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Round gobies are an important part of VHSV genotype IVb ecology in the St. Lawrence River and eastern Lake Ontario

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ABSTRACT

Although viral hemorrhagic septicemia virus (VHSV) is known to affect at least 28 species of Great Lakes fish, round gobies (*Neogobius melanostomus*) appear to be particularly affected. The first report of VHSV in New York State waters occurred in round gobies and in subsequent surveillance efforts a disproportionately high proportion of round gobies were infected with VHSV compared with other species tested. In this study, we tested the experimental susceptibility of round gobies to infection with VHSV in the laboratory, using naïve and previously exposed fish. Naïve fish were significantly more susceptible than previously exposed fish, however previously exposed fish experienced a mortality of 35% over 45 days suggesting that previous exposure did not result in complete protection. Field studies at two sites showed a significant change in prevalence over 10 weeks in the spring based on non-lethal fin and gill samples, suggesting that great care must be taken when interpreting prevalence from single sampling efforts during VHSV surveillance. There was no difference in the observed diversity of sequence types of virus from fish that tested positive during times of low or high prevalence, or during a confinement-induced laboratory epidemic. These results show that round gobies are experimentally susceptible to VHSV and that the field prevalence of VHSV in this species can vary greatly within a short period of time; these results also provide a preliminary exploration of the role round gobies may be playing in the dynamics of VHSV in the eastern Great Lakes and St. Lawrence River.

Introduction

The round goby (*Neogobius melanostomus*) is native to the Ponto-Caspian region. This species was first detected in the Great Lakes (Lake St. Clair) in 1990 (Jude et al., 1992), and subsequently spread to all Great Lakes within eight years (Hoyle et al., 2003). Round gobies overlap with other native species in terms of food sources and territory; they attack nests of mottled sculpin (*Cottus bairdii*) and overlap in diet and territory of several other cottid species (Kornis et al., 2012). Round gobies will eat the eggs of lake trout (*Salvelinus namaycush*; Chotkowski and Marsden, 1999), lake sturgeon (*Acipenser fulvescens*; Nichols et al., 2003), smallmouth bass (*Micropterus dolomieu*; Steinhart et al., 2004), and walleye (*Sander vitreus*; Roseman et al., 2006). In tributaries of Lake St. Clair, Lake Huron, and Lake Erie, seven endangered and 16 non-endangered species are potentially threatened by the presence of round gobies (Poos et al., 2010). In addition to their effects on native fish species, round gobies were implicated in increased outbreaks of avian botulism (Yule et al., 2006).

Viral hemorrhagic septicemia virus (VHSV) is a rhabdovirus that is the causative agent of a significant disease in finfish across the Northern hemisphere, viral hemorrhagic septicemia (VHS). This disease was first described in Europe in 1938 as causing kidney swelling and liver degeneration (Schäperclaus, 1938). Until the 1980s, the disease was thought to be confined to European aquaculture, but detection of the virus in spawning salmon in 1988 (Brunson et al., 1989) sparked a search for the virus that showed that it was in fact widespread in marine fish off the Pacific coast of North America (Meyers et al., 1999) and the Atlantic coast of Europe (Scholtfeldt et al., 1991). By 2004 VHSV had been isolated off the Atlantic coast of North America (Gagné et al., 2007) and by 2005 there were reports of mortality events attributed to VHSV in the Great Lakes basin (Lumsden et al., 2007). Later testing of archived samples of muskellunge suggested the presence of VHSV in the Great Lakes as early as 2003 (Elsayed et al., 2006).

The first isolation of VHSV in New York State waters occurred in round gobies (Groocock et al., 2007) and multiple years of surveillance

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data confirm a continued presence of infected gobies in Lake Ontario and the St. Lawrence River (Bain et al., 2010; Cornwell et al., 2012) with prevalence in the St. Lawrence River reaching 50% as measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR). The interaction of round gobies with native species has an effect on VHSV as well; in the St. Lawrence River, the prevalence of VHSV in round gobies is correlated with the prevalence of VHSV in smallmouth bass (Eckerlin, 2008). Little is known about infection dynamics of VHSV in wild fish populations. Because of the small home range of round gobies (Ray and Corkum, 2001), populations of this species within the Great Lakes offer a unique opportunity to monitor infection dynamics.

There is evidence for a temporal variation in prevalence of VHSV (Eckerlin et al., 2011); however, this work was undertaken over a time scale of seasons in smallmouth bass. Eckerlin et al. (2011) found a significant increase in VHSV prevalence during the spawning season in smallmouth bass. The prevalence of VHSV decreased during post-spawning, the summer, and the fall. Despite this study, little is known about the short-term temporal variation of VHSV in smallmouth bass or other fish species and this information could have serious implications for surveillance.

Overall, the genetic diversity of VHSV isolates within the Great Lakes to this point has been relatively low (Thompson et al., 2011). This study used isolates from wild fish collected from 2003–2009 and found a maximum diversity across a 669 base pair portion of the glycoprotein gene of 1.08%. However, less than 10% of the isolates studied were from round gobies and subsequent surveillance efforts (Bain et al., 2010; Cornwell et al., 2012) have shown that round gobies are disproportionately affected by VHSV.

In order to address these knowledge gaps regarding the dynamics of VHSV in round gobies, the objectives of this study were to: 1) determine the experimental susceptibility of wild captured round gobies to VHSV, 2) test whether the experimental susceptibility decreased with a history of prior exposure to VHSV, and 3) determine the short-term variation in VHSV prevalence and genetic diversity in wild round goby populations.

