Microbial Ecology (2025) 88:80
https://doi.org/10.1007/s00248-025-02566-5

RESEARCH

Eukaryotic Microbiome of Lake Sturgeon Eggs, and Identification of Chemical Thresholds for Infection Control

Kristi Gdanetz[1,2,6] · Zachary A. Noel[2,7] · Ken Saville[5] · Terence Marsh[4] · Kim T. Scribner[3] · Frances Trail[1,2]

Received: 20 February 2025 / Accepted: 5 June 2025
© The Author(s) 2025

Abstract

Eukaryotic microorganisms are an important, but understudied, component of freshwater aquatic ecosystems, and are significant sources of mortality in early life stages of fishes in natural and aquaculture systems. The eukaryotic microbiome colonizing egg surfaces of the lake sturgeon (*Acipenser fulvescens*) was characterized from eggs collected in natural stream habitats and a streamside hatchery in the Cheboygan River watershed in MI, USA. The taxonomic diversity of members of the Kingdoms Fungi and Stramenopile associated with infections of lake sturgeon eggs during spawning is contributing to lake sturgeon mortality in the hatchery. Characterization of the microbial communities from deposited eggs demonstrated heavy influence of spawning location on the diversity of *Pythium*, an Oomycete predominating in the microbiome. The Ascomycota also had a strong and distinguishing presence, with members of the Dothidiales found only on eggs from the streamside hatchery. *Aureobasidium pullulans*, a ubiquitous pigmented yeast, was present in the greatest numbers of egg samples, and Helotiales were found only on samples from the Black River. Independent isolates were collected from egg surfaces and tested for chemical sensitivity to the oomicides ethaboxam and mefenoxam, which are used for control of Oomycete agricultural pathogens. Ethaboxam inhibited mycelial growth almost completely for all *Saprolegnia* strains tested, while mefenoxam, at 20 × strength, was largely ineffective. Water prevents the natural inactivation of mefenoxam by light, thus is not advisable in aquatic systems, where it could accumulate. Alternatively, ethaboxam may be a nonpersistent, welcome control option for these fish pathogens.

Keywords Saprolegnia · Oomicides · Ethaboxan · Mefenoxam

Introduction

Eukaryotic microbes are widely abundant and taxonomically diverse in aquatic freshwater habitats [1–3]. Their roles include nutrient cycling from turnover of organic material, as well as primary production from photosynthetic species [4]. Their effect on freshwater ecosystems has rapidly changed world-wide through eutrophication of natural water systems from exposures to heat stress, contaminated water, and other consequences of human activity [5–7]. The true fungi (Kingdom Fungi) and the oomycetes (Phylum Oomycota) are members of this group and are a significant source of mortality for aquatic animals, in particular fishes, during

Kristi Gdanetz, Zachary A. Noel, Kim T. Scribner and Frances Trail contributed equally to the paper.

✉ Kim T. Scribner
scribne3@msu.edu

✉ Frances Trail
trail@msu.edu

Kristi Gdanetz
k.gdanetzmaccready@usda.gov

[1] Department of Plant Biology, Michigan State University, East Lansing, MI, USA

[2] Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI, USA

[3] Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, USA

[4] Department of Microbiology, Genetics and Immunology, Michigan State University, East Lansing, MI, USA

[5] Biology Department, Albion College, Albion, MI, USA

[6] USDA-ARS Cereal Crops Research Unit, Madison, WI, USA

[7] Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, USA

Published online: 24 July 2025

early life stages in the wild and in aquaculture [8–10]. However, despite their importance to freshwater ecosystems, the effect of these eukaryotic microbes on animals, and fish in particular, is poorly understood.

Lake sturgeon (*Acipenser fulvescens*), ancient fish native to the freshwater habitats of North America, have relatively recently experienced significant declines in abundance and distribution from historical levels [11]. Hatcheries, in particular streamside facilities using resident water [12], are widely embraced as a viable strategy to restore wild populations and to study lake sturgeon reproductive dynamics. Natural and hatchery-based recruitment can be problematic due to the frequency of fatal diseases caused by fungi and oomycetes, particularly during the vulnerable early life stages of lake sturgeon, including incubating eggs [13, 14].

In previous studies, we have shown that across the Great Lakes tributaries inhabited by sturgeon, prokaryotic taxonomic composition and diversity vary greatly between the two time periods associated with peaks in spawning activity [15]. In the Black River and elsewhere, a temperature fluctuation from 10–14 °C (early spawning adults) to 16–20 °C (late spawning adults) [16] corresponds to large variation in microbial community diversity [14]. We also compared the river to the hatchery microbial community composition, as restoration hatchery programs are widely embracing the concept of non-traditional (streamside) facilities [12] to increase the likelihood of natal imprinting that ensures a returning population [17].

Here we interrogated egg surface communities during early and late spawning in the stream and in a streamside hatchery research facility. We investigated processes of colonization and succession of eukaryotic microbial communities that associate with the chorion (outer layer) of lake sturgeon eggs. Our goal was to generate a more detailed understanding of dynamic interactions between microbial communities and lake sturgeon in natural aquatic systems, and to uncover implications of the associations for adaptive evolution and for population sustainability and conservation. Specifically, the goals of this study were to (1) characterize the taxonomic diversity of eukaryotic microbial (fungi and oomycetes) infections of lake sturgeon eggs that are believed to be contributing to lake sturgeon mortality in a hatchery, (2) characterize fungal and oomycete communities naturally colonizing egg surfaces in a stream, and (3) evaluate the efficacy of alternative and environmentally safe treatments that have the potential to reduce the prevalence of disease in hatcheries. The critical physical environmental features that we expected to result in differences in microbial taxonomy are (1) temperature — colder early in the season (early May) and warmer later in the season (late May) — and (2) samples from the river and from the reservoir — lotic (running) versus lentic (reservoir/standing) water ecosystems. Our approach was to perform community-level interrogations using DNA sequence analysis of fungi and oomycetes on healthy and moribund eggs, and to characterize the taxonomic compositional heterogeneity as a function of water source and relative time within the spawning season.

Materials and Methods

Study Site and Sample Collection

Study collections were conducted in the lake sturgeon spawning area of the Black River in the Cheboygan River watershed in MI, USA, and at the hatchery — Black River Sturgeon Stream Side Research Facility in Onaway, Cheboygan Co., MI, USA. This site and the lake sturgeon population have been described and previously researched [16], including studies of spawning and egg deposition [18] and natural stream mortality [13]. Eggs for microbiome analyses were collected during the two peaks of spawning between late April and early June 2017. An early collection of eggs was gathered on May 7 from the Research Facility (ERF), two late collections of eggs were gathered on May 27 from the Black River (LBR) and the Research Facility (LRF), and a heavily colonized, moribund sample with significant hyphal colonization (fuzzy) was collected from the Black River on May 29 (FBR). These egg collections were used for sequence- and culture-based analyses of eukaryotic microorganisms associated with the eggs.

