A correlation between chytrid abundance and ecological integrity in New Jersey pine barrens waters

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\section*{Abstract}
Zoosporic fungi (chytrid) abundance, as captured on pollen bait, was significantly higher in pristine sites than impacted sites in a survey of six water bodies of varying ecological integrity (as categorized by the NJ Pinelands Commission) in the New Jersey pine barrens (USA). Using a series of laboratory manipulations of water from a pristine and an impacted site, altered pH appeared to be the primary driver of zoosporic fungi abundance in the field. Increasing pH of the naturally acid water of the pine barrens, significantly reduced zoosporic fungal populations and a reduction of pH of impacted water ameliorated conditions and increased abundance. The addition of nitrogen or phosphorus to, or an increase in specific conductance of, pristine water reduced zoosporic fungal abundance, but a reduction in these parameters in impacted water did not induce restoration of zoosporic fungi. These results show zoosporic fungi have potential as bioindicators of pollution.

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\section*{Introduction}
Zoosporic fungi (Chytridiomycota) are found in ecosystems from the artic to the tropics in aquatic and terrestrial environments (Powell 1993), with highest species diversity in temperate zones (Shearer et al. 2007). Due to their ability to form resistant structures, zoosporic fungi can survive in a wide range of ecosystems with drastically differing biotic and abiotic parameters (Gleason et al. 2004). Thus, their distribution appears to be dependent on environmental conditions rather than geography (Shearer et al. 2007).

Due to their saprotrophic and parasitic nature, zoosporic fungi have an important, although often overlooked, role in community dynamics (Kagami et al. 2007). Aside from being hosted by a wide variety of organisms ranging from algae to invertebrates (Shearer et al. 2007), Kagami et al. (2004) found that the zoospores of these fungi are a significant food source for zooplankton. Zoosporic fungi feed on chitin, keratin and cellulose they obtain from their hosts including algae, fungi, plants, invertebrates and, as recently discovered, vertebrates (Shearer et al. 2007; Gleason et al. 2008). In aquatic systems zoosporic fungi frequently parasitize phytoplankton; in terrestrial systems some genera form obligate parasitic relationships with vascular plants (Powell 1993; James et al. 2006), although the majority of chytridiomycetes inhabit terrestrial systems as saprotrophs (James et al. 2006). The high host-specificity of many pathogenic zoosporic fungi (Van Donk & Bruning 1995) also enables them to have a significant effect on...
their host's population dynamics as well as the ecosystem food-web (Kagami et al. 2007). In each system, a few species thrive in large populations, while the majority remain infrequent and in low abundance (James et al. 2006). A comparison of zoosporic fungal species in soil between four sites in Virginia revealed that although the majority of species were found at all sites there was still a distinct pattern of distribution (Letcher & Powell 2001), but the authors did not relate community composition with edaphic variables.

Numerous studies have investigated the effect of environmental parameters on zoosporic fungi populations, specifically related to zoosporic fungi-phytoplankton interactions, and epidemics (Bruning 1991, Van Donk & Bruning 1995; Kagami et al. 2007; Wakelin et al. 2008). In a study of parasitism of the alga Asterionella formosa by Rhizophydium planktonicum, high water temperatures and low nutrient content (particularly phosphorus) favoured zoosporic fungal abundance due to rapid sporangial development at the expense of algal growth (Van Donk & Bruning 1995). In another study low water temperature favoured algal growth, whereas temperatures of 5–20 °C favoured zoosporic fungal growth, resulting in algal suppression by the zoosporic fungal pathogen (Van Donk & Ringelberg 1983).

Temperature, pH, nutrients, along with light, turbulence, and zooplankton grazing, impact zoosporic fungi related infections on phytoplankton and eutrophication, land-use changes, increasing temperature, and UV radiation may effect frequency of epidemics (Kagami et al. 2007). In soil, Gleason et al. (2004, 2005) showed survival at increased temperature was species-specific, with some tolerating higher temperatures than others.

