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Ecology of Type E Botulism Within Dreissenid Mussel Beds

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This article will discuss possible mechanisms that support the expansion of *Clostridium botulinum* type E populations within the micro-niche of dreissenid mussel beds in the Great Lakes. Type E botulism has killed thousands of fish and waterfowl since its reoccurrence in the Lower Great Lakes in 1999. Ingestion of benthic invertebrates that carry this bacterium and its toxin is suspected to be a source of type E botulism for fish and waterfowl. By solving the puzzle of how *C. botulinum* type E moves from the sediments to the intestinal tracts of benthic fish and migrating waterfowl, new mechanisms and interactions that influence disease ecology within the Great Lakes ecosystem may be revealed. Ecology of disease transmission at the sediment-water interface is an understudied area. The effect of changes in dissolved oxygen at the sediment-water interface and whether hypoxic conditions will foster proliferation of this pathogen within dreissenid mussel beds are two areas of investigation worth pursuing.

Introduction

The invasion of dreissenid mussels (*Dreissena bugensis* and *Dreissena polymorpha*) and round gobies (*Neogobius melanostomus*) has affected the diversity and interactions of the aquatic sediment biota in the Great Lakes freshwater ecosystem (Palmer et al. 2000; Stewart et al. 1998) and has coincided with the (re)emergence of avian botulism in several locations (Campbell 2003). Understanding the ecological conditions that promote or inhibit type E botulism is one of the goals of our laboratory. The invasion of these exotic species has brought about habitat transformations and the return of anoxic events (Conroy and Culver 2005) that have increased the chances that environmental conditions will be conducive for the growth and transmission of *C. botulinum* type E.

Thousands of fish and waterfowl have succumbed to type E botulism since its reoccurrence in the Lower Great Lakes in 1999. The mechanisms behind this epidemiological phenomenon have yet to be explained (Campbell 2003; Domske and Obert 2001). No one has yet described the environmental changes in the Great Lakes that support the growth of *C. botulinum* type E within the micro-niche of the dreissenid mussel beds. Further study of the physico-chemical conditions and the biotic components of this micro-niche, which include the dreissenids, round gobies, resident benthic invertebrates, and microbial populations are needed. Relating the route or routes of botulism transmission within the structural complexity of this new dreissenid-altered environment will be a significant step forward in understanding the ecology of disease transmission at the aquatic-sediment interface. Research in this area will improve the understanding of unintended health effects of invasive species, increase our capacity to forecast botulism outbreaks, and improve our understanding of how diseases (re)emerge.

Figure 1 (right). In a cooperative effort with the NYSDEC, we collected fish during spring, summer, and fall as well as sampling during active outbreaks of botulism in waterfowl. Apparently healthy, moribund, and recently dead fish were collected. One of the authors, Rod Getchell (left) is seen here discussing that day's samples with Don Einhouse, senior aquatic biologist at the Lake Erie Fisheries Unit in Dunkirk New York.

Figure 2 (above). A bottom trawl being emptied onto the deck of the R/V Argo.

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Wobeser (1981) defines disease in free-ranging wildlife populations as "any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxins, and climate; infectious agents; inherent or congenital defects, or combinations of these factors." Impairments caused by type E avian botulism that negatively affect the long-term persistence of populations and the ability of healthy populations to fulfill their ecological roles in an ecosystem are worth further study.

Since 2002, limnological data, lake sediments, benthic invertebrates (including dreissenid mussels and biota within their sediment beds), and fish samples have been collected from eastern Lake Erie. Analyses of moribund freshwater drum, smallmouth bass, and round gobies performed by Getchell et al. (2006) during 2002-2003 found that significant numbers of *C. botulinum* type E bacteria were present in the intestines and livers of these fish. Moribund fish in Lake Erie contained food in their stomachs such as dreissenid mussels and round gobies. The numbers of *C. botulinum* type E detected were similar to those measured by Nol et al. (2004a) in moribund tilapia from the Salton Sea and also close to levels detected in dying juvenile salmonids by Eklund et al. (1982). Limited numbers of dreissenid mussels, amphipods, oligochaetes, nematodes, diptera (true fly), and ephemeroptera (mayfly) larvae were carriers of the type E toxin gene at certain times and locations, as determined by Quantitative Polymerase Chain Reaction (QPCR; Pérez-Fuentetaja et al. 2006). This new QPCR assay was developed because it allows a more rapid and accurate assessment of type E toxin producing cells in fish, birds, sediments, and benthic invertebrates.