Characterization of the Eukaryotic Microbiome of Lake Sturgeon Eggs

Four collections of eggs were processed for DNA sequencing. Each collection was derived from batches of three to four eggs, resulting in two to six replicate samples per collection. The contents of the eggs were expelled and the outer egg membranes (chorions; outer acellular coats) were rinsed in several successive baths of absolute ethanol. Chorions were lyophilized overnight at − 70 °C (Labconco, Kansas City, MO, USA). Lyophilized samples were ground with a plastic pestle in the presence of glass beads, and DNA was extracted as described previously [19]. For each sample, DNA was diluted 1/10 with sterile water prior to conducting three independent PCR reactions to amplify the fungal internal transcribed spacer 2 (ITS2) region. ITS2 primers (Table 1, [22]) and reaction conditions were described previously [23] using GoTaq Master Mix (Promega, Madison, WI, USA). DNA from the oomycetes was amplified with a 15-cycle reaction using the ITS6 and ITS4 primers at an annealing temperature of 55 °C. PCR products were diluted 1/10 with water, and used as the template for the second PCR reaction, 30 cycles at an annealing temperature of 59 °C, containing the ITS6 and ITS7 primers, which amplify

Table 1 Primer and adapter sequences used to PCR-amplify fungal and oomycete from egg surfaces

Primer Name	Target Region	Sequence	Reference
ITS6	Oomycete forward	5′ GAA GGT GAA GTC GTA ACA AGG 3′	Cooke et al. [20]
ITS7	Oomycete reverse	5′ AGC GTT CTT CAT CGA TGT GC 3′	Cooke et al. [20]
ITS4	Universal reverse	5′ TCC TCC GCT TAT TGA TAT GC 3′	White et al. [21]
ITS3_KYO2	Fungi forward	5′ GAT GAA GAA CGY AGY RAA 3′	Toju et al. [22]
ITS4_KYO3	Fungi reverse	5′ CTB TTV CCK CTT CAC TCG 3′	Toju et al. [22]
Adapter for Illumina barcodes		5′ ACA CTG ACG ACA TGG TTC TAC A—[target gene forward primer] 3′	
Adapter for Illumina barcodes		5′ TAC GGT AGC AGA GAC TTG GTC T—[target gene reverse primer] 3′	

the ITS1 region in oomycetes (Table 1, [20]). Sequencing barcode adapters were added upstream of the target gene primers (Table 1). PCR products were verified by standard agarose gel electrophoresis. For each target region, a second amplification reaction was attempted for samples with failed amplification, and samples with two failed amplifications were classified as not containing the target organisms. PCR products were submitted to the MSU Research Technology Support Facility (RTSF) Genomics Core (East Lansing, MI, USA) for 250 bp paired-end sequencing on an Illumina MiSeq instrument.

DNA sequence reads were processed with the USEARCH pipeline (Version 10, [24]). Forward and reverse read pairs were merged and filtered at a minimum length of 200 base pairs. Chimera-filtering of unique sequences and assignment to amplicon sequence variants (ASVs) was conducted with UNOISE3 [25]. Taxonomy was assigned to fungal sequences using the SINTAX algorithm [26] with the UNITE fungal database [27] at the 0.5 threshold cutoff. Taxonomy was assigned to oomycete sequences using a version of the ITS database compiled by Robideau et al. [28], trimmed to the ITS2 region with ITSx [29], and formatted for use with the SINTAX algorithm. Unidentified Oomycota ASVs were further classified using the Naïve Bayesian Classifier via the "assignTaxonomy" function from DADA2 (version 1.32.0) against the V9 UNITE eukaryote database [30].

Sequence counts and taxonomic data were imported into R using the *phyloseq* package. ASVs unclassified at the Kingdom level or not identified as the target organisms were discarded. ASVs were filtered to a minimum of five reads across five independent samples. Samples with less than 20 total reads were culled. These trimmed fungi and oomycete datasets, with 218 and 416 ASVs, respectively, were used for all downstream community analyses (Table 2). Calculations of diversity indices (relative abundance, Chao, Shannon) and ordination analyses were conducted using the *phyloseq* and *vegan* packages. Plots were generated with *ggplot2* and *cowplot* packages. Files containing code used to generate ASVs and conduct community analysis are located at github.com/gdanetzk/sturgeon_egg_microbiome. Sequences can be downloaded from NCBI SRA BioProject ID PRJNA1128577.

Table 2 Sequence processing statistics describing sequence reads prior to and following filtering

Step in pipeline	Fungal sequences	Oomycete sequences
Merged read pairs	212,223	3,378,023
Quality filtered	165,593	3,347,423
Unique sequences	80,761	201,353
ASVs	1398	621
Chimeras removed	7	316
Taxonomic and abundance filtering of ASVs	218	416
Mean reads per sample	9038	92,165

Culture-Based Isolation and Characterization of Egg Surface Isolates

Microbial isolates were recovered from egg surfaces by rolling eggs across the surface of corn meal agar (CMA, Neogen Corp., Lansing, MI, USA) in 10-cm-diameter Petri dishes. Oomycete cultures were maintained on CMA slants stored at room temperature and routinely passaged on CMA amended with rifampicin (10 µg/ml) to maintain the integrity of the culture. For identification, cultures were grown on fresh CMA and crude genomic DNA extraction was performed as described in Noel et al. [31]. Briefly, a small loop containing freshly grown mycelium was transferred to a 20 µl extraction solution, incubated for 10 min at 95 °C, then diluted with an aliquot of 60 µl of 3% bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) solution. Two microliters of this solution were used for PCR with primers ITS6 and ITS4 (Table 1). Thermal cycling was performed as follows: 94 °C for 3 min; followed by 35 cycles of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min; followed by a final extension at 72 °C for 7 min, using DreamTaq Green DNA Polymerase

(ThermoFisher Scientific, USA). PCR products were purified with EXOsap-IT (ThermoFisher Scientific, USA) and sequenced at MSU RTSF Genomics Core on the Applied Biosystems 3730*xl* DNA Analyzer platform. Consensus sequences from forward and reverse reads were compared against sequences from a vouchered collection of oomycetes for identification of isolates [32, 28]. The collected strains were used for oomycete pesticide sensitivity tests.

Quantification of Oomycete Pesticide Tolerance

Oomicides are oomycete pesticides that have been historically and inaccurately called fungicides [33]. Mefenoxam (Apron XL,Syngenta Crop Protection Inc., Greensboro, NC) and ethaboxam (Valent U.S.A. L.L.C., San Ramon, CA) are oomicides commonly used against plant pathogens and were tested for their ability to reduce mycelial growth of isolates on CMA medium. Pesticide stock concentrations were prepared as previously reported [31]. Molten CMA agar medium, cooled to 50 °C, was amended with mefenoxam and ethaboxam to final concentrations of 0, 5, or 100 µg/ml. The diameter of radially expanding colonies was measured after incubation for 5 days at 27 °C.