Freshwater systems are highly susceptible to changes in environmental conditions, be they natural changes or human induced (Belden & Harris 2007) such as pollution, land-use changes, increasing UV radiation, temperature fluctuations, and habitat loss or degradation. These factors can change water conditions to the point where the entire community may become altered. This was found in acidified mountain streams of West Virginia where acidic conditions decreased zoosporic fungal species, and highest diversity was at a more neutral pH (Dubey et al. 1994).

In the New Jersey (NJ) pine barrens, urban development and agricultural run-off is affecting the once pristine water quality in sites that abut or are downstream of intensive agriculture and urban development. These water bodies, identified by the Pinelands Commission, are exhibiting changes in their water chemistry such as elevated pH, high nutrient content, and an increased specific conductance, caused by run-off, upland land use, invasive species, and habitat loss and degradation. Although zoosporic fungi are ubiquitous, observations suggest that their distribution is more dependent on environmental conditions than geography (Shearer et al. 2007). To our knowledge there have been no studies on zoosporic fungi in the NJ pine barrens so our aims were twofold: to determine (i) if zoosporic fungi were present and (ii) if zoosporic fungal populations changed as their ecosystem degraded.

We conducted a survey of pine barrens water bodies that had been categorized as either pristine or impacted (Pinelands Commission designation) with the null hypothesis that water quality, as defined in these categorizations, would not affect zoosporic fungal abundance. Through a series of laboratory manipulations, we investigated the effect that altered pH, specific conductance and nitrogen and phosphorus loading have on zoosporic fungi populations from impacted and non-impacted water bodies to identify possible causal agents in changes of zoosporic fungi abundance.

Materials and methods

Site assessment

Sites were selected using the New Jersey Pinelands Commission’s long-term ecological assessment of water bodies. This long-term environmental-monitoring program analyzed water bodies within the Mullica River, Rancocas Creek, Great Egg Harbor, and Barnegat Bay watersheds for ecological integrity. Each site was identified on a scale of 1–5, with 5 being a pristine ecosystem and 1 being a severely impacted system (Zampella et al. 2001, 2003, 2005, 2006). Integrity was evaluated through analysis of pH, specific conductance, marginal vegetation, and fish and anuran assemblages. Pristine sites were designated by possessing a low pH, low specific conductance, native and intact marginal vegetation, and healthy populations of native fish and anuran species. Impacted sites had higher, more neutral pH, high relative specific conductance, a loss of native species, and a high number of invasive species.

Three sites (Pakim Pond, Hampton Furnace, and Otter Pond) were chosen as pristine sites based on their high level of ecological integrity, having typical pineland’s water body characteristics, defined as low pH, low specific conductance, minimal invasive species, and healthy native populations. In contrast, Indian Mills Lake, Pump Branch Impoundment, and Paradise Lake were chosen as impacted water bodies, defined by high pH, high specific conductance, presence of invasive and border entrant species, and proximity to developed and impervious surfaces.

Zoosporic fungi population surveys

Population surveys assessed the abundance of zoosporic fungi, but did not assess community composition. The initial survey in 2008 was restricted to a comparison between Pakim Pond (pristine) and Indian Mills Lake (impacted). Sites were sampled a total of seven times starting in mid-Jul. and ending in mid-Oct. In 2009, all six sites were sampled three times from late Jun. to early Aug.

Sampling consisted of collecting a similar volume of water containing plant debris, or water containing a sample of mud taken from the edge of the lake into a 500 ml Mason jar and returned to the laboratory. Here, a defined weight of pollen grains (approximately 0.5 g) was added to the surface of the water. After 1 week incubation, the total number of zoosporic fungi colonizing pollen grains was counted per 50 or 100 pollen grains using a light microscope at 400× magnification. This total count of zoosporic fungi per pollen grains was used as an index of zoosporic fungi abundance. Correct observation of zoosporic fungi was confirmed through correspondence with Dr. Joyce Longcore (University of Maine at Orono). Other than identifying the presence or absence of zoosporic fungi on pollen grains, no further taxonomic identification was made.