Collaborations between investigators from Cornell University, SUNY Fredonia, SUNY Albany, the University of Pennsylvania, the New York Department of Environmental Conservation's Great Lakes Fisheries Section and Wildlife Pathology Unit, as well as the extension personnel at New York Sea Grant have fostered an intensive approach to the avian botulism problem on the Great Lakes. The results of these collaborations are evident in the publications in press in 2006 (Getchell et al. 2006; Pérez-Fuentetaja et al. 2006), the numerous botulism workshop proceedings available on the Internet through New York Sea Grant (Focazio 2006), and the enhancement of the scientific environment of all the groups involved with this issue. Further research is needed to confirm the findings in these publications and determine if *C. botulinum* type E production is taking place in certain "hot spots" in the sediment that meet the bacterial requirements for optimal growth.

Lake Erie outbreaks

Large mortalities of fish and waterfowl have recently been recorded in Lake Erie during type E botulism outbreaks (Culligan et al. 2002; Domske and Obert 2001). Since 1999, there has been a repeated pattern of annual occurrence. In late July or early August, there are

small-scale die-offs of gulls and, less commonly, of other species such as cormorants and terns, which share colony sites. Later, migrating shorebirds, such as sanderlings, plovers, and sandpipers, are affected. Large-scale die-offs begin in late September, peak in late October and November, and involve primarily predatory fish-eating species: common loons, mergansers, and grebes (Campbell 2003). More recently, long-tailed ducks (old squaw) have been involved in these events (Swift 2004). Geographically, the center of botulism activity has shifted from western to eastern Lake Erie, although sporadic events still occur in all parts of the lake as well as Lake Ontario.

Type E botulism is a neuroparalytic disease transmitted through diet caused by *C. botulinum* type E, an anaerobic bacterium found in nutrient-rich substrates. In North America, type E botulism primarily is recognized as a disease of waterfowl on the Great Lakes (Rosen 1971). In the early 1960s, there were repeated losses of thousands of common loons on Lake Michigan and western Lake Huron, and smaller scale events involving various species of gulls and grebes (Campbell 2003). The disease disappeared for almost 20 years, and later resurfaced on Lake Michigan from 1981-1983, with annual mortalities of loons again numbering in the thousands (Brand et al. 1988).

Unlike type C botulism, where a long-term research effort has addressed the ecology of avian botulism, the specific ecological conditions required for type E toxin production and transmission to birds are unknown. There are major differences in molecular biology, microbiology, and ecology between types C and E, which may explain the different epizootiological presentations of these diseases (Domske and Obert 2001). It is known that the spores of *C. botulinum* type E are abundant in many North American lakes and that the spores can readily be found in the gills and digestive tracts of fish from these lakes (Bott et al. 1966). Type E botulism occurs only under conditions when these spores germinate, the bacteria multiply, and the vegetative cells produce toxin. The ecological role of the bacterium appears to be that of a decomposer (Smith and Sugiyama 1988). The bacterium requires a rich nutrient substrate in an environment that is free of oxygen. Fish carcasses that contain the bacterial spores are suitable substrate for growth and toxin production (Eklund et al. 1984).

