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Ecological Risk Assessment of Grass Carp (*Ctenopharyngodon idella*) for the Great Lakes Basin

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

Research documents are produced in the official language in which they are provided to the Secretariat.

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TABLE OF CONTENTS

ABSTRACT

A binational ecological risk assessment was conducted to determine the extent of the risk of Grass Carp (*Ctenopharyngodon idella*) to the Great Lakes basin and to provide useful, scientifically defensible advice on prevention, monitoring, early detection, and potential management actions for managers and decision-makers in Canada and the United States. This risk assessment covered both triploid and diploid Grass Carp. It assessed the probability of occurrence (likelihood of arrival, survival, and spread) for triploid Grass Carp, and the probability of introduction (likelihood of arrival, survival, establishment and spread) for diploid Grass Carp, as well as the potential magnitude of ecological consequences within 5, 10, 20, and 50 years from 2014 (i.e., the baseline year). Arrival routes assessed were physical connections, human-mediated release (bait use, trade and stocking) and laker ballast. The most likely pathway of arrival for triploid and diploid Grass Carp into the Great Lakes basin was considered to be through the category of physical connections, specifically the Chicago-Area Waterway System into Lake Michigan. However, it is important to note that Grass Carp (both triploid and diploid) has already arrived to lakes Michigan and Erie. Based on thermal tolerance, food availability, predation, and pathogens and diseases, juvenile and adult Grass Carp will survive in the Great Lakes. Results of this risk assessment show that conditions exist to support establishment (e.g., suitable spawning and nursery habitat, potential for positive population growth, overwinter survival of early life stages) of diploid Grass Carp, and that establishment is very likely to occur within 10 years for lakes Erie, Michigan, Huron, and Ontario. However, establishment at northern latitudes of Lake Superior is less certain based on limited overwinter survival of young-of-year and ability to reach maturity. While no impediments to spread exist among the lakes, spread is of greatest concern to the other Great Lakes in the basin based on the arrival of Grass Carp in lakes Michigan and Erie; with movement from Lake Michigan to Lake Huron and from Lake Erie to Lake Ontario expected within 10 years. Should diploid Grass Carp become established, submerged aquatic vegetation will decrease or change in species assemblage, which may lead to consequences to other elements of the biotic community (e.g., birds, fishes) and abiotic environment (e.g., turbidity, nutrient cycling). These effects may be greater within localized wetlands if Grass Carp aggregate in these areas. Overall risk for triploid Grass Carp ranges from low to medium for all years and lakes. For diploid Grass Carp, overall risk is highest for lakes Michigan, Huron and Erie, followed by Lake Ontario and Lake Superior.

Évaluation du risque écotoxicologique posé par la carpe de roseau (*Ctenopharyngodon idella)* dans le bassin des Grands Lacs

RÉSUMÉ

Une évaluation binationale des risques écologiques a été menée afin de déterminer l'étendue du risque posé par la carpe de roseau (*Ctenopharyngodon idella*) dans le bassin des Grands Lacs et de fournir un avis utile et scientifiquement défendable sur la prévention, la surveillance, la détection précoce et les mesures de gestion possibles à l'intention des gestionnaires et des décideurs du Canada et des États-Unis. Cette évaluation des risques visait les carpes de roseau diploïdes et triploïdes. On a évalué la probabilité d'occurrence (probabilité de l'arrivée, de la survie et de la propagation) de la carpe de roseau triploïde, la probabilité d'introduction (probabilité de l'arrivée, de la survie, de l'établissement et de la propagation) de la carpe de roseau diploïde, ainsi que l'ampleur potentielle des conséquences écologiques dans 5, 10, 20 et 50 ans à compter de 2014 (l'année de référence). Les voies d'arrivée étudiées sont les liens physiques, l'introduction liée aux activités humaines (utilisation d'appâts vivants, commerce et empoissonnement) et le ballast de cargos hors mer. On a conclu que les liens physiques, principalement le Chicago Area Waterway System vers le lac Michigan, constituaient la voie d'arrivée la plus probable des carpes de roseau diploïdes et triploïdes dans le bassin des Grands Lacs. Cependant, il est important de noter que la carpe de roseau (triploïde et diploïde) est déjà présente dans les lacs Michigan et Érié. D'après les données sur la tolérance thermique, la disponibilité de la nourriture, la prédation, les agents pathogènes et les maladies, les carpes de roseau juvéniles et adultes survivront dans le bassin des Grands Lacs. Les résultats de cette évaluation montrent que les bonnes conditions sont présentes pour favoriser l'établissement (p. ex., habitat de frai et d'alevinage propices, potentiel de croissance positive de la population, taux de survie à l'hiver lors des premiers stades du cycle de vie) de la carpe de roseau diploïde, et qu'elle est très susceptible de s'établir au cours des dix prochaines années dans les lacs Érié, Michigan, Huron et Ontario. Toutefois, l'établissement aux latitudes nordiques du lac Supérieur est moins certain, d'après la survie hivernale limitée des jeunes de l'année et leur capacité d'atteindre la maturité. Comme il n'y a aucun obstacle à la propagation entre les lacs, la propagation constitue le risque le plus important pour les autres Grands Lacs dans le bassin, en fonction de l'arrivée de la carpe de roseau dans les lacs Michigan et Érié; et un déplacement du lac Michigan vers le lac Huron, et du lac Érié vers le lac Ontario, est attendu au cours des dix prochaines années. Si la carpe de roseau diploïde s'établit, la végétation aquatique submergée diminuera ou la communauté d'espèces sera modifiée, ce qui peut avoir des répercussions sur d'autres éléments de la communauté biotique (p. ex., oiseaux, poissons) et sur le milieu abiotique (p. ex., turbidité, cycle des éléments nutritifs). Ces effets peuvent être plus importants dans certaines zones humides si la carpe de roseau s'y regroupe. Le risque global associé à la carpe de roseau triploïde varie de faible à moyen pour toutes les années et tous les lacs. En ce qui concerne la carpe de roseau diploïde, le risque global est le plus élevé pour les lacs Michigan, Huron et Érié, suivi des lacs Ontario et Supérieur.

1.0 INTRODUCTION

The establishment of non-native aquatic species can have undesirable, sometimes substantial, consequences in the invaded ecosystem (Moyle and Light 1996, Rahel 2002, Ricciardi and MacIsaac 2011, Simberloff et al. 2013), leading to considerable challenges for resource managers. Non-native species can cause severe reduction or extirpation of native species, reduction in the abundance or productivity of sport, commercial, or culturally important species, trophic alteration, and can result in substantial habitat alteration (Rahel 2002, Dextrase and Mandrak 2006, Jelks et al. 2008, Mandrak and Cudmore 2010). Consequently, these invasive (i.e., non-native species that cause environmental or economic harm or harm to human health) species are considered a threat to aquatic biodiversity at the same level as habitat loss and alteration (Light and Marchetti 2007, Pyšek and Richardson 2010, Pimentel 2011).

The Great Lakes have not been immune to the arrival of aquatic invasive species (AIS). As of 2015, there are over 180 non-native species reported in the Great Lakes basin (GLANSIS 2015). At least 69 non-native fish species have been introduced to the Great Lakes, half of which are considered established (Mandrak and Cudmore 2010). The invasion of destructive AIS (e.g., Sea Lamprey (*Petromyzon marinus*)) into the Great Lakes, and the resulting necessity for intensive management activities and associated costs, has promoted movement towards management strategies that now focus on the prevention of establishment by new AIS (Ricciardi et al. 2011).

A responsibility of Fisheries and Oceans Canada's (DFO) is to identify potential aquatic invaders to all parts of Canada, assess their ecological risk, and provide science advice towards preventing the introduction of those species considered to be high risk. Risk assessments generate such science advice for informed decision making to prevent potential invasions, or better understand new invasions by predicting the range, and/or impact of potential invaders (Kolar 2004, Mandrak and Cudmore 2015). Asian carps, which refers collectively to Grass Carp (*Ctenopharyngodon idella*), Bighead Carp *(Hypophthalmichthys nobilis*), Silver Carp (*H. molitrix*), and Black Carp (*Mylopharyngodon piceus*), have been identified by Mandrak and Cudmore (2004), Nico et al. (2005), Conover et al. (2007), Kolar et al. (2007), Chapman and Hoff (2011), and Cudmore and Mandrak (2011), as species that threaten to invade the Great Lakes basin.

Our (DFO, U.S. Geological Survey (USGS), U.S. Fish and Wildlife Service (USFWS), and the Great Lakes Fishery Commission (GLFC)) first efforts to characterize the risk of Asian carps to the Great Lakes basin was the Binational Ecological Risk Assessment of Bigheaded Carps in the Great Lakes Basin for Bighead and Silver carps in 2011 (Cudmore et al. 2012). These two species were assessed first following recommendations of priorities of Great Lakes managers and decision makers on both sides of the border. However, the threat of Grass Carp invasion to the Great Lakes has continued to increase, with occurrence records located in close proximity to the Great Lakes basin and some even within the basin (see Figure 1 for current distribution). Recent analyses of otolith (i.e. structure of the inner ear) microchemistry and ploidy (i.e., number of sets of chromosomes in a cell) have provided evidence of natural recruitment of Grass Carp in the Sandusky River, a tributary to the U.S. waters of Lake Erie (Chapman et al. 2013), as well as in other Lake Erie tributaries (Whitledge 2014). These findings have contributed to the urgency to better understand the current status and threat of Grass Carp in and to the Great Lakes basin.

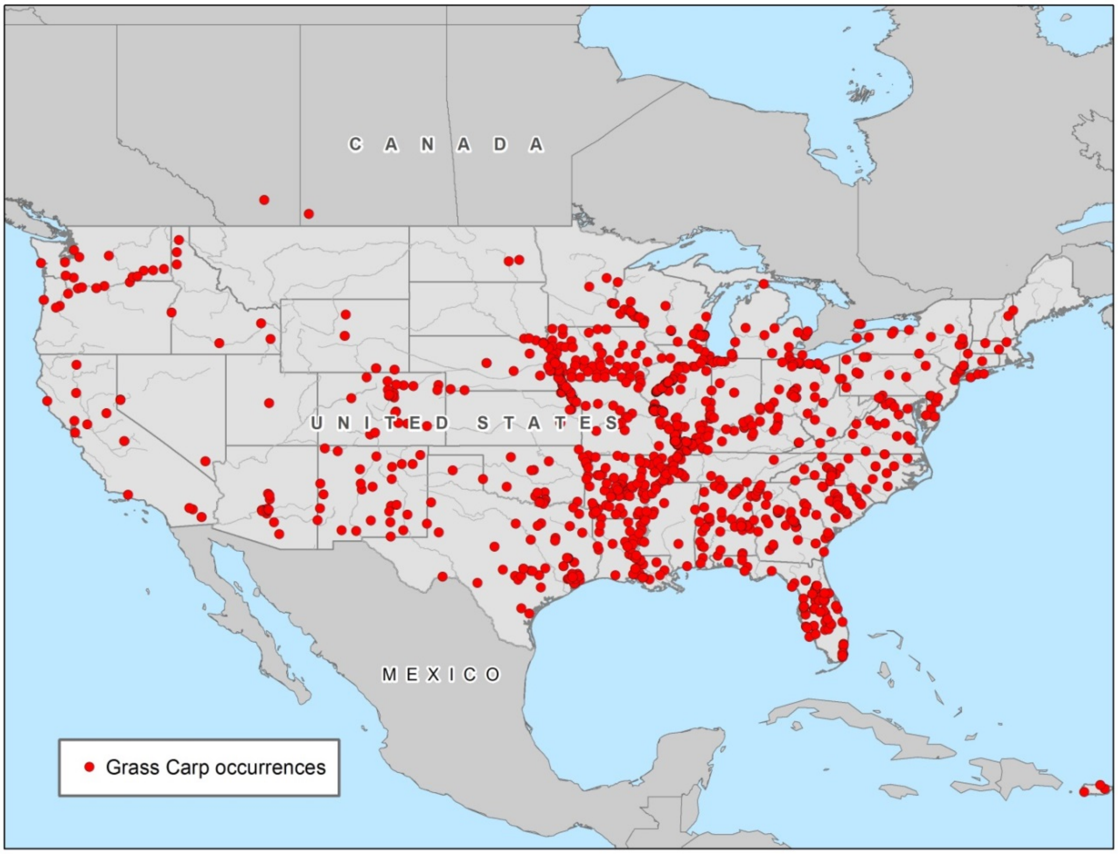


Figure 1. Non-native occurrences of Grass Carp in the U.S. and Canada (1968–2015) as reported in the [USGS Nonindigenous Aquatic Species (NAS) database](). Map courtesy of the USGS.

Grass Carp is a sub-tropical to temperate species native to the large rivers of eastern Asia, where it tends to inhabit lower and middle reaches of these rivers and connected lacustrine habitats. Its range extends across latitudes 25–65°N and from coastal waters inland. The preferred diet of Grass Carp is submerged leafy macrophytes (Bain et al. 1990, Pine and Anderson 1991), but it will also consume filamentous algae and other aquatic and terrestrial macrophytes (Opuszynski and Shireman 1995), invertebrates, and small fishes (Laird and Page 1996, Froese and Pauly 2015). Additional details about Grass Carp life history in its native range and in North America can be found in Cudmore and Mandrak (2004), Conover et al. (2007), Bogutskaya et al. (2017), Jones et al. (2017a) and Zhao and Wang (in prep.).

Grass Carp was originally brought to North America in 1963 through a joint initiative of the United Nations Food and Agriculture Organization, the USFWS, and Auburn University to evaluate their potential for biological control of aquatic vegetation. These fish successfully spawned in 1966 and some offspring are thought to have escaped from experimental enclosures (Mitchell and Kelly 2006). Feral Grass Carp were captured from the White River, Arkansas in 1970 and from the Illinois portion of the Mississippi River in 1971; these fish were aged to the 1966 year class. Private fish hatcheries in the U.S. began marketing Grass Carp for aquatic vegetation control in 1972 (Mitchell and Kelly 2006). By the late 1970s, concern about the ability of Grass Carp to reproduce in large rivers led many states to ban the use of reproductively viable (diploid) Grass Carp (Leslie et al. 1996), but stocking of diploids remains legal in some states. Numerous Grass Carp captures have since occurred in the Great Lakes basin. The first was collected from the Lake Erie basin, Michigan, in the early 1980s (Lee et al.

1980; Courtenay et al. 1984) and Grass Carp was first observed in the Canadian (Ontario) waters in 1985 (Lake Erie, west of Point Pelee; Cudmore and Mandrak 2004).

An earlier risk assessment of Asian carps in Canada, which included Grass Carp, identified broad potential risks to Canada, including the Great Lakes (Mandrak and Cudmore 2004). While this risk assessment provided insight into the risk faced by broad areas of Canada, knowledge gaps were identified. Given this, and the recent discovery of Grass Carp recruitment in Lake Erie, a binational ecological risk assessment of Grass Carp was proposed by DFO and the GLFC to the Asian Carp Regional Coordinating Committee (ACRCC) and was endorsed in early 2014. The overall purpose of this ecological risk assessment of Grass Carp is to determine the risk to the Great Lakes basin and to provide useful, scientifically defensible advice on prevention, monitoring, early detection, and management actions that are underway or could be taken.

1.1 SCOPE OF RISK ASSESSMENT

The scope of this ecological risk assessment was informed by workshop participants consisting of Great Lakes researchers, managers, and decision makers. The risk assessment considers the available information on Grass Carp to assess the likelihood of arrival, survival, establishment, and spread, as well as the magnitude of the ecological consequences within 5, 10, 20 and 50 years from 2014 (i.e., the baseline year) to the connected Great Lakes basin. The connected Great Lakes basin is defined as the Great Lakes *and* its tributaries to the first impassable barrier (Figure 2); Lake St. Clair is considered to be part of the Lake Erie basin. The geographic scope of the basin for this risk assessment was based on Neeson et al. (2015) (Figure 2). Neeson et al. (2015) evaluated the probability of migratory fish passage through tributaries across the Great Lakes basin. For this risk assessment, tributaries were deemed impassible if the probability of fish passage was 0 (red areas on Figure 2) while tributaries with probability of fish passage greater than 0 (blue and yellow areas contiguous with the Great Lakes on Figure 2) were deemed passable. The Chicago Area Waterway System (CAWS) represents a unique set of conditions where the primary flow is away from, rather than towards, the lakes; therefore, we interpreted the extent of the study area for this risk assessment to end at the Chicago Lock and O'Brien Lock and Dam, and the mouths of the Calumet and Little Calumet Rivers. The authors recognize that the use of Neeson et al. (2015) has some limitations; for example, it is missing information for large portions of the Canadian Great Lakes basin and also identified no probability of passage for canals with known passability (e.g. Trent-Severn Canal). However, these data represent the best known available information to define the Great Lakes basin to the first impassable barrier (not including the CAWS) as defined for this risk assessment.

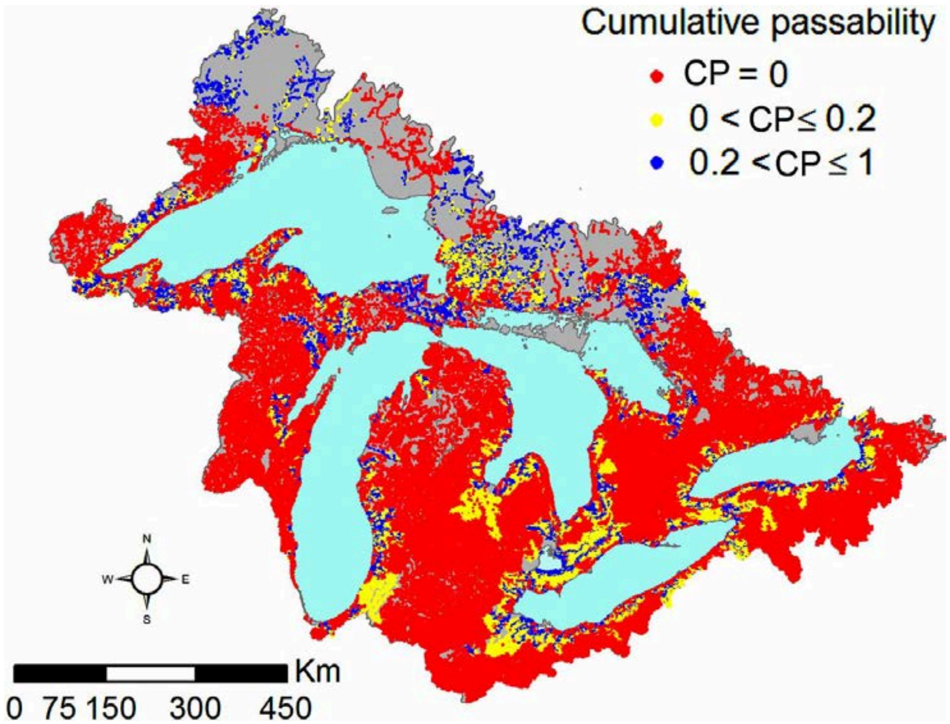


Figure 2. The cumulative passability (CP) of 6,692 dams and 232,068 road crossings in the Great Lakes basin. Nearly 87% of the total river channel length is at least partially inaccessible to adfluvial fishes (CP < 1), including 64% that is entirely inaccessible (CP = 0) (Neeson et al. 2015). Grey background represents areas without barriers or lacking barrier data. For the purposes of this risk assessment, the connected Great Lakes basin is defined as the Great Lakes and its tributaries up to the first impassable barrier (i.e., where yellow or blue changes to red). To address the unique circumstance of the Chicago Area Waterway System, the extent of the Great Lakes basin for this risk assessment ends at the Chicago Lock and O'Brien Lock and Dam, and the mouths of the Grand Calumet and Little Calumet Rivers. From Neeson et al. (2015).

Although the risk assessment targets the Great Lakes basin as a whole, to accommodate resource managers who wished to better understand the risk to a particular lake, the risk assessment also takes into account each Great Lake separately, where appropriate. This risk assessment does not address a finer geographic scale, such as ranks within a particular bay or lake sub-region.

Different life stages of Grass Carp are considered, where data are available and where appropriate, but a life-stage-specific risk assessment is beyond the scope of this risk assessment. There are two ploidies of Grass Carp found in North America: functionally sterile triploids (with three sets of chromosomes), and fertile diploids (with two sets of chromosomes). Sterile triploid Grass Carp were developed to address concerns regarding the potential for fertile diploid Grass Carp to develop self-sustaining populations in water systems where this was undesirable (Allen and Wattendorf 1987, Zajicek et al. 2011). The use of triploids allows for biological control of macrophytes, with minimal risk of reproduction (Zajicek et al. 2011). For the purposes of this risk assessment, fish that have failed triploid induction are considered as diploid fishes. For details on the technology (and failure rate) and development of triploids see Cassani and Caton (1986), Allen and Wattendorf (1987), Papoulias et al. (2011) and Zajicek et al. (2011). Where appropriate, this risk assessment takes into account differences between triploid and diploid Grass Carp.

Black Carp also poses a concern for the Great Lakes (Cudmore and Mandrak 2004, Nico et al. 2005, Cudmore and Mandrak 2011, Nico and Jelks 2011) and was identified by managers during a scoping meeting as another species of Asian carp requiring a risk assessment; however, due to resource and time limitations, it was determined that the scope of this risk assessment would focus on the Great Lakes managers' highest priority of the two species, and a Black Carp ecological risk assessment would be conducted separately at a later date.

This Grass Carp ecological risk assessment focuses only on ecological consequences; socioeconomic consequences will be assessed separately using the results of this ecological risk assessment. It also addresses only the current state of the system and management measures that were in place during the scoping of the risk assessment (baseline year = 2014). These management measures include, but are not limited to: operation of the electric dispersal barriers in the Chicago Sanitary and Ship Canal (CSSC); implementation of the USFWS National Triploid Grass Carp Certification and Inspection Program (NTGCICP); and, fish removal as part of the ACRCC Monitoring and Response Workgroup (MRWG) barrier defense program. It does not assess the effectiveness of any measures currently in place, nor the level of risks associated with any potential management measures that are not currently in place.

Targeted management questions were obtained from Great Lakes managers and decision makers at the outset of, and mid-way through, the risk assessment process. This was done to ensure the risk assessment provides the most useful advice possible to address the needs of managers and decision makers throughout the Great Lakes basin.

1.2 THE RISK ASSESSMENT PROCESS

The format of this binational ecological risk assessment for Grass Carp in the Great Lakes basin follows guidance provided in the National Detailed-Level Risk Assessment Guidelines: Assessing the Biological Risk of Aquatic Invasive Species in Canada (Mandrak et al. 2012). This process serves to summarize the best available information and identify the relative risks posed to a specified area within a specified timeframe by a non-native species. Risk assessments provide a framework for organizing and reviewing relevant information to provide scientifically defensible advice to managers and decision makers.

As a first step in conducting an ecological risk assessment, the known biological information of the species is compiled into biological synopses. New references since the publication of the biological synopsis by Cudmore and Mandrak (2004) were compiled in a new report (Jones et al. 2017a); both documents relied heavily on available English literature. Bogutskaya et al. (2017) annotated the available Russian language literature on Grass Carp, while Zhao and Wang (in prep.) summarized information from the Chinese language literature. All these documents were used as background information on Grass Carp biology for this risk assessment.

Other research documents were developed to support the risk assessment and include bioenergetics, overwinter survival, and population growth models (Jones et al. 2017b); analyses of spawning suitability of Canadian tributaries (Mandrak et al. in prep.); assessment of potential spread between lake basins via locks and dams, and spread modelling (Currie et al. 2017); and impact modelling of Grass Carp on vegetation as well as an assessment of the potential impacts on fishes and birds in the Great Lakes basin (Gertzen et al. 2017). Primary literature and publicly available reports were also used. In some cases, personal communication, personal observation, and other draft information supplied to the risk assessment authors, were used. This was done to use as much up-to-date, if not yet published, information as possible to inform the risk assessment. ***Researchers who provided draft information or personal communication/observation/data retain the intellectual property of that work and their***

information should not be cited outside of the risk assessment work presented here. Permission for use was provided for this process and product only.

Workshops were held in June and December 2014 to develop the scope of the risk assessment, obtain management questions, and to understand the research occurring on both sides of the border that could provide input into the risk assessment. From these, research needs were identified and feasibility was assessed given resources and timeframe. Development of the aforementioned research documents are a direct result of this process.

After the risk assessment parameters were scoped, the definitions for likelihood categories (Table 1), certainty of data categories (Table 2), and ecological consequence ratings (Table 3), as used in this risk assessment, were agreed upon by the authors following: guidance provided in Mandrak et al. (2012); the use in the previous risk assessment for bigheaded carps (Cudmore et al. 2012) to maintain consistency; and, input from the peer-review meeting. To ensure clarity, abbreviations for likelihood rankings and certainty levels were distinguished with two letters where necessary.

Ecological consequence ratings were based on predicted decreases in submerged aquatic vegetation (SAV) area due to increasing Grass Carp densities (Table 3). The ratings were evaluated separately for each lake based on average Grass Carp densities across the vegetated lake area, the SAV area currently in each lake (Gertzen et al. 2017), and recommended stocking densities for controlling SAV (Lynch 2009). It is assumed that SAV loss is an ecosystem change that would likely have substantial physical and ecological effects, including changes in species composition, especially negative effects on species dependent upon SAV (e.g., many fishes, waterfowl) that, in turn, would result in ecosystem changes. If change in SAV composition, or loss, is not detectable, the consequence would be negligible; if a change in SAV composition is detectable but <10% decrease in abundance, the consequence would be low; if 10–24% decrease, likely occurring at 5 Grass Carp per hectare, the consequence would be moderate; if 25–49% decrease, likely occurring at 10 Grass Carp per hectare, the consequence would be high; and, if >50% decrease, likely occurring at >15 Grass Carp per hectare, the consequence would be extreme (Table 3).

Table 1. Likelihood as probability categories

Likelihood	**Probability Category**
Very Unlikely (VU)	0.00–0.05
Low (Lo)	0.05–0.40
Moderate (M)	0.40–0.60
High (H)	0.60–0.95
Very Likely (VLi)	0.95–1.00

Table 2. Relative certainty categories reflecting the quality and quantity of data.

% Level	Certainty Category
± 90%	Very low certainty (VLo) (e.g., little to no information to guide assessment)
± 70%	Low certainty (Lo) (e.g., based on ecological principles, life histories of similar species, or experiments)
± 50%	Moderate certainty (M) (e.g., inference from knowledge of the species)
± 30%	High certainty (H) (e.g., primarily peer-reviewed information)
± 10%	Very high certainty (VH) (e.g., extensive, peer-reviewed information)

Table 3. Description of ecological consequence ratings and associated consequence thresholds (listed below each description in brackets) based on Ohio State University (OSU) recommended Grass Carp stocking densities to control different percentages of cover of preferred submerged aquatic plants in small ponds or lakes (< 5 acres) in Ohio (Lynch 2009).

Consequence Rating	Description
Negligible (N)	Undetectable changes in the structure or function of the ecosystem. *(No detectable change in composition of submerged aquatic vegetation)*
Low (Lo)	Minimally detectable changes in the structure of the ecosystem, but small enough that it would not change the functional relationships or survival of species. *(Detectable change in composition of SAV through to a <10 % decrease in vegetation)*
Moderate (M)	Detectable changes in the structure or function of the ecosystem. *(10–24% decrease in SAV at 5 Grass Carp per hectare)*
High (H)	Significant changes to the structure or function of the ecosystem leading to changes in the abundance of resident species and generation of a new food web. *(25–49% decrease in SAV at 10 Grass Carp per hectare)*
Extreme (E)	Restructuring of the ecosystem leading to severe changes in abundance of ecologically important species (those considered dominant or main drivers in the ecosystem) and significant modification of the ecosystem. *(>50% decrease in SAV at 15 Grass Carp per hectare)*

Following a similar approach to Mandrak et al. (2012), the risk assessment process was divided into two steps:

1) estimating the probability of occurrence for triploids (using likelihood of arrival, survival, and spread) or probability of introduction for diploids (using likelihood of arrival, survival, establishment, and spread); and,
2) determining the magnitude of ecological consequences if triploid Grass Carp was to occur or if diploid Grass Carp was introduced.

The evaluation of the probability of occurrence, probability of introduction, and the magnitude of the ecological consequences are based on qualitative scales (see Tables 1 and 3, respectively), and includes a corresponding ranking of certainty of data (see Table 2). For triploid Grass Carp, the overall probability of occurrence was determined for each Great Lake by taking the highest ranking between overall arrival and spread, then comparing this rank with the rank of survival, and using the lowest rank of the two. The formula was modified from that presented in Mandrak et al. (2012) to remove the element of establishment, because triploid Grass Carp are functionally sterile and considered unable to form a self-sustaining reproducing population. This is represented by the following formula:

Probability of Occurrence = Min [(Max (Arrival, Spread)), Survival]

For diploid Grass Carp, the overall probability of introduction was determined for each Great Lake by taking the highest ranking between overall arrival and spread, then comparing this rank with the ranks of survival and establishment, and using the lowest rank of the three.

This is represented by the following formula:

Probability of Introduction = Min [(Max (Arrival, Spread)), Survival, Establishment]

If either triploid or diploid Grass Carp was considered to have already arrived to a lake basin this was denoted with an asterisk in the ranking table of the overall arrival for that Great Lake.

For triploid and diploid Grass Carp, the certainty of data associated with the highest rank for overall arrival and spread was retained for Max (Arrival, Spread) or, if both elements were ranked the same, the lowest certainty associated with the tied rank was used. Certainty of data for the probability of occurrence was taken as the certainty associated with the lowest ranked element of the formula or, if both elements ranked the same, then the lowest certainty of the tied rank was used. Certainty of data for the probability of introduction was taken as the certainty associated with the lowest ranked element of the formula or, if two or more elements ranked the same, then the lowest certainty of the tied rank was used.

The magnitude of ecological consequences was based on consequence thresholds associated with estimated population sizes, the area of submerged aquatic vegetation (SAV) in each lake, and recommended stocking densities for controlling SAV (Lynch 2009). See Section 3.6 (Summary of Magnitude of Ecological Consequences) for a full explanation.

The probability of occurrence or probability of introduction and the magnitude of ecological consequences were then combined into a risk matrix to obtain an overall risk for each of triploid and diploid Grass Carp (see Figure 3 for a schematic of a species-specific overall risk assessment example). The ellipse in Figure 3 graphically illustrates the amount of certainty associated with the point (lower certainties have broader units), with the level of certainty for the probability of occurrence or introduction plotted as the height and the level of certainty for ecological consequences plotted as the width.

Each lake was assessed for four different time periods, within 5, 10, 20, and 50 years of the baseline (i.e., 2014), to show any changes in the probability of occurrence or introduction and ecological consequences over these time periods. Therefore, four overall risk matrices are presented for each of triploid and diploid Grass Carp at the end of the risk assessment (Section 4.0), one for each of the four time periods.

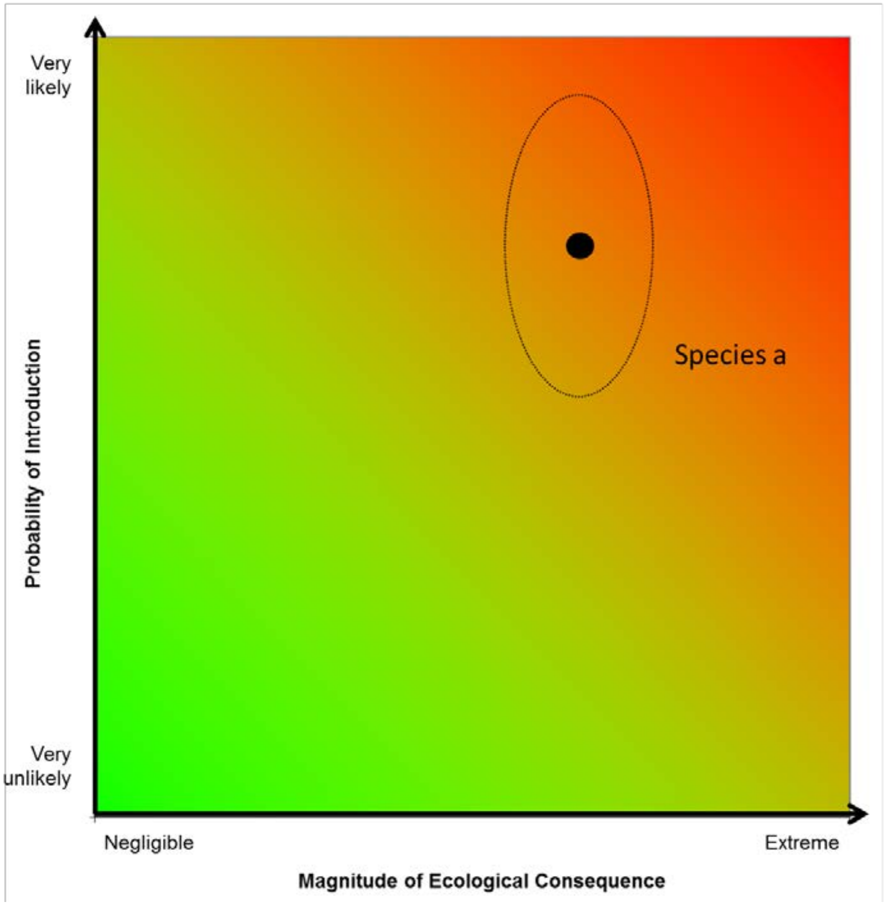


Figure 3. Schematic of a graphic representation to communicate the overall risk of a species. Matrix combined probability of introduction (High) and magnitude of ecological consequence (High) to demonstrate overall risk (High) for a species-specific risk assessment. Green = Low Risk; Yellow = Medium Risk; Orange = High Risk; Red = Extreme Risk. Relative size of ellipse denotes certainty of data. Modified from Mandrak et al. (2012).

A draft of this risk assessment document was presented to invited experts who attended a peer-review meeting June 1–3, 2015. Participants of the peer-review meeting included the authors of the risk assessment, invasive fish and/or carp experts, and experts in invasive species modelling. Participants were invited for their expertise, not to represent an agency. Some participants, who had not been strongly engaged in the scoping of the risk assessment, were also included to maximize objectivity in the process. The peer-review process followed the guidelines set out by DFOs Canadian Science Advisory Secretariat (CSAS) and met the requirements of the USGS Fundamental Science Practices. Proceedings of the peer-review meeting (DFO 2017a) and a science advisory report (DFO 2017b) have been completed in support of this risk assessment. The risk assessment document contains the body of information used to develop the overall risk, across all risk assessment elements, by consensus of the peer-review participants and, ultimately, the authors. It is the definitive science document of this process and includes science advice. The science advisory report is essentially an executive summary of the risk assessment coupled with science advice and may be most appropriate for those who do not wish to read all the details in the risk assessment (DFO 2017b). The proceedings document serves as a record of the discussions and decisions at the peer-review meeting, as well as a reconciliation of differences from the peer-review meeting and the author decisions (DFO 2017a). For further information and details about this peer-review, science advisory process, see the [Canadian Science Advisory Secretariat Website](#).

The risk assessment rankings (described above) are the product of consensus stemming from several steps. First, after reviewing the draft risk assessment research document, each author developed her/his own risk assessment tables for each element for each lake (presented in the proceedings document; DFO 2017a). The likelihood of each element along with the estimated certainty of data was then thoroughly discussed among the authors to reach consensus. This consensus output was presented at the peer-review meeting and was subsequently discussed, modified, and finalized by the authors with consensus input stemming from the peer-review meeting.

2.0 PROBABILITY OF OCCURRENCE/INTRODUCTION

To determine the probability of Grass Carp occurrence (triploid) in the Great Lakes basin, information was used related to the likelihood of arrival (Section 2.1), survival (Section 2.2), and spread (Section 2.4). For diploid Grass Carp, the probability of introduction to the Great Lakes basin was determined using information related to the likelihood of arrival (Section 2.1), survival (Section 2.2), establishment (Section 2.3), and spread (between lakes, Section 2.4). The probability of occurrence and introduction is expected to vary among lakes, therefore, where appropriate and when information was available, we considered lakes individually. Where appropriate, differences between triploids and diploids are outlined.

2.1 LIKELIHOOD OF ARRIVAL

Arrival of a non-native species to a region occurs through various pathways (i.e., the route between the source region of a non-native species and its location of release) and vectors (i.e., the manner in which a species is carried along a pathway) and, inherently, implies transit survival. Potential entry routes for Grass Carp into the Great Lakes basin were identified and assessed where information was available. Entry pathways and vectors discussed in this section are physical connections (canals and waterways, and intermittent or occasional connections around the watershed boundaries; Section 2.1.1), human-mediated release (bait use, trade, stocking of private waters; Section 2.1.2), and ballast water (Section 2.1.3). The likelihood of arrival was evaluated for each Great Lake using the available information for the identified pathways and vectors for that lake. Grass Carp already captured within the Great Lakes basin are used to inform the likelihood of arrival through the various vectors and pathways, but are themselves not directly evaluated in the ranking assessment. Arrival for a given lake was considered to be the repeated detection of at least one Grass Carp in at least one part of the lake basin within any continuous five-year period. The likelihood of Grass Carp entering one Great Lake from another Great Lake is not assessed in the Likelihood of Arrival section but rather in the Likelihood of Spread section (Section 2.4), which assesses the movement of individuals or expanding populations to one or more of the Great Lakes (inter-lake movement). We considered the likelihood of arrival separately for triploid and diploid Grass Carp.

