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Gut content analysis of Lake Michigan waterbirds in years with avian botulism type E mortality, 2010–2012

David A. Essian [a,*], Jennifer G. Chipault [b], Brenda Moraska Lafrancois [c], Jill B.K. Leonard [a]

[a] Northern Michigan University Biology Department, 1401 Presque Isle Avenue, Marquette, MI 49855, USA
[b] US Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711, USA
[c] National Park Service, 2800 Lake Shore Drive East, Ashland, WI 54806, USA

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ABSTRACT

Waterbird die-offs caused by *Clostridium botulinum* neurotoxin type E (BoNT/E) have occurred sporadically in the Great Lakes since the late 1960s, with a recent pulse starting in the late 1990s. In recent die-offs, round gobies (*Neogobius melanostomus*) have been implicated as vectors for the transfer of BoNT/E to fish-eating birds due to the round goby invasion history and their importance as prey. Dreissenid mussels (*Dreissena* spp.) are also potentially involved in BoNT/E transmission to birds and round gobies. We examined gut contents of waterbirds collected in Lake Michigan during die-offs in 2010–2012, and the gut contents of culled, presumably BoNT/E-free double-crested cormorants (*Phalacrocorax auritus*). Round gobies were found in 86% of the BoNT/E-positive individuals, 84% of the BoNT/E-negative birds, and 94% of the BoNT/E-free cormorants examined. Double-crested cormorants, ring-billed gulls (*Larus delewarensis*), and common loons (*Gavia immer*) consumed larger-sized round gobies than horned and red-necked grebes (*Podiceps auritus* and *Podiceps grisegena*), white-winged scoters (*Melanitta deglandi*), and long-tailed ducks (*Clangula hymealis*). Other common prey included dreissenid mussels, terrestrial insects, and alewives (*Alosa pseudoharengus*). Our data emphasize the importance of round gobies and mussels in diets of Lake Michigan waterbirds and suggest they may play a role in the transfer of BoNT/E to waterbirds; however, round gobies and mussels were found in BoNT/E-positive, -negative, and -free individuals, suggesting that other factors, such as alternative trophic pathways for toxin transfer, bird migratory timing and feeding locations, prey behavior, and individual physiological differences across birds may affect the likelihood that a bird will succumb to BoNT/E intoxication.

Introduction

Wild birds acquire *Clostridium botulinum* neurotoxin (BoNT) by ingesting contaminated prey (Reed and Rocke, 1992; Smith and Sugiyama, 1988). Avian botulism type C outbreaks can result in the death of large numbers of dabbling ducks and shorebirds on warm, shallow bodies of water (Newman et al., 2007). Botulism type C outbreaks have been attributed, in part, to a carcass–maggot feedback cycle, in which birds consumed contaminated invertebrates, such as maggots, from decaying carcasses. The feedback cycle exists because contaminated birds succumb to the toxin, and their carcasses provide new substrate for BoNT/C and maggots (Reed and Rocke, 1992). In contrast, botulism type E outbreaks in the Laurentian Great Lakes generally affect waterbirds that consume live fish and benthic aquatic macroinvertebrates. The food web pathways that maintain this cycle are not yet well characterized, making management of botulism type E outbreaks challenging.

Clostridium botulinum spores naturally occur throughout freshwater and marine sediments, and on plant and animal tissues, which can act as reservoirs for *C. botulinum* spores (Espelund and Klaveness, 2014). When specific environmental conditions occur (including high concentrations of *C. botulinum* spores, low concentrations of competing microbes, high temperatures, protein source, and anoxia) spores commence vegetative growth and begin to produce BoNT (Espelund and Klaveness, 2014; Rocke and Bollinger, 2007). Decomposition of dead organisms that carry *C. botulinum* spores may provide the conditions required for optimal vegetative cell growth and BoNT production (Espelund and Klaveness, 2014). Mass deposition and decomposition of *Cladophora glomerata*, a common green alga in the Great Lakes, has been proposed as a driver of *C. botulinum* neurotoxin type E (BoNT/E) production (Byappanahalli and Whitman, 2009; Chun et al., 2013; Wijesinghe et al., 2015). BoNT/E or *C. botulinum* spores have also been detected in fish (Getchell et al., 2006) and invertebrates (Perez-Fuentetaja et al., 2011) in the Great Lakes. For avian botulism to affect non-scavenging bird species, BoNT/E must either be transferred through

* Corresponding author at: Florida Atlantic University Department of Biological Sciences, 777 Glades Road, Boca Raton, FL 33431, USA. Tel.: +1 906 227 1619.
E-mail addresses: daessian@fau.edu (D.A. Essian), jileonar@nmu.edu (J.B.K. Leonard).