It seems clear that the fish-eating wild birds that have died of type E botulism have become poisoned from eating fish that contain the toxin (Stone 2003). What is not clear is whether the toxin is generated in the fish or consumed as part of the fish's diet. Waterfowl such as loons and mergansers normally capture and eat only live fish (Barr 1996). Yet, *C. botulinum* type E should not grow and produce toxin in living fish. There could be circumstances under which toxin is produced in the tissues of live and dying fish, possibly within their digestive tracts (Nol et al. 2004a). Or it may be that the

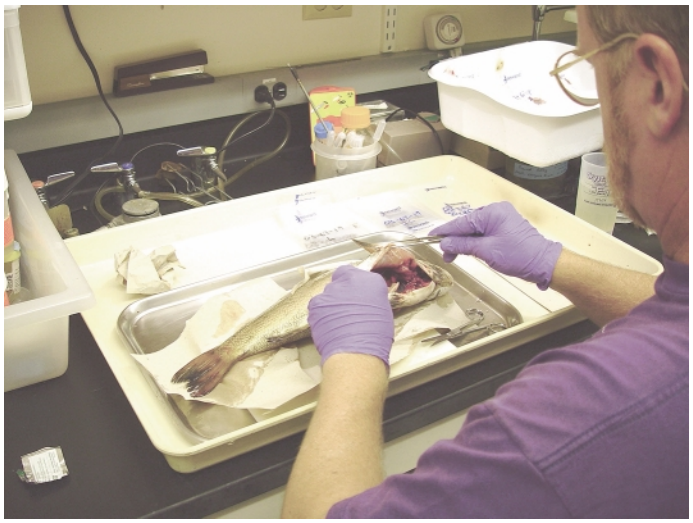

Figure 3. At the Cornell University Fish Pathology Laboratory, fish were necropsied and also examined for other pathogens besides *Clostridium botulinum* type E. Tissues were frozen for later molecular analysis. DNA was extracted from liver and intestinal content samples and then tested with a real-time PCR assay for the type E botulinum toxin gene.

fish captured alive and eaten by the birds had themselves fed on some source of type E toxin. Thus, it would be the toxin in the digestive tracts of the live fish that was the source of toxin for the birds in these outbreaks. It is even possible that the live fish captured by the birds were partially paralyzed with a sub-lethal dose of type E toxin they had recently eaten and thus were particularly easy prey for the birds (Moccia 2005). This might account for preferential feeding on toxin-containing fish by the affected birds (Campbell 2003). The source of toxin could be the benthic invertebrates that consume or filter *C. botulinum* type E spores or vegetative cells. If the spores are the microbial stage that moves from the sediments to upper trophic levels, then the location of germination is still in question.

Nol et al. (2004a) hypothesized that tilapia probably ingest type *C botulinum* spores or cells during the winter and spring, when sediment consumption was found to be highest by other investigators (Riedel and Costa-Pierce 2002). During the summer months, anoxia and high temperatures stress the large populations of tilapia and very little food material is found in their gut. This static condition of their gastrointestinal tract is ideal for the growth of anaerobic bacteria, like *C. botulinum* type C. This does not seem to be the case with type E botulism, where dreissenid mussels and round gobies are found in moribund fish displaying signs of botulism (Getchell et al. 2006). The mortality of scavengers such as ringed billed gulls could be explained by the carcass-maggot cycle during type E outbreaks in the summer, where spores germinate during post-mortem decomposition of fish, birds, and invertebrates (Reed and Rocke 1992). Birds like the long-tailed duck that feed directly on dreissenids have also suffered increased mortality from type E botulism (Skerratt et al. 2005). This would indicate that the vegetative cells and/or toxin were

present in the dreissenids prior to their consumption by the long-tailed ducks since no decomposition is involved. Therefore, spore germination took place prior to the feeding event on the dreissenid bed. Type E spores, vegetative cells, and toxin have all been detected at significant levels in fish-eating birds like the common loon and merganser (Getchell unpublished data). The source of toxin for these waterfowl is still in question.

