Chapter 34: Ecosystem Effects Workgroup Poster Abstracts

Local adaptation of *Daphnia pulicaria* to toxic cyanobacteria

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Introduction

A well-established tenet in limnology holds that the taxonomic composition of summer phytoplankton assemblages shifts with phosphorus enrichment toward greater dominance by cyanobacteria. One important consequence of this shift toward cyanobacteria with eutrophication is that summer phytoplankton assemblages in eutrophic lakes are relatively resistant to zooplankton grazing. However, one recent study showed that zooplankton from a lake in Europe adapted to tolerate bloom-forming cyanobacteria in their diet after several decades of cultural eutrophication. This project aimed to determine if adaptation by grazers to toxic prey occurs across lakes that vary in nutrient concentration.

Hypothesis

Daphnia pulicaria clones isolated from high-nutrient, cyanobacteria-abundant lakes are more tolerant of toxic *Microcystis aeruginosa* than clones isolated from low-nutrient lakes.

Methods

Twenty-two *D. pulicaria* clones were isolated from six lakes in southern Michigan. The six lakes were grouped into two categories based on the midpoint of ranges of summer total phosphorus (TP) concentration, low TP (9 - 13 μg L^{-1}) and high TP (31 - 235 μg L^{-1}). Two juvenile growth experiments were conducted and data from the two experiments were pooled for all analyses. Neonates (<24 hours old) of each clone were transferred individually into 100 ml glass beakers filled with 80 ml of glass-fiber filtered lake water, and a random subset of neonates was transferred individually to a dried and tared weighing tin for initial mass estimates (W_i). Neonates were fed either *Ankistrodesmus falcatus*, a nutritious green alga, or a single-celled toxic strain of *Microcystis aeruginosa* (microcystin quota: 36 μg mg^{-1} C) at growth-saturating concentrations (1.5 mg C L^{-1}) and transferred to new beakers with fresh medium and food daily. On day 3 (W_f) of the experiments, each animal was transferred individually to a tared weighing tin, dried, and weighed. Instantaneous somatic growth rate (g, d^{-1}) was calculated for each beaker as: $\{[\ln(W_f) - \ln(W_i)]/3\}$. We calculated a relative index of growth inhibition by *Microcystis* for each clone as: $(g_a - g_m)/g_a$, where g_a is growth rate on *Ankistrodesmus* and g_m is growth rate on *Microcystis*. Growth responses were averaged across clones for each lake, and differences between low-TP and high-TP lakes assessed via two-tailed *t*-tests, with lakes as replicates.

Results

Daphnia clones generally grew well on a diet of *Ankistrodesmus*, and there was no significant difference in growth on this diet between lake categories ($p > 0.40$), despite substantial overall variation in growth rate. As expected, all clones grew poorly on the *Microcystis* diet. More importantly, *D. pulicaria* from high-TP lakes grew significantly better, on average, than *D. pulicaria* from low-TP lakes on the *Microcystis* diet ($p < 0.02$). On average, *D. pulicaria* from low-TP lakes lost weight when fed *Microcystis* (growth < 0, *t*-test, $p < 0.04$, $n = 3$), while *D. pulicaria* from high-TP lakes did not (growth <0, *t*-test, $p > 0.30$, $n = 3$).

Conclusions

We quantified within-species variation in the tolerance of the large lake-dwelling daphnid, *D. pulicaria*, to toxic cyanobacteria in the diet. Juvenile growth rates on diets consisting of 100% *Ankistrodesmus* or 100% toxic

Microcystis were compared for *D. pulicaria* clones isolated from lakes expected to have low and high levels of bloom-forming cyanobacteria during summer. Growth rates of clones isolated from high-nutrient lakes were higher, and showed less relative inhibition, on the cyanobacterial diet, compared to clones isolated from low-nutrient lakes. Our results suggest that *D. pulicaria* populations exposed to high cyanobacterial levels over long periods of time can adapt to being more tolerant of toxic cyanobacteria in the diet.