Results

The Eukaryotic Microbiome of Eggs

We have characterized the fungal and oomycete communities of eggs from early and late spawning sturgeon, and from naturally spawned eggs that remained in the river for an extended time before collection. DNA analysis of ITS regions demonstrated that oomycete communities were largely composed of *Pythium* spp. (Fig. 1A, Supplemental Table 1). However, nearly 50% of oomycete ASVs from all collections (Fig. 1A) and nearly 75% of fungal ASVs from three of four collections (Fig. 1B) remain unidentified. Several ASVs of fungal higher taxa demonstrated location specificity: the Dothideales were found only on eggs harvested

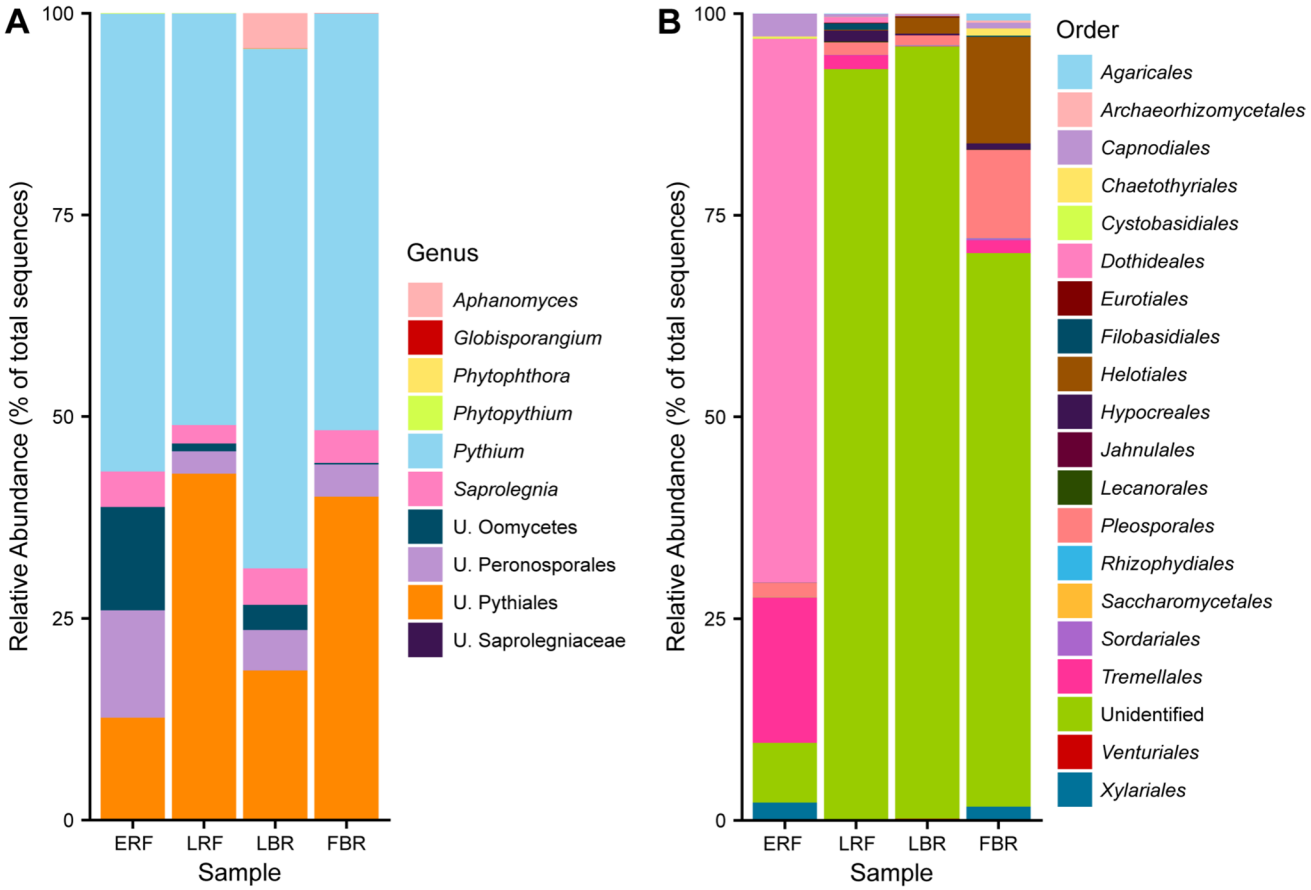

Fig. 1 Relative abundance of oomycetes (**A**) and fungal (**B**) amplicon sequence variants from lake sturgeon eggs collected from the Black Lake Research Facility during early (ERF) and late (LRF) spawning, and from the Black River during late spawning (LBR), as well as visibly colonized eggs collected from the Black River (FBR). U. Oomycota amplicon sequence variants unidentified at genus-level; higher rank–level taxonomic assignments provided

from the Research Facility samples (ERF and LRF) and the Helotiales were found only in Black River samples (LBR and FBR; Fig. 1B) indicating that egg colonization is influenced by spawning location. We also observed that ASVs of the Pleosporales were present in all samples, and the Tremellales were found in three out of four sample locations (ERF, LRF, and FBR; Fig. 1B), supporting their importance as members of the egg microbiome. The Shannon index of the oomycete communities demonstrated that the ERF and LBR samples differed from the LRF and FBR samples (Fig. 2A, Table 3), and community structure varied between spawning times (Fig. 2B, Table 3). The fungal communities of the early sample differed from both the late samples and the FBR sample (Fig. 3). The true fungi dominating the other time-points were not known to be causal agents of fish disease (Fig. 1B, Supplemental Table 2).

Culture-Based Characterizations of Isolates

We cultured 90 independent isolates from outer surfaces of sampled eggs collected in the hatchery. Of these, 43 isolates were identified as members of the Fungi or Oomycetes (Table 4). Several of the isolates were potential fish pathogens, including *Fusarium solani*, *Cladosporium* sp., and five *Saprolegnia* isolates: one *S. australis*, one *S. parasitica*, and three *S. ferax*. We tested Oomycete isolates against two oomicides, which are commonly used on plant pathogens. Six *Saprolegnia* isolates (three *S. ferax* and three *S. parasitica*) were tested for their sensitivity to ethaboxam and mefenoxam (Fig. 4). Surprisingly, all six *Saprolegnia* isolates were tolerant to mefenoxam, but not to ethaboxam. Ethaboxam inhibited observable mycelial growth almost completely (92.3% to 95.2% inhibition on average) for all *Saprolegnia* isolates tested at 5 ppm. In contrast, at 100 ppm mefenoxam, mycelial growth of the same isolates was inhibited on average only 46.3% to 56.8%.