Role of the dreissenid mussel bed

Mussel beds may serve as micro-niches for botulism. It has been hypothesized that ingestion of invertebrates that carry the bacteria and its toxin could be a source of the disease for fish and waterfowl feeding on sediment-dwelling organisms (Holeck et al. 2004). Dreissenid mussels could also play a role in botulism proliferation by providing nutrients to bacteria, as well as habitat from accumulated shells that shelter the sediments from oxygen exchange. Comparison of the diversity of microbial communities in zebra mussel tissue extract, detritus, and pseudofecal material associated with zebra mussel colonies, surrounding water, and sediment samples revealed distinct microbial assemblages associated with these environments (Frischer et al. 2000). Little attention has been focused on the relationships among dreissenids and the components of the microbial ecosystem, despite evidence that phytoplankton food resources are often limiting for zebra mussel populations and that zebra mussels may be capable of capturing bacteria-size particles. The results that Frischer et al. (2000) present suggest that zebra mussels and pseudofeces support different types of microbial communities in comparison with Hudson River sediments and water. They suggest that changes in microbial community structure induced by the presence of zebra mussels may be profound.

The ecological role of the mussel bed also may be augmented by the possibility that anoxic events may induce proliferation of *C. botulinum* type E. Dreissenid mussels have modified much of the softer sediment in eastern Lake Erie into a bed of living and dead mussels, shells, and accumulated feces and pseudofeces, creating potential *C. botulinum* type E habitat. Decomposition of dead mussels and mussel waste products may create bacterial niches where anoxia is prevalent and nutrients are abundant. Other investigators have documented the anaerobic conditions that cause dreissenid mortalities (Mikheev 1964; Matthews and McMahon 1995). At mariculture sites, biodeposition from mussel beds can result in large differences in the quality of sediment below and within mussel cultivation areas compared to surrounding sediments (Dahlback and Gunnarsson 1981; Kaspar et al. 1985; Kautsky and Evans 1987). Enrichment with organic material leads to increased bacterial respiration rates and oxygen consumption, which can lead to anoxic conditions and sulphate reduction (Dahlback and Gunnarsson 1981; Kaspar et al. 1985).

Much of the particulate material in the feces and pseudofeces is available to detritivores in the benthic community. The increase of some benthic animals, notably amphipods (Gonzalez and Downing 1999), coinciding with the spread of dreissenids suggests such a benefit. In fact, a substantial community of detritivores and decomposers develops with the dreissenids (Stewart et al. 1998). Nalepa and colleagues (2005) discuss how dreissenids may have reduced dissolved oxygen (DO) at the sediment-water interface to the point of adversely affecting *Diporeia* populations. They go on to speculate that within mussel beds, microbial decomposition of mussel biodeposits and/or dead mussel tissue may lead to areas of reduced DO. This reduced DO would mostly be a local effect, although during spring storm events dreissenid biodeposits from shallow, near shore regions may resuspend, settle into offshore, depositional zones, and create periods of reduced DO.

Holeck et al. (2004) suggest that dreissenid mussels may concentrate the type E toxin as they filter water near the nutrient-rich sediments that contain the *Clostridium* bacteria. Evidence of the type E toxin gene in *Dreissena* sediment beds and mussel tissue has been recently reported (Pérez-Fuentetaja et al. 2006). From the dreissenid mussel tissue collected in 2003, 21.7% of the samples tested positive for the toxin gene copies (bontE). Feces/pseudofeces from mussel druses tested 21.4% positive. The sediment beds underlying the mussel colonies tested 2.7% positive (Pérez-Fuentetaja pers. comm.). Dermott et al. (2005) also suggest recent increases in botulism in Lake Erie may possibly be due to bio-magnification of the *Clostridium* toxin by dreissenids or increased spore abundance among the decomposing mussels and pseudofeces in the sediments. Biomagnification may occur as small organisms ingest and bioconcentrate specific toxins, which in turn are eaten and further bioconcentrated by larger organisms. The dreissenid mussels that accumulate the toxin would be unaffected. However, round gobies that eat the toxic mussels are affected and become easy prey for piscivorous and scavenging birds, in turn poisoning them. At the present time, these connections are speculative. Research is needed to identify the components of this food web, the production of botulinum toxin within these components, and its subsequent movement through trophic levels, terminating in the annual toll of thousands of bird carcasses littering the shores of Lake Erie.