This indirect method of population assessment was adopted for simplicity as one of the overall aims of the project.
was to evaluate a simple method of use of potential bio-indicators of ecosystem degradation that could be performed by personnel with minimal training.

**Laboratory manipulations of water chemistry**

The NJ Pinelands Commission uses a suite of water quality parameters to determine the degree of degradation of water bodies in the NJ pine barrens. Of these, pH and specific conductivity are the main chemical indicators with low pH and conductivity indicating pristine conditions, where an abundance of indigenous plants and animal species exist to the exclusion of invasives (Zampella et al. 2001, 2003, 2005, 2006). We elected to use these parameters along with nitrogen and phosphorus as potential nutrient pollutants to use in water chemistry manipulation experiments to determine possible causal effects of these parameters on zoosporic fungal abundance. In an attempt to determine environmental factors that may cause changes in zoosporic fungi abundance, a non-impacted site (Pakim Pond) and an impacted site (Indian Mills Lake) were chosen as extremes of water quality. Water samples from each of these sites were compared and manipulated in the laboratory to bring the Pakim Pond water to a degraded state equivalent to that of the Indian Mills and to improve the quality of Indian Mills to the state of Pakim Pond water through appropriate manipulation of water chemistry. The abundance of zoosporic fungi was measured by baiting the samples with pollen and counting the number of fungi colonizing 50 or 100 grains as previously described. The changes in abundance as a result of these manipulations was taken as an index of importance of the pollutant in likely causing the observed differences in zoosporic fungal abundance in the field surveys. All laboratory studies were conducted in the same room at ambient temperature. A summary of water quality at each of the two sites along with target changes in water quality in the manipulation experiments is given in Table 1.

**Manipulation of nitrogen**

Nitrogen concentration of water samples from both Pakim Pond and Indian Mills Lake (Table 1) was determined colorimetrically using phenate method for ammonia and hydrazine for nitrite/nitrate with an Astoria-Pacific AutoAnalyzer 3 (Clackamus OR) (Allen 1989). Ambient nitrogen levels (μg l\(^{-1}\)) in Pakim Pond were 0.305 (NO\(_3\)) and 0.31 (NH\(_4\)); ambient levels in Indian Mills were 0.57 (NO\(_3\)) and 0.367 (NH\(_4\)).

Sediment and leaf debris from the edge of the water body was collected from Pakim Pond and divided among 24 500 ml Mason jars. All jars received debris from Pakim Pond so as to provide the same zoosporic fungi inoculum to each system. Twelve jars were filled with approximately 250 ml Pakim water and the remaining 12 jars were filled with 250 ml of Indian Mills water.

Nitrogen addition was calculated using base levels of NO\(_3\)–N and NH\(_4\)–N from Pakim Pond. In replicates of three the jars from each site were divided into 4 levels: control with no additional N loading, in Level 1 NH\(_4\) and NO\(_3\) equal to 0.5 times the initial concentration in Pakim were added, Level 2 received 1.5 times the NH\(_4\) and NO\(_3\), and Level 3 received twice the ambient Pakim level of NH\(_4\) and NO\(_3\) (Table 1). Jars were baited with pollen and every 3rd nitrogen levels were adjusted.

One week after baiting, pollen was mounted on microscope slides and the total number of zoosporic fungi on 50 or 100 pollen grains was counted at 400× magnification. Afterwards nitrogen was readjusted, and deionized distilled H\(_2\)O was added to refill jars to original volume. The jars were then bubbled with an aerator for 30 min and re-baited with pollen and assessed after another week of incubation.