Discussion

Lake sturgeon populations are not self-sustaining in many areas of the midwestern United States [35]. One approach to maintaining and restoring population numbers and distribution is to transfer eggs from native ranges to hatcheries for generation of increased numbers of young used in repopulation efforts [11]. However, aquatic fungal and oomycete populations are important members of the freshwater ecosystem and present a severe disease threat to lake sturgeon

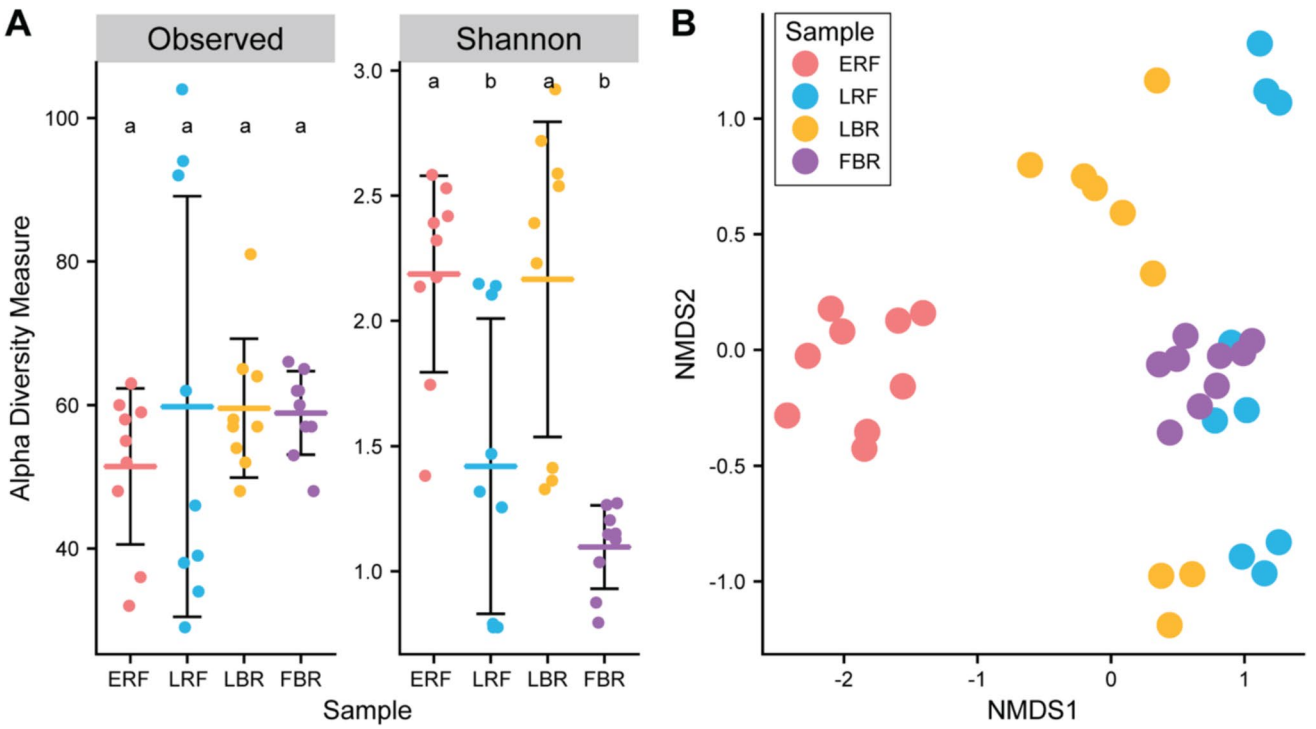

Fig. 2 Diversity of oomycete communities from lake sturgeon eggs. Eggs collected during early (ERF) and late (LRF) spawning from the Research Facility, and late spawning (LBR) and visibility colonized (FBR) from the Black River. Shannon index and richness calculated from amplicon sequence variants of the oomycete ITS region (**A**). Locations with the same letter are not significantly different following ANOVA and Tukey's HSD. Non-metric multidimensional scaling (NMDS) of Bray–Curtis dissimilarity index, stress = 0.09. ANOSIM $p = 0.01$, and $R = 0.58$, betadisper $p = 0.03$ (**B**)

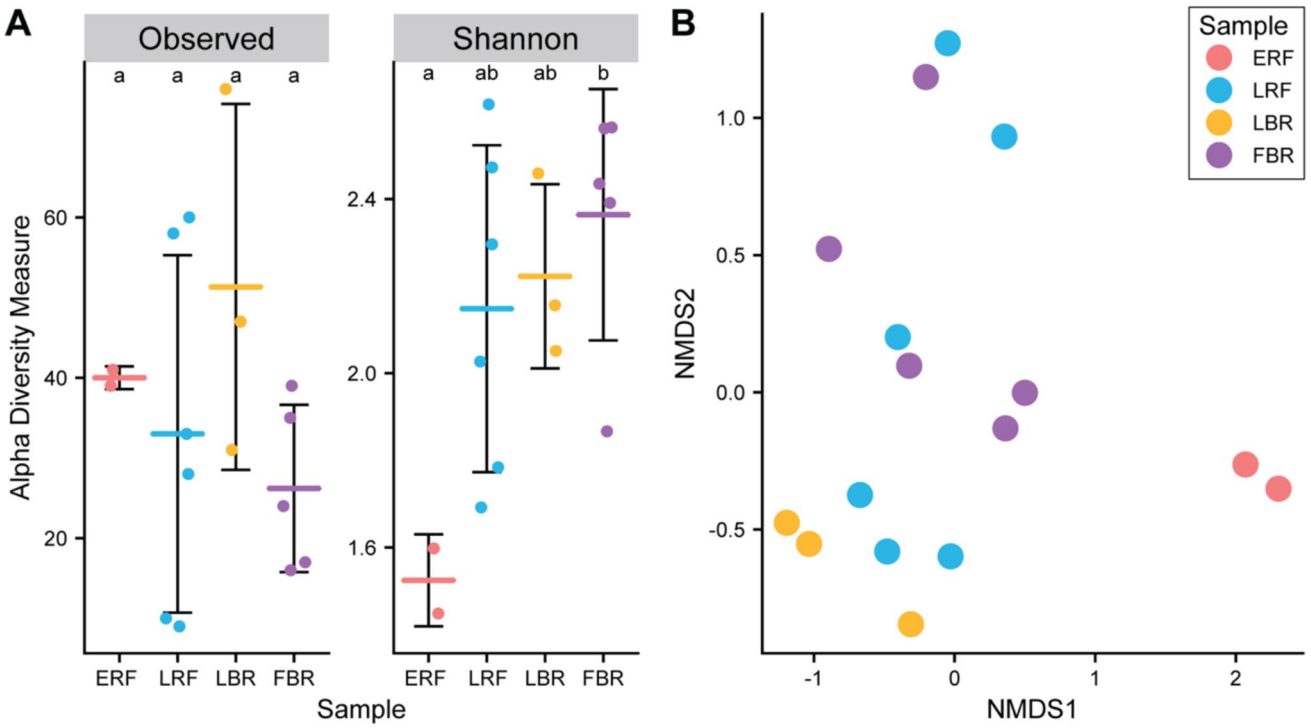

Fig. 3 Measures of diversity of fungal communities from lake sturgeon eggs. Fungal communities of sturgeon eggs collected during early (ERF) and late (LRF) spawning from the Research Facility, and eggs collected during late spawning (LBR) and visibly colonized (FBR) from the Black River. Shannon index and richness calculated from amplicon sequence variants of the fungal ITS2 region (**A**). Locations with the same letter are not significantly different following ANOVA and Tukey's HSD. Non-metric multidimensional scaling (NMDS) of Bray–Curtis dissimilarity index, stress = 0.08. ANOSIM $p = 0.04$, and $R = 0.46$, betadisper $p = 0.008$ (**B**)