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food webs from substrates containing BoNT/E to live prey and from live prey to birds, or inadvertently consumed by birds in pursuit of live prey (Espelund and Klaveness, 2014; Getchell et al., 2006). Additionally, transmission of BoNT/E through food webs must occur in locations that are used by foraging waterbirds, many of which are migratory and only temporarily reside in the Great Lakes. Avian botulism type E outbreaks are therefore driven by factors including spatiotemporal distribution patterns of *C. botulinum* toxin, prey items, and birds, as well as vulnerability to intoxication of prey and birds.

The earliest recorded avian botulism die-offs in Lake Michigan occurred throughout the 1960s (Fay et al., 1965), in 1976, and in the early 1980s (Brand et al., 1988). Outbreaks were not recorded again until the late 1990s. However, since the late 1990s, there have been regular die-offs in the Great Lakes. Recent avian botulism outbreaks in Lake Michigan began in 2006, about the same time that invasive round gobies (*Neogobius melanostomus*) appeared in annual surveys of prey fish abundance in Lake Michigan (Madenjian et al., 2014) and coincident with the recent expansion and population growth of invasive dreissenid mussels (Nalepa et al., 2010, 2014). Dreissenid mussels, including both zebra (*Dreissena polymorpha*) and quagga (*Dreissena bugensis*) mussels, have dramatically altered the Lake Michigan food web through filtration of phytoplankton and sequestering of carbon into the nearshore benthos (Heath et al., 1995; Hecky et al., 2004; Nalepa et al., 2009). Related effects include an increase in water clarity and transition of nearshore benthic substrate into extensive mussel beds which enhanced the growth of benthic algae (Auer et al., 2010). Recent studies also highlight the potential for dreissenid mussels to influence the flow of energy and nutrients (Mosley and Bootsma, 2015; Tyner et al., 2015) in Lake Michigan.

Unlike most Lake Michigan prey fish, round gobies, particularly larger individuals, consume dreissenid mussels (Barton et al., 2005; Bunnell et al., 2005; Campbell et al., 2009; Burkett and Jude, 2015). Thus, round gobies provide a potential link between mussel-bound nutrients (and toxins) and higher trophic levels (Hebert et al., 2014; Poste and Ozersky, 2013), including piscivorous waterbirds in the Great Lakes. Based on gut content examinations of birds from avian botulism type E outbreaks on lakes Erie and Ontario in 2000–2002, round gobies were an important prey item in affected birds (Hannett et al., 2011).

Several waterbird species have been affected by BoNT/E in Lake Michigan in recent outbreaks (Chipault et al., 2015). Primary species have included piscivores such as common loon (*Gavia immer*), double-crested cormorant (*Phalacrocorax auritus*), and red-breasted merganser (*Mergus serrator*), as well as molluscivorous ducks (long-tailed duck [*Clangula hyemalis*] and white-winged scoter [*Melanitta fusca*]), and generalists such as ring-billed gulls (*Larus delawarensis*) and grebes (*Podiceps* spp.), suggesting that there may be several food web linkages relevant to BoNT/E transmission to birds. Additionally, some of these species are migratory and forage in the Great Lakes for short periods of time (e.g., common loon, long-tailed duck), while others are summer residents of Lake Michigan (e.g., ring-billed gull, double-crested cormorant). This is relevant because seasonal patterns in peak reported avian mortality on Lake Michigan have varied among years, suggesting important spatial and temporal differences in transmission patterns. Transmission of the toxin is perhaps influenced by dynamics of BoNT/E production and distribution coupled with patterns of seasonal waterbird abundance on Lake Michigan (Chipault et al., 2015). In their non-native ranges, round gobies have become important prey and have caused shifts in the foraging behavior of some piscivorous bird species (Jakubas, 2004; Johnson et al., 2015; Tucker and Seefelt, 2014). After round gobies were introduced to Lake Ontario, they became the dominant prey of breeding double-crested cormorants at Pigeon and Snake Islands. Moreover, seasonal shifts in cormorant diets that corresponded with prey abundance before the introduction of round gobies were no longer observed (Johnson et al., 2010). Similarly, round gobies became the dominant prey of double-crested cormorants at the Beaver Archipelago in Lake Michigan, and other prey items became less important (Van Guilder and Seefelt, 2013). To our knowledge, the response of other waterbird species to the round goby invasion has not been published.