**Manipulation of pH**

Water and debris samples collected in Apr. 2009 from Pakim Pond and Indian Mills had initial pH of 4.16 and 7.42 respectively. The experiment was set up as described above using 3 replicates of each of a control and 2 manipulations for a total of 18 jars. The nine Pakim jars were divided into 3 control with no change in pH, 3 jars that received a NaOH solution to raise pH to a mean of both sites (5.76), and 3 jars that were raised to the pH of Indian Mills (7.42). The Indian Mills jars were divided similarly with 3 jars receiving an HCl solution to lower to the median level (5.76), and 3 unamended jars as controls and 3 receiving the acidic solution to lower the pH to the initial Pakim level of 4.16 (Table 1). NaOH and HCl were used in the alkaline and acidic solutions as per Piotrowski et al. (2004).

| Table 1 – Water quality measurements and target levels of manipulation of Pakim Pond and Indian Mills Lake (ambient levels) and target concentrations for laboratory manipulations of pH, phosphorus, specific conductance, and nitrogen loading |
|--------------------------------------------------|---------------------------|---------------------------|---------------------------|
| Manipulation level | Pakim Pond | | Indian Mills Lake | |
| Control (ambient) | 1 (median) | 2 (opposite)* | Control (ambient) | 1 (median) | 2 (opposite)* |
| pH | 4.16 | 5.76 | 7.42 | 7.42 | 5.76 | 4.16 |
| Phosphorus (mg/l P) | 0.153 NO\(_3\) | 0.305 NO\(_3\) | 0.457 NO\(_3\) | 0.153 NO\(_3\) | 0.305 NO\(_3\) | 0.457 NO\(_3\) |
| Specific conductance (mS/cm) | 48.9 | 116.7 | 184.5 | 184.5 | 116.7 | 48.9 |
| Nitrogen addition (μg/l) | Level 1 | | | Level 1 | | | |
| | 0.153 NH\(_4\) | 0.31 NH\(_4\) | 0.465 NH\(_4\) | 0.153 NH\(_4\) | 0.31 NH\(_4\) | 0.465 NH\(_4\) |

* Environmental conditions altered to those in the other water body.

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Zoosporic fungi were quantified in all jars by pollen baiting, counting, and aerating as described above. The pH was checked 3–4 times a week prior to re-baiting and adjusted accordingly.

**Manipulation of phosphorus**

Water and debris samples were obtained in Jun. 2009 from Pakim Pond and Indian Mills Lake. Ambient phosphorus for Pakim Pond was approximately 0.1 mg l\(^{-1}\) and Indian Mills was approximately 0.2 mg l\(^{-1}\). Phosphorus concentration was determined colorimetrically using the ascorbic acid method (Allen 1989). Manipulation was set up identically to the pH experiment with a control, and 2 levels of manipulation based on initial \(PO_4\) levels of Pakim and Indian Mills with a median level target concentration of 0.15 mg l\(^{-1}\) (Table 1). Phosphorus content was monitored every 3 d and adjusted with KH\(_2\)PO\(_4\). Zoosporic fungal abundance on pollen was counted weekly for 4 weeks as above.

**Manipulation of specific conductance**

Water and debris samples were collected from Pakim Pond and Indian Mills Lake in Jun. 2009. Specific conductance was analyzed with Pakim Pond having 48.9 s cm\(^{-1}\) and Indian Mills having 184.5 s cm\(^{-1}\). The experiment followed the previous set-ups with a control and 2 levels of manipulation with a median level target of 116.7 s cm\(^{-1}\) (Table 1). Specific conductance of each sample was modified through addition of NaCl or deionized distilled water to increase or decrease conductivity. Specific conductivity was adjusted every 3 d and pollen was counted weekly for 4 weeks.

**Statistical analysis**

For survey data, difference in abundance of zoosporic fungi between sites and between sample dates was analyzed by analysis of variance (SAS version 9.1) followed by Tukey’s Honestly Significant Difference test at an alpha level of 0.05. For water chemistry effects, each manipulation was considered separately as a factorial designed experiment (site \(\times\) treatment \(\times\) time) and analyzed by ANOVA or GLM (missing data) procedures followed by Tukey’s post-hoc test.