Table 3 Sample diversity for oomycetes and fungi described in Figs. 2 and 3

Sample	Abbreviation	Oomycetes Richness[z]		Shannon[z]	
Early Black Lake Research Facility	ERF	60	a	2.19	a
Late Black Lake Research Facility	LRF	60	a	2.17	a
Late Black River	LBR	59	a	1.42	b
Fuzzy (visibly colonized) Black River	FBR	51	a	1.1	b
Sample	Abbreviation	Fungi Richness[z]		Shannon[z]	
Early Black Lake Research Facility	ERF	51	a	2.36	a
Late Black Lake Research Facility	LRF	40	a	2.22	ab
Late Black River	LBR	33	a	2.15	ab
Fuzzy (visibly colonized) Black River	FBR	26	a	1.52	b

[z]Locations followed by the same letter are not significantly different following ANOVA and Tukey's HSD

repopulation efforts. These microbial populations have been shown to increase in recent decades or in association with human activity, including changes in electrical conductivity and calcium levels [9],reviewed by [36, 37], and these changes threaten amphibians and fish. We examined the fungal and stramenopile members of the lake sturgeon egg microbiome to identify those that threaten egg health. The dominant colonizers of the Late Research Facility samples (LRF2-1 and LRF2-5) are members of the Kingdom Fungi. However, the Fungi dominating the samples studied here are not known to be causal agents of fish disease. From some samples, 50% or greater of the ASVs from oomycetes and Fungi were not identified to genus, highlighting the need for better descriptions of the microbial communities in aquatic systems.

Table 4 Identification of isolates from egg collections

Collection	Culture method	Sample name	Top BLAST match	Percent identity
Early	Dissected egg on medium	diss 12A	*Trichoderma pleuroticola*	99
Early	Dissected egg on medium	diss 12B	*Fusarium solani*	99
Early	Dissected egg on medium	diss 12C	*Stagonospora foliicola*	97
Early	Dissected egg on medium	diss 165.1	*Cladosporium sphaerospermum*	97
Early	Dissected egg on medium	diss 16B.2	no match	-
Early	Dissected egg on medium	diss 16D	*Phoma bellidis*	100
Early	Dissected egg on medium	diss 170.1	*Phoma* sp.	96
Early	Dissected egg on medium	diss 17C	no match	-
Early	Dissected egg on medium	diss 17C	*Stagonosporopsis* sp.	99
Early	Dissected egg on medium	diss 19A	*Peyronellaea* sp.	100
Early	Dissected egg on medium	diss 19D.1	no match	-
Early	Dissected egg on medium	diss 1C	*Cytospora* sp.	99
Early	Dissected egg on medium	diss 1D	*Penicillium* sp.	99
Early	Dissected egg on medium	diss 2B	*Neonectria* sp.	99
Early	Dissected egg on medium	diss 5A	*Saprolegnia australis*	100
Early	Dissected egg on medium	diss 6A	no match	-
Early	Dissected egg on medium	diss 6D	*Phaeospheria pontiformis*	99
Early	Dissected egg on medium	diss 6G	*Pleosporales* sp.	99
Early	Dissected egg on medium	diss 8A	no match	-
Early	Dissected egg on medium	diss 9A	*Davidella tassiana*	98
Early	Dissected egg on medium	diss 9A	*Microdochium* sp.	99
Early	Dissected egg on medium	diss 9 A 2	*Cladosporium* sp.	99
Origins unknown	Substantial visible hyphae on egg surface	F1A	*Saprolegnia parasitica*	100
Late	Visibly colonized (fuzzy)	NTF 2A	*Saprolegnia ferax*	99
Late	Visibly colonized (fuzzy)	NTF 5B	*Saprolegnia ferax*	100
Late	Visibly colonized (fuzzy)	NTF 9A	*Fusarium* sp.	99
Early	Egg rolled across medium	roll 10A	*Mortierella alpina*	94
Early	Egg rolled across medium	roll 11C	*Acanthophysellum lividocoeruleum*	98
Early	Egg rolled across medium	roll 12A	*Alfaria terrestris*	98
Early	Egg rolled across medium	roll 12B	*Microdochium nivalae*	99
Early	Egg rolled across medium	roll 12C	*Paraphoma* sp.	98
Early	Egg rolled across medium	roll 12D	*Pleosporales* sp.	99
Early	Egg rolled across medium	roll 12E	*Pyrenochaeta* sp.	97
Early	Egg rolled across medium	roll 15 A diff	*Articulospora proliferata*	97
Early	Egg rolled across medium	roll 16A	*Mortierella alpina*	98
Early	Egg rolled across medium	roll 16B	*Pleosporales* sp.	97
Early	Egg rolled across medium	roll 16B	*Mortierella alpina*	99
Early	Egg rolled across medium	roll 16C	*Fusarium* sp.	100
Early	Egg rolled across medium	roll 16D	*Fusarium* sp.	98
Early	Egg rolled across medium	roll 16E	*Pleosporales* sp.	100
Early	Egg rolled across medium	roll 2A	*Hypocreales* sp.	96
Early	Egg rolled across medium	roll 2A	*Saprolegnia ferax*	100
Early	Egg rolled across medium	roll 5B	no match	-
Early	Egg rolled across medium	roll 9C	*Microdochium* sp.	97

Several groups of Fungi were found to colonize lake sturgeon eggs. The dominant genus in the moribund eggs was *Tetracladium*, whose members are well-known saprotrophs, including *Naganishia* sp., which can cause rare infections in humans, and *Anguillospora* sp., an endophyte of freshwater plants. Of the egg microbiome