2.1.1 Physical Connections

The likelihood of arrival of Grass Carp into the Great Lakes basin by dispersal through physical connections was assessed on a lake-by-lake basis. This section integrates information on: the collection of Grass Carp within close proximity to and within the basin, with ploidy status where available (Figure 4); the results of environmental DNA (eDNA) monitoring; state and provincial regulations regarding Grass Carp in the Great Lakes and adjacent states and provinces (Table

4); locations of Grass Carp producers and distributors (Figure 5), as well as the potential hydrologic connections between the Great Lakes basin and adjacent watersheds (Figure 6).

eDNA is dissolved DNA and/or fragments of tissue containing DNA that remain suspended and detectable in the water column for extended periods, ranging from days to weeks (Mahon et al. 2013). eDNA has been used as an early detection surveillance tool for the presence of Asian carp eDNA since 2009 with testing for species-specific DNA fragments possible; a positive detection tells researchers that Asian carp genetic material is present in the area (but not necessarily a live fish) and, thus, may be a good place to use other sampling tools to look for signs of live Asian carp (ACRCC 2015). It is important to note that the rate of false positives (detecting eDNA when not present) of eDNA for Bighead and Silver carp markers is at or near zero (US EPA 2010, ACRCC 2014a), but eDNA can degrade quickly and false negatives (no indication species is present) for both eDNA sampling and traditional capture methods may be high (see Darling and Mahon 2011, Jerde et al. 2011). However, Grass Carp qPCR markers have been developed, reducing the potential for inhibition that would cause false negatives (Mahon et al. 2013).

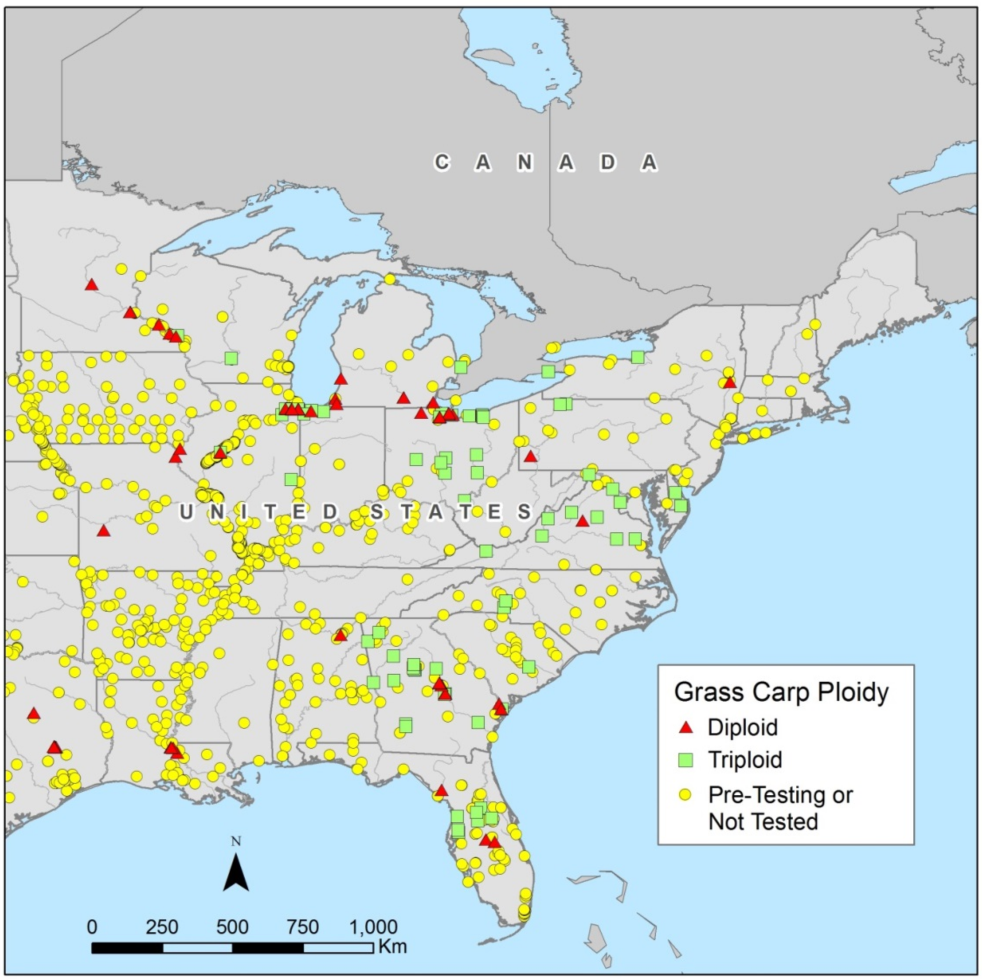


Figure 4. Non-native occurrences of Grass Carp in the eastern U.S. and Canada (1968–2015) as reported in the USGS NAS database. Ploidy (diploid or triploid) indicated when known (ploidy data courtesy of USFWS). Map courtesy of the USGS.

Table 4. Summary of activities (e.g., importation, possession, food fish sales, stocking, live transport) regulated in Great Lakes states and provinces related to Grass Carp. Adapted from Great Lakes Panel on Aquatic Nuisance Species (drafted December 17, 2014).

State/ Province	Importation	Possession	Propagation	Live transport	Stocking	Food fish sales
Illinois	Diploid: Only with aquaculture permit and letters of authorization; Triploid: allowed	Diploid: Only with aquaculture permit and letters of authorization; Triploid: allowed if owner's name is on aquaculture permit holder's restricted species transportation permit	Not on approved species list, but diploids can be held to produce triploids	Restricted species permit required for instate transport of diploid and triploid	Diploid: not allowed; Triploid: only longer than 4" with permit	Diploid: may not be transported to live fish markets for retail or wholesale sale
Indiana	Diploid and triploid allowed with aquaculture permit	Diploid: live diploid Grass Carp may be possessed to produce triploids; Triploid possession allowed by dealer with approved aquaculture permit	Aquaculture permit needed to propagate; diploid in closed systems only, and only held to produce triploids	Diploid transported allowed with aquaculture permit; Triploid: seller must deliver and stock, permit required	Diploid: prohibited, but some exceptions under aquaculture permit; Triploid: must be delivered and stocked by seller	Sales limited to permitted aquaculture facilities and stocking privately owned waters by permit
Michigan	Diploid and triploid: prohibited	Diploid and triploid: prohibited	Not on approved species list: prohibited	Diploid and triploid: prohibited	Diploid and triploid: prohibited	Diploid and triploid: prohibited
Minnesota	Diploid and triploid: prohibited	Diploid and triploid: prohibited	Diploid and triploid: prohibited for possession; permit required	Diploid and triploid: prohibited	Diploid and triploid: prohibited	Diploid and triploid: prohibited

State/ Province	Importation	Possession	Propagation	Live transport	Stocking	Food fish sales
New York	Diploid: need permit, special circumstances; Triploid: need permit	Diploid: need permit, special circumstances ; Triploid: need permit	Diploid: need permit, special circumstances	Diploid: need permit, special circumstances ; Triploid: need permit	Diploid: prohibited; Triploid: can be sold by those who have a permit to those with a permit	Diploid: prohibited; Triploid: can be sold by those who have a permit to those with a permit
Ohio	Diploid: prohibited; Triploid: need permit	Diploid: prohibited; Triploid: allowed	Diploid: prohibited; Triploid: need White Amur permit	Diploid: prohibited; Triploid: need fish transportation permit	Diploid: prohibited; Triploid: allowed	Diploid: prohibited; Triploid: allowed by permit
Ontario	Diploid and triploid: live importation prohibited	Diploid and triploid: prohibited	Diploid and triploid: prohibited	Diploid and triploid: prohibited	Diploid and triploid: prohibited	Diploid and triploid: prohibited
Pennsylvania	Diploid: prohibited; Triploid: restricted (permit required)	Diploid: prohibited; Triploid: restricted (permit required)	Diploid and triploid: prohibited	Diploid: prohibited; Triploid: restricted (permit required)	Diploid: prohibited; Triploid: restricted (permit required)	Diploid: prohibited; Triploid: restricted (permit required)
Quebec	Diploid and triploid: live importation prohibited	Diploid and triploid: prohibited	Diploid and triploid: prohibited	Diploid and triploid: prohibited	Diploid and triploid: prohibited	Diploid and triploid: prohibited
Wisconsin	Diploid and triploid: prohibited	Diploid and triploid: prohibited	Not an approved species for culture	Diploid and triploid: prohibited	Diploid and triploid: prohibited	Diploid and triploid: prohibited

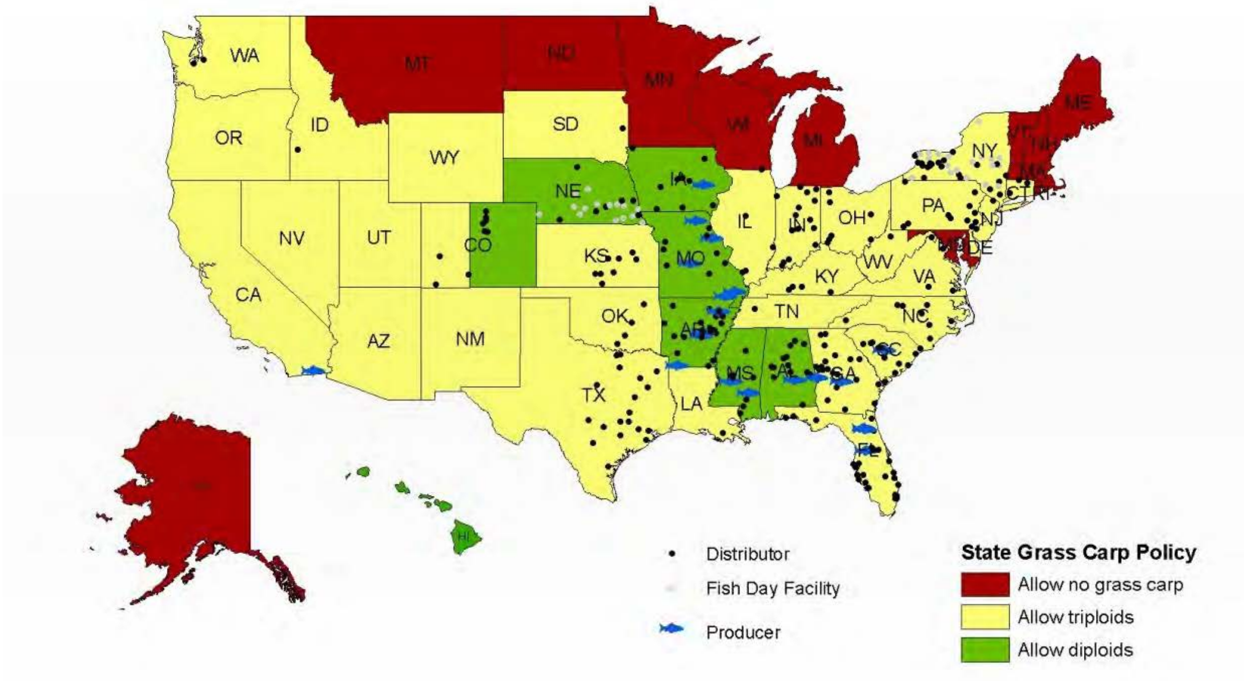


Figure 5. State policies for Grass Carp and locations of commercial Grass Carp facilities (i.e., distributors, fish day facilities, and producers) (Mississippi Interstate Cooperative Resource Association (MICRA) 2015).

Most of the potential physical connections for entry of Grass Carp to the Great Lakes basin connect to the Mississippi River basin and were assessed in the Great Lakes and Mississippi River Interbasin study (GLMRIS) by the U.S. Army Corps of Engineers (USACE) (USACE 2014a). The authors assumed that the physical connections identified in the GLMRIS report as potential pathways for bigheaded carps (*Hypopthalmichthys* spp.) to enter the Great Lakes basin are the same for Grass Carp and are considered as relevant physical connections to the Great Lakes basin as defined for this risk assessment. The GLMRIS study is separated into two focus areas:

> Focus Area 1 concerns the Chicago-Area Waterway System (CAWS) that opens to Lake Michigan (Figure 6a) and contains the only continuous aquatic connection between the basins as defined in the GLMRIS report; and,
>
> Focus Area 2, which evaluates all other aquatic pathways that exist, or are likely to form, across the divide between the Great Lakes and Mississippi River basins as defined in the GLMRIS report (Figure 6b) (USACE 2013a, 2014a).

The CAWS connection to Lake Michigan was identified as the highest risk connection, but 18 other natural and artificial hydrologic connections between the two basins were identified (USACE 2013a). Of the 18 other connections, the authors concluded that three of these connections had stronger hydrological connections with implications for Grass Carp to arrive to Lake Erie:

1) Eagle Marsh in Indiana;
2) Ohio-Erie Canal in Ohio; and,
3) Little Killbuck Creek in Ohio (pathways labeled 6, 3, and 4, respectively in Figure 6b).

The risks associated with all of these connections are discussed below in the appropriate individual lake sections.

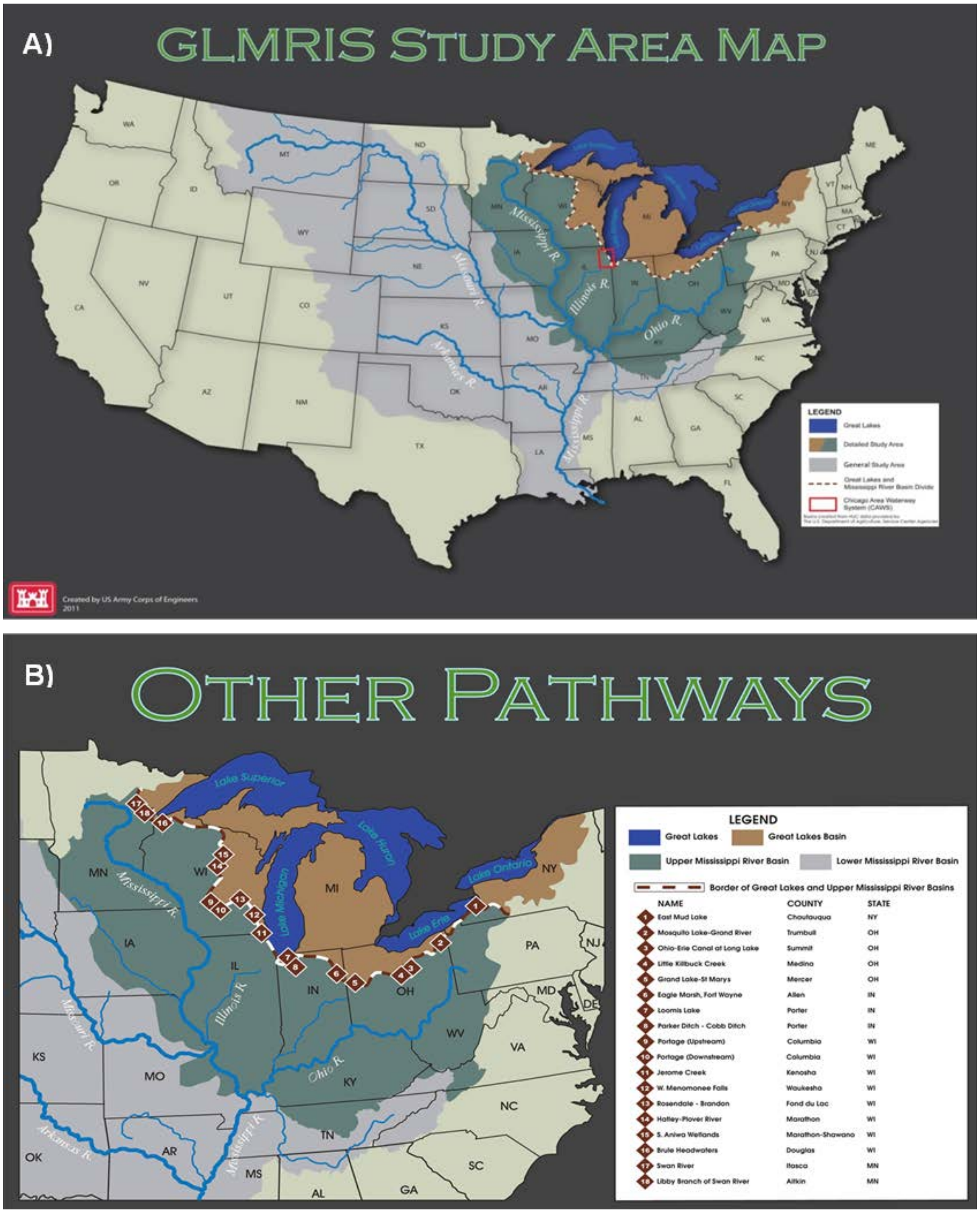


Figure 6. Identified hydrological connections for AIS transfer between the Mississippi River and Great Lakes basins as defined in the GLMRIS report in (A) Focus Area 1 (CAWS) and (B) Focus Area 2 (18 other connections) in the GLMRIS study area (USACE 2013a, 2014a).

2.1.1.1 Lake Superior

To date, no Grass Carp are known to have been collected from Lake Superior (Figure 4); the Grass Carp collected closest to the lake was in Lake Huron near Mackinac Island in 2007. No

Grass Carp are known from the tributaries of Lake Superior within the connected basin as defined for this risk assessment (Figure 2). The GLMRIS Focus Area 2 report (USACE 2013a) identified three potential aquatic pathways for AIS exchange between the Mississippi River and Great Lakes basins (draining into Lake Superior), all with a low probability of AIS spread from the Mississippi River basin to the Great Lakes basin (for swimming fishes: Brule headwaters; Swan River; and Libby Branch of the Swan River (connections 16–18 in Figure 6B). No Grass Carp eDNA samples are known to have been taken and analyzed from Lake Superior.

States and provinces surrounding Lake Superior (Michigan, Minnesota, Wisconsin, and Ontario) do not permit either triploid or diploid Grass Carp (Table 4, Figure 5). However, given its proximity to the three potential aquatic pathways noted above, it is important to note that Iowa allows stocking of both diploid and triploid Grass Carp (Figure 5) and diploid Grass Carp have been collected in the Upper Mississippi River basin (Figure 4). These are potential sources of propagules that could gain access to Lake Superior through physical connections identified in the GLMRIS Focus Area 2 report.

2.1.1.2 Lake Michigan

Two Grass Carp are known to have been collected from the southern portion of Lake Michigan: one off Navy Pier in 1990 and one triploid Grass Carp from the Port of Indiana in Burns Harbor in 2014 (Figure 7). Grass Carp have also been collected in close proximity to Lake Michigan within the Great Lakes basin. Michigan Department of Natural Resources (MDNR) reports collecting five Grass Carp from the St. Joseph River near Jasper Dairy Road Park (approximately 14.48 km from Lake Michigan) between 2007 and 2013 (N. Popoff, MDNR, pers. comm.). The MDNR also collected one Grass Carp and sighted at least three others during an intensive survey of Marrs Lake in Lenawee County in June 2012 (MDNR 2012). The Grass Carp collected from Marrs Lake was later determined to be a reproductively viable diploid. For 2010–2014, a total of 72 Grass Carp were collected upstream of the USACE electric dispersal barrier (but outside of the Great Lakes basin as defined for this risk assessment) using a variety of fish sampling techniques (rotenone, electrofishing, seining, gill netting) as part of the ACRCC Monitoring and Response Plan for Asian carps in the Upper Illinois River and CAWS; (K. Irons, Illinois Department of Natural Resources [ILDNR], pers. comm.). Ploidy was determined for 11 of these fish (Whitledge 2014).

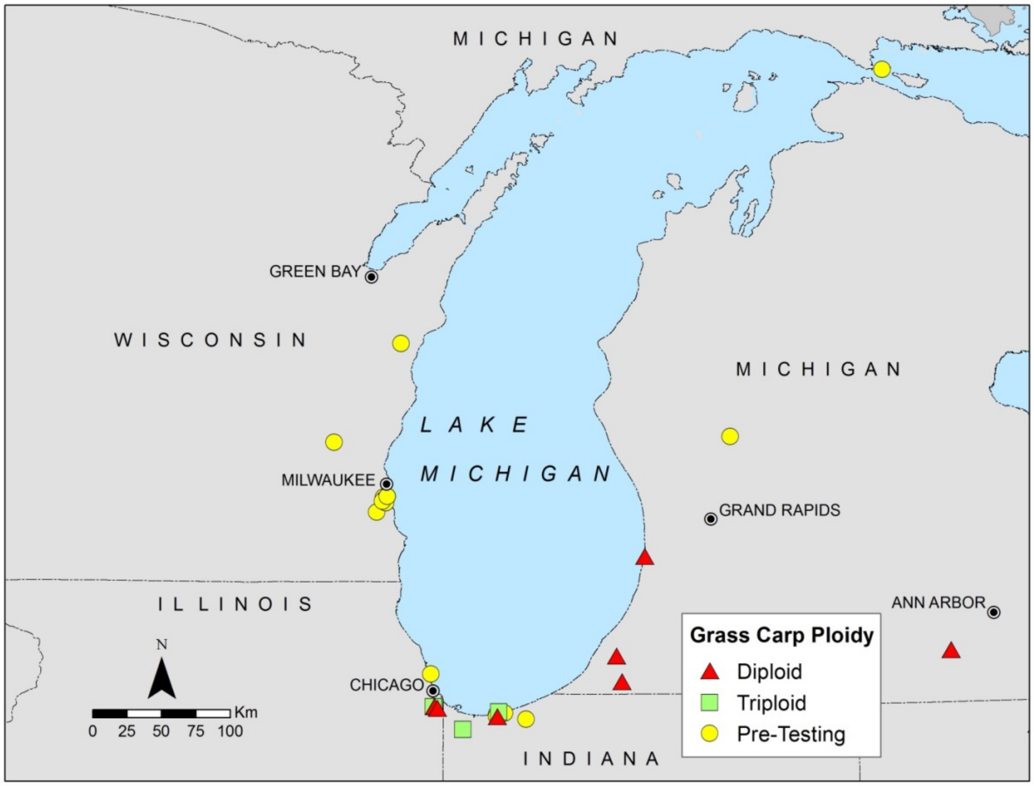


Figure 7. Collections (n = 36) of Grass Carp in Lake Michigan and surrounding area (1975–2014) as reported in the USGS NAS database. No Grass Carp have been reported since 2014. Ploidy (diploid or triploid) is indicated when known (ploidy data courtesy of USFWS).

Whitledge (2014) examined the ploidy and analyzed otolith stable isotopes of Grass Carp collected in or near the Great Lakes where they were not known to be reproducing. Ploidy was determined for 15 of the 16 Grass Carp examined from near Lake Michigan, including the 11 Grass Carp caught above the USACE electric dispersal barrier as part of the ACRCC Monitoring and Response Program: one from Burns Harbor, Indiana; one from the East Arm of the Little Calumet River, Indiana; nine from Lake Calumet, Illinois; one from the Little Calumet River, Illinois; and, three from the St. Joseph River, Michigan. Of these 15 fish, seven were diploid and eight were triploid. Data from stable isotope analysis were consistent with an aquaculture origin for all Grass Carp examined from in, or around, Lake Michigan. Whitledge (2014) surmised this finding implied escape or release of illegally imported Grass Carp and provided no evidence of natural recruitment in the Lake Michigan basin. In addition, stable isotope data from otoliths indicated that Grass Carp examined did not move through the CAWS and were not intentionally or accidentally transported from the Illinois River watershed (Whitledge 2014). More recently, one diploid Grass Carp (26–27 years old) was captured in Calumet River and otoliths were consistent with an Illinois River origin (G. Whitledge and P. Kocovksy, USGS, pers. comm.). The age of the fish indicates that it could have moved through the Chicago Sanitary and Ship Canal (CSSC) prior to the operation of the electric dispersal barrier.

The CAWS was ranked as the hydrologic connection with the highest risk for the introduction of Bighead and Silver carps for the Great Lakes basin (USACE 2014a) and presumably for Grass Carp as well. The CAWS provides a direct, artificial connection between Lake Michigan and the Mississippi River basin at Chicago, Illinois that consists of natural and artificial waterways, including locks and dams (Figure 8; Moy et al. 2011). It contains five aquatic pathways between the Great Lakes and Mississippi River basins each with a single connection point to the Great Lakes basin (Lake Michigan) (Figure 8). Grass Carp are abundant and established in the Illinois River (Raibley et al. 1995). This is further evidenced by the more than 1,700 Grass Carp collected 2010–2014 by sampling and directed removal efforts in the upper Illinois Waterway between Starved Rock and the dispersal barrier at Romeoville (ACRCC MRWG 2015).These Grass Carp were collected as part of the Barrier Defense Asian Carp Removal Project, which utilizes contracted commercial fishing efforts. To date, there has been no ploidy determination for any of these fish. During a rotenone rapid response (May 19–28, 2010) on a 2.6-mile section of the Little Calumet River immediately downstream of the T. J. O'Brien Lock and Control Works (and therefore outside of the connected Great Lakes basin), a total of 43 Grass Carp of variable size were recovered; ploidy was also not evaluated for any of these fish (ILDNR 2010). The primary purpose of the response was to determine the abundance of Bighead Carp and Silver Carp in this portion of the CAWS. Estimated standing stocks from this event suggest approximately 4.6 kg of Grass Carp per hectare within this area (K. Irons, ILDNR, pers. comm.; Figure 8). Furthermore, 30 out of 58 eDNA samples from this area were positive for presence of Grass Carp eDNA (Mahon et al. 2013). We do not know of any sampling done within Lake Michigan for Grass Carp eDNA. Because of the above evidence that substantial numbers of Grass Carp are present in the CAWS between the electric barriers and Lake Michigan, we determined that Grass Carp passage through the barriers was not a prerequisite for Grass Carp to invade Lake Michigan from the CAWS.

The GLMRIS Focus Area 2 report identified nine other potential hydrologic connections between the Lake Michigan and Mississippi River basins that could act as conduits of AIS: Aniwa Wetlands, Wisconsin; Hatley-Plover River, Wisconsin; Rosendale-Brandon, Wisconsin; Menomonee Falls, Wisconsin; Jerome Creek, Wisconsin; Portage downstream, Wisconsin; Portage upstream, Wisconsin; Parker-Cobb Ditch, Indiana; Loomis Lake, Indiana (USACE 2013a, Figure 6B). These nine connections were categorized as low risk.

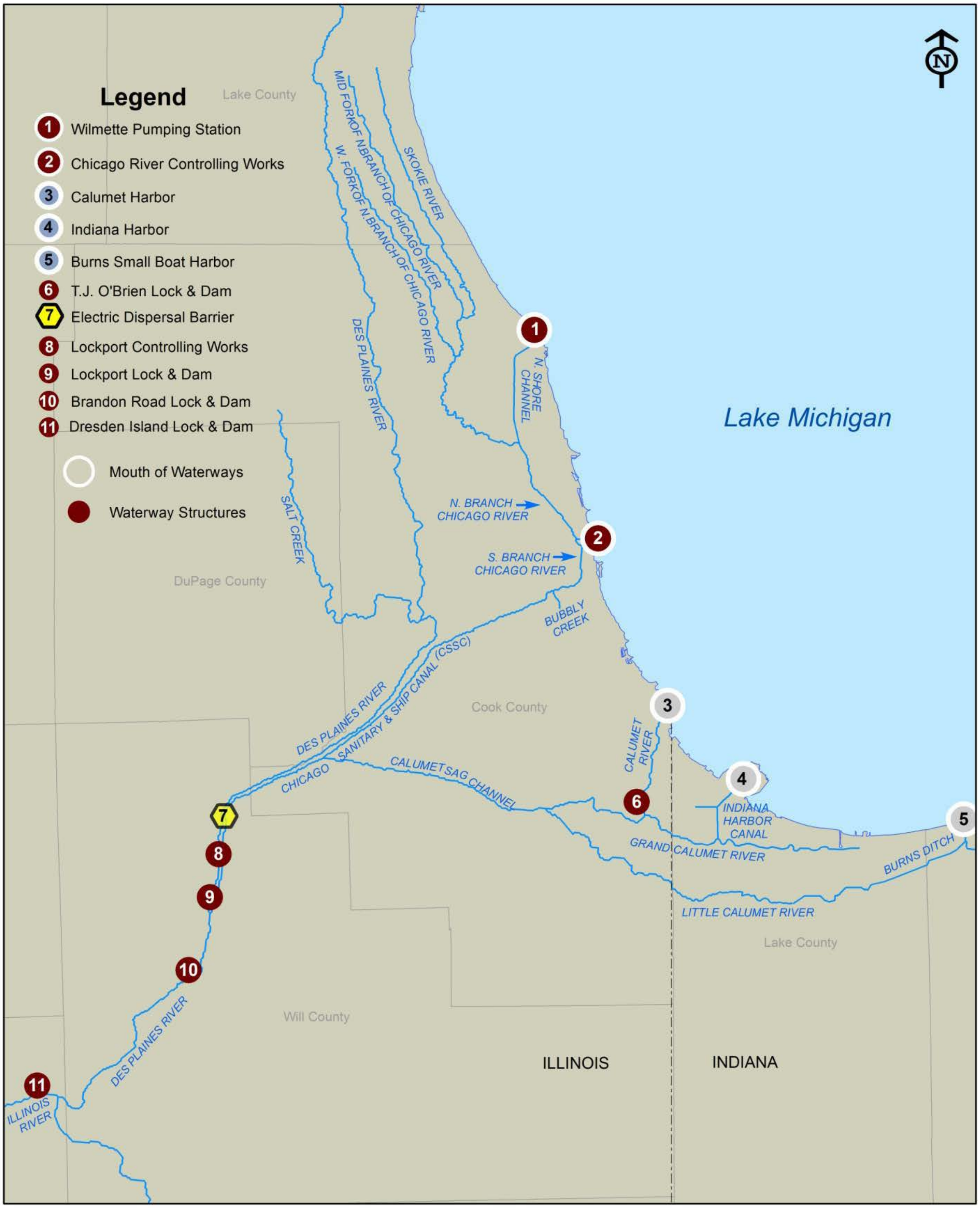


Figure 8. The Chicago Area Waterway (CAWS) system; pathways and control structures map (USACE 2014b).

A system of electric barriers (hereafter, called the electric dispersal barrier) was built near Lemont, Illinois, in the CSSC, the portion of the CAWS that connects to the Mississippi River basin (Figure 8). The electric dispersal barrier serves to deter movement of fishes upstream across the barrier. Initially constructed as a demonstration barrier that became operational on April 18, 2002, the electrical dispersal barrier now includes the original demonstration barrier and two more barriers in close proximity (Barrier IIA and Barrier IIB) with the primary purpose of preventing upstream movement of Asian carps towards the Great Lakes (Figure 9). Currently, only the narrow arrays of barriers IIA and IIB are operating, at reduced operating parameters of 1.7 V/in, 30 Hz and 2.3 ms (Col. Drew, USACE, communication to the ACRCC 2015). The demonstration barrier, while functional, is in the process of being replaced by a larger, permanent barrier (Figure 9). As such, it is operating only when nearby construction is not ongoing. Additionally, extensive maintenance of Barriers IIA and IIB will be required during May–June 2015. Barriers will be shut down sequentially to ensure that deterrent levels of electricity remain at the electric dispersal barrier.

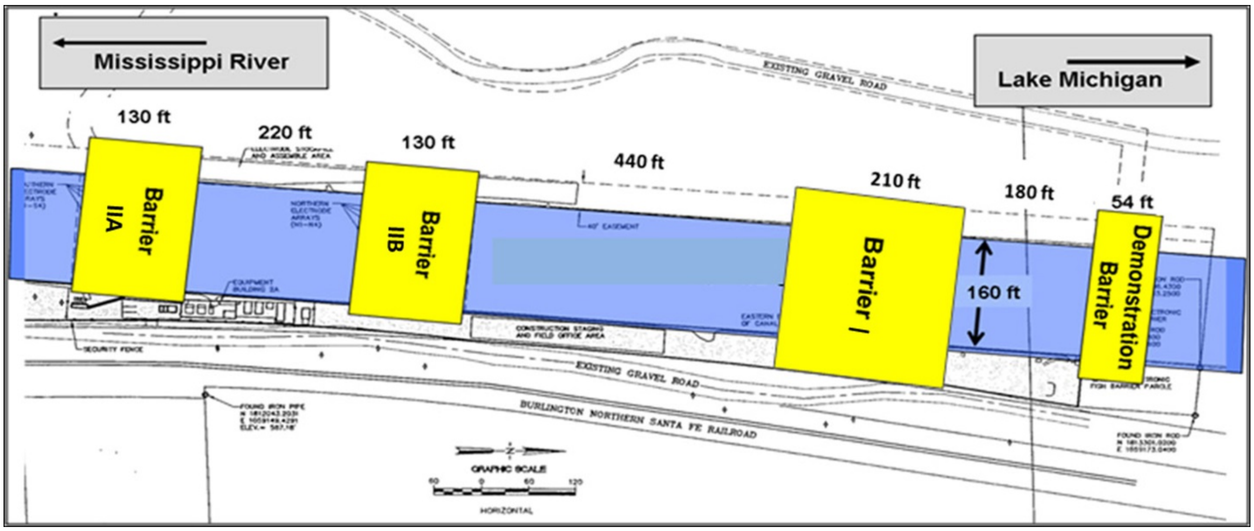


*Figure 9. The electric barrier system in the Chicago Sanitary Ship Canal (CSSC) near Lemont, Illinois where the CAWS connects to the Mississippi River basin. *Barrier 1 is under construction (USACE 2015).*

In addition, reversal of flow in the vicinity of the electric dispersal barrier is more common than originally thought; however, fishes are not likely to be swept into, or through, the electric dispersal barrier during flow reversals (Holliman 2011). Evidence to date using Common Carp (*Cyprinus carpio*) and other large-bodied surrogates for Asian carps indicates that large fishes are deterred from crossing the electric dispersal barrier (Sparks et al. 2011; ACRCC MRWG 2015.). The ACRCC's Monitoring and Response Work Group estimated that the electric dispersal barrier was 98.6% effective at deterring fish > 300 mm in length from crossing the barrier (ACRCC MRWG 2015).

Three tagged Common Carp are known to have passed through the electric dispersal barrier since 2003. One fish crossed the barrier on April 3, 2003. It was subsequently detected approximately 2.5 km upstream of Barrier I, where it did not move again (Sparks et al. 2011). Another tagged Common Carp was detected above the electric dispersal barrier in August 2011. A third tagged Common Carp was detected above the electric dispersal barrier in June 2014. In all three cases, only one barrier was operational and the transmitter did not move after it was detected upstream of the electric dispersal barrier, suggesting that the transmitter was either expelled or that the fish died. The mechanism by which these transmitters moved upstream of the barrier is not presently known, although one possible vector is entrainment by barge traffic moving upstream across the electric dispersal barrier.

Recent fine-scale positioning and surveys by DIDSON indicate that small and large fishes can penetrate the wide field of the electric dispersal barrier (ACRCC MRWG 2015). Large tagged fishes penetrated the electric field only once, but small fishes < 70 mm regularly passed across the narrow field of the barrier (ACRCC MRWG 2015).

The electric dispersal barrier only deters upstream movement through the canal at that location. Two known avenues by which Grass Carp could bypass the electric dispersal barrier include the Des Plaines River and the Illinois and Michigan Canal (I&M Canal). The Des Plaines River joins the CSSC immediately below the Lockport Lock and Dam, about 13 km downstream of the electric dispersal barrier (Figure 8). The river parallels the CSSC for about 24 km upstream, and the Des Plaines River is within about 400 m of the CSSC in this section. During flood conditions, the Des Plaines River can be overtopped and water from the Des Plaines River will flow over land into the CSSC. A flood in the Des Plaines River, determined to be a 125-year event, occurred in September 2008. Another flood could occur at any time but usually occur in the spring (USACE 2010a). The frequency and intensity of precipitation necessary for flood waters to overtop the divide north of the electric dispersal barrier is not known (USACE 2010c). During 2010, the USACE installed a combination jersey barrier and mesh fence along the nearly 21 km stretch of close proximity to reduce the potential of Asian carps entering the CSSC from the Des Plaines River during flooding (USACE 2010a). Similarly, the I&M Canal connects to the CSSC downstream of the Lockport Lock and Dam. The I&M Canal flows intermittently and there is a small drainage divide that sends water toward the Cal-Sag Channel upstream of the electric dispersal barrier (Figure 8). This divide could be overtopped during flooding (similar in frequency to that listed above) such that water from downstream of the electric dispersal barrier could move upstream of the electric dispersal barrier (USACE 2010a). The USACE enhanced the divide and plugged outflows from the I&M Canal into the CSSC upstream of the electric dispersal barrier (USACE 2010a). The USACE also installed screens on its sluice gates at the O'Brien Lock and Controlling Works (USACE 2010b). The USACE also recommended that the Metropolitan Water Reclamation District of Greater Chicago install screens on the sluice gates of the Chicago River Controlling Works and to modify operations at the Wilmette Pumping Station for diversion water intake if requested (USACE 2010b).