Environmental conditions

Previous work by Rocke and Samuel (1999) in wetlands affected by type C avian botulism identified several physico-chemical conditions prevalent at the time of outbreaks. These conditions included high temperatures, low redox potential, and pH between 7.5 and 9.0. In eastern Lake Erie, it was not known whether the wetland conditions associated with type C botulism outbreaks would apply due to the greater depth, lower productivity, lower temperatures near the sediment, and

the presence of deep water currents that characterize this basin (Schertzer 1999). Hielm et al. (1998c) studied the distribution of *C. botulinum* type E spores in sediments in the Baltic Sea. These authors positively correlated *C. botulinum* type E counts with low bottom oxygen content, depth, and absence of bioturbation activity. It has been suggested that the presence of large quagga mussel beds may also be reducing the levels of bioturbation in the sediments of Lake Erie. Sediment bacteria play an important role in catalyzing sediment chemical reactions (Wetzel 2001). When bacterial activity is high, many redox reactions take place in the sediment and, as a result, oxygen is depleted. Acid production from these reactions lowers the pH and decreases the redox potential in the sediment.

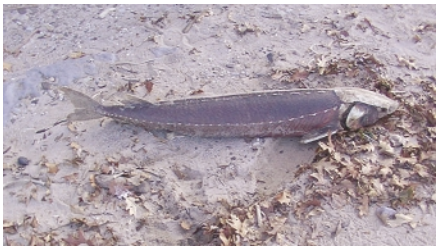

Figure 4. The focus is on species of fish that have died in recent fish-kills such as freshwater drum and lake sturgeon shown in these two photos, as well as smallmouth bass, and round goby. We hope to better understand the circumstances under which piscivorous birds become intoxicated with type E botulinum toxin from eating live or moribund fish.

Ando and Iida (1970) showed that *C. botulinum* type E spore germination might occur at various redox and oxygen concentrations. However, bacterial growth in the vegetative state can only occur under reducing conditions (redox from 198 mV to -198mV) and anaerobiosis. Therefore, low redox potential and low DO are probably essential to permit *C. botulinum* type E proliferation, and thus increase the risk of a botulism outbreak if other conditions necessary for toxin production and transfer are present.

Dreissenid mussel beds may be creating anoxic conditions for *C. botulinum* type E by modifying its own physical environment through its filtration of the water column. The highly efficient mussels clarify the water so much that sunlight can reach normally shaded aquatic plants and promote their growth. *Cladophora* and other benthic algae can benefit from the feeding activities of dreissenids, but dense growths of *Cladophora* restrict the access of dreissenids to the overlying water column and might even asphyxiate mussels owing to low oxygen conditions under *Cladophora* stands. When the unwanted proliferation of algal growth dies, its decay consumes oxygen, providing ideal conditions for the type E botulism bacteria to grow (Hecky et al. 2004). Whitman et al. (2003) have documented that *Cladophora* mats along Lake Michigan's near shore waters and beaches are a source of *Escherichia coli* and enterococci, both facultative anaerobes. Their study demonstrates that *Cladophora* harbors high densities of *E. coli* and enterococci relative to water and beach sand and that the indicator bacteria in Cladophora are ubiquitous. Further, the experiments show that *E. coli* and enterococci can survive for extended periods (over 6 months) in the algal mats.

The inability to find vegetative *C. botulinum* type E DNA in most of the benthic samples that we have collected in our previous research efforts could be the result of localized bacterial activity that may occur under very specific sediment and microbial conditions. If this is the case, it is likely that only a very intensive spatial and temporal sampling protocol would result in the detection of vegetative cells.