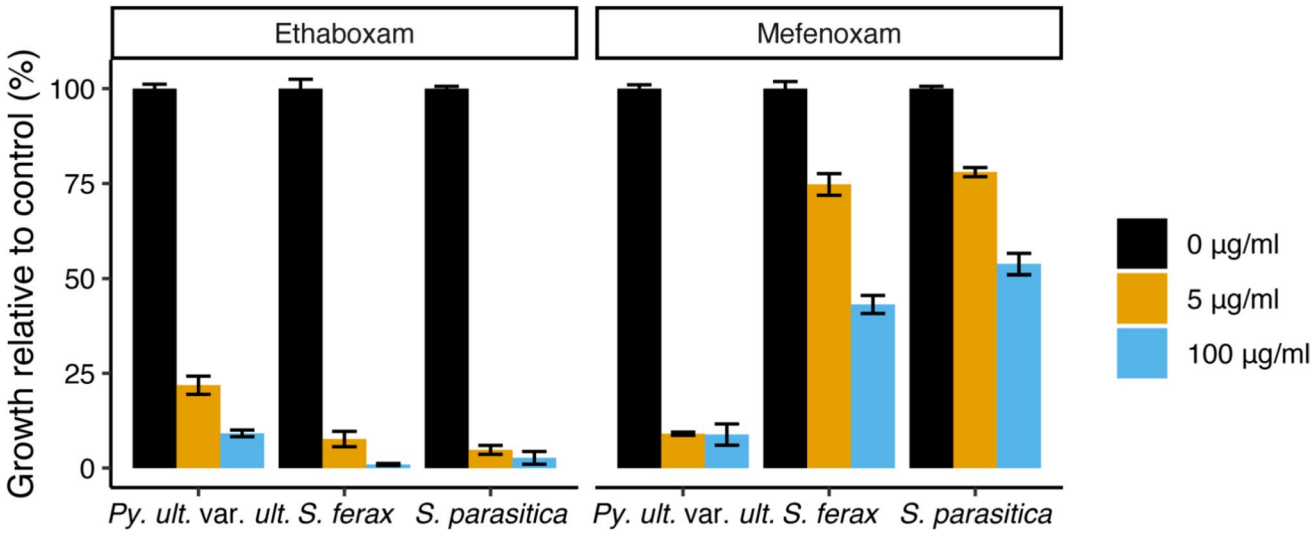

Fig. 4 Mefenoxam and ethaboxam sensitivity distribution. *S. parasitica* ($n = 3$) and *S. ferax* ($n = 3$) mycelial diameter was measured on corn meal agar medium plates containing 0, 5, or 100 µg/ml ethaboxam or mefenoxam. Values represent percent mycelial diameter relative to medium with no pesticides. One isolate of *Pythium ultimum* var. *ultimum* (*Globisporangium ultimum*) was included because it was known to be sensitive to both pesticides tested [34]

samples, the Dothideales were observed exclusively in association with eggs harvested from the Research Facility, whereas the Helotiales were associated uniquely with Black River samples. The Dothideales are a group of heavily melanized Ascomycota, and occupy niches as endophytes, pathogens, and common saprotrophs of plants. Melanization serves as a protectant against radiation damage (including solar), oxidative stress, and temperature fluctuation [38], making them physiologically more resilient to life in the water. Yeast forms of the Dothidiales are frequent colonizers of the surfaces of plants and animals. A common yeast in the Dothidiales, *Aureobasidium pullulans*, is ubiquitous in terrestrial and aquatic habitats [39]. Of all the microbes in the egg samples, *A. pullulans* provided by far the largest presence of any microbe in the egg samples, ~20,000 ASVs each in the Early Research Facility samples (ERF1-3 and ERF1-5). Members of this species were previously believed to be generalists; however, recent work indicates that this species may harbor many host-specific cryptic species [40]. Rarely, some strains of *A. pullulans* can affect humans as colonizers of hair and skin, and can cause superficial as well as serious infections, including meningitis [41]. Due to the observed cryptic diversity of *A. pullulans* in other aquatic habitats, and the demonstrated biocontrol activities of *A. pullulans* strains in plant-pathogen systems [42], the functions of this yeast species in river systems, and associations with fish eggs, need greater study. The Pleosporales, also members of the Dothideomycetes, are highly melanized as well, maintain similar niches, and were present in low numbers in the ERF and LBR samples.

The Tremellales are in the Basidiomycota, and include a large number of species which form yeasts, some of which are human pathogens, and several were also found in high numbers in the eggs. *Cryptococcus* is a yeast-forming skin pathogen of humans [43]. *C. magnus* and *C. adeliensis* have a major presence in the early egg collections. A related species, *C. uniguttulatus*, was previously identified in fish [44]. This last report also identified *Candida* spp. in a variety of fish, but *Candida* spp. were not detected in the present study. The yeasts identified here represent the dominant fungal flora of the eggs and are mainly in early samples. They may have become established on the adult lake sturgeon skin, and then, during deposition, colonized the egg. Initial colonization from the water is unlikely, since high numbers of yeast cells would have to accumulate on the surface of the eggs in a relatively short time.

Analysis of the egg microbial community demonstrated that Oomycetes are present in low numbers in recently released eggs. *Saprolegnia* is known as a problematic fish pathogen and common in hatcheries (van den [10]). *S. parasitica* is a major pathogen of wild fish populations [9]. *S. ferax* is commonly distributed with introduced fish and infects amphibian eggs [45]. *Pythium*, a genus containing common plant pathogenic species [46], is also dominant in the Early eggs, along with *Hyaloperonospora*. *Pythium* spp. are found in freshwater and marine habitats as saprobes and parasites [47].

Other studies on fish eggs have shown that Oomycetes are common and devastating pathogens. One study of salmon hatcheries in Japan found that the families Pythiaceae and Saprolegniaceae, specifically *S. australis*, *S. declina*, *S. ferax*, and *S. parasitica*, dominated infected eggs, with source water and air serving as reservoirs of the infective propagules [48]. The latter two species are known to cause high mortality in fish and *S. australis* is known to infect adult fish and eggs of both fish and amphibians [49]. *Saprolegnia* spp. have also been reported in sturgeon aquaculture [50, 51]. Thus, the predominant disease-causing organisms and probable source of mortality in the eggs are the *Saprolegnia* spp.

As expected, we determined that the cause of the fungal-appearing ("fuzzy") infections of eggs is predominantly oomycetous organisms (Phylum Oomycota). This group of organisms is not related to the Fungi, despite their vegetative growth being reminiscent of fungal hyphae. The conservation hatcheries for lake sturgeon typically use river water from resident streams [12] that flow in and out of the hatchery,therefore, we considered the effects of future pesticide treatments on the river ecosystem. Many fungicides that target true fungi are not effective treatments for oomycete diseases due to the genetic and physiological differences between organisms in these two kingdoms. Some broad-spectrum or multi-site fungicides have previously been reported to be effective at controlling oomycete diseases in plant agriculture [52]. Currently, most "fungicides" labeled for use against oomycete pathogens do not have activity against Fungi, and care should be taken to more accurately describe these products as oomicides [33]. We investigated the efficacy of two products against the Saprolegnia problem described here, with only ethaboxam showing promise as a possible control option against these isolates. Note that water prevents the natural inactivation of mefenoxam by light and is therefore not advisable for use in aquatic systems, where it can accumulate [53]. The focus for future research should be on safe delivery methods of these oomicides to reduce environmental persistence.

Reproductive success and persistence of fish populations can be threatened by taxonomic compositional shifts in microbial communities caused by natural and anthropogenic perturbations. From some samples, 50% or greater of the ASVs from oomycetes and fungi were not identified to genus, highlighting the need for better descriptions of the microbial communities in aquatic systems. The early life stages of fishes, including eggs, embryos, and larvae, are highly vulnerable to fungal and oomycete pathogenesis [3]. Identification of microbes colonizing lake sturgeon eggs and distinguishing beneficial from deleterious microbial populations will improve management practices that reduce fish mortality in natural and aquaculture settings.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-025-02566-5.