Round gobies themselves consume a variety of prey species in addition to dreissenid mussels, including chironomids, mayflies, *Mysis diluviana*, *Gammarus* spp., and various zooplankton (Brush et al., 2012; Raby et al., 2010; Walsh et al., 2007). Gut content analyses of round gobies in the Great Lakes have revealed that round gobies of a wide range of size classes ate dreissenid mussels, but the contribution of dreissenid mussels to the diets of round gobies generally increased with fish size (Burkett and Jude, 2015; Campbell et al., 2009; Ray and Corkum, 1997; Walsh et al., 2007). In studies where dreissenid mussels were absent or locally depleted, other benthic invertebrates dominated round goby diets (Barton et al., 2005; Campbell et al., 2009; Carman et al., 2006; Ray and Corkum, 1997).

Recent avian botulism die-offs offered an opportunity to examine the gut contents of waterbirds feeding in Lake Michigan in order to generate hypotheses about the location of BoNT/E in the environment and the potential pathways for the transfer of the toxin in food webs. Because BoNT/E affects birds acutely (Smith and Sugiyama, 1988), knowledge of BoNT/E positive bird diets just prior to succumbing to the toxin could provide clues about which prey items are important in the transfer of the toxin. Previous studies have identified round gobies as an important prey item for predatory waterbirds in the Great Lakes (Johnson et al., 2015), but their importance in the diets of birds that succumb to BoNT/E is not known. We examined the importance of round gobies and other prey items consumed by waterbirds collected in Lake Michigan during years with BoNT/E outbreaks by plotting percent abundance of each prey item against its frequency of occurrence. We explored differences in diet composition among bird species, by BoNT/E assay results, and by collection type (i.e., moribund birds and carcasses vs. culled birds). We also examined size-class distribution of fish prey in order to make inferences about trophic pathways of the toxin in the lower food web, with special emphasis on understanding the role of round goby and dreissenid mussels in toxin transfer.

Methods

BoNT/E-assayed bird samples

Between June and November 2010–2012, sick or dead birds were collected from fixed beach transects located in three areas around northern Lake Michigan (Door County, Wisconsin, and neighboring counties, eastern Upper Peninsula of Michigan, and Sleeping Bear Dunes National Lakeshore, Michigan) (Fig. 1). Carcasses were collected by the National Park Service and National Park Service Volunteers-in-Parks, Common Coast Research and Conservation, and the United States Geological Survey (USGS) personnel, partners, and volunteers (Chipault et al., 2015). Due to increased local awareness of avian botulism-related mortality, some bird carcasses were also reported and collected from nearby areas off-transect. To ensure that the presence of BoNT/E was indicative of toxin ingested by a live bird and not toxin produced within the carcass post-mortem, only intact carcasses that showed no signs of decomposition (i.e., no foul odor, no maggots, feathers did not pull out easily, eyes present) were collected (Chipault et al., 2015). Bird carcasses in suitable condition were sent to the USGS National Wildlife Health Center (NWHC) in Madison, Wisconsin, to be tested for BoNT/E using mouse bioassays (AOAC, 2001; Chipault et al., 2015). Birds that tested positive or negative for BoNT/E are hereafter referred to as BoNT/E-positive or BoNT/E-negative, respectively. At NWHC, gastrointestinal (GI) tracts were removed from all carcasses that were tested for BoNT/E. Botulinum toxin was inactivated with a 10% formaldehyde bath, and GI tracts were preserved in 95% ethanol.

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