Another possible pathway for moving past the electric dispersal barrier is movement of small Grass Carp in barges with damaged hulls where small fish could enter the bilge. Reports of barges pumping water from void spaces are common throughout the Illinois Waterway, including the CSSC. To investigate this potential, a study to assess the efficacy of Asian carp transport by barges was conducted (Heilprin et al. 2013). This study of water quality in barge voids found that dissolved oxygen levels and water temperatures were well within limits for fish survival, even during the hot months of the year (Heilprin et al. 2013). The study examined void spaces of more than 130 barges and found that less than 5% were filled with water. After placing early life stages of Asian carps (presumably mostly Bighead and Silver carps, but larvae were not identified to species) into barge voids and then pumping them out, 0.56% of Asian carp larvae survived one pass through either a 5 cm or 7.6 cm pump. The authors concluded that the risk of movement of Asian carp larvae in barge voids was low (Heilprin et al. 2013).

There are also many ponds and artificial lakes in the Chicago metropolitan area that are commonly stocked for fishing with Channel Catfish *Ictalurus punctatus*. Channel Catfish are often purchased from southern fish farmers, where it is possible for the stock to be contaminated with Asian carps (Conover et al. 2007, ILDNR 2011). In 2011, ILDNR reported removing seventeen Bighead Carp and five Grass Carp from Flatfoot Lake, which is on Chicago's south side, 900–1,200 feet north of the Little Calumet River (ILDNR 2011). Many urban fishing ponds in the Chicago Metropolitan area have been stocked with triploid Grass Carp to control nuisance aquatic vegetation (K. Irons, ILDNR, pers. comm.). In the summer and fall of 2011, eDNA sampling for Asian carp in Chicago-area ponds produced positive detections of Grass Carp eDNA in 15/19 sampled waterbodies: Humboldt Park, Washington Park, Gompers Park, Riis Park, Douglas Park, Powderhorn Park, Wampum Park, Horsetail Lake, Tampier Lake, Cermak Quarry, Schiller Pond, Big Bend, Beck Lake, Lake Ida, and Turtlehead (C. Jerde, University of Nevada-Reno (UNR), pers. comm.). While most of these urban fishing ponds are isolated and have no surface water connection to Lake Michigan or the CAWS upstream of the electric dispersal barrier (ILDNR 2011), it is unclear if any of these ponds could connect with the Lake Michigan watershed during flooding events. Urban fishing ponds in the Chicago Metropolitan area provide a source for the capture and illegal transport of Grass Carp within close proximity to the Great Lakes.

States bordering Lake Michigan differ in their regulation of Grass Carp (Table 4, Figure 5). Wisconsin and Michigan prohibit Grass Carp. Illinois and Indiana require a permit to possess live diploid Grass Carp and permits are approved only for the production of triploid Grass Carp in closed aquaculture facilities. Stocking of triploid Grass Carp is allowed in Illinois and Indiana with permits. Iowa, however, allows stocking of both diploid and triploid Grass Carp. Iowa does not border Lake Michigan, but its proximity and the commercial availability of diploid grass carp there provide a potential source of propagules for illegal transport and stocking.

2.1.1.3 Lake Huron

Several Grass Carp have been captured from Lake Huron (Figure 10). Four individuals were collected near Sarnia, Ontario between 1989 and 2008 and a fifth was captured near Mackinac Island, Michigan, in 2007 (USGS NAS database 2015).

Figure 10. Collections (n = 5) of Grass Carp in Lake Huron and surrounding area (1989–2008) as reported in the [USGS NAS database](). No Grass Carp have been reported since 2008. Ploidy (diploid or triploid) is indicated when known (ploidy data courtesy of USFWS).

In 2014, the Ontario Ministry of Natural Resources and Forestry (OMNRF) sampled three marinas in the Ausable River (southern basin of Lake Huron in Ontario) for eDNA of Asian carps (including Bighead, Grass, and Silver carps). One sample tested positive for Grass Carp eDNA indicating the presence of Grass Carp genetic material but follow up sampling with traditional gear revealed no Grass Carp. This is the only information provided on eDNA sampling of Lake Huron for Grass Carp.

The Lake Huron watershed does not border the Mississippi River Basin and therefore no hydrologic connections exist between Lake Huron and the Mississippi River basin (USACE 2014a). Other connections (e.g., locks and dams on St. Mary's River connecting to Lake Superior) are discussed in the Spread Section (Section 2.4) because they pertain to spread within the basin and not arrival to the basin.

Both Michigan and Ontario, the only jurisdictions bordering Lake Huron, prohibit the stocking of diploid and triploid Grass Carp; live sale and possession of Grass Carp is also banned in Ontario (Table 4, Figure 5). The states of Ohio, Illinois and Indiana, however, allow the stocking of triploid Grass Carp (Table 4).

2.1.1.4 Lake Erie

Grass Carp were recorded from the Lake Erie and Michigan basins prior to 1983 (Underhill 1986), and the first capture from a lake (rather than from a tributary) was in Lake Erie in 1985 (Crossman et al. 1987, USGS NAS database 2015). Several additional individuals were collected during the next few years and, since 2011, Grass Carp have been captured from Lake Erie and in tributaries to Lake Erie within the Great Lakes basin (as defined for this risk assessment) (Figure 11) with increasing frequency. In 2012, six Grass Carp were collected from the Sandusky River. In 2013, two triploid Grass Carp were captured and another triploid Grass Carp was caught in 2014, from the Grand River, Ontario. There is also recent evidence of successful Grass Carp recruitment in the lower 26 km of the Lake Erie tributary, the Sandusky River, Ohio (Chapman et al. 2013) and elsewhere in the basin (unknown tributaries; Whitledge 2014). In recent years, additional Grass Carp captures from the western Lake Erie basin have occurred through MDNR efforts combined with Blair Fish Co. and a reimbursement program, which came into full effect in 2014 (S. Herbst, MDNR, pers. comm.). This has resulted in 5, 7 and 22 Grass Carp captures in 2012, 2013, and 2014, respectively (ploidy not tested).

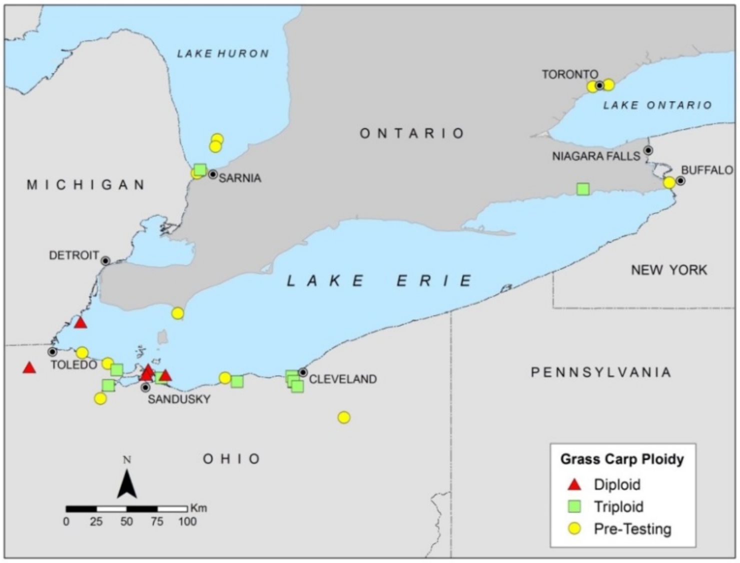


Figure 11. Collections (n = 34) of Grass Carp in Lake Erie and surrounding area (1985–2015) as reported in the USGS NAS database. No Grass Carp have been reported since 2014. Ploidy (diploid or triploid) is indicated when known (ploidy data courtesy of USFWS and DFO).

Whitledge (2014) examined ploidy and analyzed otolith stable isotopes of 14 Grass Carp collected in the Lake Erie basin. Ploidy was established for 13 of the 14 fish (six from Lake Erie, Ohio; one from Sugar Creek, Ohio; one from Muskingum River, Ohio; four from the Cuyohoga River, Ohio; one from the Maumee River, Ohio). Of these 13 fish, five were diploid and eight were triploid. Data from stable isotope analysis revealed that diploid Grass Carp were recruited from Great Lakes (presumably Lake Erie) tributaries (the descendants of escaped or illegally introduced diploid fish) and that triploid fish were escaped or intentionally introduced fish originating in aquaculture. Three Grass Carp have been collected from the Grand River, Ontario since 2013 (USGS NAS database 2015). All of these fish were triploid.

The GLMRIS Focus Area 2 Summary Report identified six potential hydrologic connections between the Lake Erie and Mississippi River basins (connections labelled 1–6 in Figure 6b). Two connections ranked medium in risk for movement of Bighead and Silver carps (and presumably Grass Carp) from the Mississippi River basin to Lake Erie (Eagle Marsh, Indiana, Little Killbuck Creek, Ohio) and four of the connections were ranked low (Grand Lake, Ohio; Mosquito Lake, Ohio; Ohio River-Erie Canal at Long Lake, Ohio; East Mud Lake, New York) (USACE 2013a).

Eagle Marsh in northwestern Indiana is an area that joins the Wabash River system with the Maumee River system (directly connected to Lake Erie) under some flood conditions. Flooding occurs from back water inundation of the St. Marys River and the Graham-McCullough Ditch with depths of inundation ranging from a 0.18–0.91 m depending on the storm event and location (USACE 2013b). This site was determined to be capable of conveying water across the basin divide for days to weeks, multiple times per year. A surface-water pathway between the basins occurs most frequently during late winter to early summer and, sporadically, during heavy rain events during other times of the year (USACE 2013b).This site has been rated the highest risk of AIS transfer among the 18 locations evaluated in Focus Area 2 of GLMRIS (USACE 2013a). Grass Carp have been reported within about 160 km of Eagle Marsh in the Wabash River west of West Lafayette, Indiana (USGS NAS database 2015). One dam upstream of Huntington, Indiana on the Little River (an older fixed crest dam approximately 2 m high) stands between Grass Carp and arrival in the Eagle Marsh area. As part of measures being put in place to decrease the risk of Eagle Marsh as a potential pathway for spread of AIS (particularly Asian carps), Indiana deployed a large-mesh fence to deter movement of adult fishes between the Wabash and Maumee drainage basins in 2010. During spring 2011 flooding, adult Common Carp attempted, but were not able, to cross this fence (IDNR 2010).

The next highest risk physical connection between the Mississippi River drainage and Lake Erie identified in the GLMRIS Focus Area 2 report was Little Killbuck Creek, Medina County, Ohio, just north of the Wayne County boundary and about 30 miles (48 km) southwest of Cleveland (Figure 6b; USACE 2013c). At this site, there is an intermittent stream that connects the basins continuously for multiple days about once every 10 years. There is also an existing agricultural ditch system at an active farm spanning the divide and connecting both basins at a 2–5% annual recurrence interval. The Grass Carp closest to this site reported to the USGS NAS database was a triploid individual collected in 2014, approximately 85 river miles away in the Muskingum River, Coshocton County (Figure 11).

Another point in the Muskingum River Watershed identified by the USACE as a potential aquatic pathway between the Mississippi-Ohio River and Lake Erie-Great Lakes basins is a connection between the Tuscarawas River and the Little Cuyahoga River at the Ohio-Erie canal (USACE 2013d). During normal weather conditions, physical barriers prevent Asian carps from crossing the watershed boundary at these locations. However the watersheds have the potential to be connected during extreme flood events. Grass Carp could move from the Muskingum River up the Tuscarawas River, to enter the Lake Erie drainage from Long Lake into the Ohio-Erie Canal and from there into the Little Cuyahoga River. Backwater flooding from the Tuscarawas River toward the Ohio-Erie Canal was observed following a storm event during the summer of 2007 in the area just south of where the railroad bridge crosses the canal. Although the flood waters came very close during the 2007 flood event, there was no direct surface water connection between the canal and the Tuscarawas River at this location (USACE 2013d).

The New York Canal System (formerly the Erie Barge Canal) connects the upper Niagara River (hence, Lake Erie) and Lake Ontario to the Finger Lakes, Lake Champlain, and the Hudson River in the Atlantic drainage (Figure 30). There are 18 known occurrences of Grass Carp in the Hudson River drainage (~200 km from Lake Ontario) and one in the Finger Lakes (USGS NAS database 2015). Therefore, it is possible that Grass Carp could arrive in Lake Erie through the New York Canal System from the Atlantic drainage.

Wilson et al. (2014) sampled eDNA and electrofished a network of 180 sites in nearshore and tributary habitats on the Canadian side of all three basins of Lake Erie and Lake St. Clair in 2012. Sites were selected on the basis of perceived risk of fish access or habitat suitability. No Grass Carp were collected by electrofishing and no positive detections of Grass Carp eDNA were made from more than 900 water samples. In October 2013, 211 water samples (including control samples) were collected (following established eDNA collection protocols (Mahon et al. 2010)) from seven locations within the Muskingum River watershed: Killbuck Creek, Tuscarawas River, Ellis lock and dam, Philo lock and dam, McConnelsville lock and dam, Luke Chute lock and dam, and Devola lock and dam (C. Jerde, UNR, pers. comm.). No Grass Carp eDNA was detected in any of the composite samples from these locations.

Regulation of Grass Carp differs among the states and provinces bordering Lake Erie (Table 4). Michigan and Ontario prohibit the stocking of both diploid and triploid Grass Carp. Ohio, Pennsylvania and New York allow stocking of triploid but not diploid Grass Carp.

2.1.1.5 Lake Ontario

Five Grass Carp have been reported from the Lake Ontario basin: one in each of 1985, 1987, 1999, 2003, and 2010 (triploid) (Figure 12). Two of these captures occurred in Canada (unknown ploidy): one in a pond in an urban park just steps from Lake Ontario(1999), and another in a tributary of Lake Ontario (2003) (Cudmore and Mandrak 2004).

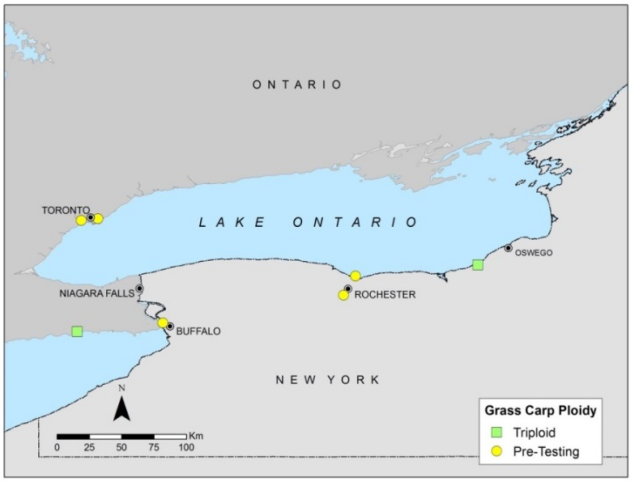


Figure 12. Collections (n = 5) of Grass Carp in Lake Ontario and surrounding area (1985–2010) as reported in the USGS NAS database. No Grass Carp have been reported since 2010. Ploidy (diploid or triploid) is indicated when known (ploidy data courtesy of USFWS).

The Lake Ontario basin is not contiguous with the Mississippi River basin, thus the GLMRIS study identified no connections between the two basins (USACE 2014a).

Since its opening in 1959, the St. Lawrence Seaway continues to be an important route for the introduction of AIS, such as the copepod, *Eurytemora affinis*, and White Perch *Morone americana* (Mills et al. 1993). Should Grass Carp gain access to the St. Lawrence River, this would provide a direct route into Lake Ontario. However, there are currently no known occurrences of Grass Carp in the upper St. Lawrence River.

The New York Canal System connects the upper Niagara River and Lake Ontario to the Finger Lakes, Lake Champlain, and the Hudson River in the Atlantic drainage (Figure 30). There are 18 known occurrences of Grass Carp in the Hudson River drainage and one in the Finger Lakes (USGS NAS database 2015). Therefore, it is possible that Grass Carp could arrive in Lake Ontario through the New York Canal System from the Atlantic drainage.

We know of no eDNA sampling for Grass Carp in Lake Ontario.

Jurisdictions bordering Lake Ontario vary in their regulation of Grass Carp. Ontario and Quebec prohibit the stocking of diploid and triploid Grass Carp, while New York allows the stocking of triploids. The neighboring jurisdictions of Ohio and Pennsylvania also allow the stocking of triploid Grass Carp. (Table 4, Figure 5).

2.1.2 Human-mediated Release

The potential for purposeful, human-mediated releases of Grass Carp into the Great Lakes basin exists. There is a strong demand to control aquatic macrophytes in waterbodies using Grass Carp in jurisdictions where this activity is permitted (Conover et al. 2007; Table 4) and motivation for illegal use for that purpose where the activity is not permitted. It is important to note that diploid Grass Carp are less expensive to purchase than triploids. As such, there is likely a greater incentive to purchase diploid fish than triploids, where both are available, increasing the risk that diploid fish or their offspring could escape into Great Lakes tributaries. Such legal (and illegal) stocking practices of triploid or diploid Grass Carp may lead to accidental escape of Grass Carp (e.g., during flooding events) from these targeted waterbodies.

Furthermore, humans have illegally released freshwater fishes for numerous reasons, such as sport opportunities (Crossman and Cudmore 1999a, Bradford et al. 2008, Drake et al. 2015b) or spiritual/ethical reasons (Crossman and Cudmore 1999b, Severinghaus and Chi 1999, Shiu and Stokes 2008). This human behaviour of illegally releasing non-native fishes into the aquatic environment is difficult to characterize and quantify (Bradford et al. 2008). For this reason, we are unable to assess the risk of illegal intentional release, but should note its existence as a possible source of Grass Carp introduction into the Great Lakes basin. Within this risk assessment, we assessed the human-mediated release of Grass Carp from bait use and trade, which are likely to be most important in the southern portion of the Great Lakes basin where these activities are more prevalent.

2.1.2.1 Bait

The live baitfish pathway is a potential entry route for the arrival of small Grass Carp into the Great Lakes basin. Baitfishes are used for angling in all states and provinces surrounding the Great Lakes, although specific regulations and the degree of baitfish activity vary by state/province (Table 5). The term 'baitfish' generally refers to a variety of small fishes, with species dependent on local regulations, supply, and angler preference. Within most Great Lakes jurisdictions, baitfish are supplied by angler self-harvesting (i.e., angler capture of small baitfishes using minnow traps, seines, or dip nets), commercially harvest or aquaculture. Although culture does not normally occur within Great Lakes jurisdictions due to limited growing seasons, cultured baitfishes from U.S. states outside of the basin (e.g., Arkansas, parts of Minnesota, North Dakota, South Dakota; G. Whelan, MDNR, pers. comm. in Cudmore et al. 2012) may be transported to Great Lakes states for sale to retailers and anglers. It is illegal to import live baitfishes from the U.S. into Canada; however, it is not illegal to import baitfish into the U.S. from Canada. Despite regulations prohibiting bait release for most states and provinces, anglers may release undesirable or leftover baitfishes into the destination waterbody following angling (Litvak and Mandrak 1993, 1999, Dextrase and MacKay 1999, Kulwicki et al. 2003, Drake and Mandrak 2014a, c), although the prevalence of live release may be declining (Drake et al. 2015b).

The likelihood of the baitfish trade as an entry route for Grass Carp is dependent upon:

1) the distribution and intensity of baitfish harvest activity in relation to the distributional co-occurrence of Grass Carp and target baitfishes in the wild and in baitfish culture facilities;
2) the ability of commercial harvesters, baitfish retailers, and anglers to effectively sort or 'cull' Grass Carp (presumably juveniles) from target catches; and,
3) the nature and prevalence of angling activities (e.g., long-distance transport from invaded ranges and subsequent baitfish release).

All states and provinces within the Great Lakes basin designate certain baitfish species, usually deemed to be of low ecological risk, for angling use. Bait species designations provide a legal mechanism to prohibit the capture, use, and movement of invasive fishes, such as Grass Carp, during baitfish operations (Table 5). Most states prohibit the use of "carp" as baitfish with Michigan and Ontario specifically prohibiting the use of "Asian carps" (Table 5; OMNRF 2015). Most states and Ontario have restrictions on the within-jurisdiction movement of baitfishes, with Ontario prohibiting the importation of baitfishes (Table 5). For most jurisdictions, knowledge is lacking about: the degree to which these regulations are followed; which bait originates in areas of Grass Carp populations; angler use, movement, and release patterns; and annual volume and distribution of live bait angling events.

Yet, because the industry in Ontario and other jurisdictions within the Great Lakes basin relies on wild harvest, potential for non-target fish by-catch exists. A study of the Ontario baitfish pathway indicated invasive and other non-target fish by-catch occurs during baitfish harvest operations in Great Lakes nearshore waters and tributaries (Drake 2011, Drake and Mandrak 2014b).The prevalence of invasive and other non-target fishes within retail tanks and angler purchases of bait was generally much lower than the prevalence of these species within harvest operations, indicating that a substantial degree of non-target species culling occurs following harvest (Drake and Mandrak 2014c). However, even low prevalence of non-target species in angler purchases was sufficient for non-target species to be introduced to the wild, as the low prevalence of purchasing, transporting, and releasing non-target fishes was offset by a large number of angler trips (4.24 million yearly events involving live baitfishes) (Drake and Mandrak 2014a), leading to non-target species introductions that occur relatively frequently. Drake and Mandrak (2014a) found that, when facilitated by angler release behaviour, the rate of invasive fishes sold as bycatch to anglers could lead to as many as 3,715 Round Goby (*Neogobius melanostomus*) introduced annually into Ontario lakes. Angler harvesting of bait could also lead to a high number of non-target fishes commonly encountered as bycatch that are introduced beyond their range (Drake and Mandrak 2014c). The spatial distribution of live bait angling events in Ontario indicated that even the shortest trips involving live baitfishes were sufficient to surpass drainage basin boundaries, with the longest trips further enhancing the overland spread potential of invasive and non-target fishes (Drake and Mandrak 2010). Both these mechanisms (wild harvest operations and angler self-harvest) are plausible methods for inadvertent introduction of young Grass Carp to the Great Lakes. Results from the Drake and Mandrak (2014c) study of the baitfish industry and AIS in Ontario suggest that the entry route of Grass Carp into the Great Lakes basin through the baitfish pathway will be largely dependent on the specifics of baitfish activity within each jurisdiction such as: characteristics of harvest activity in relation to Grass Carp source populations; angler use, movement patterns, release rates; and, the yearly volume and spatial distribution of angling events within and outside of the Great Lakes basin. Use of live bait, and hence the risk of introduction of Grass Carp through bait, is most common in regions that are heavily fished for percids, especially Lake Erie, southern Lake Michigan, and Saginaw Bay.

Samples from six bait retailers from around the U.S. Great Lakes basin indicated no Grass Carp visually or through meta-genetic (eDNA) analyses (Mahon et al. 2014). Sampling of baitfish retailers in southern Ontario (n = 50 retail tanks and n = 68 baitfish purchases made from these tanks) between August 2007 and February 2008 identified several non-target and invasive fishes, but no Grass Carp were documented (Drake and Mandrak 2014c). Follow-up sampling in 2011 and 2012 conducted by the OMNRF also failed to detect Grass Carp in 58 bait purchases (A. Drake, University of Toronto Scarborough, pers. comm.) and in 29 water samples from Ontario bait retailers during the same time period. Although these studies provided no evidence that Grass Carp are part of the bait trade within the Great Lakes basin, they were a brief snapshot in time with few samples.

Table 5. Summary of 2015 recreational angling regulations for states and provinces in the Great Lakes basin related to potential Asian carp entry through the baitfish pathway (Prepared by J. Wingfield, GLFC). Although each jurisdiction has specific movement regulations (e.g., certain significant waterbodies may exhibit 'no-baitfish' rules to protect sensitive game stocks), movements listed below concern noteworthy baitfish movement restrictions within each jurisdiction as they relate to pathway operations and the potential for Asian carp movement or entry into the pathway. In many cases, generic restrictions against 'carp' (presumably Common Carp) were made; these are listed simply as 'carp' unless specifically defined as Asian carps.

State / Province	Regulation (Possession / Use)	Regulation (Movement)
Illinois	General white/permissible list of species includes 'minnows,' but does not specifically preclude 'carp' (ILDNR 2015, p. 2). Definition of 'minnow' excludes 'carp' (ILDNR 2015, p. 4). Grass Carp not listed as injurious species so no restrictions on possession; stocking prohibitions on diploid and hybrid, restrictions on triploid (permit required) (ILDNR 2015, p. 3).	Collected live bait may not be transported between waterbodies (IL DNR 2015, p. 2) Statewide movement restrictions in response to viral hemorrhagic septicemia (VHS) concerns; removal of designated "VHS-Susceptible Species" from their waters prohibited (may be caught and kept, but not transported live). Use of wild-trapped fishes from within state as bait restricted to waters where legally captured (ILDNR 2015, p. 67).
Indiana	Generic list of legal baitfish species; specific prohibition against 'carp' as live bait at any location. Collection and use of certain species based upon waterbody (IDNR 2015, p. 9). Grass carp not included in list of illegal species so possession not prohibited (IDNR 2015, p. 7).	Regulations governing movement of baitfish based upon species and waterbody where collected (e.g. Alewives must be collected, used and killed on Lake Michigan) (IDNR 2015, p. 9).

State / Province	Regulation (Possession / Use)	Regulation (Movement)
Michigan	List of species that are illegal to possess, transport, or use as baitfish; specifically includes all species of live carp and goldfish (MDNR 2015, p. 12–13).	Regulations governing the movement of baitfish species and their roe listed as "Susceptible Fish Species for VHS" are based upon defined management areas (VHS-free, -surveillance, -positive areas, exclusion zones) and bait harvest types (personal, commercial-uncertified, commercial-certified) (MDNR 2015, p. 12–13, 32–33). Designated species must be certified disease free prior to importation into MI; minnows harvested in Michigan cannot be exported (Fisheries Order 245; *Public Act* 324, Part 487). General Statewide Provisions prohibit movement of fish, and release of baitfish, outside original waterbody (MDNR 2015, p. 33).
Minnesota	List of permissible bait species with prohibition on using whole or parts of 'carp' for bait (MNDNR 2015, p. 21). Specific prohibition about possessing or transporting Asian carps; must be reported to DNR office within 7 days of catch (MNDNR 2015, p. 27).	Statewide movement restrictions in response to VHS concerns; anglers required to exchange bait water when leaving infected zone. Importation of live minnows and leeches into MN prohibited (MNDNR 2015, p. 21).
New York	Green/permissible list of legal species for widespread angler use in the state. Additional list of species for use in waters only where currently found (e.g., Alewife in Great Lakes). Specific prohibition about 'carp' collection or use as bait (NYDEC 2015, p. 57–58).	Commercial catch VHS-free certification process determines the degree of allowable movement. Uncertified bait (some permitted commercial catches and all self-harvested fishes) may only be transferred overland in defined transportation corridors within the Great Lakes drainage basin (NYDEC 2015, p. 58–59).
Ohio	List of legal baitfish species; Ohio prohibits use of fish species that are not already established in Ohio waters (Ohio Administrative Code 1501:31–13–04 Bait and bait dealers). Specifically prohibits use of bighead and Silver Carp as live bait (ODNR 2015, p. 20). Grass, Bighead, Silver, and Black Carp designated as 'forage fish'; take allowed by multiple methods (ODNR 2015, p. 17).	Intra-state transportation, sale, and distribution out of the affected region of northern Ohio of 28 fish species designated as susceptible to VHS prohibited; importation restrictions exist (ODNR VHS Proclamation). Release of wild-caught or purchased baitfish in waters other than those from which they were collected is prohibited statewide (ODNR 2015, p. 2, 17). Transport of any aquatic species from one waterbody to another is prohibited (ODNR 2015, p. 16).

State / Province	Regulation (Possession / Use)	Regulation (Movement)
Ontario	List of live fish permitted for use as bait (contains 48 species of "baitfish", contains no carp species) (OMNRF 2015, p. 10). Possession (live or dead) of all Asian carps specifically prohibited (OMNRF 2015, p. 8).	Movement of Great Lakes commercial baitfish catches to inland waters is prohibited in response to VHS concerns; no regulation of angler movement of live baitfish; however, anglers are encouraged to comply with commercial movement restrictions.
Pennsylvania	List of permissible bait species includes 'minnows,' and specifically precludes 'Common Carp' (PAFBC 2015, p. 8). Eggs, only originating from trout and salmon (unpreserved, refrigerated, or frozen) may be used as bait. No other eggs, regardless of origin or method of preservation, may be used. (58 PA Code 63.54) Possession of diploid Grass Carp prohibited and triploid Grass Carp restricted (permit required) (58 Pennsylvania (PA) Code 71.7).	Importation of any fish from another state is prohibited (without consent) and transferring any fish from one PA watershed to another where that species is not always present is also prohibited (without consent from the Commission) (PAFBC 2015, p. 8). Transport of any baitfish taken from within the Commonwealth out of the state is restricted (license/permit required) (PAFBC 2015; p. 9) Importation of designated "VHS Susceptible Species" into the state, and transportation of "VHS Susceptible Species" from the Lake Erie watershed is restricted (conditions apply) (58 Code 73.3, 69.3) Transportation, importation, introduction of diploid Grass Carp prohibited and triploid Grass Carp restricted (permit required) (58 PA Code 71.7).
Québec	In general, possession/use of baitfish is prohibited; some exceptions exist and are determined by zone; includes species *not* on a prohibited list - 'carp' is specifically included on the list of "Fish Prohibited as Bait"; and, typically require bait to be dead (very limited exceptions exist). The use of live baitfish is prohibited during the summer season (QC MFFP 2016, p. 11, 13). The use and possession for use as bait of all finned freshwater and saltwater fish that are not indigenous to Quebec (with several exceptions) is prohibited (QC MFFP 2016, p. 13).	Movement of baitfish is generally prohibited, with some exceptions that are defined by zone, determined by species (includes only those species *not* included on the prohibited list), generally require the bait to be dead (very limited exceptions exist) (QC MFFP 2016, p. 11, 13).
Wisconsin	Baitfish (specifically defined as minnow family species) collection and use allowed from VHS-free waters, use restricted to that trip on that waterbody. All baitfish collection prohibited on VHS-known and -suspected waters (WDNR 2015, p. 16, 19). Possession of live fish (other than baitfish acquired by bait dealer) away from waterbody prohibited. (WDNR 2015, p. 8). Asian carp cross designated as 'invasive species'; possession exemption allowed through 'rough fish species' cross-designation (WDNR 2015, p. 17; Invasive Species Rule NR40; Chapter NR20.20).	Movement of any live fish away from waterbody prohibited with one exception: transport of baitfish purchased from licensed bait dealer permitted only if no lake or river water, or other fish were added to their container (WDNR 2015, p. 18). Asian carp cross-designated as 'invasive species' and 'rough fish species' - exceptions to possession, transportation, and transfer (not live) prohibitions exist (WDNR 2015, p. 17; NR40; NR20.20).

2.1.2.2 Trade and Stocking

Grass Carp is shipped and sold live for stocking private waters to control nuisance aquatic vegetation in the U.S. and for sales in U.S. live food fish markets and Canadian food fish markets. The intentional stocking of Grass Carp into private waterbodies for vegetation control, recreational opportunities, or other unknown reasons is a potential entry route for their arrival into the Great Lakes basin. Accidents that occur during transport, contamination of Grass Carp in stocking of other farm-raised species (e.g., Channel Catfish), and aquarium and internet trade are all potential sources of intentional or unintentional Grass Carp releases in the Great Lakes basin. These components of trade are discussed in further detail below.

Unlike Bighead Carp, Silver Carp, and Black Carp *Mylopharyngodon piceus*, Grass Carp is not listed under the injurious wildlife provisions of the *Lacey Act* and, within the U.S., is regulated only at the state level. Grass Carp regulations among the eight Great Lakes states are varied and complicated by differing regulations for diploid and triploid forms within several states (Table 4, Figure 5). All eight Great Lakes states prohibit the release of diploid Grass Carp. Michigan, Minnesota, and Wisconsin prohibit the possession and release of diploid and triploid Grass Carp. Possession of diploid Grass Carp is also prohibited in Ohio and Pennsylvania and a permit is needed for diploid possession in Illinois, Indiana, and New York (Table 4). It is legal to stock triploid Grass Carp by permit in Illinois, Indiana, Ohio, Pennsylvania, and New York.

In Canada, federal regulations to manage and control aquatic invasive species are in place prohibiting import, possession, transport and release of aquatic species that pose a significant invasion risk and includes Asian carps. In Ontario, OMNRF banned the live sale of Asian carps through the *Fish and Wildlife Conservation Act* in 2004 and banned the possession of live Asian carps through the Ontario Fishery Regulations in 2005.

Regulations that permit the legal possession of live Grass Carp increase the chance that a person could intentionally or unintentionally release Grass Carp into the Great Lakes or a tributary. This threat is greatest for lakes Erie, Michigan, and Ontario because these lakes are bordered by states that permit the possession, sale, and stocking of triploid Grass Carp. While it is not legal to stock Grass Carp directly into any of the Great Lakes, Grass Carp may escape from stocked locations and enter into open tributary systems that connect to the Great Lakes. For example, triploid Grass Carp stocked in confined embayments to control submerged macrophytes escaped through different barriers; potentially between 35 and 42% of Grass Carp escaped through gated and V-shaped barriers, respectively (Maceina et al. 1999). The threat to the Great Lakes from stocked Grass Carp increases with the number of fish stocked, the number of stocking locations within the basin, and the proximity of stocking locations to the Great Lakes and tributaries. Furthermore, in the U.S., Grass Carp are also stocked in ponds with other species on private fish farms. Grass Carp is stocked in Fathead Minnow (*Pimephales promelas*) ponds along with Black Carp for vegetation and snail control to prevent infestations of yellow grub (Conover et al. 2007). Black Carp and Grass Carp are also stocked into foodfish ponds with Channel Catfish, hybrid temperate basses (*Morone* spp.), and Largemouth Bass (*Micropterus salmoide*s) (Conover et al. 2007). Farm-raised catfish are also purchased by state agencies and stocked in lakes and ponds for recreational fishing. In an investigation of Bighead Carp in urban fishing ponds in Illinois, the ILDNR (2011) collected both Bighead Carp and Grass Carp from Flatfoot Lake in the Chicago area. The ILDNR caution that any producers rearing catfish and Asian carps together in culture ponds could be a source of Asian carps in waters stocked with catchable-sized Channel Catfish.

In a national analysis of Grass Carp regulation, production, triploid certification, shipping and stocking, HDR Inc. identified 393 producers and distributors of Grass Carp in the U.S. (MICRA 2015). No producers were identified in Great Lakes states and no distributors were located in

Michigan, Minnesota, or Wisconsin (Figure 5). Multiple Grass Carp distributors are located in each of the five Great Lakes states that allow stocking of triploid Grass Carp (Table 6). Many distributors in New York State are in close proximity to lakes Erie and Ontario (Figure 5). Within these five states, triploid fish are not produced from diploid fish contained in the state, but rather triploid fish may be brought into these states for growout (Table 6).

Table 6. Summary of types of Grass Carp facilities by state as reported by HDR, Inc. in MICRA (2015). All growout facilities are also distributors, but are only marked as growout facilities to prevent double counting.

Distributor Type	Illinois	Indiana	New York	Ohio	Pennsylvania
Producer					
Growout*	2	2		1	
Food Fish Market	1				
Food Fish Distributor			1	1	
Warehouse	1				
Distributor No Holding Facility		2	1		1
Truck With Holding Facility	1	2	10	2	5
Fish Day Facility			18		

HDR identified several risk factors for accidental or illegal introductions of Grass Carp as a result of live fish distribution, including: not all live Grass Carp distributors are held to operating standards, or even permitting and licensing requirements in some states; more than 50% of the distributors use a holding facility for their operations which requires additional transfer of fish and the increase risk of accidents or human error; and at least 15% of distributors distribute Grass Carp out of state. In addition, few commercial fish haulers have written Standard Operating Procedures (SOPs) or Best Management Practices (BMPs) to prevent contamination of diploid fish in triploid shipments unless it is part of a written permit provided by the state (MICRA 2015). The activities of commercial fish haulers are regulated by the receiving state. Without written SOPs and BMPs the possibility for introductions of diploid Grass Carp by distribution/stocking or facility escape increases.