Acknowledgements This material is based on work supported by the Michigan State University Environmental Sciences and Policy Program Water Cube project awarded to T.M., K.T.S., and F.T. We also acknowledge the support of Michigan State University AgBioResearch. Funding supporting the lake sturgeon field and hatchery operations was provided by the Michigan Department of Natural Resources through the State Wildlife Grants Program. We acknowledge the Alabama Supercomputer Authority for usage of the High-Performance Computer for parts of this analysis. Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The USDA is an equal opportunity provider and employer.

Author Contributions K.G.: experimental design and analysis of microbial collection, figure design, and wrote manuscript. Z.A.N: testing the fungicides and identifying the Saprolegnia cultures, figure design, wrote manuscript K.S.: experimental design and analysis, edited manuscript T.M.: Initiated research, design and collection of field-based material, edited manuscript K.T.S.: Initiated research, design and collection of field-based material, analysis and interpretation of data, wrote manuscript F.T.: Initiated research; design and collection of field-based material and in-lab study of microbial isolates, analysis and interpretation of data, wrote manuscript

Funding Michigan State University Environmental Sciences and Policy Program Water Cube Project, Michigan Department of Natural Resources through the State Wildlife Grants Program, Michigan State University AgBioResearch

Data Availability Sequences can be downloaded from NCBI SRA BioProject ID PRJNA1128577.

Declarations

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

References

1. Ittner LD, Junghans M, Werner I (2018) Aquatic fungi: a disregarded trophic level in ecological risk assessment of organic fungicides. Front Environ Sci 6:105–122
2. Krauss G-J, Sole M, Krauss G, Scholsser D, Wesenberg D, Barlocher F (2011) Fungi in freshwaters: ecology, physiology,

and biochemical potential. FEMS Microbiol Rev 35:620–651. https://doi.org/10.1111/j.1574-6976.2011.00266.x
3. Llewellyn MS, Boutin S, Hoseinifar SH, Derome N (2014) Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. Front Microbiol 5:1–17
4. Rani A, Ranjan R, Bonina SMC, Izadmehr M, Giesy JP, Li A, Sturchio NC, Rockne KJ (2023) Aqueous geochemical controls on the sestonic microbial community in Lakes Michigan and Superior. Microorganisms 11(2):504. https://doi.org/10.3390/microorganisms11020504
5. Fones HN, Bebber DP, Chaloner TM et al (2020) Threats to global food security from emerging fungal and oomycete crop pathogens. Nat Food 1:332–342. https://doi.org/10.1038/s43016-020-0075-0
6. O'Beirne MD, Werne JP, Hecky RE, Johnson TC, Katsev S, Reavie ED (2017) Anthropogenic climate change has altered primary productivity in Lake Superior. Nat Commun 8:15713
7. Yuan T, Zhang H, Feng Q, Wu X, Zhang Y, McCarthy AJ et al (2020) Changes in fungal community structure in freshwater canals across a gradient of urbanization. Water 12:1917
8. Lokesh J, Siriyappagouder P, Fernandes JMO (2024) Unravelling the temporal and spatial variation of fungal phylotypes from embryo to adult stages in Atlantic salmon. Sci Rep 14:981
9. Pavić D, Grbin D, Hudina S, Zmrzljak UP, Miljanović A, Košir R et al (2022) Tracing the oomycete pathogen *Saprolegnia parasitica* in aquaculture and the environment. Sci Rep 12:16646
10. van den Berg AH, McLaggan D, Dieguez-Uribeond J, van West P (2013) The impact of the water moulds *Saprolegnia diclina* and *Saprolegnia parasitica* on natural ecosystems and the aquaculture industry. Fungal Biol Rev 27(2):33–42. https://doi.org/10.1016/j.fbr.2013.05.001
11. Peterson DL, Vecsei P, Jennings CA (2007) Ecology and biology of the lake sturgeon: a synthesis of current knowledge of a threatened North American Acipenseridae. Rev Fish Biol Fisheries 17(1):59–76
12. Holtgren JM, Ogren SA, Paquet AJ, Fajfer S (2007) Design of a portable streamside rearing facility for lake sturgeon. N Am J Aquac 69:317–323
13. Forsythe PS, Scribner KT, Crossman JA, Ragavendran A, Baker EA (2013) Experimental assessment of the magnitude and sources of lake sturgeon egg mortality in a natural stream setting. Transactions American Fisheries Society 142:1005–1011
14. Fujimoto M, Marsh T, Scribner KT (2021) Effects of filtration and temperature on microbial colonization of lake sturgeon eggs, survival, and development in aquaculture. N Am J Aquac 83:26–37. https://doi.org/10.1002/naaq.10169
15. Sanfilippo GE, Homola JJ, Ross J, Kanefsky J, Kimmel J, Marsh T, Scribner KT (2021) Seasonal and spatial variation in stream bacterial communities across the Lake Michigan basin. J Great Lakes Res 47:862–874. https://doi.org/10.1016/j.jglr.2021.02.009
16. Forsythe PS, Scribner KT, Crossman JA, Ragavendran A, Baker EA, Davis C et al (2012) Environmental and lunar cues are predictive of the timing of river entry and spawning-site arrival in lake sturgeon *Acipenser fulvescens*. J Fish Biol 81:35–53
17. Kimmel JG, Buchinger TJ, Larson DL, Baker EA, Zorn TG, Scribner KT, Li W (2023) Behavioral evidence of olfactory imprinting during embryonic and larval stages in lake sturgeon. Conserv Physiol 11(1). https://doi.org/10.1093/conphys/coad045
18. Finley AO, Forsythe PS, Crossman JA, Baker EA, Scribner KT (2018) Assessing impact of exogenous features on biotic phenomena in the presence of strong spatial dependence: a lake sturgeon case study in natural stream settings. PLoS ONE 13:e0204150
19. Abd-Elsalam KA, Schnieder F, Guo JR (2003) A modified DNA extraction minipreparation protocol for Fusarium isolates. J Rapid Methods Autom Microbiol 11(1):75–79
20. Cooke DEL, Drenth A, Duncan JM, Wagels G, Brasier CM (2003) A molecular phylogeny of Phytophthora and related oomycetes. Fungal Genet Biol 30(1):17–32
21. White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications, vol 18, pp 315–322
22. Toju H, Tanabe AS, Yamamoto S, Sato H (2012) High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLoS ONE 7(7):e40863. https://doi.org/10.1371/journal.pone.0040863
23. Gdanetz K, Trail F (2017) The wheat microbiome under four management strategies, and potential for endophytes in disease protection. Phytobiomes J 1:158–168
24. Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26(19):2460–2461. https://doi.org/10.1093/bioinformatics/btq461
25. Edgar RC (2016a) UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *bioRxiv*, 1–21, https://doi.org/10.1101/081257.
26. Edgar RC (2016b) SINTAX: a simple non-Bayesian taxonomy classifier for 16S and ITS sequences. *bioRxiv*, 1–20, https://doi.org/10.1101/074161.
27. Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Duenas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Luecking R, Martin MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U et al (2013) Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 22:5271–5277. https://doi.org/10.1111/mec.12481
28. Robideau GP, De Cock AWAM, Coffey MD, Voglmayr H, Brouwer H, Bala K, Chitty DW, Désaulniers N, Eggertson QA, Gachon CMM, Hu C-H, Küpper FC, Rintoul TL, Sarhan E, Verstappen ECP, Zhang Y, Bonants PJM, Ristaino JB, André Lévesque C (2011) DNA barcoding of oomycetes with cytochrome *c* oxidase subunit I and internal transcribed spacer. Mol Ecol Resour 11:1002–1011. https://doi.org/10.1111/j.1755-0998.2011.03041.x
29. Nilsson RH, Veldre V, Hartmann M, Unterseher M, Amend A, Bergsten J, Kristiansson E, Ryberg M, Jumpponen A, Abarenkov K (2010) An open source software package for automated extraction of ITS1 and ITS2 from fungal ITS sequences for use in high-throughput community assays and molecular ecology. Fungal Ecol 3:284–287. https://doi.org/10.1016/j.funeco.2010.05.002
30. Abarenkov K, Zirk A, Piirmann T, Pöhönen R, Ivanov F, Nilsson RH, Kõljalg U (2023) UNITE general FASTA release for eukaryotes. UNITE Community. https://doi.org/10.15156/BIO/2938069
31. Noel ZA, McDuffee D, Chilvers MI (2021) Influence of soybean tissue and oomicide seed treatments on oomycete isolation. Plant Dis 105(5):1281–1288. https://doi.org/10.1094/PDIS-03-20-0642-RE
32. Lévesque CA, De Cock AWAM (2004) Molecular phylogeny and taxonomy of the genus Pythium. Mycol Res 108(12):1363–1383. https://doi.org/10.1017/S0953756204001431
33. Govers F (2001) Misclassification of pest as "fungus" puts vital research on wrong track. Nature 411:633
34. Noel ZA, Rojas AJ, Jacobs JL, Chilvers MI (2019) A high-throughput microtiter-based fungicide sensitivity assay for oomycetes using Z′-factor statistic. Phytopathology 109:1628–1637
35. Bruch RM, Haxton TJ, Koenigs R, Welsh A, Kerr SJ (2016) Status of lake sturgeon (*Acipenser fulvescens* Rafinesque 1817) in North America. J Appl Ichth 32:162–190
36. Derevnina L, Petre B, Kellner R, Dagdas YF, Sarowar MN, Giannakopoulou A et al (2016) Emerging oomycete threats to plants and animals. Philosophical Transactions of the Royal Society B: Biological Sciences 371:20150459