**Results**

**Abundance survey 2009**

All six sites were sampled three times during the summer of 2009. Significant differences (\(P \leq 0.001\)) were found for all factors (site, time, and substrate) and all interactions. There were significant differences in zoosporic fungal abundance between sites (\(P \leq 0.0001\)) with Pakim Pond (PP) having significantly higher zoosporic fungal abundance than any of the other sites and Indian Mills (IM) having the lowest zoosporic fungal abundance. Overall the pristine sites had significantly higher zoosporic fungal abundance than the impacted sites (\(P < 0.0001\) from analysis of combined pristine and impacted sites). Time (week) had a significant effect on zoosporic fungal abundance as each site responded differently each week, resulting in a significant site \(\times\) time interaction (Table 2).

It should be noted that in week 1 Otter Pond (OP) and Hampton Furnace (HF) had samples of water instead of debris. However, the only significant substratum effect resulted from a significantly lower abundance of zoosporic fungi in the water samples than in either mud or debris (Table 2); therefore, there is no significant difference in zoosporic fungal abundance between mud and debris samples, as found in 2008.

**Laboratory manipulations of water quality**

**Nitrogen**

Zoosporic fungal abundance was significantly (\(P \leq 0.001\)) different between sites, treatments and over time and for all interactions. The increase of nitrogen in Pakim Pond was negatively related to zoosporic fungal abundance as habitat quality decreased (Fig 1A). In contrast, increasing nitrogen in Indian Mills did not show a strong trend in zoosporic fungal abundance but levels fluctuated, being at their highest at Level 1 and lowest at Level 2 (Fig 1A). ANOVA results for both sites showed zoosporic fungal abundance significantly different between the sites (\(P = 0.0016\)) with Pakim having a higher
abundance. The significant site × treatment interaction implies that increasing N loading of Pakim Pond reduces zoosporic fungal abundance, but the attempted cleaning of Indian Mills did not affect fungal abundance. Pakim Pond experienced a significant (P ≤ 0.05) and sequential decrease in zoosporic fungal abundance over time (from 53.7 ± 2.7 to 2.6 ± 0.7 over 4 weeks), whereas the abundance in Indian Mills was not significantly (P > 0.05) different between weeks 2 and 3, but having a final value at week 4 of 1.5 ± 0.3 compared with a first week abundance of 52.8 ± 3.1, resulted in a significant treatment × time interaction.

**pH**

Altering pH significantly (P ≤ 0.05) affected zoosporic fungal abundance in both Pakim Pond and Indian Mills (Fig 1B). Increasing pH in the Pakim samples simulated an increase in ecological degradation and caused a significant decrease in zoosporic fungal abundance. As pH was lowered back to optimal Pinelands levels in Indian Mills samples, zoosporic fungal abundance increased. Zoosporic fungal abundance increased over time in both Pakim Pond (P ≤ 0.001) and Indian Mills (P ≤ 0.01).

**Phosphorus**

Pakim Pond showed a steady and significant (P ≤ 0.05) decline in zoosporic fungal abundance as phosphorus content increased and habitat quality decreased. In Indian Mills, a change in phosphorus status had no significant (P = 0.885) effect on zoosporic fungal abundance (Fig 1C). Overall, Pakim Pond constantly had a higher zoosporic fungal abundance (P = 0.0027) than Indian Mills and in general fungal abundance decreased in both sites over time (P ≤ 0.0001).

**Specific conductance**

Zoosporic fungal abundance in Pakim Pond decreased as specific conductance increased (P ≤ 0.05) but fungal abundance in Indian Mills did not respond (P = 0.460) to a decrease in specific conductance (Fig 1D). Zoosporic fungal abundance decreased over time in Indian Mills (P ≤ 0.001), but fluctuated in Pakim Pond with high abundance in weeks 1 and 3 and low in weeks 2 and 4 (P ≤ 0.0001).

**Discussion**

The abundance of zoosporic fungi, as measured by the indirect method of pollen baiting, is significantly lower in polluted NJ pine barrens water bodies than in pristine waters. Abundance surveys from 2008 on paired sites, Pakim Pond (pristine) and Indian Mills (impacted), showed that Pakim Pond had significantly higher zoosporic fungal population than Indian Mills. In the more extensive 2009 survey of three pristine and three impacted sites, similar results on fungal abundance...
were found. Our surveys did not extend to taxonomic identification of these fungi, so we cannot comment on the community composition of these fungal communities.