Over the last 10 years, the National Triploid Grass Carp Inspection and Certification Program (NTGCICP) has prevented 33 lots of Grass Carp that did not meet the standards of the program from entering the certified triploid Grass Carp supply chain. The NTGCICP requires that the producer check the ploidy of every Grass Carp in the lot designated for certification prior to the USFWS inspection. The USFWS inspector witnesses the retesting of an approved sample size of fish for verification of ploidy. If there is a failure for any reason, the entire lot fails and a penalty and/or suspension is imposed by the USFWS. Producers participating in the NTGCICP report that the number of Grass Carp on their farms prior to 100% farm testing (i.e., producer individually blood tests each fish in a lot) contain less than 1% diploid Grass Carp. In a preliminary investigation of detection limits of NTGCICP, it was determined that populations containing 99% triploids would fail 60% of inspections if they were not 100% farm tested prior to inspection. However, following implementation of 100% farm testing in August 2010, only 0.64% of Grass Carp inspected lots failed NTGCICP inspections (MICRA 2015).

In a project with Illinois and Indiana DNR law enforcement personnel, 25 Grass Carp were seized and analyzed for ploidy (Whitledge 2014). One fish confiscated from a fish hauling truck in Illinois was diploid and two fish seized from a fish hauling truck in Illinois were mosaic (containing both diploid and triploid cells). Illinois requires a permit to possess live diploid Grass Carp and permits are approved only for the production of triploid Grass Carp in closed aquaculture facilities; stocking of triploid Grass Carp is allowed by permit (Table 4). While only one diploid Grass Carp was found being illegally transported during this study, the discovery of several illegally introduced, diploid Grass Carp in the Lake Michigan watershed and in Marrs Lake, Michigan indicate the potential for introduction through this vector (Whitledge 2014). HDR concluded that the risk from live Grass Carp distribution is greatly diminished in states that have strong regulations in place. However, despite regulations prohibiting possession and sale of all Grass Carp in Michigan, an out-of-state fish distributor was arrested in June 2012 and admitted to possessing and selling over 100 live Grass Carp in Michigan (State of Michigan 2012).

In Ontario, it is illegal to possess or sell live Asian carps. Despite this regulation, reports from January to December 2014 revealed that live Grass and Bighead carps were documented in shipments for import into Ontario from the U.S. through the Queenston (Niagara Falls) and Windsor ports of entry (OMNRF, unpubl. data). Mixed shipments of these two species amounted to 27,685 kg, with 22,822 kg coming into Niagara Falls, and 4,863 kg coming into Windsor (Figure 13). All shipments originated from five U.S. states: Michigan (69.35%), Illinois (20.67%), Arkansas (4.92%), Ohio (3.62%), and Maryland (1.34%) (Figure 14). Caution must be used with analyzing import records into Canada as the full database was not available and source regions may not be accurately reported to authorities. Only Harmonization System codes were available, which group live fish imports into very broad categories (e.g., “Carp”, fresh or live). Importers are relied upon to accurately place their imports into the correct categories and to correctly identify place of origin and species; however, during border inspections of live aquatic species being brought into Toronto and Niagara Falls, several discrepancies were noted among import records, import invoices, and the actual specimen/commodity being imported (B. Cudmore, N. Mandrak, DFO, pers. obs.).

The possession and sale of live Asian carps within the province of Quebec is illegal. Import records into Canada in 2014 (B. Cudmore, DFO, unpubl. data) indicate that only “fresh” (dead) Common Carp are entering into the province from the U.S. at the Lacolle port of entry.

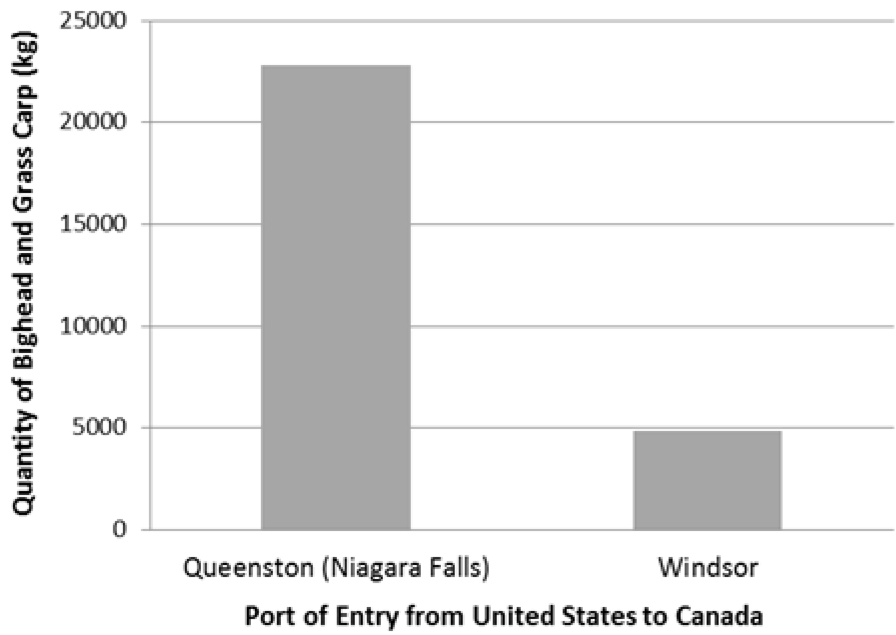


Figure 13. Quantity (kg) of Bighead and Grass carps imported into Ontario, Canada by port of entry from January–December 2014 (B. Cudmore, DFO, unpubl. data).

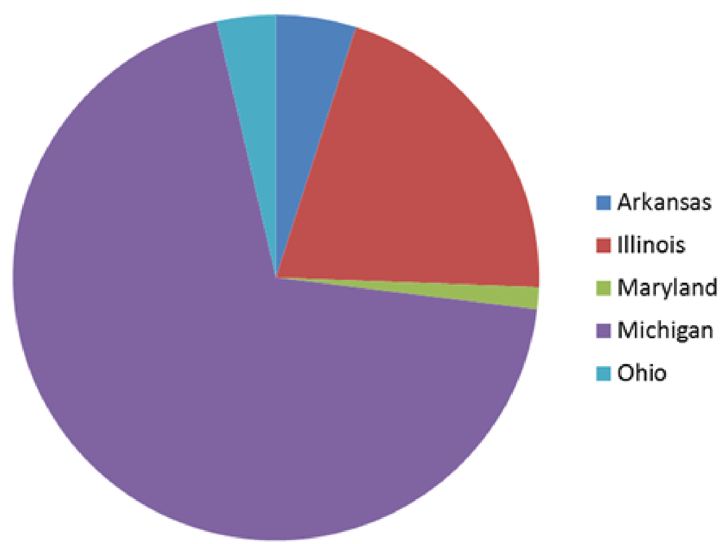


Figure 14. Proportion of Bighead Carp and Grass Carp shipments by last U.S. state in prior to Canadian entry from January–December 2014 for ports of entry Queenston and Windsor (B. Cudmore, DFO, unpubl. data).

Since 2005 (after the provincial Asian carp ban was enacted), Ontario has had 18 convictions for possession of Asian carps, with over $340,500 CDN in fines and at least 18,303 kg of fish seized (OMNRF Enforcement Unit, pers. comm.). These occurred throughout the Greater Toronto Area and border crossings of Windsor and Sarnia, Ontario.

Feeder fishes (typically Goldfish (*Carassius auratus*) or “rosy reds” (colour variant of Fathead Minnow [*Pimephales promelas*]) shipped into the Great Lakes basin could be contaminated with

Grass Carp if they originated from fish farms in the Mississippi River basin. Fathead Minnows found in the bait industry in Michigan are known to originate from culture in Arkansas, Minnesota, North Dakota, and South Dakota (G. Whelan, MDNR, pers. comm. in Cudmore et al. 2012). However, the volume of such movement and the extent of contamination, if any, is unknown. Based on a subsample of live fish import records to Canada for 2006–2007, Fathead Minnows (likely rosy reds) imported for the aquarium trade originated primarily from Missouri and secondarily from North Carolina (B. Cudmore, DFO, unpubl. data).

Grass Carp is more in demand for live sale in specialty food markets than Bighead Carp, and sells for a higher price (Stone et al. 2000). Live fish haulers view Grass Carp as more profitable than Bighead Carp (Engle and Brown 1998). Consumers in specialty markets prefer to purchase live Asian carps rather than dead or even freshly killed fish (Kerr et al. 2005). The sale of live Asian carps in food fish markets has created considerable concern about potential unauthorized releases; there is enough concern that some large cities have promulgated local laws that require Asian carps to be slaughtered at the time of sale (Higbee and Glassner-Schwayder 2004). Live Grass Carp sold in food fish markets can be either wild-caught or farm-raised. In Ontario and Quebec, live Grass Carp were found in three of six live fish markets visited between October 2002 and July 2003 (Rixon et al. 2005); however, this was prior to legislative changes to prohibit possession of live Asian carps. Transportation of live Grass Carp by truck within the Great Lakes basin presents the possibility for a large introduction event as the result of an accident or other human error. Although the likelihood of such an introduction may be low, the threat to the environment is high. This type of release is known to have occurred with Bighead Carp outside the Great Lakes basin (Conover et al. 2007). Sales of live fish at food fish markets also create opportunities for individuals to release Grass Carp for spiritual/ethical reasons.

Release of aquaria or hobby fish is a potential source of Grass Carp within the Great Lakes basin. Although uncommon as an aquaria or water-garden fish, Grass Carp are available for purchase over the Internet (Great Lakes Commission 2014). Internet trade by aquaria owners and hobbyists is a possible, but unlikely, pathway for the introduction of Grass Carp in the Great Lakes basin. Internet trade is unregulated and the extent of this pathway is unknown.

2.1.3 Laker Ballast

Unlike the ballast water in freighters that originate outside of the Great Lakes-St. Lawrence River basin, ballast water in freighters that remain in the basin (known as “lakers”) is not treated for AIS in any way (see Section 2.4.3 for more details on laker ballast as a vector for spread). If Grass Carp were to become established first in the St. Lawrence River, laker movement may facilitate the arrival of the species into the Great Lakes basin, particularly for small early life stages such as eggs, larvae, and juveniles. To date, there have been no empirical studies on the role of laker ballast water in the movement of fishes.

To determine the potential for between-lake ballast movement, Drake et al. (2015a) developed models describing the probability of spread and establishment of AIS as a result of domestic ballast-water movement based on the data from Rup et al. (2010), describing all combined U.S. and Canadian laker traffic within the Great Lakes-St. Lawrence River basin, 2005–2007 (Figures 15 and 16). Drake et al. (2015a) developed both relative risk models (i.e., probability of spread from a source port relative to all other source ports), and absolute risk models (i.e., the rate of spread between ports or lakes compared to natural dispersal). The models were run for several invasiveness scenarios related to ballast uptake and establishment probability based on propagule density (Figures 17 and 18). The invasiveness scenarios were not specifically developed for Grass Carp but represent generic scenarios that can be applied to reflect the establishment characteristics of a given species. Therefore, uncertainty exists about which scenario best reflects the characteristics of Grass Carp spread as a result of ballast and represents a knowledge gap. Nonetheless, by evaluating the suitability of each invasiveness scenario against what is known about Grass Carp, the likelihood of Grass Carp arrival through laker ballast from the upper St. Lawrence River to the Great Lakes basin can be estimated.

Grass Carp would most likely be entrained into ballast as lentic young-of-year (YOY), for only a very short period (< one month) of their life, in areas adjacent to spawning tributaries that provide appropriate nursery habitat, would have to survive pump mortality and transit, and avoid predation upon discharge. This assumes that suitability of spawning tributaries for Grass Carp includes the presence of an adjacent nursery habitat. Ports do not generally occur in nursery habitats where juvenile Grass Carp would most likely occur. Therefore, assuming that uptake probability is ≤ 0.01 and establishment probability ≤ 0.0001, the low invasiveness scenarios of Drake et al. (2015a) would apply to Grass Carp. As a result, the movement of Grass Carp between ports (Figure 17) or between lakes (Figure 18), through ballast water is likely negligible, thus the arrival of Grass Carp from potentially invaded source ports (Ontario: Cardinal, Morrisburg, Prescott; Quebec: Becancour, Contrecoeur, Montreal, Portneuf, Quebec, Sorel, Trois Rivieres, Valleyfield; New York: Ogdensburg) in the upper Saint Lawrence River to other ports or to one of the Great Lakes is not likely (Figure 17 and 18; ports identified with vertical black lines). Further, given the low invasiveness scenario from Drake et al. (2015a) and taken over a cumulative 10-year period, modelling indicates the species fails to enter the basin 80% of the time when Montreal, Quebec acts as a source port. However, 20% of the time, the species enters the basin and the most likely destinations are Thunder Bay, Ontario followed by Hamilton, Ontario. When Sorel, Quebec acts as a source port, the species fails to enter the

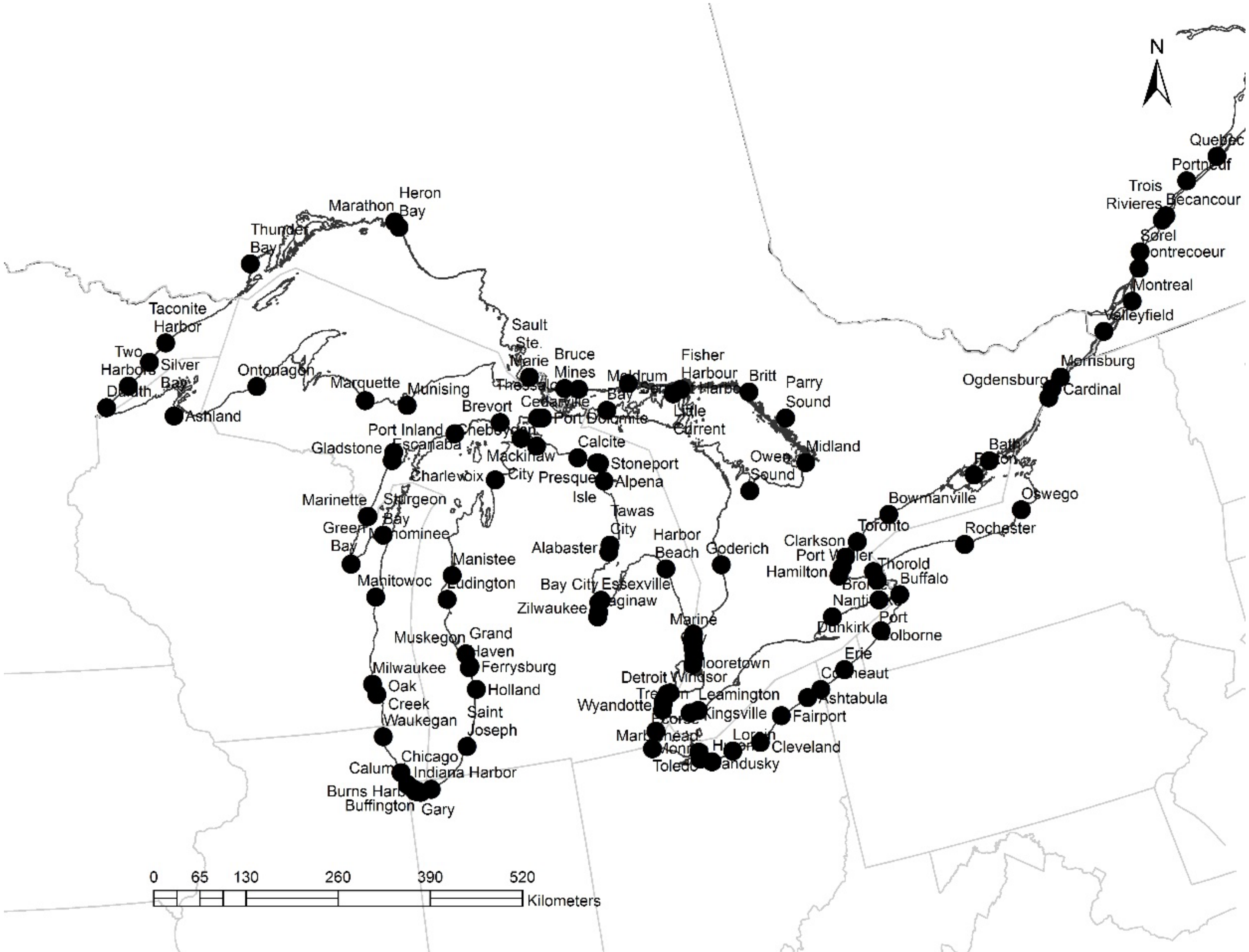


Figure 15. Ports acting as ballast water sources, ballast water recipient areas, or jointly as sources and recipient areas from domestic shipping activity in the Great Lakes basin (shipping years 2005, 2006, and 2007). Only ports with freshwater conditions were considered in the global set, with Quebec City, QC forming the eastern boundary of the study area. From Drake et al. (2015a).

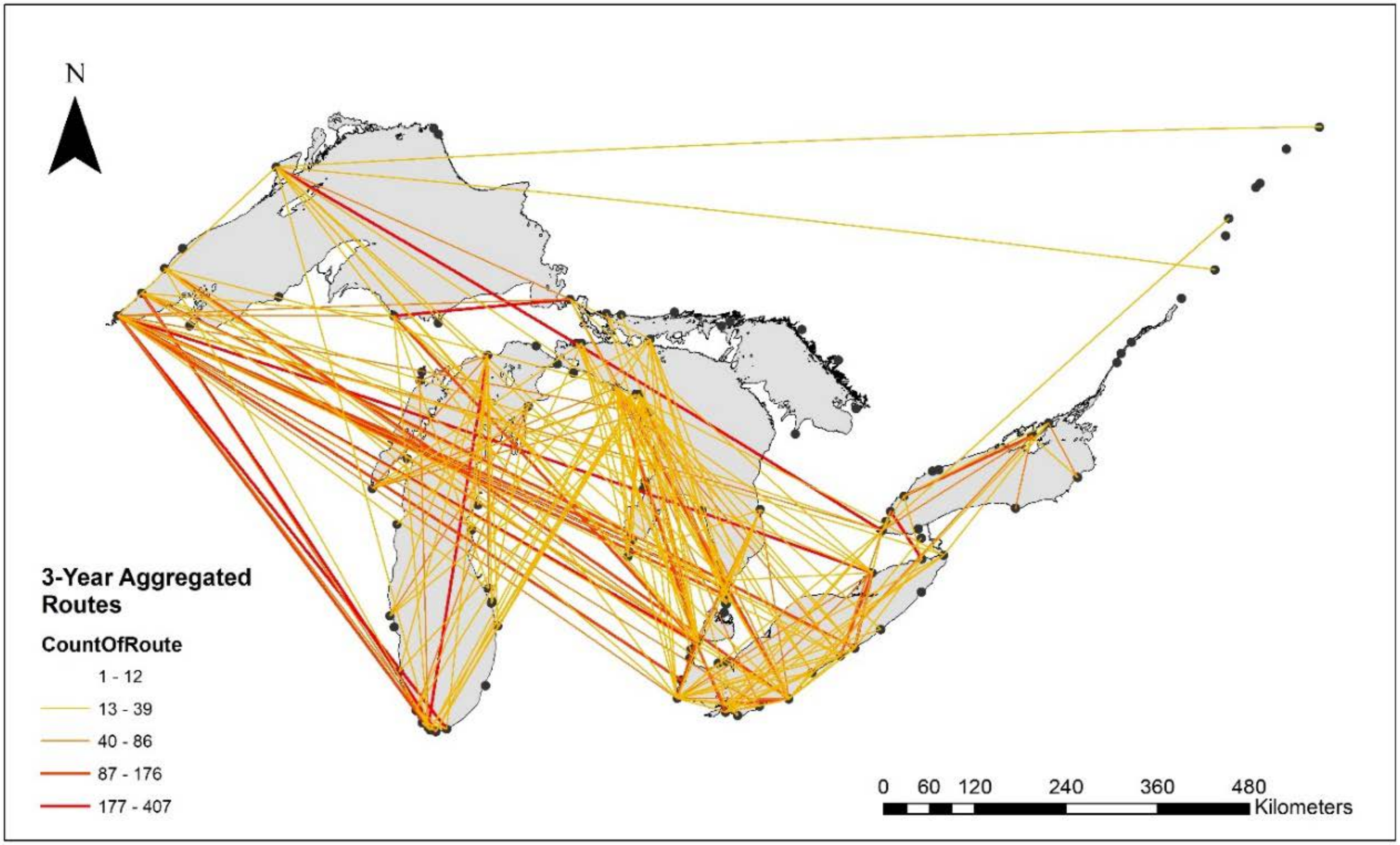


Figure 16. Aggregated routes within the Great Lakes Basin resulting from domestic shipping activity, but with only dominant routes (>40 trips out of global set) shown to provide clarity about dominant trip patterns. From Drake et al. (2015a).

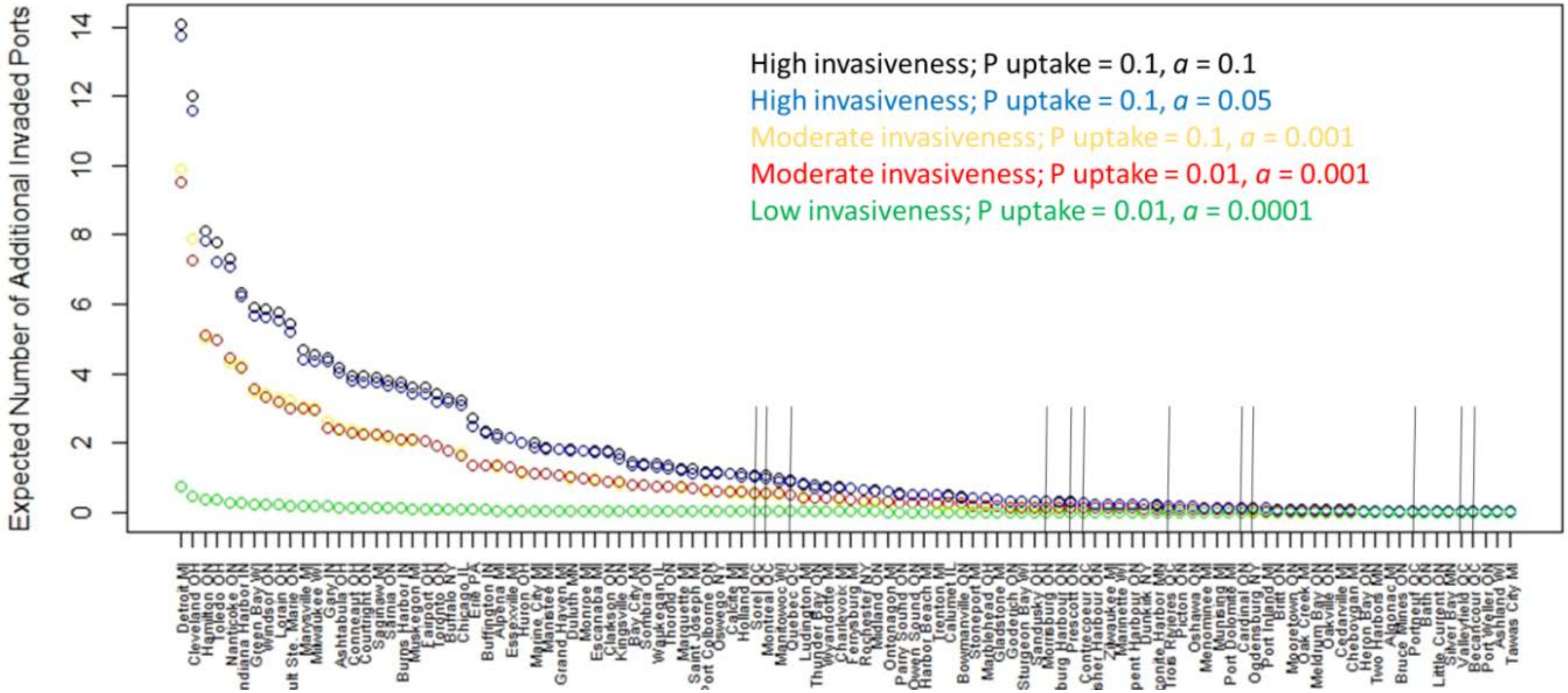


Figure 17. Results of the absolute risk scenario describing the expected number of additional ports invaded within a single year following the independent invasion of each single source port listed along the x-axis. The 12 ports representing potential arrival points from the St. Lawrence River to the Great Lakes basin are shown with vertical black lines. Invasiveness scenarios (e.g., high vs moderate) describe absolute risk as a function of different organism characteristics (physical and biological attributes) and represent generic scenarios that can be applied to reflect the establishment characteristics of a given species; authors identified the low invasiveness scenario to be most representative of Grass Carp. Parameter values involve per-trip probabilities of uptake (P uptake) and the establishment parameter, a, which describes the probability that a single propagule will establish reproducing populations. From Drake et al. (2015a).

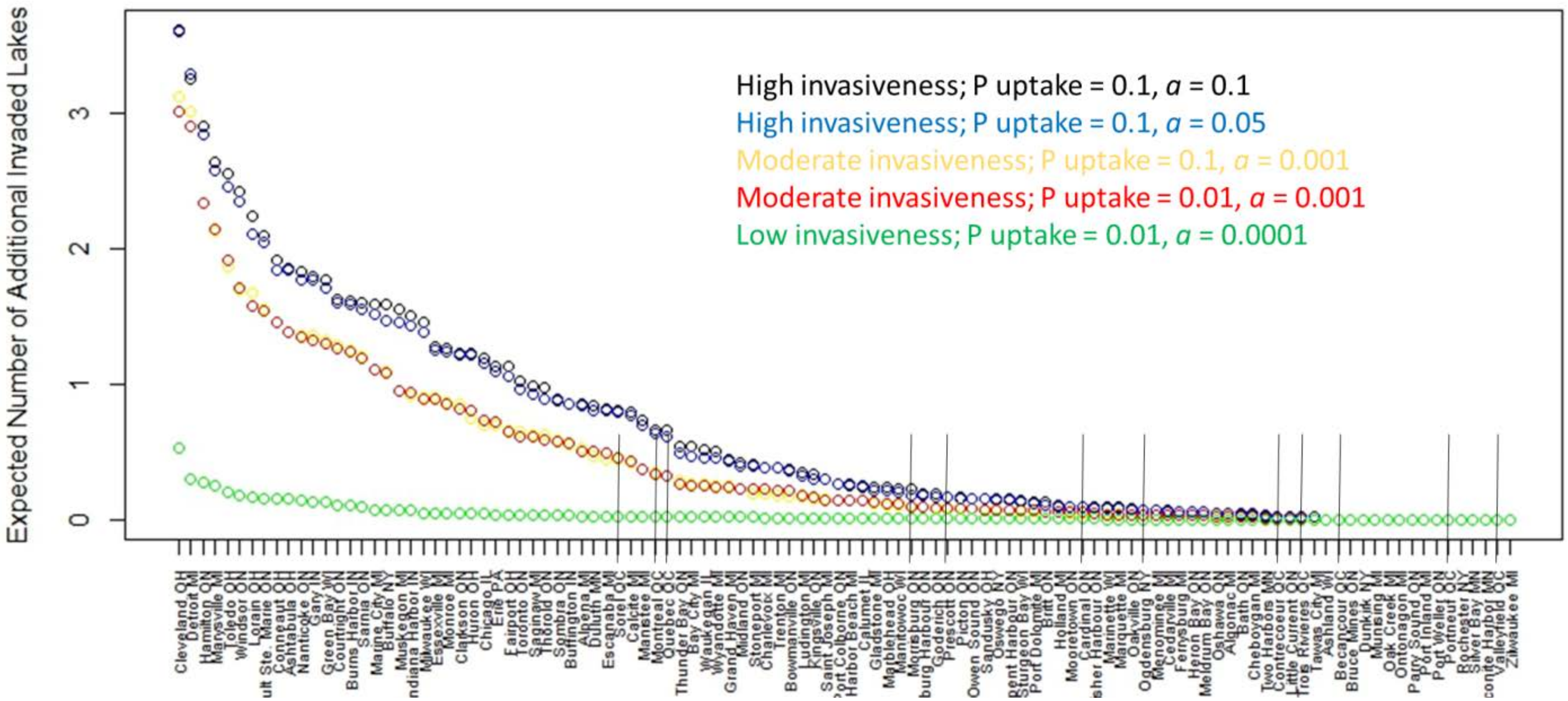


Figure 18. Results of the absolute risk scenario describing the expected number of additional Great Lakes invaded within a single year following the independent invasion of each single source port listed along the x-axis. The 12 ports representing potential arrival points from the St. Lawrence River to the Great Lakes basin are shown with vertical black lines. Lake values describe the number of new lakes (i.e., those beyond the initial lake of introduction) expected to experience establishment under each scenario. Invasiveness scenarios (e.g., high vs moderate) describe absolute risk as a function of different organism characteristics and represent generic scenarios that can be applied to reflect the establishment characteristics of a given species; authors identified the low invasiveness scenario to be most representative of Grass Carp. Parameter values involve per-trip probabilities of uptake (P uptake) and the establishment parameter, a, which describes the probability that a single propagule will establish reproducing populations. From Drake et al. (2015a).

basin 74% of the time, but 26% of the time the species is expected to enter the basin and the most likely destination is Hamilton, Ontario followed by Duluth, Minnesota. As Montreal and Sorel are the two prominent freshwater shipping hubs in the St. Lawrence River, these arrival results represent a “worst-case” scenario for ballast-mediated movement when the St. Lawrence River acts as a source population.

2.1.4 Summary of Likelihood of Arrival

Three vectors of potential entry into the Great Lakes basin were identified and assessed: physical connections; human-mediated release; and, laker ballast (Table 7). Here, the authors’ rankings are summarized, noting that the current status is a result of monitoring efforts and the recent inclusion of ploidy testing. Therefore, the current status may be underestimated in those areas where monitoring has not occurred. For triploid and diploid Grass Carp, overall human-mediated release was determined by taking the maximum rank of bait, stocking and trade, and overall arrival was subsequently determined by taking the maximum rank of physical connections, laker ballast and overall human-mediated release. The certainty of data associated with the maximum rank was retained; if tied ranks occurred, then the lowest certainty of the tied rank was used.

For this risk assessment, the invasion process for triploid and diploid Grass Carp is considered at ‘arrival’ for lakes Erie and Michigan as repeated detections of at least one Grass Carp in at least one part of the lake basin within a continuous five-year period has occurred in each of these lakes (Table 7); however, the vector/pathway of arrival remains unknown. For Lake Erie, it remains unclear as to whether the arrival of diploid Grass Carp occurred through vectors and pathways considered in arrival or through spread from another Great Lake. For the remaining Great Lakes, the invasion process is considered at ‘pre-arrival’ for both triploid and diploid Grass Carp (Table 7).

The most likely point of direct arrival into the Great Lakes basin is through the CAWS to Lake Michigan due to the proximity of established and invading Grass Carp populations within this connection, including in locations above the electric barrier. Sampling from within the CAWS 2010 through 2014 resulted in the collection of 72 Grass Carp above the electric dispersal barrier, including five confirmed diploid fish collected in, or near, Lake Calumet (K. Irons, ILDNR, pers. comm.). The documented collection of numerous Grass Carp above the electric dispersal barrier in recent years leads the authors to believe that it is very likely that triploid and diploid Grass Carp will invade Lake Michigan from the CAWS for all time periods. The authors also concluded that it is very unlikely that triploid or diploid Grass Carp will invade through physical connections to Lake Superior or Lake Huron. For Lake Superior, this rank increased to a low likelihood over time for both triploid (50 years) and diploid (20 years) Grass Carp given the proximity of Grass Carp to Lake Superior basin. Invasion through physical connections to lakes Erie and Ontario was considered to be of low likelihood for both triploid and diploid Grass Carp (Table 7). The low likelihood for Lake Erie, reflects that Eagle Marsh (with fence installed to prevent adult and juvenile transfer) and Little Killbuck Creek were considered medium risk and do not represent permanent direct connections and fish already present are not considered to have arrived through physical connections. Given that the probability of a rare event occurring increases over time and the potential for increasing numbers of triploid and diploid Grass Carp in close proximity to the basin, the ranks for lakes Superior and Huron increased to low likelihood, and to moderate likelihood for lakes Erie and Ontario.

Of the six hydrological connections considered in the GLMRIS Focus Area 2 that are associated with Lake Erie (USACE 2013a), Eagle Marsh, Indiana and Little Killbuck Creek, Ohio were considered by the authors as a low to moderate potential for triploid and diploid Grass Carp transfer to Lake Erie. Both Eagle Marsh and Little Killbuck provide conditions suitable for Grass

Carp movement and are in proximity to Grass Carp populations. However, these areas are not suitable for spawning; therefore, the potential movement is limited to adults and advanced juveniles only.

In the eastern portion of the basin, Grass Carp are in proximity to the New York Canal System, which could facilitate movement into lakes Erie and Ontario. Author certainty for physical connections for all lakes was high, with the exception of Lake Ontario, where certainty was judged to be low given the lack of information on the distribution of Grass Carp in and around the physical connections to the Lake Ontario basin (Table 7).

With respect to the human-mediated release pathway, potential for movement to the Great Lakes basin varies for each lake (Table 7). Legal trade of Grass Carp, whether diploid or triploid, is not allowed in Michigan, Minnesota, Ontario, or Wisconsin; whereas, only triploid Grass Carp are permitted in Illinois, Indiana, Ohio, New York, and Pennsylvania. It also is important to note that possession of diploid Grass Carp is permitted in Iowa and Missouri, two states that border at least one Great Lakes state. Because of this variation in regulations across the basin, and because diploid and triploid Grass Carp are likely to be part of human-mediated pathways, we evaluated the likelihood of arrival differently for each ploidy group.

The likelihood of triploid Grass Carp arriving through the baitfish vector was ranked very unlikely as triploid Grass Carp of the size range associated with the baitfish industry are unlikely to be present but, given the lack of information, certainty was ranked very low. Assuming a consistent rate of harvest, the likelihood of arrival through bait was slightly higher for diploid Grass Carp given the potential for small individuals to be present and part of by-catch with wild-caught bait, but Lake Superior remained as very unlikely given the overall lack of bait activity in the surrounding areas. Lakes Michigan and Erie were ranked higher than the other lakes, up to moderate by 50 years and up to high by 20 years, respectively, given the presence of a higher number of anglers and the frequent use of live bait in the areas around these lakes. Ranks increased over time for lakes Michigan and Erie given the probability of a rare event increasing over time and increasing likelihood of established populations which would increase the likelihood of small Grass Carp being present and available for harvest in areas around these lakes.

There is no trade of diploid or triploid Grass Carp identified within the Lake Superior watershed so the risk of arrival through this vector was ranked very unlikely but with very low certainty given the lack of information (Table 7). All other lakes were ranked as low likelihood, with Lake Erie increasing to moderate likelihood at 50 years given the probability of a rare event increasing over time, such as accidental release from illegal trucking of Grass Carp to Toronto from border crossings in southwestern Ontario. The likelihood of arrival through stocking includes the direct stocking of private lakes and ponds within the basin and accidental or intentional release from stocked waterbodies into the basin. Therefore, if a stocked fish escapes and enters into the Great Lakes basin, this was considered as arrival through stocking. The likelihood of arrival to Lake Superior through stocking increased from very unlikely (5 years) to low (10–50 years) for both triploid and diploid Grass Carp given the probability of a rare event increasing over time, the longevity of Grass Carp and the potential for fish accumulating in surrounding areas with consistent stocking. For Lake Huron, the likelihood of arrival through stocking was ranked low for both triploid and diploid Grass Carp because both Michigan and Ontario do not permit possession of live Grass Carp of any sort and there is no evidence to expect an increase in propagule pressure over time that would increase the likelihood of arrival from stocking. For Lake Michigan, arrival through stocking was ranked high for both triploid and diploid Grass Carp. While most of the watershed is in Michigan and Wisconsin where Grass Carp are prohibited, there is a small portion in Illinois and Indiana where stocking is likely to occur and increase propagule pressure over time given the probability of a rare event increasing over time and the longevity of Grass Carp. In addition, given the availability of both diploid and triploid Grass Carp in close-by states, the demonstrated motivation for illegal stocking, and the capture of fish in the Lake Michigan basin that likely resulted from illegal importation, some stocking is likely to occur in Michigan and Wisconsin, and, similarly to legal stocking in Illinois and Indiana, propagule pressure may increase over time. However, direct transfer to the Great Lakes basin from Chicago-area ponds (which are located on flat land with no substantial watershed) would be difficult due to the lack of natural connections. For Lake Erie, the likelihood of arrival of triploid Grass Carp through stocking was ranked as very likely (higher than Lake Michigan) because Lake Erie is connected to more states with stocking and there is more area around the lake basin with triploid fish being legally stocked. Diploid Grass Carp were ranked lower, at moderate (and lower than Lake Michigan), because no diploid Grass Carp caught from Lake Erie have been the direct result of stocking and the proximity of diploid Grass Carp to Lake Michigan is higher than those found in proximity to Lake Erie. For Lake Ontario, arrival through stocking of triploid Grass Carp was ranked from low (5 years) to moderate (10–50 years) and from very unlikely (5 years) to low (10–50 years) for diploid Grass Carp. Triploid Grass Carp are still stocked in states connected to Lake Ontario and agriculture is prevalent in the surrounding area, which is unlike the landscape surrounding Lake Superior and is why Lake Ontario is ranked higher. While there is the potential for illegal stocking, we do not have evidence that the rate of illegal stocking would increase through time.