Cytotoxicity of microcystin-LR to primary cultures of channel catfish hepatocytes and to the channel catfish ovary cell line

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Introduction

We observed responses of channel catfish (*Ictalurus punctatus*) hepatocytes in primary culture to microcystin-LR (MC-LR) in order to develop biomarkers of effect for cyanobacterial hepatoxins. Livers in live animals and freshly isolated liver cells (hepatocytes) are highly sensitive to microcystins because the toxin is concentrated into hepatocytes by the bile acid transporter system. Work with rat hepatocytes has shown that microcystin MC-LR is taken into the cell cytoplasm where it inhibits protein phosphatases leading to hyperphosphorylated cytoskeleton proteins leading to collapse of the cell structure, followed later by cell death. Although fish have not appeared to be as sensitive to the microcystin class of hepatotoxins as are rats, the following data indicate that channel catfish hepatocytes share some MC-LR toxic mechanisms with rat hepatocytes.

Hypothesis

We propose that (1) primary cultures of channel catfish hepatocytes respond to the presence of MC-LR with the formation of abnormal morphology before loss of viability; and (2) there is a significantly greater sensitivity of hepatocytes to MC-LR in comparison with the channel catfish ovary (CCO) cell line, which would not be expected to possess the bile acid transporter.

Methods

Hepatocyte culture Hepatocytes from channel catfish (75-120 g) maintained in aquaria at 20˚C were prepared essentially as described by Seddon and Prosser (1999) except that cells were washed by centrifugation in phosphate buffered saline and resuspended in serum-free EMEM at a con-

centration of 1 million cells/mL after a selection for viable cells by centrifugation though 30% Percoll. The hepatocytes were incubated in Falcon Primaria 96-well plates, 100,000 cells per well in serum-free EMEM medium buffered with 25 mM HEPES in the dark at 20-22˚C. The medium was not changed throughout an experiment.

Channel catfish ovary (CCO) cell culture. CCO cells obtained from the American Type Culture Collection were seeded into 96-well plates in EMEM supplemented with 10% fetal calf serum at a confluency of 20% and exposed to doses of MC-LR for 96 hours at 30°C.

XTT cytotoxicity assay. XTT, a tetrazolium salt that is converted to its formazan derivative by metabolic reduction due to cellular dehydrogenases, was used to measure the viability of hepatocytes. The assay was performed according to the manufacturer's instructions except for the extended incubation times at 20-22˚C. Formazan was measured at 450 nm wavelength on a Synergy HT microplate reader. Hepatocytes cultured in 96-well plates were observed and photographed with the aid of a bright-field, inverted microscope.

Results

Viability measured as XTT reduction in primary cell cultures of channel catfish hepatocytes following exposure to microcystin for 5 days resulted in approximately 30% less XTT reduction for each 1 μg microcystin/mL compared with control cells without MC-LR. In addition, a dose response was evident for the concentrations of microcystin used in this experiment. After a 3-day exposure to 10 μg/mL microcystin, channel catfish hepatocytes in cell culture had rounded. This morphological change was observed before a change in XTT reduction was detectable. CCO cells required approximately 50 μg/mL MC-LR to attain a similar amount of XTT reduction as did 1 μg/mL in the hepatocyte culture.

Conclusion

Exposure of channel catfish hepatocytes in primary culture to microcystin-LR elicits a similar pattern of toxicity to that reported for rat hepatocytes, suggesting that there are common toxic mechanisms between the two systems upon which biomarkers of effect may be developed.

Mortality of bald eagles and american coots in southeastern reservoirs linked to novel epiphytic cyanobacterial colonies on invasive aquatic plants

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Introduction

Invasive species compromise habitat and degrade the quality of the environment for many species and can even accelerate the decline of endangered species in these sites. Our field surveys and feeding studies implicate invasive aquatic plants and an associated epiphytic cyanobacteria species in an emerging avian disease to herbivorous waterfowl and their avian predators. The disease, Avian Vacuolar Myelinopathy (AVM), was first noted in 1994 and has been the cause of death for at least 100 American bald eagles (*Haliaeetus leucocephalus*) and 1000's of American coots (*Fulica americana*) and other waterfowl. The disease causes neurological dysfunction in the birds prior to death but no known neurotoxins or disease agents have been detected at the sites or within the birds.

Hypotheses

We propose that the agent for the disease is a neurotoxin produced by a novel cyanobacterial epiphyte of the order Stigonematales growing on invasive aquatic plants. Our working hypothesis is that the neurotoxin travels through the food chain from hydrilla and other aquatic plants to waterfowl who are consumed by predators including the American Bald Eagle and Great Horned Owls.