Materials and methods

Collection of fish for laboratory infection trials

Round gobies were collected from Youngstown (43° 15′ 37.1″ N, 79° 3′ 42.6″ W; median total length = 68 mm, range 43–146 mm; collected 13 June 2011) and Clayton (44° 14′ 36.6″ N, 76° 4′ 44.7″ W; median total length = 89 mm, range 62–162 mm; collected on 31 May 2011) New York via seine netting during the day. Approximately 200 fish were collected from each site. Fish were transported to Cornell University and acclimated to the laboratory in separate flow-through 700 L Living Stream tanks (Frigid Units, Toledo, Ohio) kept at 10 °C for at least two weeks prior to use. Fish were fed bloodworms once daily. The use of all animals in experimentation was performed under a protocol approved by the Institutional Animal Care and Use Committee of Cornell University. During acclimation, 36 fish from Youngstown died in the first four days. These fish all tested negative for VHSV and because no definitive cause of death could be determined at necropsy, capture stress was assumed to be the most likely cause. Because these 36 fish tested negative for VHSV and because round gobies have never tested positive for VHSV at this site despite extensive surveillance over the past four years, we assumed that all fish from Youngstown had likely not been exposed to VHSV (Bain et al., 2010; Cornwell et al., 2012). Sixty-seven fish from Clayton died during acclimation, all of which tested positive for VHSV and many showed signs of VHSV infection (multiple areas of dermal hemorrhage). These fish served as a confinement-induced laboratory epidemic for the genetic diversity study described below. The acclimation period for this tank was thus extended to two weeks past the last VHSV-infected mortality (maximum time from exposure to end of acclimation period 30 days; range 14–30 days) and it was assumed that all fish in this tank had been naturally exposed to VHSV. Direct testing for previous exposure using serology was not possible due to the small size of the fish used.

A subset of the fish collected from Youngstown (naïve fish) were randomly divided into 6 groups of 28 fish and exposed to VHSV genotype IVb isolate MI03 by intraperitoneal (IP) injection at doses of 10^6, 10^5, 10^4, 10^3, 10^2 plaque forming units (pfu) per fish, or with a sterile media control IP injection. A subset of the fish from Clayton (previously exposed fish) were randomly allocated into two groups of 20 fish each and exposed to VHSV isolate MI03 at 10^4 pfu per fish (re-exposure group) or a sterile media control (control for mortality due to previous exposure). Injection volumes for all groups were 0.1 mL per fish. Fish from each dose group were housed in separate tanks for 35 days and monitored twice daily for signs of infection. A plaque assay was performed to confirm the infectious dose. Any moribund fish or fish remaining at the end of the trial were euthanized using 500 ppm tricaine methanesulfonate (Western Chemical Inc., Ferndale, Washington), buffered 1:1 (weight:weight) with sodium bicarbonate (Sigma-Aldrich, St. Louis, Missouri).

Fish marking and sample collection

Round gobies were collected from two sites in Clayton (44° 14′ 36.6″ N, 76° 4′ 44.7″ W; Upper St. Lawrence Watershed, HUC 04150301) and Fair Haven (43° 20′ 40.8″ N, 76° 42′ 16.3″ W; Lake Ontario Watershed, HUC 04150200), NY every other week over 10 weeks (6 May 2011 to 8 July 2011). A HOBO temperature logger (Onset, Pocasset, Massachusetts) was deployed at each site to monitor water temperature every hour throughout the study. At each collection date, fish were collected by boat electrofishing during the day for a maximum of four hours. A fin and gill biopsy was taken from each fish and stored in 200 μL RNALater (Ambion, Carlsbad, California) on every collection date. On the last day of sampling at each site, all collected gobies were euthanized and a pooled sample of heart, liver, kidney, and spleen along with a separate sample of brain was collected in addition to a fin and gill biopsy and stored as described above to allow comparison of sensitivity between lethal and non-lethal sample types. To avoid contamination between samples, biopsy and necropsy instruments as well as the working surface were disinfected with a 10% solution of household bleach and dried between each fish (3–6% sodium hypochlorite, final solution approximately 10,000 mg/L active chlorine, contact time at least 30 s). Additionally, a new sterile scalpel blade was used to dissect each fish during necropsy at the final sampling point.

Virus testing

Fish from both laboratory trials and from field samples were tested for VHSV using the qRT-PCR assay described by Hope et al. (2010) with viral RNA and DNA extracted using a MagMax magnetic bead extraction (Life Technologies, Carlsbad, California) and a MagMax-96 viral isolation kit following the protocols described in the kit and the manufacturer's extraction program AM1836_DW_50_V2. RNA quantity and quality was measured using a NanoVue spectrophotometer (GE Healthcare, Piscataway, New Jersey). This qRT-PCR assay detects the Nucleoprotein (N) gene of VHSV. Every qRT-PCR plate contained a no template control and a series of positive control standards to generate a standard curve. Amplification was never observed in the no template control. Results were standardized to VHSV N gene copies per 50 ng RNA.

The fin, gill, brain, and a pooled visceral organ sample (liver, anterior and posterior kidney, spleen and heart) were tested in both laboratory trials. Fin and gill biopsies were tested for VHSV from all field fish and, for fish collected on the final day of sampling, a pooled visceral organ and brain sample were also tested for VHSV.