The likelihood of arrival through laker ballast is ranked very unlikely with moderate certainty for both triploid and diploid Grass Carp (Table 7). Currently, there are no Grass Carp in or near the St. Lawrence River. Should they gain access to the St. Lawrence River, laker ballast water or natural dispersal would provide a direct route to Lake Ontario. The opportunities for the introduction of Grass Carp to the St. Lawrence River are not well understood but given the low invasiveness scenario deemed by the authors to be most applicable to Grass Carp, and the likelihood of arrival over time, the probability of uptake and establishment is ranked very unlikely.

Overall arrival for triploid Grass Carp was ranked very likely for lakes Michigan (physical connection to the CAWS where fish are resident) and Erie (stocking) with high certainty, noting that the authors considered arrival to have occurred in these two lakes, and very unlikely to moderate likelihood for the remaining lakes with very low to low certainty (Table 7a). For diploid Grass Carp, Lake Michigan (physical connections) was again ranked as very likely with high certainty for overall arrival and Lake Erie (bait and stocking) was ranked from moderate (5–10 years) to high likelihood (20–50 years) with moderate (5 years) to very low certainty (10–50 years), noting that the authors considered arrival of diploid Grass Carp to also have occurred in these two lakes (Table 7b). The remaining lakes were ranked from very unlikely to moderate likelihood with very low to low certainty (Table 7b).

Table 7. Overall likelihood of arrival rankings and certainties of data for each lake for (A) triploid, and (B) diploid Grass Carp, 5, 10, 20, and 50 years from the risk assessment baseline (i.e., 2014). Arrival is defined as the repeated detection of at least one Grass Carp in at least one part of the lake basin (within any continuous 5 year period) and does not include the likelihood of Grass Carp entering one Great Lake from another; this is addressed in Spread (Section 2.4). Overall arrival is the combination of physical connections, laker ballast and overall human-mediated release; the highest rank of these three elements is retained with the associated certainty of data. If a tied ranking occurs, the lowest associated certainty level is retained. If no anticipated change in rankings and certainty over time, then years are not shown in the individual boxes. Likelihood (Rank): Very Unlikely (VU), Low (Lo), Moderate (M), High (H), Very Likely (VLi); Certainty of data (Cert.): Very Low (VLo), Low (Lo), Moderate (M), High (H), Very High (VH) (see Tables 1 and 2 for definitions of rank and certainty of data).

A) TRIPLOID ARRIVAL

	Superior		**Michigan**		**Huron**		**Erie**		**Ontario**	
Element	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
Physical Connections	5,10,20= VU 50=Lo	H	VLi	H	VU	H	5,10,20 =Lo 50=M	H	5,10,20= Lo 50=M	Lo
Laker Ballast	VU	M	VU	M	VU	M	VU	M	VU	M
Bait	VU	VLo	VU	VLo	VU	VLo	VU	VLo	VU	VLo
Stocking	5=VU 10,20,50 =Lo	Lo	H	M	Lo	Lo	VLi	M	5=Lo 10,20,50 =M	Lo
Trade	VU	VLo	Lo	VLo	Lo	VLo	5,10,20 =Lo 50=M	VLo	Lo	VLo
Overall Human-Mediated Release	5=VU 10,20,50 =Lo	5=VLo 10,20, 50=Lo	H	M	Lo	VLo	VLi	M	5=Lo 10,20,50 =M	5=VLo 10,20, 50=Lo
OVERALL ARRIVAL (Combined Physical Connections, Laker Ballast, and Overall Human-mediated Release)	5=VU 10,20,50 =Lo	5=VLo 10,20, 50=Lo	VLi*	H	Lo	VLo	VLi*	H	5=Lo 10,20,50 =M	5=VLo 10,20, 50=Lo

** Grass Carp is considered to have already arrived to the lake basin in question; arrival vector/pathway unknown.*

B) DIPLOID ARRIVAL

	Superior		**Michigan**		**Huron**		**Erie**		**Ontario**	
Element	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
Physical Connections	5,10= VU 20,50= Lo	H	VLi	H	VU	H	5,10,20 =Lo 50=M	H	5,10,20 =Lo 50=M	Lo
Laker Ballast	VU	M	VU	M	VU	M	VU	M	VU	M
Bait	VU	VLo	5,10,20 =Lo 50=M	VLo	Lo	VLo	5=Lo 10=M 20,50= H	VLo	Lo	VLo
Stocking	5=VU	Lo	H	M	Lo	Lo	M	M	5=VU	Lo

	Superior		Michigan		Huron		Erie		Ontario	
Element	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
	10,20, 50=Lo								10,20, 50=Lo	
Trade	VU	VLo	Lo	VLo	Lo	VLo	5,10,20 =Lo 50=M	VLo	Lo	VLo
Overall Human-Mediated Release	5=VU 10,20, 50=Lo	5=VLo 10,20, 50=Lo	H	M	Lo	VLo	5,10=M 20,50= H	5=M 10,20,50 =VLo	Lo	VLo
OVERALL ARRIVAL (Combined Physical Connections, Laker Ballast, and Overall Human-mediated Release)	5=VU 10,20, 50=Lo	5=VLo 10,20, 50=Lo	VLi*	H	Lo	VLo	5,10=M 20,50= H*	5=M 10,20,50 =VLo	5,10,20 =Lo 50=M	5,10,20 =VLo 50=Lo

* *Grass Carp is considered to have already arrived to the lake basin in question; arrival vector/pathway unknown.*

2.2 LIKELIHOOD OF SURVIVAL

The likelihood of Grass Carp survival (i.e., individuals do not die upon arrival and adults live through winter months in the Great Lakes basin) was based on existing records of Grass Carp captures and available scientific knowledge of the species' biological requirements. The biological requirements considered included thermal tolerance and food resources, and the availability of such conditions within the Great Lakes basin, as well as potential predation pressure (on Grass Carp > 20 mm in length) and disease occurrence. Along with potential predation pressure on adults, potential predation on juveniles (20–200 mm) is considered in this section because Grass Carp of this size could arrive through the bait trade. However, predation of eggs and larvae < 20 mm is addressed in the establishment section (Section 2.3.4) because it is unlikely these early development stages will arrive to a lake basin. Overwinter survival of YOY Grass Carp is also addressed in the establishment section (Section 2.3.4) as it is relevant to the likelihood of establishment.

Triploid Grass Carp might differ somewhat from diploids in their likelihood of survival, because triploid fish may grow at different rates (Tiwary et al. 2004), may have higher rates of skeletal deformity (Grimmett et al. 2011), and may have lower energetic demands as adults since they do not need to divert energy for sexual maturation (Tiwary et al. 2004). However, inadequate information exists to make a substantive comparison in likelihood of survival between diploid and triploid fish; thus, we treat both diploid and triploid fish together in this section.

2.2.1 Grass Carp Occurrence in the Great Lakes Basin

Live Grass Carp have been captured from all of the Great Lakes except Lake Superior, providing evidence that Grass Carp do not die upon arrival (USGS NAS database 2015). Grass Carp have been reported from the Great Lakes basin since at least the early 1980s (USGS NAS database 2015). Chapman et al. (2013) found four 1-year-old Grass Carp had been spawned and survived their first year of life in the Sandusky River (an Ohio tributary of Lake Erie) and two more Grass Carp of that same year class, also with a Sandusky River provenance, were acquired since that publication (D. Chapman, USGS, unpubl. data). Otolith evidence of three additional diploid Grass Carp captured in the Lake Erie basin not only indicates that these fish originated in a Lake Erie tributary (other than the Sandusky) but that they survived multiple years in the basin (Chapman et al. 2013, Whitledge 2014). Together, data from these captures provide evidence that conditions within Lake Erie are sufficient for survival (i.e., do not die upon arrival and can live through winter months), not only of young fish, but also of adults.

Furthermore, Grass Carp captured from the Great Lakes basin were robust individuals, ranging in age from 1 to approximately 26 years, with high body condition and various states of maturity (Whitledge 2014, USGS NAS database 2015, P. Kocovsky, USGS, pers. comm.). Collection of larger, older fish (both diploid and triploid) is also evidence of long-term survival because most pathways of entry to the basin (e.g., bait buckets, fellow travelers with aquaculture fishes, legal and illegal stocking) are limited to small fish of one or two years of age, although larger individuals could have been introduced through the live food trade.

2.2.2 Thermal Tolerance

Grass Carp has a broad thermal tolerance, with a native range extending from northern Vietnam as far north as the southern portion of Kamchatka, Russia. Lethal upper temperatures for Grass Carp in the literature range from 35–41 °C (Fedorenko and Fraser 1978, Chilton and Muoneke 1992). Preferred temperatures for Grass Carp are reported to range 10–26 °C (Conover et al. 2007). Of greater concern to the Great Lakes basin is tolerance to cold temperatures. Lower temperature tolerance (permanent loss of balance) occurs at 0–0.1 °C for fry, but older fish may overwinter at 1–2 °C (Chilton and Muoneke 1992) as evident by their presence in the Missouri River, which often goes below 1 °C in the winter (D. Chapman, USGS, pers. comm.). To date, adult Grass Carp have been captured in all of the lakes except Lake Superior, with captures primarily occurring from the southern portion of the lake basins (Figure 4). Based on otolith microchemistry, it is known that fish captured from Lake Erie and the Lake Michigan basin have survived and overwintered in at least the southern portion of these basins. However, it is not known whether the absence of captures in Lake Superior and northern portions of lakes Huron and Michigan are related to temperature tolerance, distance from sources of fish, or other factors. Thus, we address the thermal-tolerance-related survival of Grass Carp through existing climatological models.

Existing environmental niche models (ENM) for Grass Carp in the Great Lakes use climatological variables from the native Asian range (Herborg et al. 2007; Chen 2008; DeVaney et al. 2009) or a combination of native and non-native (including “established” populations) range data (Wittmann et al. 2014, Wittmann et al. 2016) to estimate suitability of different regions for Grass Carp. All of these models predict the potential distribution of Grass Carp based on suitable areas for establishment. However, Asian carps, including Grass Carp, have exacting requirements for spawning and survival of early life history stages (Nico et al. 2005) that are not addressed by these climate models. We include these models in our discussion of the likelihood of survival rather than in the likelihood of establishment section because they do not address the requisite habitat variables for spawning and recruitment necessary for establishment.

Herborg et al. (2007), Chen (2008), and DeVaney et al. (2009) used slightly different data to train models from the native range in Asia, climatic data and modelling methods in their ENMs, but all three models predicted either that the entire Great Lakes basin would be suitable for Grass Carp survival (Herborg et al. 2007, DeVaney et al. 2009) or, in the case of Chen (2008), which assessed only the tributaries of the Great Lakes, that all tributaries would be suitable for survival (Figure 19a, b, and c).

Wittmann et al. (2014) and Wittmann et al. (2016) used both native range and global established range data to train their models. Wittmann et al. (2014) used the sum of seven different types of niche model algorithms and used more stringent data acceptance parameters compared to Herborg et al. 2007, Chen (2008), and DeVaney et al. (2009) by excluding data without relatively precise location estimates. This resulted in few model training points from the northern portion of the native and established range and an associated prediction of low probability of survival of Grass Carp in Lake Superior and northern portions of lakes Huron and Michigan (Figure 19d). Wittmann et al. (2016) used a different modelling procedure and expanded its occurrence data sources and criteria for inclusion of data, which resulted in greater geographic coverage and an ENM prediction that included Lake Superior as providing a high chance of survival based on climatic variables (Figure 19e) and aligned with predictions from the other ENMs. In general, Wittmann et al. (2014) and Wittmann et al. (2016) found that all four southern lakes provide suitable climate conditions for Grass Carp to establish, and that there is more uncertainty about survival in Lake Superior.

It should be noted that all of the ENMs assessed here, predicted survival of Grass Carp in even the northern-most areas of the Great Lakes (with the exception of Wittmann et al. (2014)) even without anticipating future climate scenarios. Therefore, including climate change (assuming a warming trend) in these models would not have changed their general prediction of survival of Grass Carp in all of the Great Lakes.

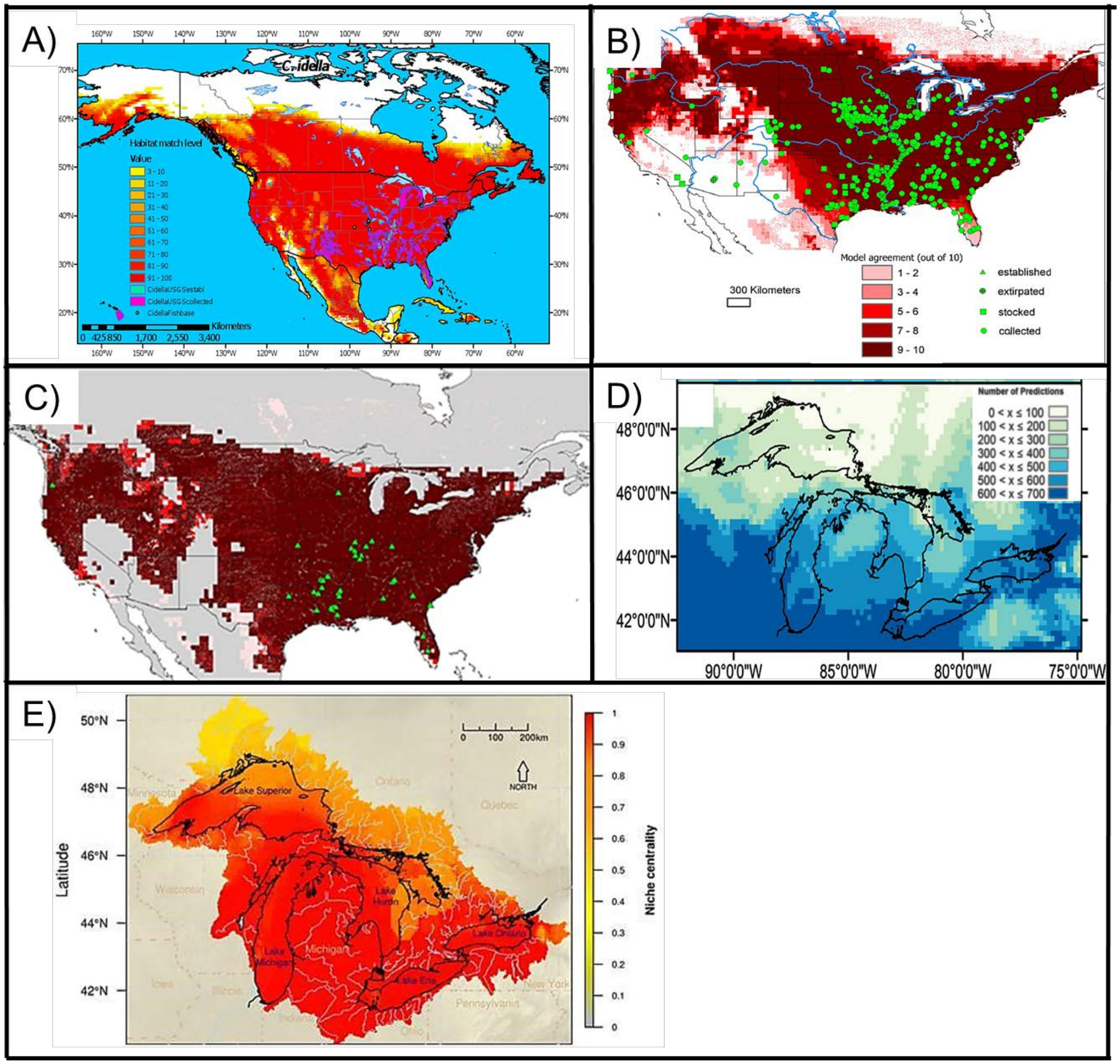


Figure 19. Environmental niche models predicting the potential suitable Grass Carp extent in the Great Lakes region from A) Herborg et al. (2007), B) Chen (2008), C) DeVaney et al. (2009) (shading indicates predicted suitability brick red = 7–10 models, canary red = 4–6 models, pink = 1–3 models; occurrence points as independent validation data = green triangles), D) Wittmann et al.(2014), and E) Wittmann et al. (2016).

2.2.3 Food Availability

Grass Carp feed primarily on macrophytes as adults. However, Grass Carp diets change with age, size, and availability of plants. Juvenile Grass Carp feed mainly on plants but, like other small fishes native to, or present in, the Great Lakes basin, they also consume animal food (chironomids, cladocerans, copepods, insects and their aquatic larvae, crustaceans, and small fishes) (Chilton and Muoneke 1992, Opuszynski and Shireman 1995). Therefore, it seems unlikely that food availability would limit survival of young Grass Carp that may arrive to the Great Lakes basin.

Adult Grass Carp are selective in their consumption of plant species (see Table 1 in Cudmore and Mandrak 2004), preferring submerged plants with soft leaves (Bain et al. 1990, Pine and Anderson 1991) and consuming the most preferred species first until they become scarce (Bain 1993). Other plant species, such as filamentous algae and firmer-leaved macrophytes (e.g., Eurasian Water Milfoil *Myriophyllum spicatum*) are consumed when they are the only species available (Opuszynski and Shireman 1995). Adult Grass Carp may also consume other food sources, including benthos, zooplankton, small fishes, earthworms, water beetles, and crayfishes (Laird and Page 1996). However, some studies have found Grass Carp lose weight when kept in unvegetated ponds with sufficient animal food sources (van Zon et al. 1977). Tree leaves and twigs from banks have also been found in the stomachs of Grass Carp deprived of aquatic plants but no food of animal sources was found, indicating that they did not shift to animal sources in the absence of aquatic macrophytes (Bailey and Boyd 1971 in Shireman and Smith 1983).

Based on these reports, the likelihood of survival of Grass Carp adults in the Great Lakes is, in part, dependent on the presence of available plant diets. Gertzen et al. (2017) used existing wetland inventories and bathymetry modelling to estimate the location and abundance of wetland macrophytes in the Great Lakes basin. While the Great Lakes as a whole are not dominated by such habitats, substantial areas with macrophytes that could be consumed by Grass Carp are present in all of the Great Lakes (Figure 20), with a total wet weight biomass of approximately 2.5 to 4.5 million metric tonnes across the lakes (Gertzen et al. 2017). In addition, Grass Carp is known to readily consume the colonial benthic alga *Cladophora* (Pípalová 2002; D. Chapman, USGS, unpubl. data), which has become highly abundant in all of the Great Lakes, except Lake Superior, since the invasion of dreissenid mussels (MTRI 2015).

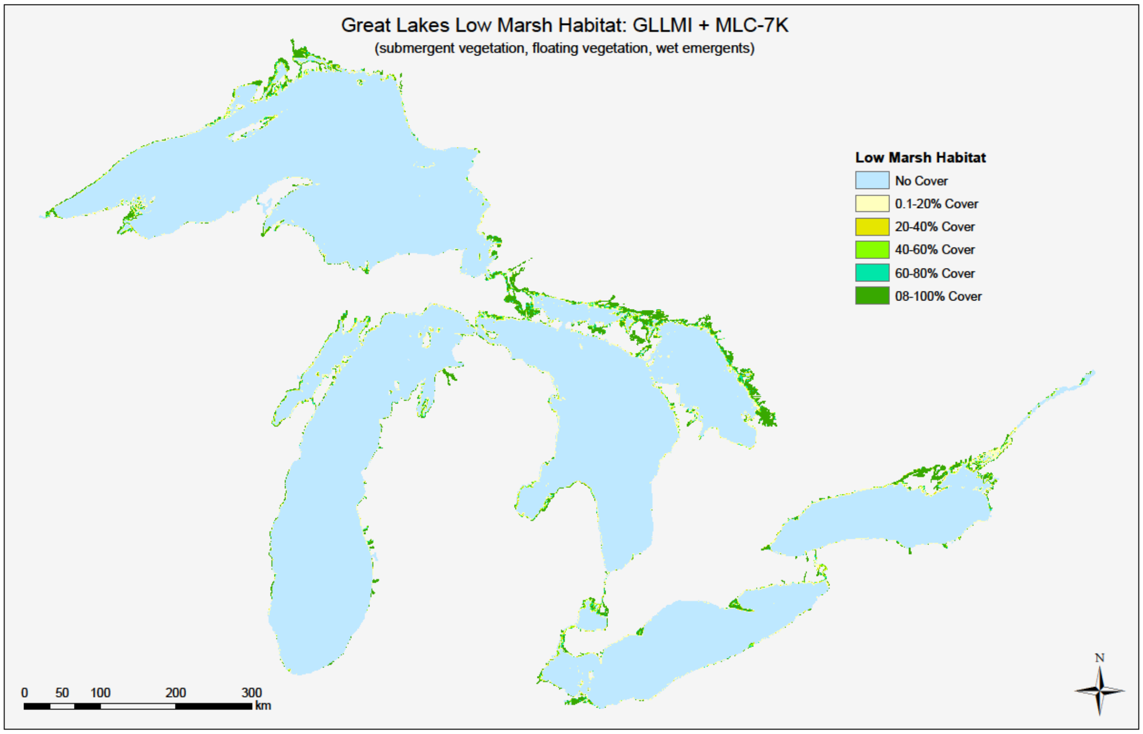


Figure 20. Percent cover of low marsh habitat (including emergent, floating, and submergent vegetation) throughout the Great Lakes, representing 2.5–4.5 million tonnes (wet weight) of nearshore vegetation. This map uses data from the Great Lakes Low Marsh Inventory and modelled percent cover of submerged aquatic vegetation. See Gertzen et al. (2017) for further details.

A bioenergetics model (van der Lee et al. model in Jones et al. 2017b) was prepared to assess the ability of Grass Carp to survive in the Great Lakes given available food resource conditions and to determine the quantity of food that would be consumed by Grass Carp. Based on this model, both *Cladophora* and macrophytes present in the Great Lakes would provide adequate food resources for Grass Carp to survive, at least in localities where they would most likely occur (i.e., nearshore areas) (Figure 20). *Cladophora*, widely present in the lower four lakes, was energetically equivalent to approximately the mean value for macrophytes consumed by Grass Carp (Jones et al. 2017b). *Cladophora* is not restricted to the zones where macrophytes have been historically abundant in the Great Lakes, which could substantially increase the area in which Grass Carp would be able to find adequate food resources and potentially create pathways of travel for Grass Carp to move between macrophyte beds without leaving zones of abundant food.

Overwinter survival of Grass Carp is also dependent on bioenergetics. Food requirements of Grass Carp are closely related to temperature (Shireman and Smith 1983). Grass Carp requires more food at warmer temperatures (Fedorenko and Fraser 1978), but Grass Carp feed at a reduced rate, or not at all, in very cold water (Cudmore and Mandrak 2004), thus, Grass Carp must feed sufficiently during the warm-water period to build up sufficient metabolic reserves to survive the metabolic demands of overwintering. In general, Grass Carp rarely feed at temperatures below 3 °C, with active feeding beginning at 7–8 °C, and intensive feeding occurring only when water temperature is at least 20 °C (Chilton and Muoneke 1992). Fischer and Lyakhoich (1973) noted that overwintering Grass Carp did not feed at all, and D. Chapman (USGS, pers. obs.) observed that, in the Missouri River, winter-captured Grass Carp always had empty guts. The bioenergetics model developed by van der Lee et al. in Jones et al. (2017b), parameterized through the primary literature with environmental data representative of the Great Lakes, specifically Lake Erie, suggests it is likely that juvenile Grass Carp will attain sufficient size for winter survival and be able to reach reproductive size using a variety of diets, including solely *Cladophora* spp. (see Section 2.3.4 for YOY overwinter survival assessment pertaining to likelihood of establishment). Required annual consumption (i.e., the amount of food necessary to maintain weight with no spawning effort) was as low as 3.4 kg for an age 1 (290 g) fish, which is used to approximate the minimum requirement for winter survival. Results were based on a winter temperature of 2 °C, which assumes that Grass Carp will move from cooler areas to deeper waters; the overwinter temperature had little effect on model results.

Wittmann et al. (2016) combined an ENM (Figure 19e) with the presence of remotely sensed submerged macrophytic food to limit the extent of the available habitat. In the combined model, some suitable habitat existed in all of the Great Lakes, but was much reduced compared to the ENM alone, especially in Lake Superior (Figure 21a). That model likely underestimates the potential area in which Grass Carp could survive because the remote sensing method would not capture macrophytes present in water deeper than the limits of the remote-sensing method (controlled by water clarity, but not over 20 m). Wittmann et al. (2016) also modelled the potential range of *Hydrilla verticillata*, and then modelled the potential change in habitat availability for Grass Carp that would occur if *Hydrilla* were to become established in the lakes. The model predicted very large increases in habitat availability for Grass Carp if *Hydrilla* was to establish and spread through the lakes (Figure 21b).

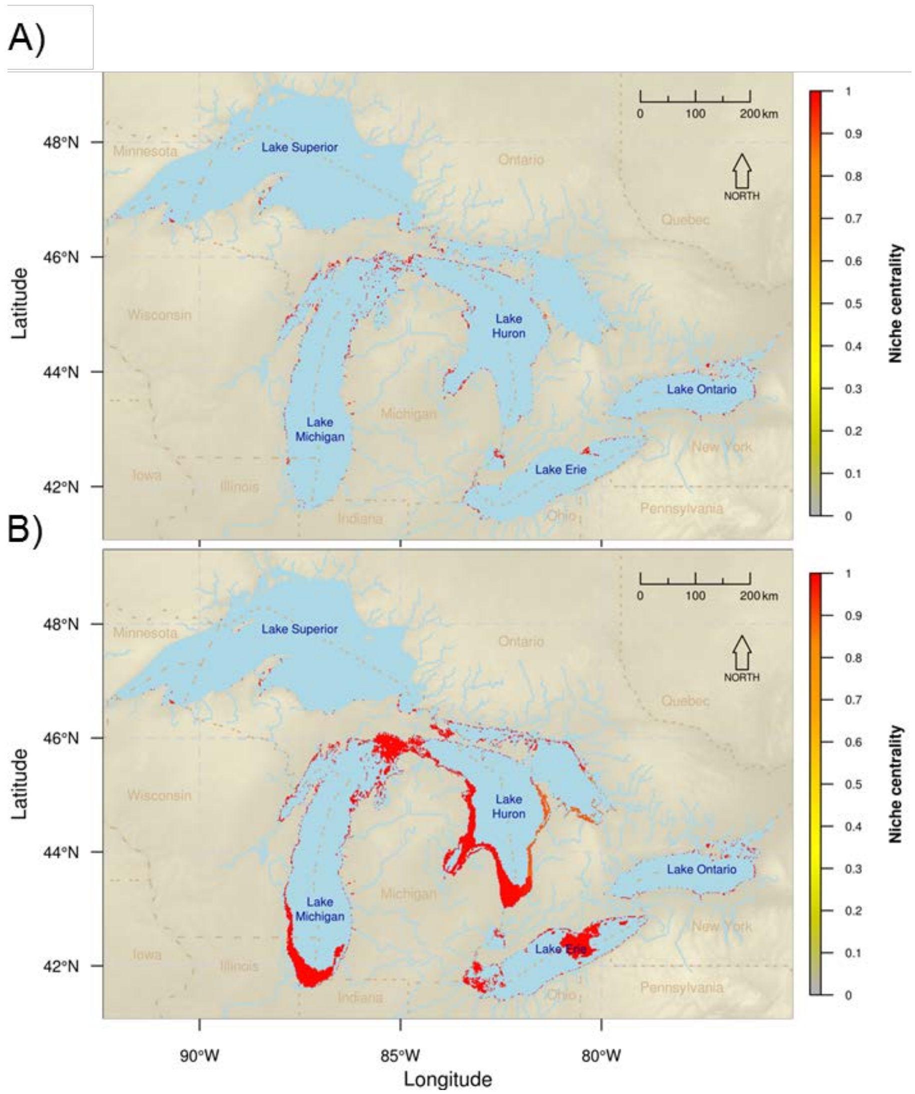


Figure 21. Niche centrality for Grass Carp for the comprehensive Great Lakes watershed region (A) clipped using a submerged aquatic vegetation (SAV) and wetlands data layer, and (B) combined with SAV, wetlands and predicted Hydrilla verticillata *niche. High values of niche centrality indicate climate conditions in the Great Lakes basin fall generally within the predicted niche. From Wittmann et al. (2016).*

2.2.4 Predators

No research has directly assessed predation on Grass Carp in North America but Grass Carp are unlikely to be susceptible to most predators for very long, relative to lifespan, given their rapid growth rates; reaching up to 1 kg by age one in temperate climates (Shireman and Smith 1983). Based on information available from other regions and from the aquaculture industry (Cudmore and Mandrak 2004, Jones et al. 2017a) some predators of Grass Carp include Largemouth Bass *Micropterus salmoides*, Northern Pike *Esox lucius*, and other fauna such as great cormorant *Phalacrocorax carbo*, osprey *Pandion haliaetus*, egrets (e.g., *Ardea alba*) and herons (e.g., *Butorides striatus*), all of which are present in the Great Lakes basin (Gertzen et al. 2017; Table 8).

Predation on adult Grass Carp in the Great Lakes would most likely come from humans (e.g., recreational capture) and large predatory birds because adult Grass Carp are unlikely to be susceptible to predation by piscivorous fishes in the Great Lakes due to rapid growth of Grass Carp and gape-limitation of Great Lakes piscivorous fishes (Table 8). For example, Grass Carp less than two years old captured in the southern Great Lakes basin measured 450 to more than 500 mm (Chapman et al. 2013), a size that is likely to be consumed by few, if any, piscine predators in the Great Lakes. The primary impact of predators is most likely to occur on juvenile Grass Carp up to 200 mm in size, as they prefer shallow water for feeding and are easily seen swimming in small groups or individually at the surface of ponds (SC DNR 2014). In the Great Lakes, several piscivorous fishes could feed on juvenile Grass Carp (Table 8)

When considering potential differences in predation pressure among the Great Lakes, a combination of lake trophic status, bathymetry, and mean water temperature must be considered. Generally, predation pressure on Grass Carp should be greater in areas that are relatively warmer and more productive because more piscivorous fishes will be present and feed at higher rates for a longer period of time than will fishes in relatively colder and less productive systems. Although colder systems would keep small Grass Carp available to predators longer because of reduced growth, these same systems tend to be more oligotrophic and dominated by coldwater fishes that do not overlap substantially with preferred Grass Carp habitat. For example, coldwater fishes such as Atlantic Salmon *Salmo salar*, Brown Trout *S. trutta*, Brook Trout *Salvelinus fontinalis*, Lake Trout *S. namaycush*, Chinook Salmon *Oncorhynchus tshawytscha*, Coho Salmon *O. kisutch*, Pink Salmon *O. gorbuscha*, and Rainbow Trout *O. mykiss* could be predators of juvenile Grass Carp but may not overlap in habitat based on temperature differences. Therefore, in warmer-water areas, such as Lake Erie, Saginaw Bay (Lake Huron), Green Bay (Lake Michigan), and the Bay of Quinte (Lake Ontario), the effects of predation pressure may be more significant than in other cooler regions within the basin.

Depending on the vector through which Grass Carp arrive to the Great Lakes, predation pressure may vary because of vector-associated fish size differences. For example, Grass Carp introduced through release of live bait are likely to be between 40 and 125 mm, whereas, those arriving through natural dispersal or escape from ponds are likely to be larger than the size piscivorous fishes can consume. Grass Carp are generally stocked for aquatic vegetation control at sizes of at least 200–250 mm (up to 450 mm), which is large enough to minimize a predation effect (Cassani et al. 2008, MSU 2014). Furthermore, if differences in distribution and abundance of wetland and submerged aquatic vegetation (SAV) exist between lakes, predation pressure on juveniles may also vary because SAV may provide cover from predation (Savino et al. 1982). No difference among lakes is expected for predation effects on adult Grass Carp.

Table 8. Potential predators of Grass Carp in the Great Lakes basin.

Prey on Juvenile (20–200 mm) Grass Carp	**Prey on Sub-adult and Adult > 200 mm Grass Carp**
Birds (e.g., egrets, cormorants, herons)	Humans
Largemouth Bass (*M. salmoides*) Rock Bass (*A. rupestris*) Smallmouth Bass (*M. dolomieu*)	Large predatory birds (e.g. bald eagles, osprey, white pelicans)
White Bass (*M. chrysops*) Bowfin (*A. calva*) Black Bullhead (*A. melas*) Brown Bullhead (*A. nebulosus*) Yellow Bullhead (*A. natalis*) Burbot (*L. lota*) Channel Catfish (*I. punctatus*) Black Crappie (*P. nigromaculatus*) White Crappie (*P. annularis*) Freshwater Drum (*A. grunniens*) Longnose Gar (*L. osseus*)	Piscivorous fishes (predation limited on adult Grass Carp due to gape limitation): Muskellunge (*E. masquinongy*) Northern Pike (*E. lucius*)

Prey on Juvenile (20–200 mm) Grass Carp	Prey on Sub-adult and Adult > 200 mm Grass Carp
Spotted Gar (*L. oculatus*)	
Muskellunge (*E. masquinongy*)	
Trout Perch (*P. omiscomaycus*)	
White Perch (*M. americana*)	
Yellow Perch (*P. flavescens*)	
Northern Pike (*E. lucius*)	
Green Sunfish (*L. cyanellus*)	
Northern Sunfish (*L. peltastes*)	
Orangespotted Sunfish (*L. humilis*)	
Pumpkinseed (*L. gibbosus*)	
Walleye (*S. vitreus*)	
Warmouth (*Lepomis gulosus*)	

2.2.5 Disease

A large number of diseases and parasites are known to infect Grass Carp (Cudmore and Mandrak 2004). Most of the diseases and parasites reported are from pond-raised Grass Carp in aquaculture where high densities and enriched waters are common (Cassani et al. 2008). These are essentially the same diseases that affect Common Carp and many other cyprinids (A. Goodwin, USFWS, pers. comm.). The primary exception is that Common Carp is susceptible to the koi herpes virus and Grass Carp is not. Since Common Carp do very well in the Great Lakes, there is no reason to believe that infectious diseases would strongly limit Grass Carp survival.

If there is a pathogen in the Great Lakes to which Grass Carp is naïve, it is likely that it would have an impact on incipient Grass Carp populations. However, Grass Carp, like other fish species, would likely adapt in a few generations and fish kills would become very rare. This was observed with viral hemorrhagic septicemia (VHS) virus, which was found in the Great Lakes in 2005 and 2006 with occurrences of large fish kills in lakes Erie, Huron, Michigan, Ontario, St. Clair, and the St. Lawrence River, but no major fish kills have occurred since (GLIN 2015).

With Grass Carp, or any organism, infectious disease outbreaks may be expected to occur if host populations are crowded or the environment is compromised. While disease outbreaks might sporadically occur if Grass Carp populations reach very high densities, there are unlikely to be diseases already present in the Great Lakes that would pose a serious limitation to the expansion of Grass Carp populations (A. Goodwin and N. Heil, USFWS, pers. comm.). Furthermore, Grass Carp in the Illinois and Mississippi River basins have not been reported to have suffered from any serious consequences from diseases or parasites and, in North America, no Grass Carp tested through the USFWS National Wild Fish Health Survey (1996–April 2015) have tested positive for any diseases (USFWS National Wild Fish Health Survey 2015).

The two diseases likely to be of greatest concern to survival of Grass Carp in the Great Lakes are VHS and Spring Viremia of Carp (SVC) (N. Heil., USFWS, pers. comm.). In the U.S., SVC (caused by *Rhabdovirus carpio*) was first detected in 2002 (Goodwin 2002) and is currently present in the states of Wisconsin, Illinois, Missouri, and Washington and in areas of the upper Mississippi River (Wisconsin, Minnesota) and Lake Michigan. In Wisconsin, a Common Carp die-off of wild fish tested positive for SVC in 2002 at Cedar Lake (Dikkeboom et al. 2004). The virus was also found in a Common Carp caught from the Cal-Sag Channel near Calumet, Illinois in 2003 and, while there was no Common Carp mortality observed, the fish were carriers of *R. carpio* (USGS 2013). The Cal-Sag Channel provides these infected fish with a direct connection into Lake Michigan and its tributaries. In 2004, *R. carpio* was found in Common Carp from Missouri and Washington (USGS 2013). In 2006, 18 of 30 tissue pools (five fish per pool) of wild

Common Carp sampled from Hamilton Harbor, Lake Ontario, tested positive for SVC and represented the first detection in Canada (Garver et al. 2007). No Grass Carp die-offs due to VHS or SVC have been confirmed to date in North America and neither VHS nor SVC has been isolated from Grass Carp in North America.

