epilimnion and metalimnion. Thermal stratification occurs in Crater Lake at the same time as pollen input. During this time, a warmer epilimnion floats on colder deep water, and prevents deep water mixing. The upper 200m of Crater is mixed by wind (5, 25). Nutrients derived from pollen rafts will diffuse and be assimilated quickly in the epilimnion and/or leach slowly into deep water. Wintertime mixing to the very bottom of the lake occurs every 2-5 years in Crater Lake, due to a reversal of thermal stratification. During reverse stratification, colder surface water is forced downward, causing nutrient upwelling (12). If chytrid fungi were absent from Crater Lake, undecomposed pollen grains would likely sink to the lake's bottom and be effectively removed from the aquatic food web. Pollen decomposition by chytrids and other microorganisms in the summer-stratified epilimnion ensures that much of the organic and inorganic nutrients from pollen remain in the upper water column, supporting the growth of phytoplankton, heterotrophic microorganisms and larger organisms. Pollen-derived nutrients that leach into water below 200 meters are removed from the food web until an upwelling event brings them back up to the more productive epilimnion and metalimnion.

Organisms that live in Crater Lake contend with a nutrient poor environment. Low levels of nitrate in the euphotic zone limit the growth of phytoplankton and food is scarce for organisms at higher trophic levels (26, 43). Low levels of primary production increase the impact of allochthonous nutrients on food webs. The seasonal pulse of pollen input supports Crater Lake organisms at several trophic levels. Release of mineral nutrients and DOC from pollen supports microorganisms at the base of food webs. Pollen particles with decomposed exine walls are high quality food for

zooplankton, as are chytrid zoospores that are generated during chytrid growth on pollen (22). Atmospheric deposition of particulate nutrients such as pollen has not been quantified for Crater Lake (25). To fully understand nutrient dynamics in Crater Lake it will be important to estimate the amount of pollen entering the lake. Long-term studies that track pollen abundance and *Daphnia* abundance might reveal a correlation between them. Additionally, studies that trace flow of carbon and nitrogen from pollen through aquatic food webs should be considered.

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