In all environmental manipulations when Pakim Pond water was degraded through increasing nitrogen, pH, phosphorus, and specific conductance zoosporic fungal abundance decreased significantly (Fig 1). These results were expected since endemic microflora are frequently sensitive to environmental conditions (Hafez & Elbestawy 2009) and the total abundance and species of fungi decreases in the presence of pollution (El-Sharouny 1992).

The response from Indian Mills was not clear. When nitrogen, phosphorus, and specific conductance were lowered, simulating increasing water quality, zoosporic fungal abundance was not significantly different from the control level (Fig 1). If the zoosporic fungal communities in degraded waters, such as Indian Mills, were endemic then we would have expected increasing abundance as water quality improved during experimentation. However, since there was no clear trend in zoosporic fungal abundance it may be that the community in Indian Mills has shifted from native to invasive species or the sites were so degraded that the remediation attempted could not restore zoosporic fungal abundance. This scenario seems likely when compared to Tsui et al. (2001) where in heavily degraded water common species of fungi were absent and had been replaced with species that had not previously been seen in that area. Hafez & Elbestawy (2009) also found that in pollution-contaminated soils, fungal communities shifted from native populations to invasive species that were resistant to pollution; this change in community also had an adverse effect on soil quality as nutrients were depleted.

Of the effects in zoosporic fungi population response to altered water conditions, the response to changes in specific conductance was smallest. The difference in zoosporic fungal abundance between specific conductance levels in Pakim Pond was only just significant and there was no response in Indian Mills. It may be that the manipulation of other parameters is more important to zoosporic fungi, or that the actual specific conductance in water is dependent on the balance of a number of chemical ions which may not be truly represented by changes in NaCl concentration alone. Of all water quality parameters, pH was the one that had the most marked effects on zoosporic fungal abundance and had some degree of remediation in the impacted Indian Mills site. This appears contradictory to Dubey et al. (1994) who found higher fungal diversity at more neutral pH. However, when pH is viewed in the context that native fungi are adapted to the highly acidic waters of the pine barrens, it seems probable that increasing pH would have adverse effects on the natural community.

Lower zoosporic fungal abundance in degraded sites is further evidence that pollution changes community structure and lowers diversity (Tan & Lim 1983; El-Sharouny 1992; Tsui et al. 2001; Hafez & Elbestawy 2009). Previous studies have found that native microflora in soil communities were more sensitive to environmental conditions and died in the presence of contamination (Hafez & Elbestawy 2009). Changes in fungal communities due to nutrient enrichment pollution have also been observed in rivers (El-Sharouny 1992; Tsui et al. 2001). Furthermore, as decreases in native species were observed, a community shift towards resistant species, not previously found in the area, was recorded (Tsui et al. 2001; Hafez & Elbestawy 2009). These changes in community structure had an adverse effect on habitat quality as nutrients were depleted and decomposition slowed (Tsui et al. 2001; Hafez & Elbestawy 2009).

As our surveys did not include taxonomic identity of the fungi, a logical and necessary next step is therefore to obtain information on community compositional changes in these sites, to identify changes due to level of impact. This work should be conducted using both morphological (Chen et al. 2000; Letcher & Powell 2001) and molecular means of identification (James et al. 2006; Euringer & Lueders 2008). Some functional aspect of potential community changes could also be quantified, such as rates of leaf litter decomposition (Gleason et al. 2008) and the interaction between fungal and algal abundance (Van Donk & Ringelberg 1983; Van Donk & Bruning 1995). In this regard, recent evidence has suggested just how far-reaching the impact of pollutants can be. Hafez & Elbestawy (2009) suggest that environmental conditions can affect the utilization of resources, increase or decrease a community’s stress tolerance, limit available ecosystem services, and increase a community’s susceptibility to biological invasions, which in turn may lower biodiversity and endangering native organisms.

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