2.2.6 Summary of Likelihood of Survival

Information on thermal tolerance, food availability, predation, and pathogens and diseases was used to assess the likelihood of survival of Grass Carp in each of the Great Lakes (Table 9). Given a lack of information on differences between triploids and diploids pertaining to factors influencing likelihood of survival, both triploids and diploids were treated together, and the associated rankings and certainties are the same (Table 9). Modelling of environmental characteristics from the native range of Grass Carp indicates that there is a strong match to environmental characteristics for the entire Great Lakes basin. Based on the estimated availability of macrophytes in the Great Lakes basin and Grass Carp consumption estimates, it is likely that there is sufficient food for Grass Carp to survive, including younger individuals that do not exclusively feed on macrophytes. Based on occurrence data for Grass Carp in the basin along with bioenergetics modelling, there is no reason to believe that Grass Carp will not be able to overwinter in the Great Lakes basin. However, occurrence data are limited to southern portions of the basin and bioenergetics values are generated from a Lake Erie temperature regime; therefore, values may differ for colder water temperature regimes farther north in the basin and survival estimates may decrease for Lake Superior and northern portions of Lake Huron and Lake Michigan (see Establishment Section 2.3.4 for further discussion). Predation pressure is not likely to be a significant factor except for smaller-sized Grass Carp that may be released through bait fishing activity, and effects of predation pressure in Lake Erie, Saginaw Bay (Lake Huron), Green Bay (Lake Michigan), and the Bay of Quinte (Lake Ontario), may be more significant than in other regions within the basin. Currently, there are no known significant diseases or pathogens present in the Great Lakes basin that would prevent the survival of Grass Carp. Together, the information on these factors resulted in the likelihood of survival being ranked very likely with very high certainty for all of the Great Lakes except for Lake Superior, which was ranked high with very high certainty (Table 9).

Table 9. Likelihood of survival rankings and certainties of data for each lake for (A) triploid, and (B) diploid Grass Carp, 5, 10, 20, and 50 years from the risk assessment baseline (i.e., 2014). Survival is defined as individuals do not die upon arrival and adults live through winter months in the Great Lakes basin. Likelihood (Rank): Very Unlikely (VU), Low (Lo), Moderate (M), High (H), Very Likely (VLi); Certainty of data (Cert.): Very Low (VLo), Low (Lo), Moderate (M), High (H), Very High (VH) (see Tables 1 and 2 for definitions of rank and certainty of data).

A) TRIPLOID SURVIVAL

	Superior		**Michigan**		**Huron**		**Erie**		**Ontario**	
Time step (yr)	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
5	H	VH	VLi	VH	VLi	VH	VLi	VH	VLi	VH
10	H	VH	VLi	VH	VLi	VH	VLi	VH	VLi	VH
20	H	VH	VLi	VH	VLi	VH	VLi	VH	VLi	VH
50	H	VH	VLi	VH	VLi	VH	VLi	VH	VLi	VH

B) DIPLOID SURVIVAL

	Superior		Michigan		Huron		Erie		Ontario	
Time step (yr)	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
5	H	VH	VLi	VH	VLi	VH	VLi	VH	VLi	VH
10	H	VH	VLi	VH	VLi	VH	VLi	VH	VLi	VH
20	H	VH	VLi	VH	VLi	VH	VLi	VH	VLi	VH
50	H	VH	VLi	VH	VLi	VH	VLi	VH	VLi	VH

2.3 LIKELIHOOD OF ESTABLISHMENT

Assessment of the likelihood of establishment is based on the presence of a self-sustaining population, which is defined as occurring when individuals spawned within the Great Lakes basin, have subsequently successfully reproduced. The establishment of Grass Carp in the Great Lakes is dependent upon availability of suitable spawning and nursery habitats, enough individuals for positive population growth, stock size required for effective recruitment, and survival of early life stages (considers predation, food availability and overwinter survival).

This section primarily addresses diploids, because triploids are considered functionally sterile for management purposes and essentially provide a very unlikely likelihood of establishment (Allen and Wattendorf 1987, Cassani et al. 2008, Zajicek et al. 2011). Some uncertainty exists regarding sterility of triploid Grass Carp. Triploid males may produce substantial testes and can be induced to spermiate and, if a diploid female population is present, reproduction may occur; however, this is very unlikely and may even act to reduce diploid populations due to the majority of triploid Grass Carp gametes produced being unviable (Allen and Wattendorf 1987, (Cassani et al. 2008, Zajicek et al. 2011). Failed induction of triploidy resulting in fertile Grass Carp individuals, are considered diploid for this risk assessment.

Since 2010, 51 Grass Carp collected from the Great Lakes basin and reported to the USGS NAS database have been tested for ploidy: 22 were diploid; 21 triploid; 1 mosaic (containing both diploid and triploid cells, likely from incomplete induction of triploidy); and, 7 indeterminate/inconclusive/unknown (USGS NAS database 2015). During late 2013 and throughout 2014, an additional 22 Grass Carp collected from near the Raisin River (a tributary to Lake Erie) were tested for ploidy, but have not yet been reported to the USGS. Of those fish, 16 were diploid, 5 triploid, and 1 mosaic (A. Mahon, Central Michigan University, pers. comm.).

2.3.1 Spawning and Nursery Habitat

In northern regions of its native and established range, such as the Amur basin of eastern Russia and in Germany, age of maturity in Grass Carp is 6–10 years and 4–8 years, respectively (Shireman and Smith 1983). In temperate areas of the United States, maturity was reached in 4–5 years (Cudmore and Mandrak 2004). Males generally mature one year younger than females (Abdusamadov 1989). Size at maturity is generally 50–86 cm total length (TL) (Cudmore and Mandrak 2004). Once mature, Grass Carp require minimum of 800 degree-days within a single year to reach spawning condition (Aliyev 1977 in Bogutskaya et al. 2017), water temperatures of 15–17 °C to initiate migration to spawning locations (Chilton and Muoneke 1992) and a rising hydrograph (flood event) to initiate spawning (Chilton and Muoneke 1992, Wang et al. 2013, Bogutskaya et al. 2017), although, it is important to note that a rising hydrograph may not always be necessary for some Asian carp spawning to occur (Deters et al.

2013, Coulter et al. 2013, Jones et al. 2017a). Onset of spawning occurs at 18 °C (Chilton and Muoneke 1992, Jones et al. 2017a, Bogutskaya et al. 2017) and mass spawning occurred at 20–22 °C in the former Soviet Union and 26–30 °C in China (Chilton and Muoneke 1992).

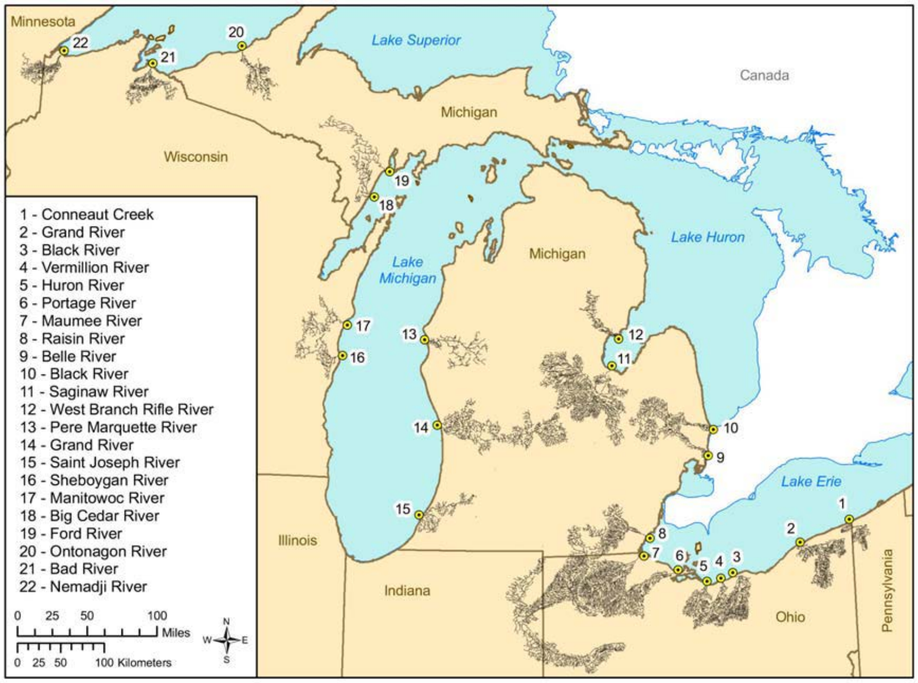
Grass Carp spawn in rivers and canals (Shireman and Smith 1983). It requires an average of 2,685 total annual degree-days (ADD; sum of mean daily water temperatures for all days above 0 °C) each year over several years to mature (Gorbach and Krykhtin 1981; Gorbach and Krykhtin 1980 in Bogutskaya et al. 2017), and a minimum number of total annual degree-days based on water temperature above 15 °C (ADD15) to reach spawning condition: 565–633 ADD15 for onset of spawning (650 ADD15 in low-water years); and, 919 ADD15 for mass spawning (Gorbach and Krykhtin 1980, 1981 in Bogutskaya et al. 2017). Grass Carp has been documented to spawn late April-early June in Oklahoma (Hargrave and Gido 2004), May–July in Missouri (Zhang et al. 2012), late April–early July in China (Duan et al. 2009), and predicted to spawn in late June–September in western Lake Erie and its major tributaries where reproduction has been found (Kocovsky et al. 2012, Chapman et al. 2013).

Fecundity is directly proportional to length, weight, and age and ranges from 1,000 eggs to 2 million eggs, but generally averages 0.5 million for a 5 kg brood stock (Shireman and Smith 1983; Chilton and Muoneke 1992). In the Amur basin, fecundity ranged from 0.2 to 1.7 million eggs with an average of 0.8 million (Fedorenko and Fraser 1978). Geographic location does not appear to affect fecundity (Shireman and Smith 1983). Grass Carp eggs are 2.0–2.5 mm in diameter when released, but quickly swell to a diameter of 5–6 mm as water is absorbed (Lin 1935 from Chilton and Muoneke 1992). George and Chapman (2015) conducted embryonic and larval development studies on Grass Carp collected in Missouri and found mean water-hardened egg diameters of 4.02–4.42 mm under varying water temperatures. Grass Carp eggs are semi-buoyant and non-adhesive, requiring well-oxygenated water and a current to keep them suspended until hatching (Stanley et al. 1978, Chilton and Muoneke 1992, Cudmore and Mandrak 2004). Once eggs are released and fertilized, the semi-buoyant, fertilized eggs of Asian carps may need to remain suspended in current until they hatch (Kolar et al. 2007, George and Chapman 2015). Hatching time is related to temperature and may take between 33–70 hours (Guo 1980, Yi et al. 2006; George and Chapman 2015). Larvae then move to productive habitats (e.g., wetlands) for feeding and/or refuge (Nico et al. 2005, Kolar et al. 2007).

Asian carp spawning has been documented in tributaries generally longer than 100 km (Nico et al. 2005, Kolar et al. 2007). Kolar et al. (2007) identified 22 U.S. tributaries of the Great Lakes that were unimpounded from the mouth to at least 100 km upstream of lakes Superior (three tributaries), Michigan (seven tributaries), Huron (four tributaries), and Erie (eight tributaries) (Figure 22a). There were no U.S. tributaries of Lake Ontario identified by Kolar et al. (2007). However, recent evidence suggests that Asian carp may need less river length to spawn (e.g. Chapman et al. 2013, Murphy and Jackson 2013). Mandrak and Cudmore (2004) identified over 80 Canadian tributaries to the Great Lakes that were unimpounded from the mouth to at least 50 km upstream [Superior (30 tributaries), Huron (28), Erie (9), Ontario (19)]. Fifty-two Canadian tributaries are unimpounded from the mouth to at least 80 km upstream [Superior (22 tributaries), Huron (16), Erie (6), Ontario (8)], and forty-one tributaries to at least 100 km upstream [(Superior (16 tributaries), Huron (14), Erie (5), Ontario (6)] (Cudmore and Mandrak 2011) (Figure 22b).

Four recent studies have examined the suitability of Great Lakes tributaries for Asian carp (or for bigheaded carp) spawning based on more detailed considerations of reproductive biology. Kocovsky et al. (2012) examined eight U.S. tributaries (Maumee, Portage, Sandusky, Huron, Vermilion, Black, Raisin, and Grand rivers) in the central and western basins of Lake Erie (see Figure 22a for river locations; Sandusky River not included). They considered: the thermal

A)



B)

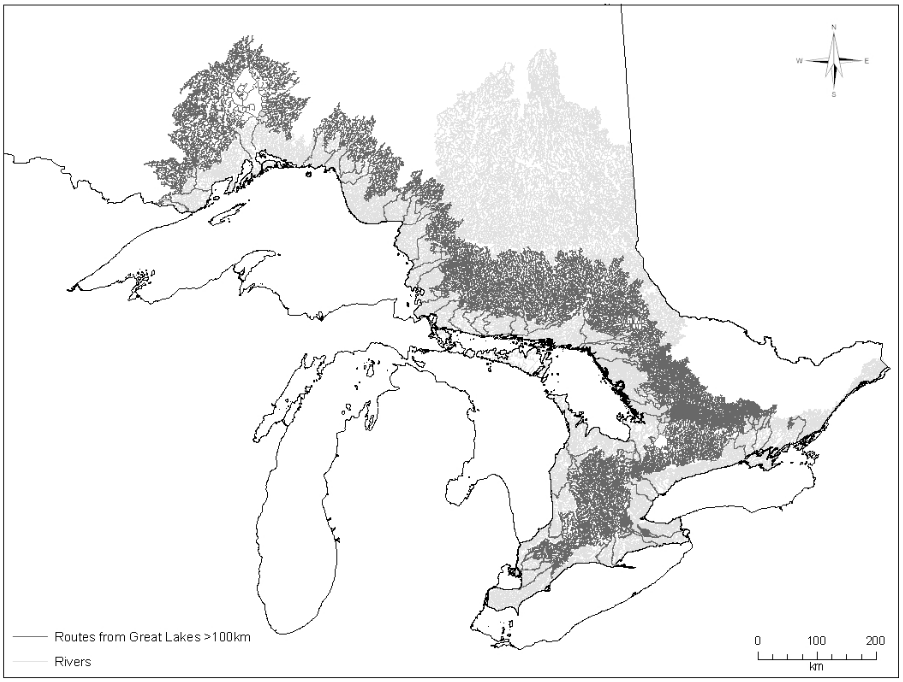


Figure 22. Distribution of suitable (A) U.S. spawning tributaries (Kolar et al. 2007) and (B) Canadian spawning tributaries (Mandrak and Cudmore 2004) for Grass Carp in the Great Lakes basin based on unimpounded length of tributaries.

conditions of the tributaries and Lake Erie; the minimum total degree-days required for maturation; onset of spawning and mass spawning; timing of flood events as triggers for spawning; length of stream required for egg hatching based on stream velocity; and, estimated incubation time. They concluded that the three larger tributaries (Maumee, Sandusky and Grand rivers) were thermally and hydrologically suitable to support spawning of Asian carps, while four tributaries (Black, Huron, Portage and Vermilion rivers) were less suited, and one (Raisin River) was ill suited due to an impassable dam. Mandrak et al. (in prep.) conducted a similar analysis for the Canadian tributaries of the Great Lakes; sufficient data to produce suitability graphs were available for 98 tributaries. They concluded suitable spawning conditions, including growing degree-days required for maturation, were present in 20 of 29 tributaries to Lake Huron, 12 of 14 tributaries to Lake Erie, and 18 of 39 tributaries to Lake Ontario (Figure 23). Mandrak et al. (in prep.) also concluded suitable spawning conditions were present in six of 12 tributaries to Lake Superior with sufficient data (Figure 23); however, only one of the 12 tributaries had a mean annual total degree-days exceeding 2,685. Therefore, Asian carps are unlikely to mature within Lake Superior tributaries, but may encounter sufficient growing degree-days to mature in some parts of Lake Superior such as nearshore and bays. Further analysis is required to identify such areas.

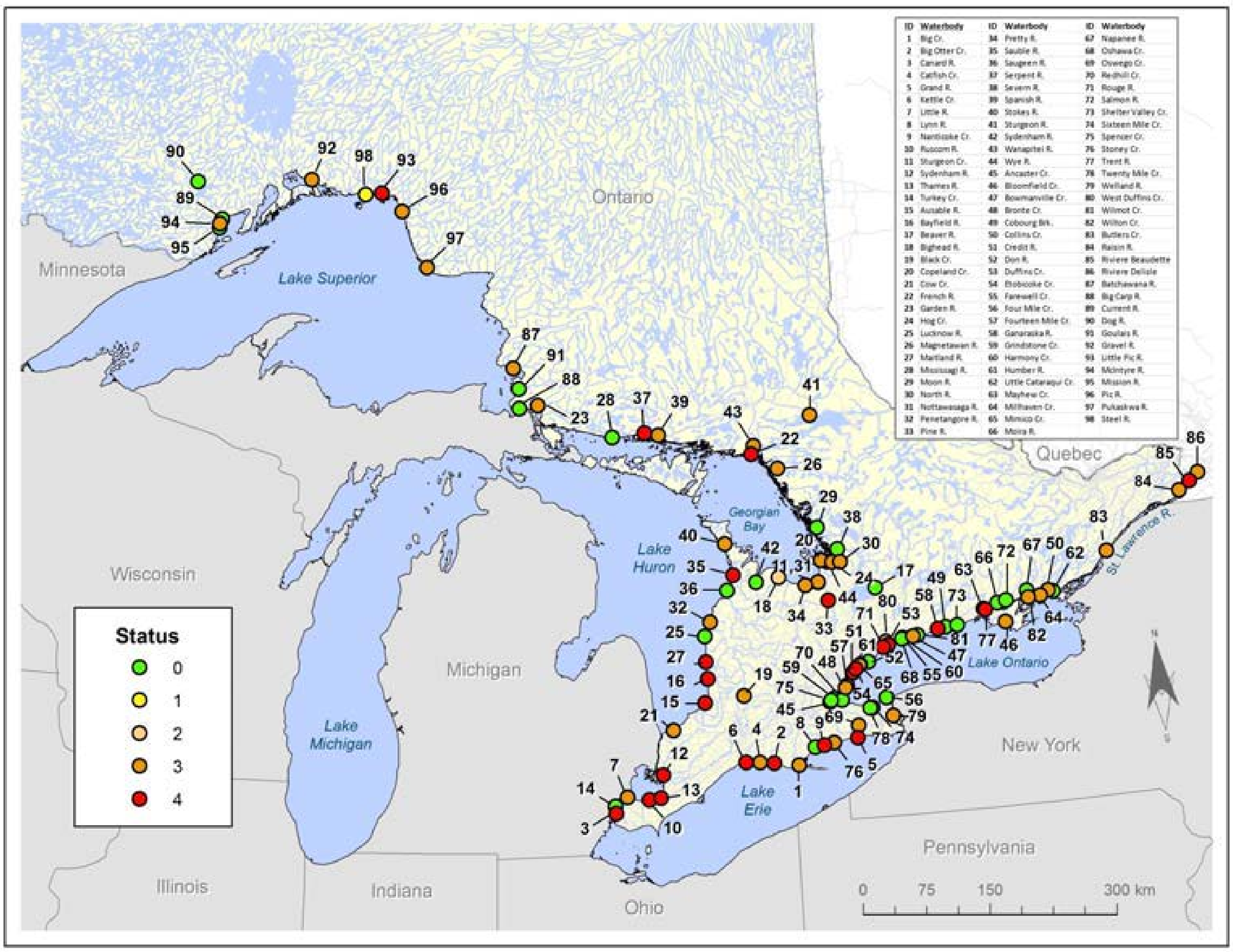


Figure 23. Suitable Canadian spawning tributaries for Asian carp in the Canadian Great Lakes basin based on thermal and hydrologic suitability (Mandrak et al. [in prep.]) Suitability Status: 0 = not suitable; 1 = minimally suitable; 2 = suitable; 3 = very suitable; 4 = highly suitable.

Neither Kocovsky et al. (2012) nor Mandrak et al. (in prep.) incorporated minimum stream width, turbulence or other factors known to affect spawning. However, most, if not all, streams assessed had a width greater than the documented minimum width (8–10 m) during low flow of a Grass Carp spawning stream (D. Chapman, USGS, pers. comm.). Furthermore, Kocovsky et al. (2012) and Mandrak et al. (in prep.) relied on linear velocity measurements at few locations and are, thus, coarse drift models. They did not consider shear velocity, lateral velocity, turbulence diffusion, and water density related to temperature, which may influence egg settling velocities and travel times and, hence, distance to hatch (Garcia et al. 2015).

Murphy and Jackson (2013) incorporated shear velocity into a model of Asian carp spawning suitability and concluded that all four of the tributaries assessed (two in Lake Michigan: the Milwaukee and St. Joseph rivers and two in Lake Erie: the Maumee and Sandusky rivers) are suitable for bigheaded carp spawning and that river reaches as short as 25 km may allow bigheaded carp eggs sufficient time to develop to hatching. Garcia et al. (2013) developed a fluvial egg drift model of Silver Carp spawning suitability that incorporated flow velocity, shear dispersion, and turbulent diffusion. The model was evaluated using experimental data from China and applied to data for the Sandusky River. They concluded that the Sandusky River would be suitable for Silver Carp spawning, which is consistent with the results of Kocovsky et al. (2012) and Murphy and Jackson (2013), and the conclusion of Chapman et al. (2013) that Grass Carp have successfully spawned and recruited in the Sandusky River. However, the frequency and duration of flood events is variable; between 1990 and 2009 there were 13 years in the Sandusky River without suitable high flood events for spawning. The Garcia et al. (2013) model developed for Asian carps has recently been updated with new Grass Carp developmental data (George and Chapman 2015). Grass Carp has slightly different developmental rates and egg sinking rates from the bigheaded carps (George and Chapman 2015), but these differences have not yet been assessed in relation to the Sandusky River.

A particle tracking model and estimated hatching times were used to examine the spawning suitability of St. Clair and Detroit rivers for bigheaded carps (N. Mandrak, DFO, unpubl. data). The model indicated that fertilized eggs in the St. Clair River would be deposited in Lake St. Clair before hatching, and those in the Detroit River would be deposited in Lake Erie before hatching. Because there are no verified records of Asian carp eggs hatching in lentic waters and the currents of lakes St. Clair and Erie are generally less than 0.1m/s (Ibrahim and McCorquodale 1985; León et al. 2005), the eggs would likely not survive. There is evidence of some hatching success on sediment (Kolar et al. 2007), but there is also some evidence that settling is detrimental to Grass Carp eggs (D. Chapman, USGS, unpubl. data). The potential for lentic spawning (i.e., where eggs fall to substrate) is a knowledge gap that needs to be further investigated, particularly for locations with strong currents, clean substrates, and few Round Goby populations (e.g., Lake Superior). Round Goby is a predator of fish eggs and could limit any opportunity for successful development of early life stages. Results from this tracking model may be conservative (predicting mortality when survival may occur) because Chapman and George (2011) developed models showing faster hatching rates.

Cudmore and Mandrak (2011) concluded that there are ample wetlands throughout the Great Lakes basin, including those associated with unimpounded tributaries greater than 50 km, which would be suitable nursery habitats for Asian carps. Mandrak et al. (in prep.) similarly concluded that many of the Canadian tributaries with suitable spawning habitat for Asian carps also had suitable nursery habitat. Numerous coastal wetlands exist throughout the Great Lakes basin, in both the United States and Canada, and would likely provide accessible nursery habitat to the suitable tributaries (Table 10; Figure 24).

Table 10. Summary of low marsh habitat available in the Great Lakes (Low Marsh Inventory by Great Lake; Gertzen et al. 2017).

Lake	Total Area (ha)	Max. Low Marsh Area (ha)	# Wetlands > 500 ha
Erie	27,840	11,976	6
Huron	41,668	5,781	6
Michigan	13,426	1,748	2
Ontario	21,720	1,740	9
Superior	10,166	557	1

A)

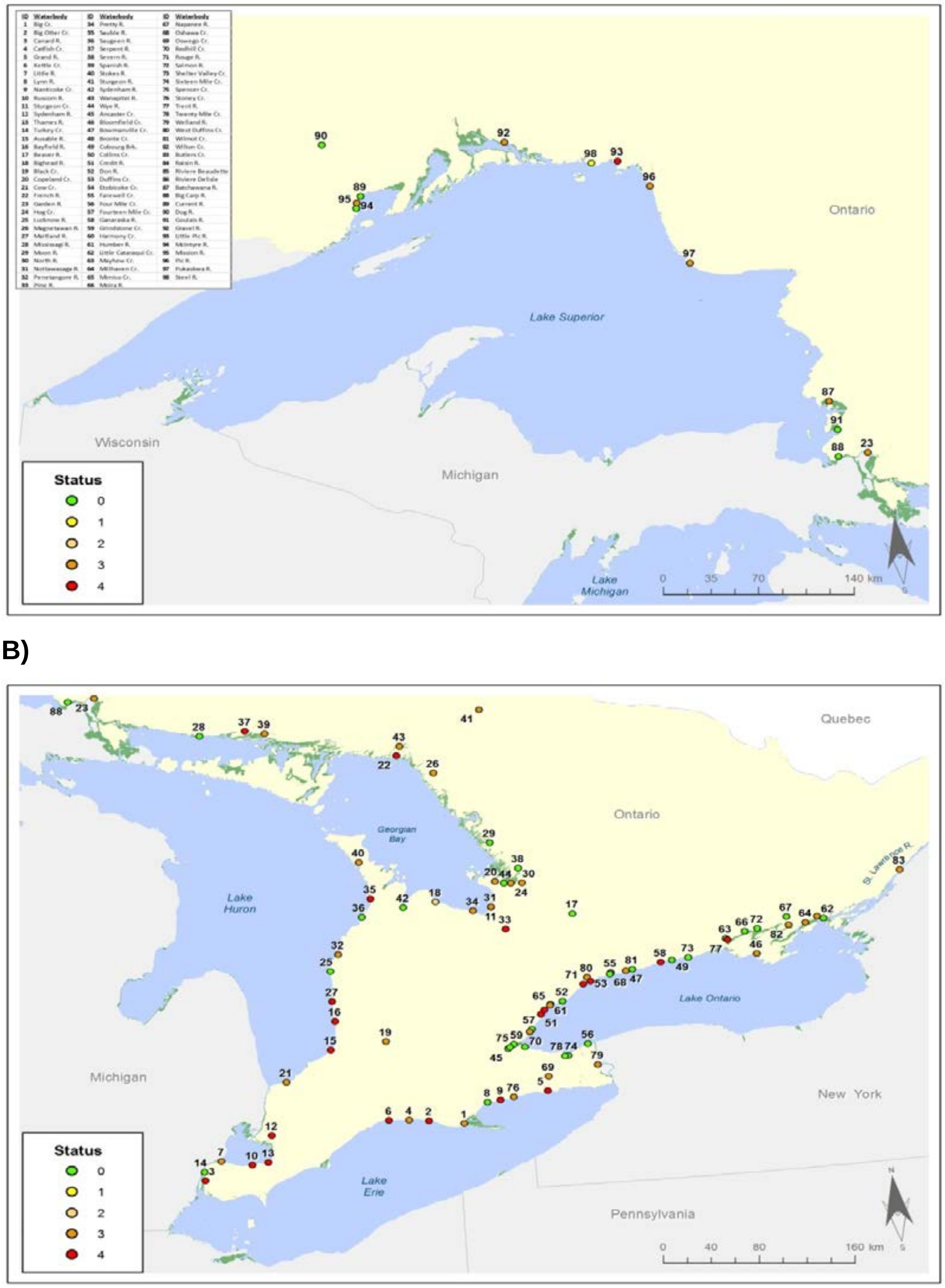


Figure 24. Suitable Canadian spawning tributaries for Asian carps in A) Lake Superior, and B) lakes Huron, Erie, and Ontario for the Canadian Great Lakes basin based on thermal and hydrologic suitability combined with the distribution of coastal wetlands, including submerged aquatic vegetation areas (Mandrak et al. [in prep.], Gertzen et al. 2017).

2.3.2 Estimated Spawning Population Needed for Establishment

There are no published data on the minimum number of Grass Carp needed to establish a population. Jones et al. (2017b) developed a stage-structured life history model for Grass Carp establishment in the Great Lakes using data from the primary literature collected under natural or unassisted conditions (e.g., wild or naturalized populations) and was similar, where possible, to environmental conditions of the Great Lakes basin. Based on 1,000 simulations, median net reproductive rate (R_0; number of females produced by a single female in her lifetime) is 24.8 (ranging between a minimum of ~0 and a maximum of 515.4). An R_0 that is greater than 1 is required for positive population growth. R_0 was less than 1 in only 9.3% of the simulations, indicating that there is 91% probability that Grass Carp will become established in the Great Lakes if introduced, based on the assumptions of the model, including no stochasticity and age of maturity of 4 years. Even if the age of maturity in the Great Lakes is older, the probability that R_0 is greater than 1 is still high (Figure 25). Incorporating demographic stochasticity, the predicted probability of establishment of a population 20 years after the introduction of 0+ age-class (J_1) individuals is low unless a very large number is introduced (e.g., 40,000 individuals for a 75% chance of establishment); whereas, very few 1+ age-class (J_2) individuals might need to be introduced to establish a population in 20 years (Figure 26). The probability of finding mates would have to be very low (<0.026) for R_0<1 (negative population growth).

To account for the ability of females to find suitable spawning rivers, Jones et al. (2017b) estimated the probability of establishment based on the probability that the river is suitable for spawning (i.e., the total number of suitable spawning rivers in a basin/total number of rivers with attractant flow in a basin) and the number of reproductively viable individuals. Relatively low numbers of individuals are required regardless of probability of spawning suitability (Figure 27). This does not take into account any chemical attractants (i.e., pheromones) released, spawning interference from triploid Grass Carp, or a need for a minimum number of spawners to initiate spawning behaviour, as these are unknowns.

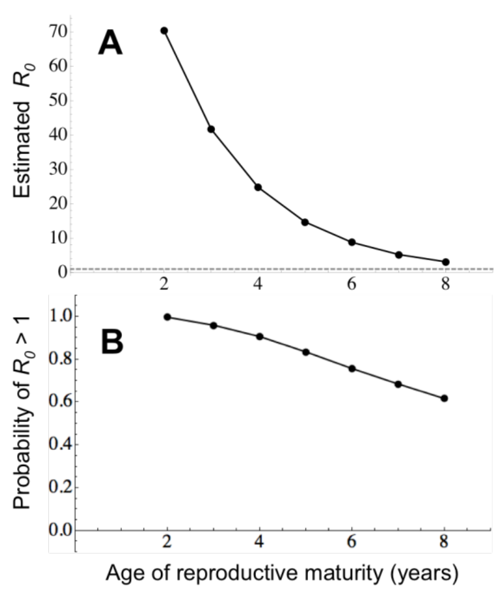


Figure 25. R_0 for variable times of reproductive maturity (A) and the probability of R_0 > 1 for variable times of reproductive maturity (B).The gray dashed line indicates R_0 = 1. Regardless of the timing of reproductive maturity for females in the Great Lakes, Grass Carp populations would be expected to establish (Jones et al. 2017b).

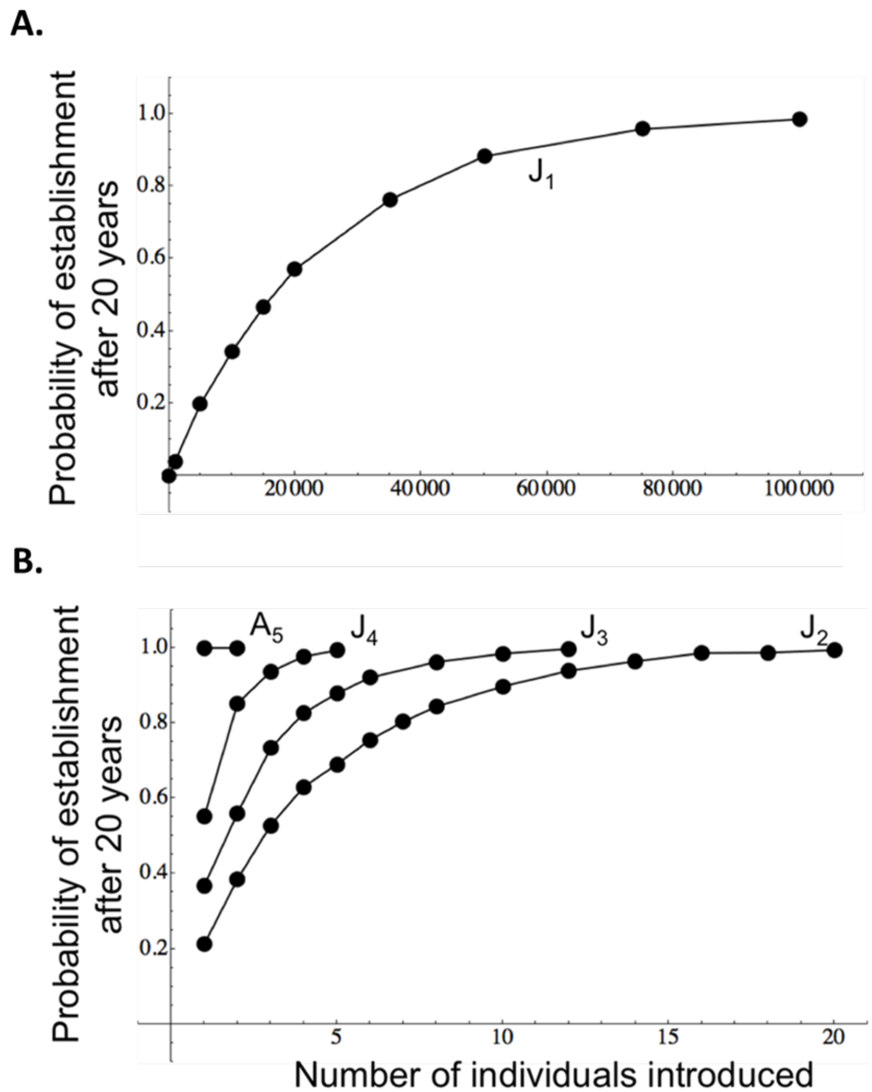


Figure 26. Probability of establishment after 20 years for J_1 stage (A) and the remaining J_2 to A_5 (B) age classes (Js are juvenile states and A is the adult state). Because the transition probability for J_1 to J_2 is so small, it would take many thousand juvenile individuals introduced to likely cause population establishment. In contrast, only 10s of individuals of J_2 to A_5 could be introduced to create an establishment event. This relationship is driven by demographic stochasticity and does not account for reduced survival or fecundity due to environmental stochasticity (Jones et al. 2017b).

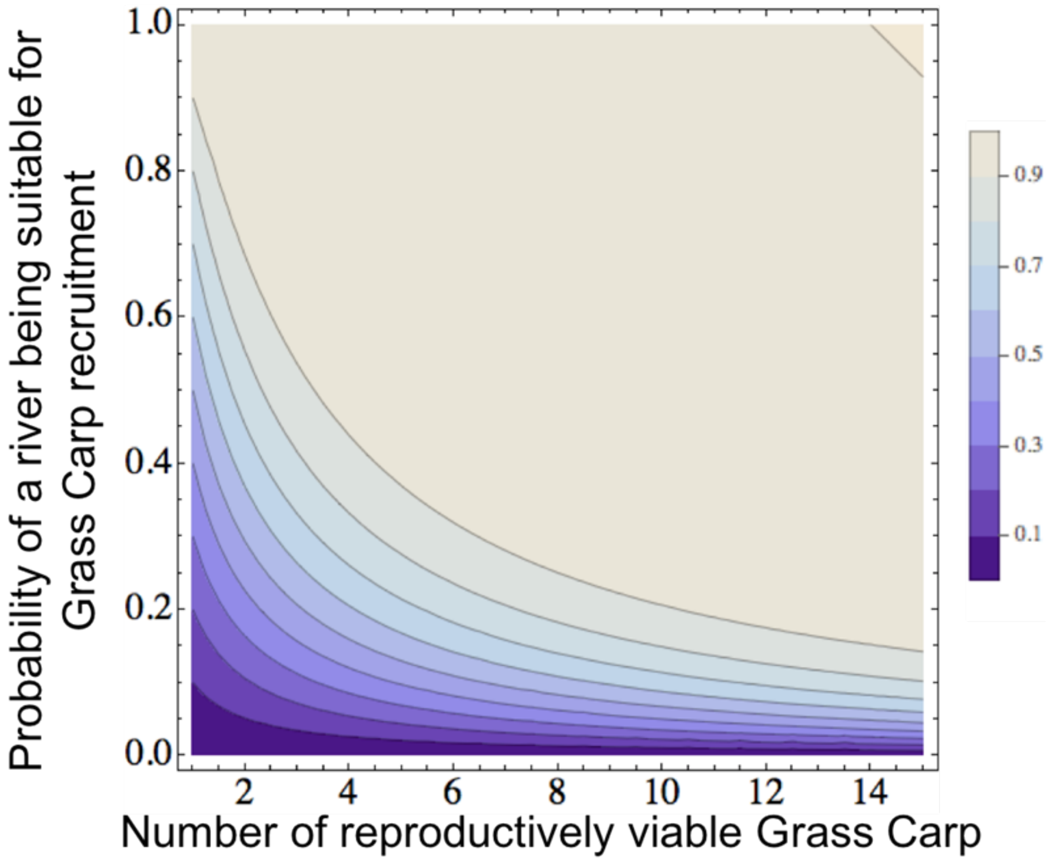


Figure 27. Probability of at least one Grass Carp occurring in a suitable spawning tributary (Jones et al. 2017b). If we assume that any female Grass Carp will be capable of spawning, then the threshold would be very low, M = 1 (where M is a defined threshold of the minimum number of fish necessary to induce spawning). We can look across the parameter space, $0 < p_s/k < 1$ and n = 1, 2, 3,.. ∞, at this function; here the figure shows that even if there are proportionally few streams in a system, (i.e., $p_s/k = 0.05$) capable of supporting reproduction, the probability of at least one female finding a stream when there are 15 female fish in the system is 0.53. In contrast, if there was a threshold of M = 2 or M = 3, then the probability would decrease to 0.17 and 0.026, respectively (Jones et al. 2017b).