Quantitative PCR

The ability to quantify the number of vegetative cells of *C. botulinum* type E while avoiding the enumeration of the ubiquitous spores in the aquatic sediments has been a key part of our work. The dormant spores can remain viable for decades and are widely distributed in aquatic sediments (Smith and Sugiyama 1988). Most researchers in the past have tried to enumerate the prevalence of spores in the sediments of lakes and marshes by first culturing them in enrichment media and then extracting the DNA and amplifying by PCR. Williamson et al. (1999) were one of the first to recognize that the key to quantifying the naturally occurring *C. botulinum* populations was to detect the toxin gene without prior enrichment. It is the vegetative cells that are the toxin producing populations that need to be measured. The extraction method is the key to selectively isolating the DNA of vegetative cells, while not lysing the hardy spores. Scientists at the National Wildlife Health Center in Madison, WI suggested a commercially available kit that accomplishes this and have subsequently published the finding (Nol et al. 2004b). The MoBio Soil DNA extraction kits (Solana Beach, CA) are isolating the DNA of *C. botulinum* vegetative cells only (Pérez-Fuentetaja et al. 2006). The presence of type E spores in the sediments and biota of Lake Erie can be detected by incorporating a bead-beater into the processing of samples. In addition, the QPCR assay allows the amplification of the type E toxin gene fragment and not other related *Clostridium* species (Getchell et al. 2006).

A QPCR technique to identify specific *C. botulinum* genes has been developed by a few other research teams (Kimura et al. 2001; Akbulut et al. 2004; Nol et al. 2004b). The presence of the toxin gene is an indication of the presence of the *C. botulinum* bacterium. Molecular detection of clostridial populations has been de-

scribed for the human intestinal tract and the landfill environment (Van Dyke and McCarthy 2002). Improved understanding of the microbial populations of dreissenid mussel beds, and especially the groups involved in type E botulism, can lead to an overall scheme to monitor bacterial populations involved in anaerobic degradation in sediments. The real-time amplification of the type E toxin gene can be used to monitor changing conditions in the sediments, and give some measure of microbial activity. The techniques would also have application to other areas of anaerobic ecology, such as soils, compost, anaerobic digestors, and the gastrointestinal tract. The application of molecular biological techniques will therefore contribute to the understanding of the ecology of an important bacterial group that has been difficult to isolate and monitor (Van Dyke and McCarthy 2002).

The focus has been on the type E toxin gene, which is the most common type of botulinum toxin found in fish and the type identified in the waterfowl and fish mortalities in 2001-2002 (Getchell et al. 2006; Ward Stone, personal communication). A small fragment of the type E toxin gene has been cloned, at the junction of the light and heavy chains, which is the key to creating a standard curve in the QPCR assay. With dilutions of the plasmid DNA as a standard, the results can be compared with the unknown samples and the number of *C. botulinum* bacteria present quantified. DNA sequence analysis has confirmed that the amplification products generated with the QPCR primers contained the correct type E toxin gene sequence. The presence of *C. botulinum* type E genome equivalents can be determined from sediment and invertebrate samples as well as fish intestinal content and liver samples. The assay detects as few as four copies of the type E toxin gene in this QPCR assay.

Vectors in the type E botulism food chain

One question that needs to be addressed is whether it is the smaller or larger round gobies that are the vector for type E botulism. Vanderploeg et al. (2002) explain that small round gobies (<70 mm) eat a variety of benthic macroinvertebrates (e.g., amphipods, isopods, insect larvae, benthic cladocerans) and zebra mussels, whereas large round gobies eat mainly zebra mussels (e.g., Ray and Corkum 1997; French and Jude 2001). Round gobies are gape-limited, so that the size of mussels ingested increases with goby length; however, in general, small mussels (<10 mm) are selectively cropped (Ray and Corkum 1997).

Quagga mussels were found first in Lake Erie in 1989 (Mills et al. 1999), Lake Michigan in 1999 (Nalepa et al. 2001), and four years later in the St. Lawrence River. Quagga mussels are competitively replacing zebra mussels, as they become the dominant species in the eastern basin of Lake Erie and Lake Ontario where zebra mussels had initially colonized (Mills et al. 1996, 1999). Quagga mussels compete with zebra mussels in the near shore zone and have expanded their range to deeper hypolimnetic waters (Dermott et al. 1998). Quagga mussels are expected to colonize greater portions of the hypolimnetic areas of the Great Lakes depending on food availability and temperature regimes (Jude et al. 2002).