Methods

This research incorporated field site monitoring, laboratory experiments, and field trials. An extensive survey of documented AVM sites was conducted from 2001–2005 to monitor the field occurrence of the disease, the abundance of the invasive plant species and the density of the target cyanobacterial species. Laboratory and field trials were conducted to test hydrilla with an abundance of the targeted cyanobacteria would cause AVM lesions in the experimental mallards and triploid grass carp.

Results

In reservoirs where eagles and waterfowl deaths have been most prevalent, the novel Stigonematales species was dominant; and diversity of other groups (primarily diatoms and green algae) was lower. In reservoirs where bird deaths from AVM have not been diagnosed, epiphytic assemblages were diverse and abundant, but the suspect Stigonematales species was either rare or not present. Mallard and grass carp fed hydrilla dominated by the novel Stigonematalean species developed AVM lesions in the laboratory tanks and in the field trial. Those receiving the control hydrilla which contained abundant epiphytic algae, but none of the target Stigonematales species did not develop AVM lesions.

Conclusion

The agent responsible for AVM is associated with the hydrilla and other aquatic plants in the sites where the disease occurs. The most probable theory is that the epiphytic algae growing on these plants are producing a neurotoxin that causes brain lesions and death in the birds who consume it. The invasive potential of these exotic plants make it likely that the disease will expand to new sites and the impact of this disease on waterfowl and eagles will continue to increase.

Investigation of a novel epiphytic cyanobacterium associated with reservoirs affected by avian vacuolar myelinopathy

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Introduction

The recovery and conservation of bald eagles (*Haliaeetus leucocephalus*) has been contested due to a newly identified fatal bird disease: Avian Vacuolar Myelinopathy (AVM). Since the discovery of the disease in 1994, AVM has caused mortality in at least 100 bald eagles, thousands of American coots, and other various species of bird throughout the southeastern US. AVM has been found in AR, TX, NC, SC, and GA. The cause of the disease has yet to be identified.

A strong association has been observed between the occurrence of AVM, *Hydrilla verticillata* (hydrilla), and a novel potentially toxic, epiphytic cyanobacterium on hydrilla. The correlation has led to the hypothesis that this epiphyte is the source of the neurotoxin causing AVM. During 2001–2004, the Stigonematales species was present on the surface of hydrilla at every site where AVM had been diagnosed, but was absent or scarcely found in areas where AVM was not observed.

Hypotheses

It is hypothesized that the proposed toxin of the cyanobacteria is bioaccumulated through the food chain from waterfowl (e.g. coots) ingesting the Stigonematales species growing on the hydrilla. The goals of the study included; establishing a culture of the targeted Stigonematales species, ex-

panding morphological descriptions of the species, determining gene sequence data from material collected in the field, and developing a Real Time–PCR assay specific to the cyanobacterium.

Methods

A monoculture of the cyanobacterium was established on BG–11 medium at 27°C. The 16S rRNA sequence identity was determined from environmental isolates using DGGE and then "ground–truthed" with culture isolates. The 16S rRNA sequence data were aligned with additional cyanobacteria sequences to determine designations for probe development, and to use in phylogenetic analysis. Real–Time PCR assays were developed specific to the Stigonematales species.

Results

The Stigonematales species has been cultured in order to aide in development of species identity, genetic research, feeding trials, and toxin analysis. 16S rRNA sequence data were aligned with additional cyanobacteria sequences to advance understanding of the species' phylogeny, and to lay groundwork for its formal description. Phylogeny data confirmed that the species is in section V, order Stigonematales. Phylogeny also inferred that the species is novel and most genetically similar to a *Stigonema* sp. Based on sequence variability, a Real Time–PCR assay has been developed for rapid, specific detection of the Stigonematales species from environmental samples.

Conclusion

The dominant epiphyte found on collected *Hydrilla* is an undescribed species of cyanobacterium in the order Stigonematales. The genetic probe and taxonomic information produced by this study will help test the hypothetical link between these cyanobacteria and AVM, and therefore help guide decisions on managing hydrilla and other invasive macrophytes in AVM–affected waters.