2.3.3 Stock Required for Effective Recruitment

No data were located on stock size for effective recruitment of Grass Carp in environments similar to the Great Lakes. A Ricker stock-recruitment model has been developed, but is for Grass Carp in the LaGrange reach of the Illinois River (M. Hoff, USFWS and K. Irons, ILDNR, unpubl. data). Data from 1992–2013 were used to develop the model, which explained 47% of recruitment variation using stock size. Assuming the stock-recruitment relation in the Illinois River is similar to one that would develop in the Great Lakes, the risk of establishment of Grass Carp would increase rapidly with small increases in adult stock size. Using a different approach than did Jones et al. (2017b), the results from this model independently arrive at the same conclusion as those from the stage-structured life history model (Jones et al. 2017b; see Section 2.3.2), lending greater support to these model conclusions.

2.3.4 Survival of Early Life Stages

High fecundity, as found in Grass Carp (Chilton and Muoneke 1992), is typically associated with high mortality in early life stages. To date, there have been no specific studies on mortality rates of early life stages in wild Grass Carp, but survival would be related to feeding, predation, and overwinter mortality (see Section 2.2.1 and Section 2.2.3 for juvenile and adult overwinter survival based on occurrence data and bioenergetics).

Given high fecundity rates, newly hatched Grass Carp larvae may encounter high intraspecific competition which would result in an initial high density-dependent mortality. Competition for food resources with other species has not been studied for early life stages of Grass Carp, but it is highly unlikely to be a limiting factor given the success of Grass Carp in other regions in the United States. Larval Grass Carp begin to feed on rotifers three or four days after hatching and begin to eat crustacean zooplankton 11–15 days post-hatch (Fedorenko and Fraser 1978, Opuszynski and Shireman 1995). By 4–6 weeks post-hatch, plants dominate the diet, but like most other small fishes native to, or present in, the Great Lakes basin, young Grass Carp also consume animals (chironomids, cladocerans, copepods, insects and their aquatic larvae, crustaceans and small fishes) (Chilton and Muoneke 1992, Opuszynski and Shireman 1995). Therefore, in these early life stages it seems unlikely that food availability would limit survival of very young Grass Carp in the basin. In addition, based on Grass Carp consumption estimates (van der Lee et al. in Jones et al. 2017b), it is likely that there is sufficient food for Grass Carp individuals to survive, including for smaller individuals that do not exclusively feed on macrophytes until 4–6 weeks of age.

Grass Carp larvae younger than the gas bladder inflation stage do not exhibit horizontal directional swimming in the drift, but newly hatched Grass Carp larvae can move vertically and, as a result, do not need to rely on turbulence to remain in the water column (George and Chapman 2015). Swimming capacity increases with ontogeny and, at the gas bladder emergence stage, Grass Carp can hold their position in the water without swimming and can swim horizontally (George and Chapman 2015). At this stage, which is approximately 35 h post-fertilization at temperatures 19–23 °C, Grass Carp larvae begin to move toward wetland nursery habitats (George and Chapman 2015).

A number of fishes in the Great Lakes basin could prey upon Grass Carp eggs and larvae (up to 20 mm in length) including Alewife *Alosa pseudoharengus*, White Bass *Morone chrysops*, Round Goby, Tubenose Goby *Proterohinus semilunaris,* and White Perch *M. americana*. Invertebrate predators may also be a source of predation for very young Grass Carp in wetland habitat. There have not been any direct North American studies of predation on YOY and juvenile Grass Carp (Jones et al. 2017a). However, given the spawning behaviour of Grass Carp, it is more likely that pelagic river fishes (e.g., small cyprinids, centrarchids) and invertebrate predators would consume Grass Carp eggs and early post-hatch larvae than would benthic and lake fishes. Predation pressure on eggs and larvae in spawning tributaries may vary with turbidity; predation by sight feeding predators may be limited by their ability to detect drifting eggs and larvae in those rivers with higher levels of turbidity (Carreon-Martinez et al. 2014). Effects of predation on eggs, larvae, and juveniles may also be greater in the warmer more productive southern regions of the Great Lakes basin, such as in Lake Erie, because more piscivorous fishes will be present and feed at higher rates for a longer period of time than will fishes in relatively colder and less productive systems. Given the high fecundity of Grass Carp, and that most native species must go through a similar predation-prone period, it is unlikely that such predation would preclude Grass Carp population growth and establishment. Grass Carp has rapid growth, averaging 217.6 mm at age 1 (back-calculated) in fishes caught in the tributaries of southern Lake Erie (Chapman et al. 2013, USGS NAS database 2015). Growth would likely be slower in more northern regions of the Great Lakes but, even with slower growth within the first year, Grass Carp would quickly exceed the gape size of predators in the Great Lakes.

Overwinter mortality is an important factor limiting temperate fishes (e.g., Shuter et al. 1980) and it typically results from prolonged starvation or extended periods of low dissolved oxygen (known as winterkill, which does not typically occur in the Great Lakes and its tributaries). Overwinter mortality, as a result of starvation, occurs when fishes have accumulated insufficient energy reserves (typically correlated with size) to survive their first winter (Holm et al. 2009). Because overwinter mortality is correlated to length of winter, it becomes more important with increasing latitude. Overwinter mortality is not known to be an issue for Grass Carp in tributaries to southern Lake Erie and the CAWS. Diploid Grass Carp with otolith chemistry indicative of spending their entire lives in this area have been captured, some with ages up to age 10 (G. Whitledge and P. Kocovksy, USGS, pers. comm.).

In temperate populations, growth of YOY fishes varies with temperature and variations in first-year growth affect survival over the first winter because the ratio of energy stored to basal metabolic rate increases with size. Thus, larger fishes can withstand winter starvation better than smaller fishes (Shuter et al. 1980) because high metabolic demands of small age-0 fishes exhaust energy supplies more quickly during winter, making smaller fishes less tolerant of starvation conditions. Starvation endurance is constrained by the same size-dependent effects that shape the metabolic functions of most organisms, creating a critical length at which Grass Carp will either survive or die depending on the duration of winter. Numerous factors influence the size distribution of fishes at the onset of winter, most being dependent on growing season water temperatures: onset of spawning (in part, hydrology is also important); early development rate (hatching date); daily growth; and, onset of winter.

Using an approach based on Shuter et al. (1980), Jones et al. (2017b) estimated the critical lengths at winter onset and the proportion of the length distribution that would die overwinter (cohort mortality) for each of the Great Lakes using multiple years of daily water temperature series from locations within the Great Lakes basin. Locations were selected to represent a ‘southern’ and ‘northern’ region of each Great Lake and all locations were chosen from the lower reaches of U.S. and Canadian tributaries (<10 km from mouth of lake) or from nearshore areas within the Great Lake proper. The model was parameterized using values (onset of spawning, duration of spawning, hatching time, length at hatch, onset and end of winter, and larval growth rate) from the literature that were (where possible) reflective of similar temperature conditions to the Great Lakes (Jones et al. 2017b). Model results revealed that the average critical length (L_{crit}) of Grass Carp at onset of winter differed between lakes and between locations within each lake (Figure 28). The smallest mean L_{crit} values occurred in the warmer waters of the southern locations of lakes Michigan and Erie. For all lakes the L_{crit} value was smaller for southern locations, indicating that small Grass Carp have a higher probability of overwinter survival in these regions. The average cohort mortality ranged from 0.42 to 0.99 for all locations (Figure 28), except for Nipigon River (Lake Superior, northern location), Mississagi River and Still River (Lake Huron, northern location and Georgian Bay), and Big Creek (Lake Erie, northern location), which all had 100% mortality. This was because spawning failed to occur due to insufficient temperatures in these locations. Despite the high fraction of cohort mortality in a given year across many locations (e.g., mean cohort mortality of 0.98 in southern Lake Superior), all populations that initiated spawning exhibited relatively high ($P \geq 0.75$) probabilities that at least one cohort, out of a group of cohorts hatched across a 20-year period, would survive within the Great Lakes. Overwinter survival of YOY varies with location but establishment of Grass Carp in more northern latitudes is less probable given the general pattern of increasing overwinter mortality in northern regions.

Although validating the overwinter model is difficult without observing spawning, growth, and mortality of Grass Carp within the basin, the fall cohort-length distributions produced in this model for southern Erie populations (95% CI of all lengths = 0.62 cm, 28.3 cm for Vermillion

River) exhibited values that are consistent with back-calculated length at the end of the first growing season for Grass Carp caught in the tributaries of lakes Michigan and Erie (Chapman et al. 2013, USGS NAS database 2015, P. Kocovsky, USGS, pers. comm.).

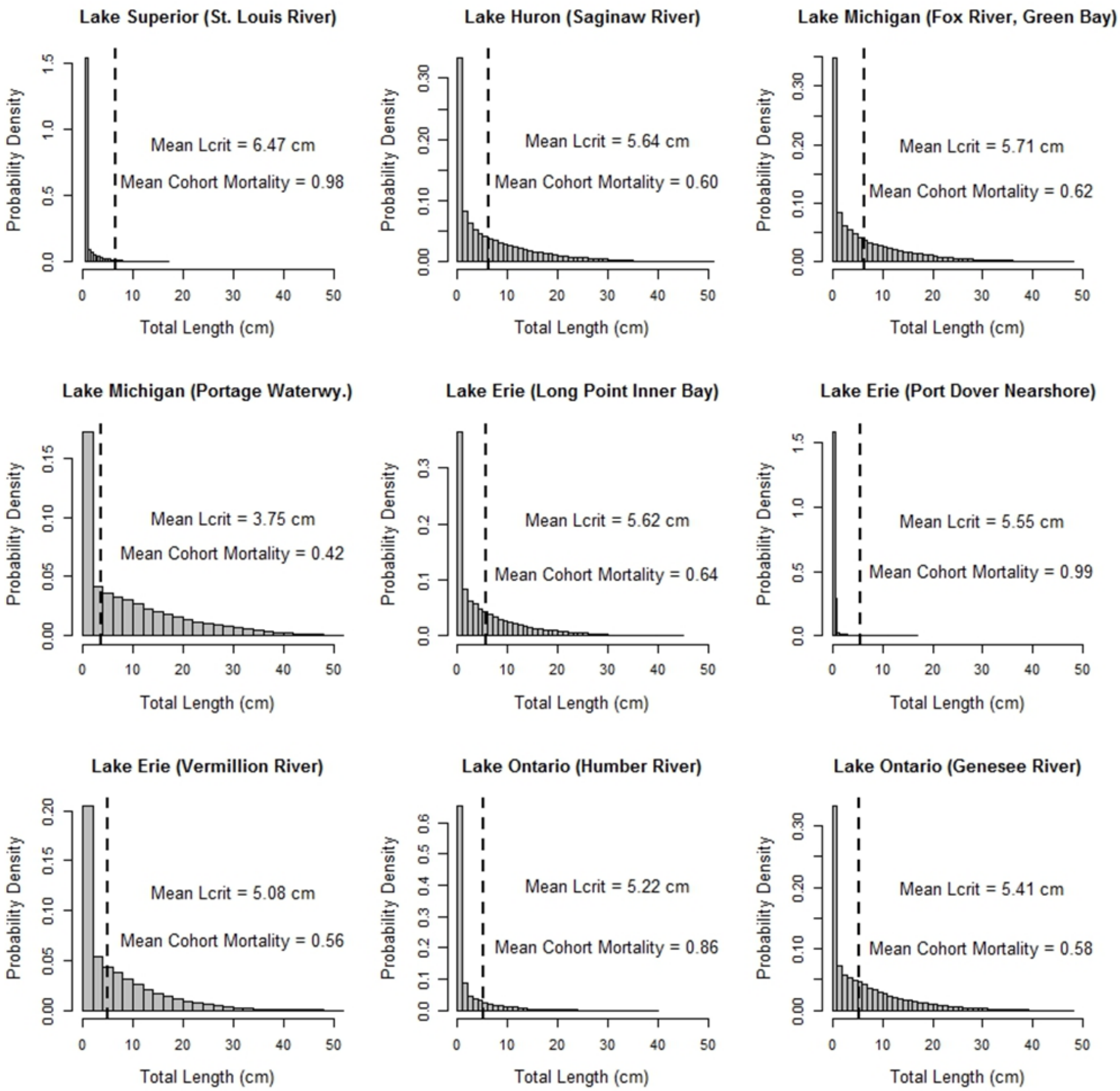


Figure 28. Overall length-frequency distributions of the fall cohort at the onset of winter derived by aggregating 1,000 permutations of yearly temperature regimes and daily growth increments. The dashed vertical line represents the mean L_{crit} (e.g., length that must be attained to survive overwinter). Length classes to the left side of the line are predicted to die as a result of starvation; length classes to the right side of the line have attained sufficient reserves to persist past the winter starvation period (Jones et al. 2017b).

2.3.5 Summary of Likelihood of Establishment

Given the functional sterility of triploid Grass Carp, they would likely not become established in any of the Great Lakes regardless of the amount of time into the future. Therefore, triploid Grass

Carp likelihood of establishment is ranked very unlikely for all time periods with high certainty in all lake basins (Table 11a).

The likelihood ranks for establishment of diploid Grass Carp reflect the definition of establishment with the suitability of conditions for establishment as the basis for assessment at 10, 20 and 50 years, while the current state and age of maturity used in the population model (Jones et al. 2017b) were also taken into account for the first time period of 5 years. The likelihood of establishment of diploid Grass Carp for each lake was assessed independently of other elements in the process and reflects the availability of suitable spawning and nursery habitat, population growth, and survival of early life stages. Two independent models (Jones et al. 2017b; M. Hoff, USFWS and K. Irons, ILDNR, unpubl. data) based on different methods and data both indicated that few Grass Carp would be needed for successful establishment, thus, providing greater certainty in the general output of these models. Suitable conditions (e.g., thermal and hydrologic regimes of tributaries) for recruitment were determined to exist in at least one location in all lakes. However, the full extent of availability of these conditions remains to be determined because the frequency of suitable conditions for overwinter survival and spawning is not known for many locations and many tributaries have yet to be assessed.

Diploid Grass Carp can reproduce in the Great Lakes basin, but within the 5-year time period reproduction is most likely to occur in Lake Erie, given the recent evidence of Grass Carp recruitment in Lake Erie (Table 11b). All other lakes were ranked low (Lake Michigan) to very unlikely (lakes Superior, Huron and Ontario) at the 5-year time period given the current status of known Grass Carp occurrence in these lakes. Given the availability of suitable spawning and overwinter conditions the likelihood of establishment for later time periods (10, 20 and 50 years) was ranked very likely for all lakes except for Lake Superior (Table 11b). Although little published information exists, competition, predation, and overwinter mortality (due to starvation) of early life stages were not thought to limit establishment of Grass Carp in the Great Lakes basin over time with the exception of limited YOY overwinter survival and ability to reach maturity in Lake Superior, which was ranked low for likelihood of establishment.

Table 11. Likelihood of establishment rankings and certainties of data for each lake for (A) triploid, and (B) diploid Grass Carp, 5, 10, 20, and 50 years from the risk assessment baseline (i.e., 2014). Establishment is assessed independently of other elements in the introduction process and is evident by a self-sustaining population which is defined as the occurrence of individuals spawned within the Great Lakes basin subsequently reproducing. Likelihood (Rank): Very Unlikely (VU), Low (Lo), Moderate (M), High (H), Very Likely (VLi); Certainty of data (Cert.): Very Low (VLo), Low (Lo), Moderate (M), High (H), Very High (VH). Note: triploid Grass Carp are considered functionally sterile for management purposes and as such are ranked as VU with H certainty (see Tables 1 and 2 for definitions of rank and certainty of data).

A) TRIPLOID ESTABLISHMENT

	Superior		**Michigan**		**Huron**		**Erie**		**Ontario**	
Time step (yr)	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
5	VU	H	VU	H	VU	H	VU	H	VU	H
10	VU	H	VU	H	VU	H	VU	H	VU	H
20	VU	H	VU	H	VU	H	VU	H	VU	H
50	VU	H	VU	H	VU	H	VU	H	VU	H

B) DIPLOID ESTABLISHMENT

	Superior		Michigan		Huron		Erie		Ontario	
Time step (yr)	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
5	VU	VH	Lo	Lo	VU	H	H	M	VU	H
10	Lo	M	VLi	H	VLi	M	VLi	H	VLi	M
20	Lo	M	VLi	H	VLi	M	VLi	H	VLi	M
50	Lo	M	VLi	H	VLi	M	VLi	H	VLi	M

2.4 LIKELIHOOD OF SPREAD

The likelihood of spread (i.e., movement of individuals or expanding populations into new areas within the basin, between lakes; but not into the basin, as this is arrival) was assessed based on the best available scientific information about natural dispersal (i.e., volitional swimming of individual fish) and movement through canals, laker ballast, or human-mediated vectors (i.e., baitfish introductions). Each of the Great Lakes was considered separately since the likelihood of spread via these vectors may differ among lakes.

Triploid Grass Carp may differ somewhat from diploids in their likelihood of spread because triploid fish may not undertake spawning movements and this may enable triploid fish to expend additional energy in extended daily movement patterns (Tiwary et al. 2004). However, inadequate information exists to make a substantive comparison in likelihood of spread between triploid and diploid fish based on individual movement, thus, we treat both triploid and diploid fish together in this section. However, the likelihood of spread may differ between triploid and diploid Grass Carp based on the likelihood of uptake in laker ballast water or as baitfish.

2.4.1 Natural Dispersal

It is difficult to predict Grass Carp movement and dispersal rates within the Great Lakes basin, because there are no similar areas in which Grass Carp movements have been measured. Otolith data from a diploid Grass Carp of at least ten years of age, collected in Lake Erie near Monroe, Michigan, suggest it had likely briefly entered the Sandusky River as an adult. A younger diploid fish (3+) captured in the same area had also entered either the Sandusky or the Maumee River, not in the year of its birth (G. Whitledge, Southern Illinois University, unpubl. data). The straight line distance from the collection point to the Maumee River is approximately 20 km, and the straight line distance from the collection point to the Sandusky River is approximately 70 km. This is the only currently available data on movement of Grass Carp within the Great Lakes basin.

Grass Carp movement has been assessed by telemetry in the U.S., and some Grass Carp are currently tagged in Lake Erie. Available telemetry studies of Grass Carp have used triploid fish. It is not known whether triploid fish will mimic the movements of diploid fish associated with reproductive activities and if other differences exist. Differences and similarities in diploid and triploid behaviour, foraging efficiency, and endurance swimming, have been shown in other fish species (e.g., Czesny et al. 2002, Cotterell and Wardle 2004, Tiwary et al. 2004, Preston et al. 2014). Therefore, we do not differentiate between triploid and diploid movement. We examine available estimates of Grass Carp movement based on telemetry and on a constrained random-walk style model.

Bain et al. (1990) assessed the movement of triploid Grass Carp in Guntersville Lake, Tennessee and found that adult Grass Carp movement was highly variable but averaged 33 km over a 4-month period (minimum of 0.7 km, and maximum of 71 km). One fish moved 53 km in a 9-day period. Immature fish moved much less than adult fish in that study, which is consistent both with the behaviour of juvenile Grass Carp and their weaker swimming ability (Bain et al. 1990, Webb 1990). Chilton and Poarch (1997) tracked larger (1.5 kg) subadult Grass Carp in Texas reservoirs with abundant *Hydrilla* and other macrophytes and found that after an initial settling period, Grass Carp, on average, moved only a few hundred meters over the four months of the study. Cassani and Maloney (1991) tracked adult triploid Grass Carp in Florida reservoirs, finding very high initial rates of movement in most fish, followed by establishment of home ranges for at least three months of the year in which the fish were tracked. Home range size was not estimated in that study. Mean movement rate during tracking, after the initial settling-in period, was over 100 m/h. Nixon and Miller (1978) tracked large (3.68–12.72 kg) triploid Grass Carp in a Florida lake. They also showed highly variable rates of movement, with one fish moving 18 km over 4 days, and the smallest measured rate of movement being 600 m over 5 days. In that study, movement occurred mostly during the daylight hours and was faster at warmer temperatures, but the report did not distinguish movement that occurred soon after stocking from the rate of movement occurring months following release. Kirk et al. (1996) tracked the movement of adult triploid Grass Carp in South Carolina reservoirs. In that study, movements averaged 0.1 to 0.3 km/d. Kirk et al. (2001) tracked adult triploid Grass Carp in a South Carolina coastal river. The length of time that individual fish were tracked is not reported in the manuscript; fish were stocked over two years (1998 and 1999) and tracking of all fish was terminated in the spring of 2000. In addition substantial mortality apparently occurred in this study. However, total distance of fish tracked ranged from less than 3 to 44 river km. Olive et al. (2010) tracked adult triploid Grass Carp in a large impoundment in Arkansas. Of the 48 stocked fish, 39 (82%) were consistently located in the reservoir while one fish moved upstream and then returned and five fish were located upstream of the reservoir in the Ouachita and Saline rivers. The average home range movement was 5.75 km^2 and maximum movement from the release site averaged 5.7 km and 51.5 km for Grass Carp that remained in and left the reservoir, respectively. Another study in Lake Gaston (North Carolina and Virginia), followed movement of tagged triploid Grass Carp released for *Hydrilla* control for two years (Stich 2011). The average rate of movement for subadult Grass Carp (333–467 mm, TL) was about 137 m/d, with rapid dispersal after stocking followed by long periods of no movement. When time after stocking was held constant in models of behavior, fish moved about 200 m/d more in the second year after stocking than in the first year and were found closer to shore (Stich 2011). This behaviour is suggested to reflect juvenile Grass Carp spending early years in rearing habitats before adopting behavioural characteristics of adults or the loss of post-stocking home ranges that may last from 3 months to one year (Stich 2011).

Currie et al. (2017) modelled movement within the Great Lakes, assuming two different arrival points (southern Lake Michigan near the CAWS and southwestern Lake Erie near the mouth of the Maumee River). The model assumed that the fish would remain in nearshore areas (because Grass Carp are not pelagic and they feed primarily on vegetation present only in nearshore areas) and does not account for the presence of *Cladophora* or include reproduction. This may result in an underestimate of spread for diploid Grass Carp over time because the potential for the abundance of established populations to increase is not included. The model was predicated on the idea that fish would move less if quality food was present, assigning a movement of 1/4 the normal speed, but otherwise move randomly. In this model, two potential speeds of movement were assumed, both of which the authors considered conservative. The “fast rate” was 2,000 m (or 500 m in high food) every time step (2 h) and a very “slow” movement rate of 800 m (or 200 m in high food) every time step. The “slow” speed gives a searching movement rate of 10 cm/s, while the “fast” rate has a searching movement of 22 $cm{\cdot}s^{-1}$ and foraging movement of 5 cm/s. Both of these values are below the U_{crit} value of 3 body $lengths{\cdot}s^{-1}$ that Grass Carp can easily sustain (Cai et al. 2014) and still below the movement rates of other fishes. Random-walk models are recurrent, so even though an individual may have these large steps, the overall displacement is much less since individuals tend to remain in the same location due to the random direction of movement. There is much dispute regarding the estimate of Grass Carp movement since there are very few direct measurements of real-time telemetry and movement rates are usually estimated by measuring activity or passage whereby the time between passing one receiver is compared to the next, which erroneously assumes that the individual swims constantly directly from point to point (Currie et al. 2017). Cassani and Maloney (1991) indicated a large variability in movement rate, but averaged ~180 m/hr ± 113 SD, which is in-between the fast and slow rates of this model. The presence of *Cladophora* in the nearshore of most lakes is reflected in the slow rate of dispersal in the model.

Given modest movement characteristics for Grass Carp (0.1–0.3 body lengths$\cdot s^{-1}$), individuals were expected to reach another basin from the one in which they were introduced within 5–10 years (Figure 29). With the arrival scenario of Grass Carp in southwestern Lake Michigan (near the CAWS) the “fast” movement rate resulted in a small percentage of individuals leaving Lake Michigan for Lake Huron by 5 years and by 20 years a few individuals moved into Lake Erie (Figure 29a). At the “slow” rate of movement the second lake basin (Lake Huron) was not moved into until Year 7 and a small percentage of individuals moved into Lake St. Clair and Lake Erie by 20 and 50 years, respectively (Figure 29b). With an arrival into Lake Erie near the mouth of the Maumee River, the “fast” movement rate resulted in a small percentage of individuals leaving Lake Erie for Lake Ontario by 5 years (Figure 29c), and at the “slow” rate of movement the second lake basin was not moved into until Year 10 (Figure 29d). For both movement scenarios with an arrival in Lake Erie, the nearshore of Lake Erie was completely or nearly completely visited by Year 5. For the “fast” scenario, the movement speed of individuals was capable of moving a few individuals upstream into Lake St. Clair but most stayed in the high quality habitat in Lake Erie. After 10 years, the model predicted that the second lake basin was likely to have multiple individuals, almost 20% (Lake Michigan arrival) of the population and 7–10% (Lake Erie arrival) of the population; this was true for both the “slow” and “fast” movement scenarios of the model. The extensive presence of highly suitable wetland habitat was very important in slowing the spread of Grass Carp in the model because the model constrains Grass Carp to nearshore areas and assumes lower likelihood of movement away from suitable habitats once they are encountered.

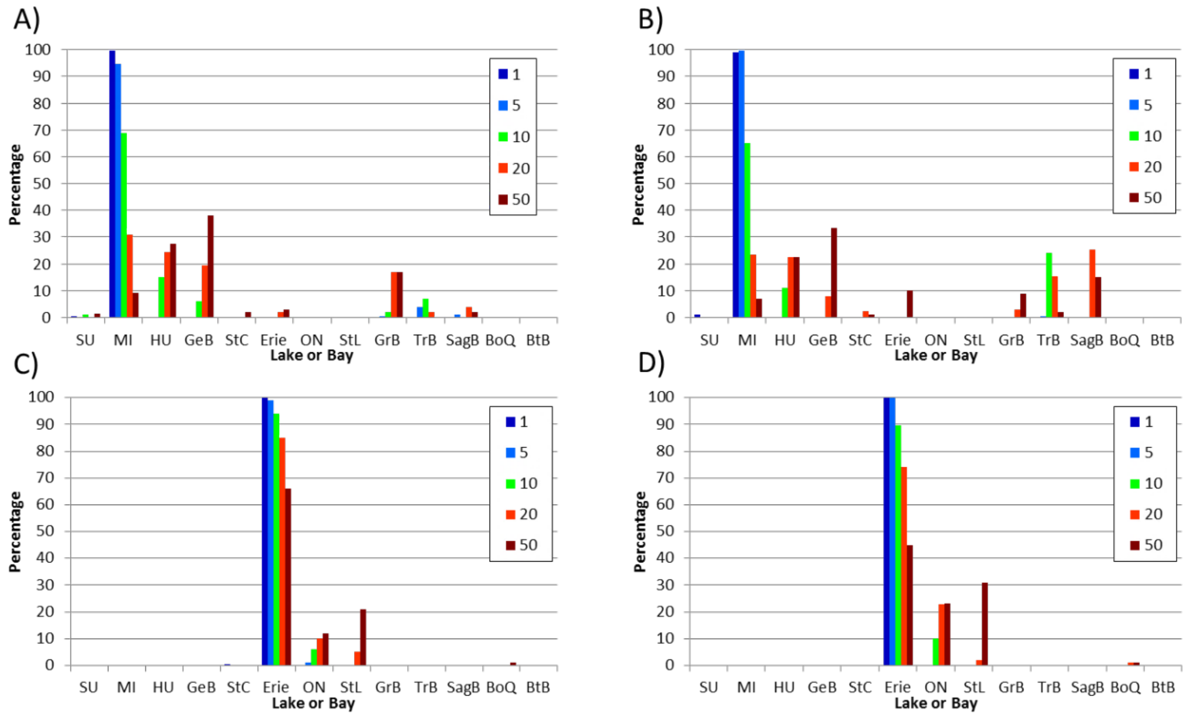


Figure 29. Grass Carp dispersal simulation with arrival from southwestern Lake Michigan (near the CAWS) (top row) under (A) "fast" and (B) "slow" movement scenarios; and with arrival from Lake Erie (near the mouth of the Maumee River) (bottom row) in both a (C) "fast" and (D) "slow" movement scenario (Currie et al. 2017). Bar graph depicts percent occupied by lake or large embayment for 1, 5, 10, and 20 years from the lake basin of introduction. Lakes or Bays are: Superior (SU), Michigan (MI), Huron (H), Georgian Bay (GeB), St Clair (StC), Erie, Ontario (ON), Saint Lawrence River (StL), Green Bay (GrB), Traverse Bay (TrB in MI), Saginaw Bay (SagB in HU), Bay of Quinte (BoQ in ON), Black/Thunder Bays (BtB in SU).

None of the above studies incorporate movements specifically related to reproductive activities of adults, although it is possible that such movements may be to some degree incorporated in some of the telemetry studies if triploid Grass Carp mimic spawning movements made by fertile diploid fish. It is impossible to predict at this time whether reproductive movements would enhance spread, or perhaps limit spread because of the need to remain close to spawning rivers or due to aggregation of fish because of reproductive behaviour or response to reproductive pheromones. This is an identified knowledge gap. Also, Grass Carp in the above studies were not constrained in large-scale dispersion by the low temperatures present in the northern Great Lakes and seasonality of movement that may occur due to the colder temperatures over winter was also not included. These factors introduce uncertainty into estimation of the rate of spread within and between basins. Nevertheless, these studies provide a useful starting point to assess spread.

2.4.2 Canals

Artificial waterway connections, canals, are important pathways that facilitate the spread of AIS between waterbodies within the Great Lakes basin (Mills et al. 1993, Mandrak and Cudmore 2010; Figure 30). Such connections may allow direct dispersal of species or indirect movement (e.g. ballast). Sea Lamprey, Alewife, Bigmouth Buffalo *Ictiobus cyprinellus* and White Perch are examples of species that are thought to have expanded their range to the upper Great Lakes through the Welland Canal, the portion of the St. Lawrence Seaway connecting Lake Ontario to Lake Erie (Mandrak and Cudmore 2010).

Figure 30. Canal systems within the Great Lakes basin considered in the likelihood of arrival and spread of Grass Carp.

Relatively few studies have directly examined the movement of fishes through lock and dam complexes, such as those found in the Mississippi River basin, and in the Welland Canal and St. Marys River in the Great Lakes basin. In the Mississippi River basin, Brooks et al. (2009) tagged several fish species, including bigheaded carps, between 2006 and 2008, to assess passage of fishes through the lock and dam complexes of the Upper Mississippi River System using stationary data logging receivers. They documented both upstream and downstream passages of bigheaded carps through each lock and dam complex between lock and dams 19 (Keokuk, IA) through 26 (Alton, IL). Studies have also documented movement of other fish species (Sauger *Sander canadensis* (Knights et al. 2003, Pegg et al. 1997) and Silver Carp (Calkins et al. 2012)) through lock structures.

In the Great Lakes basin, the movement of fishes through lock and dam structures in the St. Marys River and Welland Canal were examined (Figure 30; Currie et al. 2017). The St. Marys River flows from Lake Superior to Lake Huron and has U.S. shipping locks, Canadian recreational locks, and compensation gates in Sault Ste. Marie to allow movement of vessels between the two Great Lakes basins. In 2013, 128 fishes representing 11 species (but not Common Carp, because few are present) were caught below the lock and dam complex, tagged with Vemco V9 acoustic tags, and tracked with 28 stationary acoustic receivers above, below, and throughout the lock and dam complex, from May 27 to the end of October. During that period, two White Sucker *Catostomus commersonii*, one Chinook Salmon, one Atlantic Salmon, and one Smallmouth Bass moved upstream completely through the lock and dam complex (Currie et al 2017). The Chinook Salmon subsequently returned downstream through the complex. In 2014, an additional 24 fishes representing 4 species were caught below the lock and dam complex, tagged, and tracked from May 7, 2014 to early January 2015. During that period, two Atlantic Salmon, one White Sucker, and another single White Sucker that was tagged in 2013 (which had moved upstream in 2013) moved upstream completely through the lock and dam complex (Currie et al. 2017).

The Welland Canal is thought to have played a role in the dispersal of various invasive fishes into Lake Erie and upper Great Lakes (e.g. Mills et al. 1993), although there is some debate to what extent (Daniels 2001). The Welland Canal extends from Lake Ontario to Lake Erie and has eight locks, including a flight of three locks with a total lift of 50 m over the Niagara Escarpment. The Welland Canal, completed in 1959, allows ocean-bound ships and lakers to navigate past Niagara Falls. In 2012, 79 fishes representing seven species were caught above and below the flight locks, tagged with Vemco V9 acoustic tags, and tracked with 21 acoustic receivers placed throughout the Welland Canal, from May to the end of December (Currie et al. 2017). One Common Carp and one Freshwater Drum were documented moving towards Lake Erie and moved through 1 or 2 locks and up to 20 km upstream towards Lake Erie (Currie et al. 2017). Two Common Carp and one Freshwater Drum were documented moving from above to below the flight locks. In 2013, 100 fishes representing 10 species were caught above and below the flight locks, tagged, and tracked with 34 acoustic receivers placed throughout the Welland Canal, from May to the end of December (Currie et al. 2017). Two Freshwater Drum were documented moving through one lock and travelled 25 km upstream toward Lake Erie. Two Freshwater Drum and one Common Carp were documented moving through two locks and moved 7 km downstream towards Lake Ontario. No fishes were documented moving through the flight locks. In 2014, 11 fishes tagged in 2012 and 55 fishes tagged in 2013 were tracked with 34 acoustic receivers placed throughout the Welland Canal, from May to the end of December (Currie et al. 2017). Two Freshwater Drum were documented moving through one lock and 25 km into Lake Erie. One Freshwater Drum and one Common Carp were documented re-entering the Welland Canal from Lake Ontario, and two Freshwater Drum were documented re-entering from Lake Erie. Based on detections by the Great Lakes Acoustic Telemetry Observation System, two Freshwater Drum moved from the Welland Canal across Lake Erie to the western basin and back. No fishes were documented moving through the flight locks.

The results of Currie et al. (2017) movement studies suggest that migratory fishes can move through the St. Marys lock and dam complex and, thus, movement between Lake Superior and Huron basins appears possible. Conversely, there was no documented movement of fishes between the Lake Erie and Ontario basins through the Welland Canal. Although fishes did move between the canal and each lake, very few (3 of 179 tagged fishes) moved through the flight locks.

Alternate routes between the Erie and Ontario basins include the New York Canal System and the Niagara River (Figure 30). The New York Canal System (formerly the Erie Barge Canal) connects the upper Niagara River (hence, Lake Erie) to Lake Ontario, the Finger Lakes, Lake Champlain, and the Hudson River. No published studies have been undertaken to determine the extent of fish movement through the New York Canal System. There is uncertainty as to whether or not fishes (at any life stage) could move from Lake Erie to Lake Ontario through the Niagara River and survive the descent over Niagara Falls.

The Trent-Severn Waterway, a 386 km, 45 recreational lock and dam complex through central Ontario, joins the Lake Huron and Lake Ontario basins (Figure 30). Movement from Lake

Ontario to Lake Huron is possible; however, the reverse is not possible as a result of a waterfall (Big Chute) that flows into Lake Huron, which boats by-pass via a marine railway. Marson (2008) examined fish movement in the vicinity of three locks on the Trent-Severn Waterway and found eight species in the lock chambers and 20 species in the immediate vicinity. In a mark-recapture experiment, two of 626 recaptured marked fishes had gone through the locks (Marson 2008), indicating that fishes do move through the locks. However, there are no published studies on the extent of fish movement through the entire Trent-Severn Waterway.

2.4.3 Laker Ballast

Laker ballast water is not treated for AIS, therefore, lakers may facilitate the movement of organisms between ports and Great Lakes, particularly small early life stages such as eggs, larvae, and juveniles (see Section 2.1.3 for discussion of laker ballast as an arrival route). To date, there have been no empirical studies on the role of laker ballast water in the movement of fishes.

To determine the potential for between-lake ballast movement, Drake et al. (2015a) developed models describing the probability of spread and establishment of AIS as a result of domestic ballast water movement based on the data from Rup et al. (2010), describing all combined U.S. and Canadian laker traffic within the Great Lakes-St. Lawrence River basin, 2005–2007 (Figures 15 and 16). Drake et al. (2015a) developed both relative risk models, i.e., probability of spread from a source port relative to all other source ports, and absolute risk models, i.e., the rate of spread between ports or lakes compared to natural dispersal. The models were run for several invasiveness scenarios related to ballast uptake and establishment probability based on propagule density. The uptake probability of Grass Carp is likely ≤ 0.01 and establishment probability is likely ≤ 0.0001, which corresponds to the low invasiveness scenarios of Drake et al. (2015a) (see Section 2.1.3 for further discussion on invasiveness scenarios). As a result, the movement of Grass Carp between ports (Figure 17), or between lakes (Figure 18), through ballast water is likely negligible.

