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Assessing the risk of diploid grass carp *Ctenopharyngodon idella* in the certified triploid supply chain in Ohio

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ABSTRACT

Non-native grass carp (*Ctenopharyngodon idella*) have been stocked in the United States for vegetation control since the 1970s, and recent evidence suggests some natural reproduction in the Great Lakes basin. Despite all states and provinces bordering Lake Erie either banning grass carp or requiring imports of only sterile, U.S. Fish and Wildlife Service (USFWS)-certified triploids, the majority of grass carp captured and analyzed from Lake Erie are diploid, or reproductively viable. Potential sources of diploid grass carp include illegal importation, compromises in the USFWS-certified triploid shipments, migration from legal diploid states, or natural reproduction resulting from diploid stockings prior to the 1988 Ohio law requiring only certified triploids. The goal of this study was to explore the risk that diploid grass carp occur in the USFWS-certified triploid supply chain destined for Ohio. During 2015 and 2016, undercover Ohio Department of Natural Resources–Division of Wildlife law enforcement purchased 1200 grass carp from 16 distributors, and overnighted 80 dissected grass carp head and eyeball sample shipments ($n = 15$ fish per shipment) for ploidy analysis by flow cytometry. Standardized methods for both field and laboratory processing were established. No diploid grass carp were detected in these collections, indicating fidelity of the USFWS-certified triploid grass carp supply chain in Ohio. Thus, these shipments are not a likely source of diploid grass carp in Lake Erie. This study is the first large-scale evaluation of the potential for ecological risk from diploid grass carp occurrence in USFWS-certified shipments of triploids for national distribution.

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Introduction

Of the four species of Asian carp considered an ecological threat to the Laurentian Great Lakes (Conover et al., 2007; Cudmore et al., 2012; Wittmann et al., 2014; Cudmore et al., 2017), only grass carp (*Ctenopharyngodon idella*) are encountered consistently in this system. Grass carp are most often encountered in Lake Erie and its major tributaries where >200 individuals have been reported since 2010 (USGS, 2018). Grass carp were introduced into the United States (Arkansas) in the 1960s to provide an inexpensive, more environmentally-friendly alternative to chemical aquatic vegetation control (Stevenson, 1965; Mitchell and Kelly, 2006). Stocking of diploid grass carp took place in lakes, ponds, and reservoirs throughout the 1970s, particularly in the Mississippi River Basin (Guillory and Gasaway, 1978; Mitchell and Kelly, 2006), thus they commonly occurred in the Mississippi and Missouri Rivers during this decade (Guillory and Gasaway, 1978;

Pflieger, 1978). Concerns over the potential for grass carp to reproduce in large rivers and tributaries prompted efforts in the 1970s and early 1980s to produce sterile fish for stocking (Mitchell and Kelly, 2006); triploid grass carp, considered sterile for management purposes (Nico et al., 2005), have been produced on a commercial scale since the mid-1980s (Malone, 1984). Currently, eight states in the U.S. still allow for the importation of diploids (Colorado allows diploids east of the Continental Divide), 12 states have completely banned grass carp importation, and 30 states allow the importation of only triploid grass carp (MICRA, 2015).

In the Laurentian Great Lakes, there is concern about the potential for grass carp establishment. Because of their consumption of aquatic vegetation, grass carp have the potential to undesirably impact water quality and habitat in the Great Lakes, including wetlands (Wittmann et al., 2014; Cudmore et al., 2017; van der Lee et al., 2017). Currently, all eight states bordering the Great Lakes, as well as Ontario, have either banned the commercial sale of grass carp entirely or allow only certified triploid fish. However, through January 2018, the majority of the ~112 grass carp captured and analyzed from Lake Erie and its tributaries have been diploid (over 70%; Wieringa et al., 2017; U.S. Fish and Wildlife Service (USFWS), personal communication) and grass carp eggs have

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been found in at least one Lake Erie tributary (Embke et al., 2016). While aging techniques, ploidy analysis, and otolith microchemistry indicated that four grass carp were naturally reproduced within the Lake Erie basin (Chapman et al., 2013), the origin of most of the diploids captured in the Lake Erie Basin remains uncertain.

One potential source of these diploids in Lake Erie could be compromises in the USFWS-certified triploid grass carp supply chain (from production, shipment, and sale; Conover et al., 2007; MICRA, 2015). As an assurance to the receiving states that shipments of triploid grass carp contain no diploids (within the confidence limits of the program), the USFWS began inspecting shipments of grass carp at the point of fish production in 1985 (National Triploid Grass Carp Inspection and Certification Program; NTGCICP), and the program was formalized by the 104th U.S. Congress in 1995 (Mitchell and Kelly, 2006; Zajicek et al., 2011). In order for a triploid grass carp producer to participate in the NTGCICP, a site inspection and Memorandum of Agreement with the USFWS are required. Certified producers are then required to follow strict program standards to ensure only triploid grass carp are shipped (MICRA, 2015; Glennon, 2014). As a requirement of the NTGCICP, prior to shipment, producers must test the ploidy of each individual grass carp in the batches induced for triploidy and remove any diploids discovered prior to USFWS inspection and certification (Zajicek et al., 2011; Glennon, 2014). Subsequently, a USFWS inspector then oversees the testing of 120 randomly selected fish from batches determined to be 100% triploid by the producer. If all tested fish are triploid, certificates are then issued for each scheduled shipment and the batch of triploid grass carp can be shipped to vendors within permitting states. These certificates accompany each shipment of certified triploid grass carp and shipments are subject to inspection by state law enforcement (MICRA, 2015). If any grass carp fails the NTGCICP inspection, producers must re-test every fish in that batch before another USFWS inspection will be conducted. In addition to the NTGCICP certificate accompanying the shipment, the appropriate regulatory agency in each receiving state is alerted to the incoming shipment by duplicate triploid certificates sent by the USFWS inspector. Shipments must occur within six days or the certificate expires and the batch must be re-inspected (MICRA, 2015). Nine commercial triploid grass carp producers located in five different states participate in the NTGCICP; however, nearly all triploid grass carp in the United States originate from four major Arkansas producers (MICRA, 2015). Not all commercial grass carp producers participate in the NTGCICP (MICRA, 2015), as participation is voluntary, but NTGCICP certification is required by many triploid-only states. The USFWS certifies >400,000 triploid grass carp annually (Mitchell and Kelly, 2006; MICRA, 2015).