Vanderploeg et al. (2002) also point out that round gobies may change toxic substance transfer routes. For example, accumulations of polychlorinated biphenyls (PCBs) have been recorded in round gobies in the PCB-contaminated Raisin River, a tributary to Lake Erie (Jude 2001). Presumably the gobies acquire high PCB concentrations from contaminated zebra mussels, and this transfer leads to contamination of gamefishes that feed on gobies. Round gobies and dreissenids may have changed the type E botulinum toxin route in Lake Erie as well.

Vanderploeg (2003) mentions that *Dreissena*, round gobies, and *Echinogammarus* form an 'invasional meltdown' assemblage in the Great Lakes that is becoming an important part of the food web in many areas of the Great Lakes. That is, rather than restricting invasion by other species, zebra mussels facilitated invasion of the Great Lakes by their Ponto-Caspian associates. Dreissenids provide substrate (shell) and food (pseudofeces and feces) to *Echinogammarus*. Round gobies and other fishes feed on *Echinogammarus*. The detritivore *Echinogammarus* may serve as a vector in the movement of *C. botulinum* type E up the food chain. But our ability to understand present impacts and to predict future impacts may be limited by insufficient monitoring and understanding of Great Lakes food web (Vanderploeg et al. 2002).

Future investigations

Future investigations should focus on the role of food chain organisms in the movement of type E botulism from dormant spores in the sediments within and below dreissenid mussel beds through a hypothesized invertebrate vector to higher vertebrates such as fish and waterfowl. The overall effort could comprise a number of interrelated objectives involving collection of data in the field as well as laboratory-based trials to determine whether hypoxic conditions in dreissenid beds will provide suitable conditions for the proliferation of *C. botulinum* type E. To test the hypothesis that hypoxic conditions will foster proliferation of this pathogen within dreissenid mussel beds, dissolved oxygen data loggers could be placed at designated dreissenid colonies in Lake Erie and visited periodically to measure environmental parameters at the sediment-water interface and collect resident benthic organisms. Changes in the local environmental conditions, particularly a decrease in dissolved oxygen, are anticipated and may be correlated with an increase in the prevalence of *C. botulinum* type E both in the sediment and in the resident benthic organisms within and below dreissenid colonies. There are many variables that could affect the outcome of the

experiments. Among them are water temperature, quagga density, quagga age and size, nutrient levels, sediment type, and *C. botulinum* type E spore density. In subsequent years of the study, depending on the outcomes measured in the field, the effects of different levels of hypoxia and temperature may be tested in the dreissenid microcosm experiments following the procedures described by Johnson and McMahon (1998).

About the Authors

Rod Getchell, PhD is a research associate in the Department of Microbiology and Immunology at Cornell University. He works for Dr. Paul Bowser in the Aquatic Animal Health Program. His research interests most recently have been applying real-time PCR technology towards important pathogens in the aquatic environment such as Largemouth Bass Virus, Koi Herpesvirus, Viral Hemorrhagic Septicemia Virus, and *Clostridium botulinum* type E.

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The diagnostic service of the Aquatic Animal Health Program assist New York State residents who are commercial aquaculturists or who use fish as biomedical research models. The Program also provides fish disease diagnostic support to the New York State Department of Environmental Conservation for fish kills in the natural fisheries resources of the state.

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Exploring the coexistence of the hydroid Cordylophora caspia and the zebra mussel Dreissena polymorpha: counterbalancing effects of filamentous substrate and predation

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Abstract

As invasive species continue to immigrate, we are more likely to find multiple introduced species co-occurring in new habitats. The invasive hydroid, *Cordylophora caspia* and the zebra mussel, *Dreissena polymorpha* are Ponto-Caspian associates that are becoming prevalent in freshwater systems in the United States. Since *C. caspia* commonly grows on the shells of *D. polymorpha*, their population dynamics in new habitats may be interlinked. One important interaction is the influence of *Cordylophora caspia* on the settlement of zebra mussel larvae. There is evidence that marine bivalve larvae preferentially settle on filamentous substrates such as hydroid colonies and algae; however, similar studies are rare in freshwater systems. We examined the importance of filamentous substrate to settlement of zebra mussel (*Dreissena polymorpha*) larvae by deploying PVC settlement plates with and without polypropylene filaments in the Bark River,