37. Phillips AJ, Anderson VL, Robertson EJ, Secombes CJ, van West P (2008) New insights into animal pathogenic oomycetes. Trends Microbiol 16:13–19
38. Cordero RJB, Casadevall A (2017) Functions of fungal melanin beyond virulence. Fungal Biol Rev 31:99–112
39. Gunde-Cimerman N, Zalar P, de Hoog S, Plemenitaš A (2000) Hypersaline waters in salterns – natural ecological niches for halophilic black yeasts. FEMS Microbiol Ecol 32:235–240
40. Gostinčar C, Turk M, Zajc J, Gunde-Cimerman N (2019) Fifty *Aureobasidium pullulans* genomes reveal a recombining polyextremotolerant generalist. Environ Microbiol 10:3638–3652. https://doi.org/10.1111/1462-2920.14693
41. Mittal J, Szymczak WA, Pirofski L, Galen BT (2018) Fungemia caused by *Aureobasidium pullulans* in a patient with advanced AIDS: a case report and review of the medical literature. JMM Case Reports 5:e005144
42. Zeng Q, Johnson K, Mukhtar S, Nason S, Huntley R, Millet F et al (2023) A*ureobasidium pullulans* from the fire blight biocontrol product, Blossom Protect, induces host resistance in apple flowers. Phytopathology 113:1192–1201
43. Cano EJ, Yetmar ZA, Razonable RR (2020) Cryptococcus species other than *Cryptococcus neoformans* and *Cryptococcus gattii*: are they clinically significant? *Open Forum Infectious Diseases*, 7, ofaa527.
44. Tartor Y, Taha M, Mahboub H, Ghamery ME (2018) Yeast species associated with diseased fish: occurrence, identification, experimental challenges and antifungal susceptibility testing. Aquaculture 488:134–144
45. Romansic J, Diez K, Higashi E, Johnson J, Blaustein A (2009) Effects of the pathogenic water mold *Saprolegnia ferax* on survival of amphibian larvae. Dis Aquat Org 83:187–193
46. Lee JS, Lee HB, Shin H-D, Choi Y (2017) Diversity, phylogeny, and host-specialization of hyaloperonospora species in Korea. Mycobiology 45:139–149
47. Shearer CA, Descals E, Kohlmeyer B, Kohlmeyer J, Marvanová L, Padgett D et al (2007) Fungal biodiversity in aquatic habitats. Biodivers Conserv 16:49–67
48. Sakaguchi SO, Ogawa G, Kasai H, Shimizu Y, Kitazato H, Fujikura K et al (2019) Molecular identification of water molds (oomycetes) associated with chum salmon eggs from hatcheries in Japan and possible sources of their infection. Aquacult Int 27:1739–1749
49. Tedesco P, Saraiva M, Sandoval-Sierra JV, Fioravanti ML, Morandi B, Dieguez-Uribeondo J, van West P, Galuppi R (2021) Evaluation of potential transfer of the pathogen *Saprolegnia parasitica* between farmed salmonids and wild fish. Pathogens 10:926. https://doi.org/10.3390/pathogens10080926
50. Conte F (1988) *Hatchery manual for the white sturgeon (Acipenser transmontanus Richardson): with application to other North American Acipenseridae*. University of California.
51. Jalilpoor J, Masouleh AS, Masoumzadeh M (2006) Fungal flora in *Acipenser persicus* eggs with particular emphasis on *Saprolegnia* sp. (Oomycetes) and mortality during mass incubation at the Shahid Beheshti hatchery. J Appl Ichthyol 22:265–268
52. Quesada-Ocampo LM, Parada-Rojas CH, Hansen Z, Vogel G, Smart C, Hausbeck MK et al (2023) *Phytophthora capsici*: recent progress on fundamental biology and disease management 100 years after its description. Annu Rev Phytopathol 61:185–208
53. Sukul P, Spiteller M (2000) Metalaxyl: persistence, degradation, metabolism, and analytical methods. Rev Environ Contam Toxicol 164:1–26

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.