Despite the meticulous operations of NTGCICP, some risks exist for diploids to enter the triploid grass carp supply chain. For instance, diploid grass carp may contaminate the chain at the point of production if they were not identified as diploids during the producer-level testing of the batch, and were not sampled during the USFWS inspection (MICRA, 2015). Additionally, many of the certified triploid grass carp are shipped from the participating producer facilities to distributors (vendors) in receiving states by third-party shippers (fish haulers), some of whom transport both diploid and certified triploid grass carp. Shippers and distributors have been identified as potential sources of diploid grass carp in the supply chain (MICRA, 2015). NTGCICP oversight ends with the issuance of the triploid certification to the producer and notification of the receiving state; the NTGCICP does not include standards for shippers or distributors of certified triploids. As diploids are available in the commercial supply chain due to some states allowing stocking of diploid grass carp, mixing of diploids and triploids, accidentally or deliberately, is possible (MICRA, 2015; Jenkins et al., in review). Adding to this risk, few shippers or distributors have written standard operating procedures or best management practices to prevent diploid contamination (MICRA, 2015).

Because the NTGCICP does not have a law enforcement component, inspection of certified grass carp shipments and enforcement of

regulations are dependent on the receiving states. Record checks of the documentation accompanying the shipments are sometimes conducted by destination states, but ploidy testing of USFWS-certified shipments is rarely performed (MICRA, 2015). Until now, no verifications have been published on whether diploid grass carp are being imported into states and sold to the public by way of the USFWS-certified triploid supply chain, despite the recognized need for such a study (Conover et al., 2007; MICRA, 2015). To investigate this, we evaluated this risk by targeting grass carp at the point of retail sale in Ohio. Through undercover law enforcement, NTGCICP-certified triploid grass carp were purchased from distributors selling grass carp directly to the public in Ohio. Eyeballs were excised from each fish and ploidy was determined using flow cytometry (Jenkins and Thomas, 2007; Jenkins et al., 2017; Jenkins et al., in review). As we purchased fish from the end of the supply chain, we evaluated the combined risks from the production and distribution components of the chain. In addition to providing a better understanding of the possible origin of diploid grass carp in Lake Erie and the Great Lakes Basin in general, this study provides the first large-scale evaluation of the fidelity of the USFWS-certified triploid supply chain at the point of retail sale in a receiving state.

Methods

Experimental design

The study design and scheduling was informed by the triploid certificates provided by the USFWS inspector to the Ohio Department of Natural Resources (ODNR). The notification identifies the grass carp distributor receiving the shipment, the number of certified triploid individuals shipped, and the approximate shipment date. These notifications are typically received 3–5 days prior to the expected shipment date. According to historical NTGCICP notifications, approximately 15–20 distributors in Ohio import and sell triploid grass carp annually to the public (ODNR, unpublished data). Most of these distributors are fish propagators that provide pond fish to the public for stocking. Ohio produces no triploid grass carp; all triploid grass carp sold in Ohio are produced in and imported from other states. These grass carp are imported at a size that is appropriate for stocking in private ponds (150–400 mm; ODNR, unpublished data). After being imported into Ohio (typically via third-party shipper or transported by the distributor themselves), grass carp are held on site at the Ohio distributor's facility until being sold directly to the public. Some distributors sell grass carp off a truck at advertised fish sales. After being sold, the pond-owner then transports the fish via a bucket or bag to their pond for stocking. The distributor is not required to stock or supervise the stocking of the grass carp in the pond, though some distributors will deliver fish to pond owners. Pond owners are not required to obtain a permit in Ohio.

Based on their annual average number of grass carp imported and the site location of their operation, distributors were divided into three strata according to 2011–2013 historical data. Large distributors (LD) were those whose base of operations is in Ohio, and whose average annual imports exceeded 1000 grass carp. Small distributors (SD) were based in Ohio but imported fewer than 1000 grass carp. The out-of-state distributors (OD) were not located in Ohio, but they brought grass carp into Ohio to sell to the public directly off a truck at various locations throughout the state (Table 1). During 2011–2013, LDs and SDs imported approximately 33,000 (~76% of Ohio total) and 4800 (~11%) certified triploid grass carp per year, respectively. The ODs imported approximately 5600 (~13%) grass carp per year. Overall, for each of the years from 2011 to 2013 across the three strata, slightly >43,000 certified triploid grass carp were imported into Ohio annually.

For this study, 600 grass carp per year were purchased from distributors and were tested for ploidy during both 2015 and 2016. We obtained 450 grass carp from LDs, 75 grass carp from SDs, and 75 grass carp from ODs in 2015. Thus, in 2015, ploidy was determined from approximately 1.4% of fish imported by LDs, 1.5% imported by SDs, and

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