2.4.4 Human-mediated Dispersal

If Grass Carp became established in some portion of the Great Lakes basin, its spread to other areas of the basin could be facilitated by human-mediated dispersal mechanisms. For the purposes of this risk assessment, baitfish introductions are the main focus for human-mediated dispersal as they can be qualified to some extent.

There is the potential for Grass Carp, after arrival in the Great Lakes basin, to be spread through the use of baitfish given the possible bycatch of a wide variety of non-target species during commercial and angler-based baitfish harvest (Drake and Mandrak 2014b, c; see Section 2.1.2.5 for discussion of baitfishes as an arrival route). Despite recreational angling regulations for states and provinces in the Great Lakes basin (Table 5), for most jurisdictions, knowledge is lacking on the degree to which baitfish harvest is concentrated in areas of likely Grass Carp occurrence, as is knowledge relating to aspects of angler use, movement, and release patterns. Such information is necessary to understand the potential for Grass Carp dispersal resulting from baitfish activity. Drake (2011) and Drake and Mandrak (2010, 2014a, c) examined aspects of the baitfish pathway in Ontario, which can be used to assess the potential spread of Grass Carp through baitfish pathways in the Great Lakes basin.

Lake Ontario was the most popular destination for Ontario anglers using live baitfishes, while Lake Erie was ranked the third most popular destination, and Georgian Bay, Lake Huron, Lake Superior, and Lake St. Clair, were ranked fourth, fifth, sixth, and fourteenth, respectively (Drake and Mandrak 2010). The current commercial baitfish distribution network in Ontario indicates that baitfishes used in these waterbodies would have originated in the wild primarily from the Canadian nearshore waters and tributaries of lakes Erie, Huron, and Ontario, and secondarily from inland lakes in southern Ontario (Drake and Mandrak 2014a, c). These results indicate that potential exists for Grass Carp to undergo human-mediated dispersal among the Great Lakes in Canadian jurisdiction, assuming that Grass Carp bycatch occurs and that the species remains undetected within the pathway. Although this scenario seems unlikely, given the substantial volume of baitfishes captured from the wild (100 million harvested/year; Drake and Mandrak 2014b) and the tremendous volume of yearly live bait angling events (4.24 million; Drake and Mandrak 2014a), even low probabilities of bycatch and movement and release by anglers can lead to a substantial number of non-target fish introductions each year (see Drake and Mandrak 2014a for similar mechanisms involving Round Goby). In Ontario, management zones have been created to restrict movement of commercial baitfishes within the province, although these zones will likely have a minimal influence on the movement of fishes among the Great Lakes as a whole because of joint commercial and angler activity (A. Drake, University of Toronto Scarborough, pers. comm.). No studies on angler behaviour related to baitfish movement along the U.S coasts of the Great Lakes were identified.

2.4.5 Summary of Likelihood of Spread

Based on history of movement of fishes in the Great Lakes, there is evidence that fishes can move from lake to lake (both upstream and downstream) (Mandrak and Cudmore 2010). Habitat and food are two factors to be taken into consideration regarding fish movement, along with availability of suitable physical routes for movement. Tagged Grass Carp studies and modelling conducted by Currie et al. (2017) found significant movement rates by Grass Carp. Currie et al. (2017) did not incorporate how movement between basins may be impeded by waterfalls, canals, and locks; however, fishes can pass freely across the Erie, Huron, and Michigan basins, with the connecting channels between these basins having suitable wetland habitat for Grass Carp. Based on evidence from telemetry studies, fish movement could also occur through canals between the Huron and Superior basins, but less likely between the Erie and Ontario basins (Currie et al. 2017). Given the low likelihood of uptake and release, inter-lake ballast water transfer movement between lakes is an unlikely potential vector of spread for Grass Carp. Ballast and bait are even more unlikely vectors for triploid Grass Carp given the low likelihood of small triploid individuals being present in the basin.

Given the variability in Grass Carp movement in ponds and rivers and the lack of knowledge of Grass Carp movement in a large-lake system, the rankings for the likelihood of spread into a lake were mainly informed by the current knowledge of Grass Carp occurrences in and around the Great Lakes basin and the spread model, which had the two most likely starting points for spread in the basin as southern Lake Michigan (at the CAWS) and south-western Lake Erie (the Maumee River). The spread model does not incorporate reproduction and may, therefore, underestimate spread of diploid Grass Carp over time. Triploid and diploid Grass Carp were treated the same for individual movement behaviour. However, lower rankings for triploid Grass Carp occur due to a lower likelihood of uptake in bait and ballast vectors and that spread is limited to the lifespan of the individual from source points in the spread model because establishment will not occur.

The likelihood of triploid Grass Carp spreading to lakes Superior, Michigan and Erie from another Great Lake was ranked very unlikely given the lack of triploid Grass Carp in adjacent lakes (Table 12a). Lake Huron was ranked moderate (5 and 10 years) to high (20 and 50 years) given the occurrence of triploid Grass Carp in lakes Michigan and Erie and the results of the spread model by Currie et al. (2017). Spread to Lake Ontario was ranked very unlikely for 5 and

10 years and increased to low likelihood at 20 years given the occurrence of triploid Grass Carp in Lake Erie (Table 12a).

Spread of diploid Grass Carp to lakes Superior, Michigan, Erie, and Ontario was ranked very unlikely to moderate, given the low opportunity for diploid fishes to spread to these lakes from the adjacent lake basins (Table 12b). Although the spread model predicted spread to Lake Ontario from Lake Erie within 10 years and diploid Grass Carp have been caught in Lake Erie, the likelihood was not ranked any higher due to the low likelihood of movement through the Welland canal system. The likelihood of spread of both triploids and diploids to Lake Huron was ranked higher (Table 12) given its proximity to the increasing occurrences of both triploids and diploids (similar ploidy ratio of captures fishes) within western Lake Erie and southern Lake Michigan.

Table 12. Likelihood of spread (between lakes, e.g., into Lake Superior from other lakes) rankings and certainties for each lake for (A) triploid, and (B) diploid Grass Carp, 5, 10, 20, and 50 years from the risk assessment baseline (i.e., 2014). Spread is defined as the movement of individuals or expanding populations into new areas within the basin, between lakes; but not into the basin, as this is arrival. Rankings mainly informed by the spread model which used two of the most likely entrance points to the basin: the CAWS for Lake Michigan and Maumee River for Lake Erie (Currie et al. 2017. Likelihood (Rank): Very Unlikely (VU), Low (Lo), Moderate (M), High (H), Very Likely (VLi); Certainty of data (Cert.): Very Low (VLo), Low (Lo), Moderate (M), High (H), Very High (VH) (see Tables 1 and 2 for definitions of rank and certainty of data).

A) TRIPLOID SPREAD

	Superior		**Michigan**		**Huron**		**Erie**		**Ontario**	
Time step (yr)	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
5	VU	M	VU	M	M	M	VU	M	VU	M
10	VU	M	VU	M	M	M	VU	M	VU	M
20	VU	M	VU	M	H	M	VU	M	Lo	M
50	VU	M	VU	M	H	M	VU	M	Lo	M

B) DIPLOID SPREAD

	Superior		**Michigan**		**Huron**		**Erie**		**Ontario**	
Time step (yr)	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
5	VU	M	VU	M	M	M	VU	M	VU	M
10	VU	M	VU	M	H	M	VU	M	Lo	M
20	Lo	M	VU	M	H	M	Lo	M	Lo	M
50	Lo	M	Lo	M	VLi	M	M	M	Lo	M

2.5 SUMMARY OF PROBABILITY OF OCCURRENCE/INTRODUCTION

In summary, the likelihood of arrival, survival, establishment (diploid only) and spread of Grass Carp within the Great Lakes basin were assessed using the best available information. As the Great Lakes are interconnected, the overall probability of occurrence and introduction was ascertained by first determining the highest ranking between overall arrival and spread (Table 13).

Table 13. Maximum rank of overall arrival and spread (Max(Arrival, Spread)) for each lake for (A) triploid, and (B) diploid, Grass Carp, 5, 10, 20, and 50 years from the risk assessment baseline (i.e., 2014). The certainty of data category associated with the maximum rank is retained; however, if tied ranks occur, the lowest certainty of data is retained. Likelihood (Rank): Very Unlikely (VU), Low (Lo), Moderate (M), High (H), Very Likely (VLi); Certainty of data (Cert.): Very Low (VLo), Low (Lo), Moderate (M), High (H), Very High (VH) (see Tables 1 and 2 for definitions of rank and certainty of data). Note: If no anticipated change in rankings and certainty over time, then years are not shown in the individual boxes.

A) TRIPLOID MAX(ARRIVAL, SPREAD)

	Superior		**Michigan**		**Huron**		**Erie**		**Ontario**	
Element	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
Overall Arrival	5=VU 10,20,50 =Lo	5=VLo 10,20,50 =Lo	VLi*	H	Lo	VLo	VLi*	H	5=Lo 10,20,50 =M	5=VLo 10,20,50 =Lo
Spread	VU	M	VU	M	5,10=M 20,50=H	M	VU	M	5,10=VU 20,50=Lo	M
Max(Arrival, Spread)	5=VU 10,20,50 =Lo	5=VLo 10,20,50 =Lo	VLi*	H	5,10=M 20,50=H	M	VLi*	H	5=Lo 10,20,50 =M	5=VLo 10,20,50 =Lo

** Grass Carp considered to have already arrived to the lake basin in question; arrival pathway/vector unknown.*

B) DIPLOID MAX (ARRIVAL, SPREAD)

	Superior		**Michigan**		**Huron**		**Erie**		**Ontario**	
Element	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
Overall Arrival	5=VU 10,20,50= Lo	5=VLo 10,20,50 =Lo	VLi*	H	Lo	VLo	5,10 =M 20,50 =H*	5=M 10,20,50 =VLo	5,10,20 =Lo 50=M	5,10,20 =VLo 50=Lo
Spread	5,10=VU 20,50=Lo	M	5,10,2 0=VU 50=Lo	M	5=M 10,20 =H 50=VLi	M	5,10 =VU 20=Lo 50=M	M	5 =VU 10,20,50 =Lo	M
Max(Arrival, Spread)	5=VU 10,20,50 =Lo	5=VLo 10,20,50 =Lo	VLi*	H	5=M 10,20 =H 50=VLi	M	5,10 =M 20,50 =H*	5=M 10,20,50 =VLo	5,10,20 =Lo 50=M	5,10,20 =VLo 50=Lo

** Grass Carp considered to have already arrived to the lake basin in question; arrival pathway/vector unknown.*

For triploid Grass Carp, this rank was then compared to the rank of survival, and the lowest rank of the two was retained (Table 14a). The formula was modified from that presented in Mandrak et al. (2012) to remove the element of establishment as triploid Grass Carp are functionally sterile and considered unable to form a self-sustaining reproducing population. This is represented by the following formula:

Probability of Occurrence = Min [(Max (Arrival, Spread)), Survival]

For diploid Grass Carp, the highest ranking between overall arrival and spread was then compared to the ranks of survival and establishment, and the lowest rank of the three was retained. This is represented by the following formula:

Probability of Introduction = Min [(Max (Arrival, Spread)), Survival, Establishment]

If either triploid or diploid Grass Carp was considered to have already arrived to a lake basin this was denoted with an asterisk in the ranking table of the overall arrival for that Great Lake.

In determining the maximum rank of overall arrival and spread, the certainty associated with the highest rank was used or, if tied ranks occurred, the lowest certainty associated with the tied rank was used (Table 13). For probability of occurrence and introduction, the certainty associated with the lowest ranked element was retained or, if tied ranks occurred, the lowest certainty of that tied rank was used (Table 14).

The probability of occurrence for triploid Grass Carp was considered to be least likely for lakes Superior and Ontario, most likely in lakes Michigan and Erie, and of moderate to high likelihood for Lake Huron (Table 14a). The increase in rank for lakes Superior and Ontario reflect the potential for arrival over time through stocking, while the increase for Lake Huron reflects the potential for spread from lakes Erie and Michigan.

For diploid Grass Carp, the probability of introduction by 5 years was driven mainly by the likelihood of establishment and ranked from very unlikely to low, except for Lake Erie (moderate) which was driven by the likelihood of arrival (arrival is considered to already have occurred in this lake) (Table 14b). By 10 and 50 years, lakes Michigan and Huron were ranked very likely, respectively (Table 14b). By 50 years, Lake Erie was ranked as high given the likelihood of arrival, while lakes Ontario and Superior were ranked moderate and low, respectively. Compared to lakes Michigan and Huron, the probability of introduction for Lake Erie was lower at 10–50 years due to the lower likelihood of spread to Lake Erie than to Lake Huron and the lower likelihood of arrival to Lake Erie (ranks were based on the probability of arrival occurring, not the probability of arrival having already occurred). Overall, the increase in ranks over time reflects the potential accrual of Grass Carp through arrival, establishment and spread

Table 14. Overall rankings and certainties for each lake for (A) the probability of occurrence of triploid, and (B) the probability of introduction of diploid, Grass Carp, 5, 10, 20, and 50 years from the risk assessment baseline (i.e., 2014). For triploid Grass Carp, the minimum ranking of Max (Arrival, Spread) and Survival is retained for the probability of occurrence and the associated certainty; however, if tied ranks occur, the lowest certainty is retained. For diploid Grass Carp, the minimum ranking of Max (Arrival, Spread), Survival, and Establishment is retained for the probability of introduction and the associated certainty; however, if tied ranks occur, the lowest certainty is retained. Likelihood (Rank): Very Unlikely (VU), Low (Lo), Moderate (M), High (H), Very Likely (VLi); Certainty of data (Cert.): Very Low (VLo), Low (Lo), Moderate (M), High (H), Very High (VH) (see Tables 1 and 2 for definitions of rank and certainty of data). Note: If no anticipated change in rankings and certainty over time, then years are not shown in the individual boxes.

A) TRIPLOID PROBABILITY OF OCCURRENCE

	Superior		**Michigan**		**Huron**		**Erie**		**Ontario**	
Element	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
Max(Arrival, Spread)	5=VU 10,20, 50=Lo	5=VLo 10,20, 50=Lo	VLi*	H	5,10=M 20,50= H	M	VLi*	H	5=Lo 10,20,50= M	5=VLo 10,20,50 =Lo
Survival	H	VH	VLi	VH	VLi	VH	VLi	VH	VLi	VH
P(Occur)=Min [Max(Arrival, Spread), Survival]	5=VU 10,20, 50=Lo	5=VLo 10,20, 50=Lo	VLi*	H	5,10=M 20,50= H	M	VLi*	H	5=Lo 10,20,50= M	5=VLo 10,20,50 =Lo

** Grass Carp is considered to have already arrived to the lake basin in question; arrival vector/pathway unknown.*

B) DIPLOID PROBABILITY OF INTRODUCTION

	Superior		Michigan		Huron		Erie		Ontario	
Element	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
Max(Arrival, Spread)	5=VU 10,20, 50=Lo	5=VLo 10,20, 50=Lo	VLi*	H	5=M 10,20=H 50=VLi	M	5,10 =M 20,50 =H*	5=M 10,20 ,50= VLo	5,10,20= Lo 50=M	5,10,20= VLo 50=Lo
Survival	H	VH	VLi	VH	VLi	VH	VLi	VH	VLi	VH
Establish.	5=VU 10,20, 50=Lo	5=VH 10,20, 50=M	5=Lo 10,20 ,50= VLi	5=Lo 10,20 ,50= H	5=VU 10,20,50 =VLi	5=H 10,20 ,50= M	5=H 10,20 ,50= VLi	5=M 10,20 ,50= H	5=VU 10,20,50 =VLi	5=H 10,20,50 =M
P(Intro)=Min [Max (Arrival, Spread), Survival, Establish]	5=VU 10,20, 50=Lo	5=VLo 10,20, 50=Lo	5=Lo 10,20 ,50= VLi*	5=Lo 10,20 ,50= H	5=VU 10,20=H 50=VLi	5=H 10,20 ,50= M	5,10 =M 20,50 =H*	5=M 10,20 ,50= VLo	5=VU 10,20=Lo 50=M	5=H 10,20= VLo 50=Lo

** Grass Carp is considered to have already arrived to the lake basin in question; arrival vector/pathway unknown.*

3.0 MAGNITUDE OF ECOLOGICAL CONSEQUENCES

Magnitude of ecological consequences was assessed at a lake-wide scale and ratings were based on predicted decreases in submerged aquatic vegetation due to modeled increasing carp densities. This assumes that triploid Grass Carp have arrived and survived in the Great Lakes basin and that diploid Grass Carp have successfully arrived, survived, and established in the Great Lakes basin. Although triploid Grass Carp would not reproduce and create a self-sustaining population, they could have effects for the duration of their lifespan. While triploid and diploid Grass Carp have been found to have similar standardized energy balances, triploid Grass Carp may feed more slowly than diploid Grass Carp (Wiley and Wike 1986), thus, the magnitude of ecological consequences may differ depending on ploidy. However, inadequate information exists on the magnitude of ecological consequences associated with triploid and diploid Grass Carp to make a substantive comparison and, thus, we treat both triploid and diploid fish together in this section.

Cudmore and Mandrak (2004), Jones et al. (2017a), Bogutskaya et al. (2017), and Zhao and Wang (in prep.) reviewed the effects of Grass Carp on invaded environments and several research efforts were carried out specifically for this risk assessment based on identified managers questions and knowledge gaps (Gertzen et al. 2017). For the purposes of this risk assessment, ecological consequences addresses any ecological change that may occur due to Grass Carp in the basin; the risk assessment does not make a value judgment of desirable or undesirable effects as a result of this change but rather identifies the potential change and its direction.

There is little known about the ecological consequences of Grass Carp introduction on large lake systems such as the Great Lakes. Wittmann et al. (2014) conducted a meta-analysis of Grass Carp impacts on biotic (amphibians, birds, invertebrates, fishes, macrophytes and phytoplankton) and abiotic (chlorophyll *a,* dissolved oxygen, nutrients, pH, turbidity, and sediment metals) factors for water bodies where Grass Carp was stocked or introduced. These systems included ponds, lakes, reservoirs, tanks, and canals in North America, South America, Europe, and Asia. Wittmann et al. (2014) reviewed 193 papers from all across the world on Grass Carp and obtained 18 papers with 111 unique data points that quantitatively described the impact of Grass Carp on biotic and abiotic factors. The meta-analysis summarized the cumulative effect size (E) of Grass Carp presence on the biotic and abiotic environments compared to control systems without Grass Carp, and used Hedges' *d* to account for small sample sizes.

The results of the meta-analysis show highly variable cumulative effects of Grass Carp (Table 15; Wittmann et al. 2014). Positive (increasing values) and negative (decreasing values) cumulative effect sizes were observed with Grass Carp presence; however, no relationship between Grass Carp biomass (kg/ha) and effect size was detected (Table 15). Grass Carp presence had a significantly negative effect size on the overall biotic community which may be attributed in a large part to its negative effect size on macrophyte abundance or biomass (Table 15). Grass Carp presence resulted in a negative but non-significant effect size on amphibians and invertebrates, and a positive but non-significant effect size on birds and fishes. On the other hand, Grass Carp presence had a significantly positive effect size on the overall abiotic environment (Table 15). Grass Carp had a significantly positive effect size on physiochemical parameters (hardness, alkalinity, conductivity and salinity); a positive but non-significant effect size on dissolved oxygen, nutrients (nitrogen and phosphorus), sediment metals, and turbidity, and a negative but non-significant effect size on pH and phytoplankton/chlorophyll *a* (Table 15).

Overall, the effects of Grass Carp on the biotic and abiotic environments were variable (Table 15; Wittmann et al. 2014). While this meta-analysis focused on impacts in small systems, which may not accurately describe the potential impacts in a large lake system, it provides a basis on which to begin considering potential ecological consequences in the Great Lakes. Generally, submerged aquatic vegetation, the preferred food source for Grass Carp, provides habitat and refuge for a wide range of biota, including fishes, birds, and invertebrates. Studies have shown that macroinvertebrate and zooplankton communities are more abundant and diverse in vegetation beds (Harrod 1964; Krull 1970). These invertebrates are important prey species for fishes, birds and amphibians. Further, amphibians deposit egg masses on aquatic vegetation and amphibians have aquatic life stages such as tadpoles that use vegetated habitat. Approximately 33 species of amphibians occur on the U.S. side of the Great Lakes basin and two-thirds of these species have conservation concerns due, in large part, to habitat loss and pollution (Sierszen et al. 2012). In an experimental enclosure, Grass Carp presence reduced the survival of Green Frog and Northern Cricket Frog tadpoles (Ade et al. 2010). In addition to providing important ecological services for biota, aquatic vegetation also plays an important role in abiotic processes such as nutrient cycling. In the sections below, the potential ecological consequences of Grass Carp on vegetation, birds, fishes, abiotic factors, and pathogens/diseases are described in more detail.

*Table 15. Results of meta-analysis of the ecological impacts of Grass Carp on biotic and abiotic variables (Wittmann et al. 2014). * denotes significant difference from zero based on 95% Confidence Intervals (CI). df = degrees of freedom, A = heterogeneity statistic, P_Q = probability level of X^2 distribution with n-1 df.*

Grouping	Cumulative effect size (E)	95% CI	df	Q	P_Q
Biotic (all) *	-0.12	-0.20, -0.03	84	15E04	<0.001
Amphibians	-1.14	-3.26, 0.99	2	11	0.004
Birds	0.03	-0.12, 0.17	12	1E04	<0.001
Fishes	0.11	-0.21, 0.43	17	170	<0.001
Invertebrates	-0.07	-0.76, 0.63	5	50	<0.001
Macrophytes *	-0.29	-0.41, -0.14	47	6E04	<0.001
Abiotic (all) *	0.36	0.09, 0.63	25	193	<0.001
Dissolved oxygen	0.10	-1.55, 1.75	2	25	<0.001
Hardness, alkalinity, conductivity, salinity *	1.18	0.46, 1.90	5	98	<0.001
Nutrients (N, P)	0.27	-0.64, 1.17	3	22	<0.001
pH	-0.32	-1.78, 1.14	2	16	<0.001
Phytoplankton-chl *a*	-0.51	-1.59, 0.56	3	17	<0.001
Sediment metals	7.31	-11.17, 25.80	1	1	0.317
Turbidity	1.07	-5.58, 7.71	1	10	0.002

3.1 VEGETATION

Grass Carp can be effective environmental engineers, causing changes primarily by removal of aquatic vegetation. This attribute of Grass Carp constitutes the primary reason for its introduction and use in North America as a biological control agent (Kelly et al. 2011) and also represents the most likely mechanism for unwanted effects in the Great Lakes basin. Grass Carp effectively control many types of vegetation and do so much less expensively than can be accomplished with mechanical or chemical controls (Kelly et al. 2011). Grass Carp is often successfully used to control non-native, invasive plants such as *Hydrilla* in ponds and other waterbodies. However, Grass Carp does not distinguish between plant types, abundances, or locations that are defined as desirable or undesirable to humans. When Grass Carp populations reach critical densities, they can cause nearly complete removal of aquatic plants (Sills 1970). Aquatic vegetation in the Great Lakes provides ecosystem services such as provision of spawning and recruitment habitat for native fishes, food and habitat for waterfowl, high biological productivity, shore erosion protection, nutrient-cycle control, accumulation of sediment, supply of detritus (Herdendorf 1987), and mitigation of nonpoint source pollution (Mitsch 1992). Coastal wetlands in many parts of the Great Lakes have declined due to anthropogenic effects (Herdendorf 1992). If large populations of Grass Carp occur in the Great Lakes, they would likely further degrade vegetated wetlands.

Numerous studies have been carried out on the effect of Grass Carp on aquatic macrophytes, because the species is extensively stocked for macrophyte control. The majority of these studies reported the stocking intensity and the success of aquatic macrophyte reduction or removal. For example, in New Zealand, Grass Carp stocked (40–80 kg/ha) in a canal dominated by Coontail *Ceratophyllum demersum* reduced aquatic macrophyte coverage within 7 months by about 80% (Hicks et al. 2006). In a Saudi drainage, Grass Carp fingerlings (1–5 fish/m^2) completely eliminated filamentous algae within 5 months and significantly reduced the abundance of *Phragmites australis* (Belal 2007). In a small pond in the Czech Republic, stocked Grass Carp (29 kg/ha) reduced the biomass of aquatic macrophytes from 109 g/m^2 to 33 g/m^2 in one growing season (Pípalová et al. 2009). Grass Carp significantly decreased the biomass of *Cladophora globulina*, *Eleocharis acicularis,* and *Potamogeton pusillus*. The most preferred plant was the filamentous alga *Cladophora globulina*, the biomass of which decreased from 66 g/m to 0.4 g/m in the pond stocked with Grass Carp (Pípalová et al. 2009). In an attempt to control mosquitoes in one of the lakes of the Karakum Canal system (Turkmenistan), 375 Grass Carp were released in the lake in May 1961. Following one season post introduction, no aquatic vegetation remained in the lake (Aliyev and Bessmertnaya 1968 in Bogutskaya et al. 2017). In subsequent years, the estimated consumption rate was 10–15 tonnes of macrophytes per vegetation season. By 1974, the entire 850 km long canal bed and reservoirs were free from submerged vegetation, which was previously extremely abundant (Vinogradov and Zolotova 1974 in Bogutskaya et al. 2017). Grass Carp has also been shown to be successful at suppressing the growth or eradicating Water Hyacinth *Eichhornia crassipes* (Gopalakrishnan et al. 2011). In earthern ponds in Turkey, Grass Carp was experimentally stocked for macrophyte control and *Cladophora* and *Zygnema* species of aquatic plants were consumed and eliminated within about a month, and *Chara* was eliminated within three months after stocking; overall plant biomass was reduced by more than 80% in ponds stocked at 145 kg Grass Carp per hectare (Kirkagac and Demir 2004). In this study, all vegetation except *Phragmites australis* was eliminated. In Dianchi Lake (China), the loss of *Ottelia acuminate*, a dominant macrophyte, was likely caused by the massive introduction of Grass Carp (Yang et al. 2013). In the Lower Terek and Arakum drainages of Dagestan, the plant composition and community phytomass changed sharply after two years of Grass Carp stocking. Diversity of aquatic vegetation declined from 71 species of flowering plants to 58. This was mainly attributable to the consumption of submerged macrophytes preferred by this fish. The area of reed aggregations declined 80%, to 30% foliage cover, and the biomass of reed beds were reduced by 80–90% (Vinogradov and Zolotova 1974 in Bogutskaya et al. 2017). A meta-analysis that included 48 data points from 13 studies found Grass Carp stocking strongly reduced macrophyte abundance or density (Wittmann et al. 2014).

Grass Carp can also influence macrophyte composition through selective feeding behaviour. Grass Carp has a preference for plants with soft tissues and long, thin morphology (Wiley et al. 1986; Pine and Anderson 1991) because those plants are most easily consumed. Pharyngeal dentition enables the biting off, and grinding of, coarse stems of plants. Food preference may also be related to macrophyte chemical composition (Bonar et al. 1990). Krupska et al. (2012) reported changes in the composition of charophyte communities following Grass Carp introduction to a lake in western Poland, as well as a general decline in the number of aquatic macrophytes species. In an earthen pond (Georgia, U.S.) stocked with >100,000 juvenile triploid Grass Carp, selective feeding by Grass Carp eliminated most palatable plants from the community and promoted the persistence of the chemically defended and unpalatable *Micranthemum umbrosum* (Parker et al. 2006). In Arkansas, following Grass Carp stocking for *Hydrilla* control, the macrophyte community shifted in biomass dominance from American Lotus (*Nelumbo lutea*), *Hydrilla*, Egeria (*Egeria densa*), Coontail, and Duckweed (*Lemna* L.) to being dominated by American Lotus, Fragrant Water Lily (*Nymphaea odorata*), Coontail, Duckweed, and *Hydrilla* after stocking (Timmons 2012). Selective impact on the plant community was demonstrated by studies in irrigation systems and reservoirs in Krasnodar Krai, Russia (Vinogradov and Zolotova 1974 in Bogutskaya et al. 2017). Following depletion of preferred food items in one feeding ground, Grass Carp moved onto another feeding ground. Within a few years after introduction, plants such as pondweed, hornwort, water milfoil, and duckweed disappeared, and toxic plants and nuisance hydrophytes became more abundant.

Temporal variation in the influence of Grass Carp on aquatic vegetation may also be expected. Feeding intensity has been found to vary by season; the highest feeding intensity was observed in spring and autumn (Karpov et al. 1989 in Bogutskaya et al. 2017). Data from the middle Syr Darya River indicated that Grass Carp continue to feed during migration and spawning, and feeding was only suspended in winter when fish aggregated in deep river pockets (Mitrofanov et al. 1992 in Bogutskaya et al. 2017).

While Grass Carp consumes large amounts of aquatic vegetation, it processes it inefficiently (Fedorenko and Fraser 1978). The van der Lee et al. bioenergetic model in Jones et al. (2017b) predicted that a 5-year-old (~7.5 kg) Grass Carp would consume approximately 50 kg of vegetation per year, and a 10 year-old fish (~16 kg fish) would consume approximately 90 kg/yr. Using estimates from the bioenergetics model, Gertzen et al. (2017) evaluated the potential effect of Grass Carp on wetlands classified as "low marsh" (areas that are permanently inundated, support submerged aquatic vegetation (SAV) and floating leaf vegetation). Gertzen et al. (2017) estimated that approximately 2.5–4.5 million metric tonnes of aquatic vegetation exist in the Great Lakes at peak annual abundance (approximately August) (Figure 31). Using this estimate, the potential effects of Grass Carp on low marsh habitat in the Great Lakes was modelled (Figure 32). Annual vegetation die-back and spring regrowth was incorporated in the model, because vegetation growth must overcome Grass Carp foraging starting at a near-zero baseline, or vegetation growth would not occur. A variety of Grass Carp densities and sizes were tested in the model, and two different estimates of current vegetation biomass were used. In the model, over all scenarios, complete elimination of vegetation occurred in few areas (typically less than 5% of areas) but substantial reductions in peak aquatic biomass were predicted in many scenarios. A tipping point seemed to occur at a density of ten 13.2 kg Grass Carp/ha (Figure 33). For example, predicted proportion of sites with a 50% decrease in vegetated biomass with ten 9.5 kg Grass Carp/ha ranged from 2 to 8%. When size of the ten Grass Carp/ha were increased to 13.2 kg, 20 to 31% of sites were reduced in vegetated biomass by 50%. Notably, ten Grass Carp/ha is within the range of Grass Carp recommended stocking rates for successful vegetation control in ponds (e.g., Lynch 2009); complete eradication of macrophytes has occurred after 2 years following a stocking of 16 and 30 fish per vegetated acre but results are highly variable (Cassani et al. 2008).

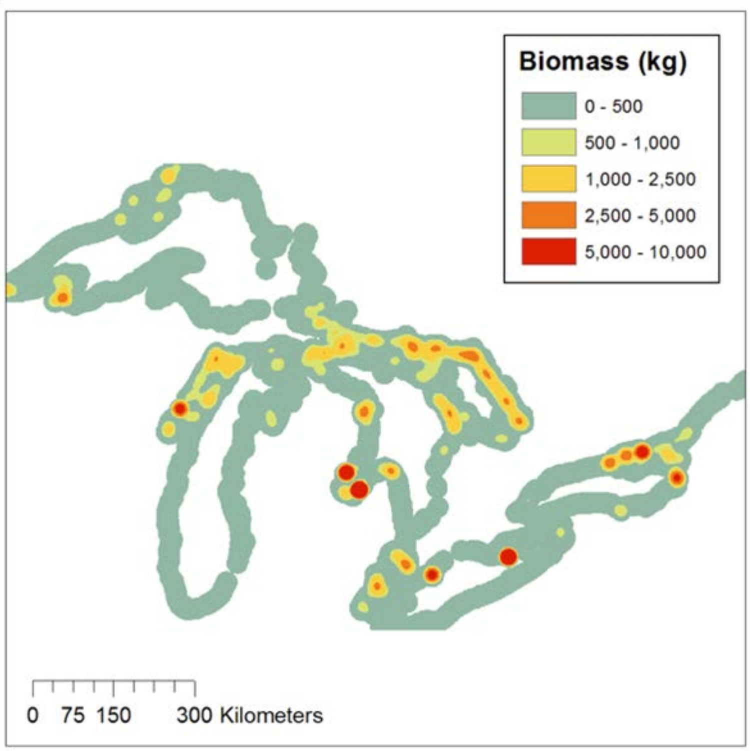


Figure 31. Distribution of areas with high biomass throughout the Great Lakes. This map uses data from the Great Lakes Low Marsh Inventory (GLLMI) and the CK85-Base biomass model. See Gertzen et al. (2017) for further information.

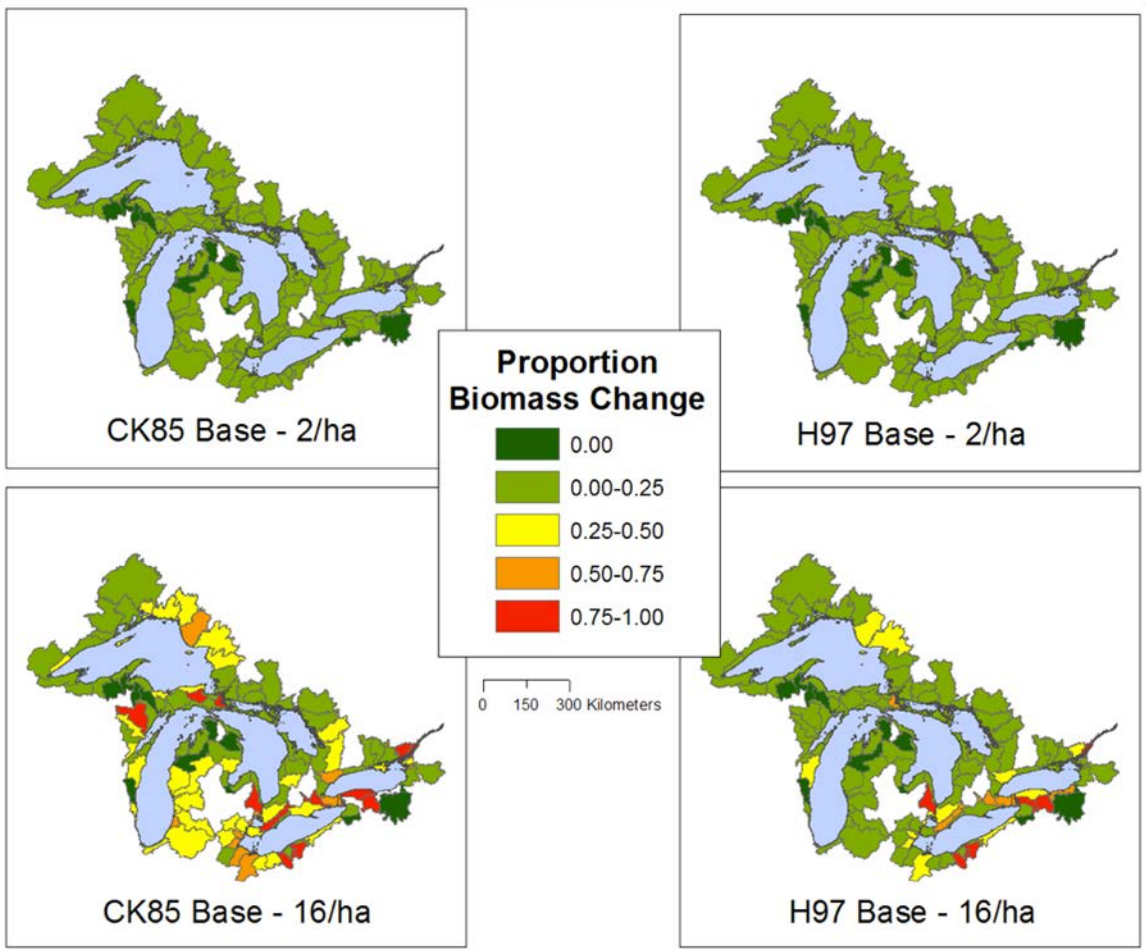


Figure 32. Watershed-level proportional changes in biomass for the Great Lakes Low Marsh Inventory (GLLMI). The values refer to only wetlands in direct contact with a Great Lake or connecting waterway, but the entire watershed is shaded to better show differences in the basin. Two different vegetation biomass estimates for the GLLMI are shown; the CK85 Base model shows the largest changes and the H97 Base model typically shows the smallest changes. The predicted change in proportion biomass is shown for two different Grass Carp densities: 2/ha (top row) and 16/ha (bottom row).See Gertzen et al. (2017) for further information.

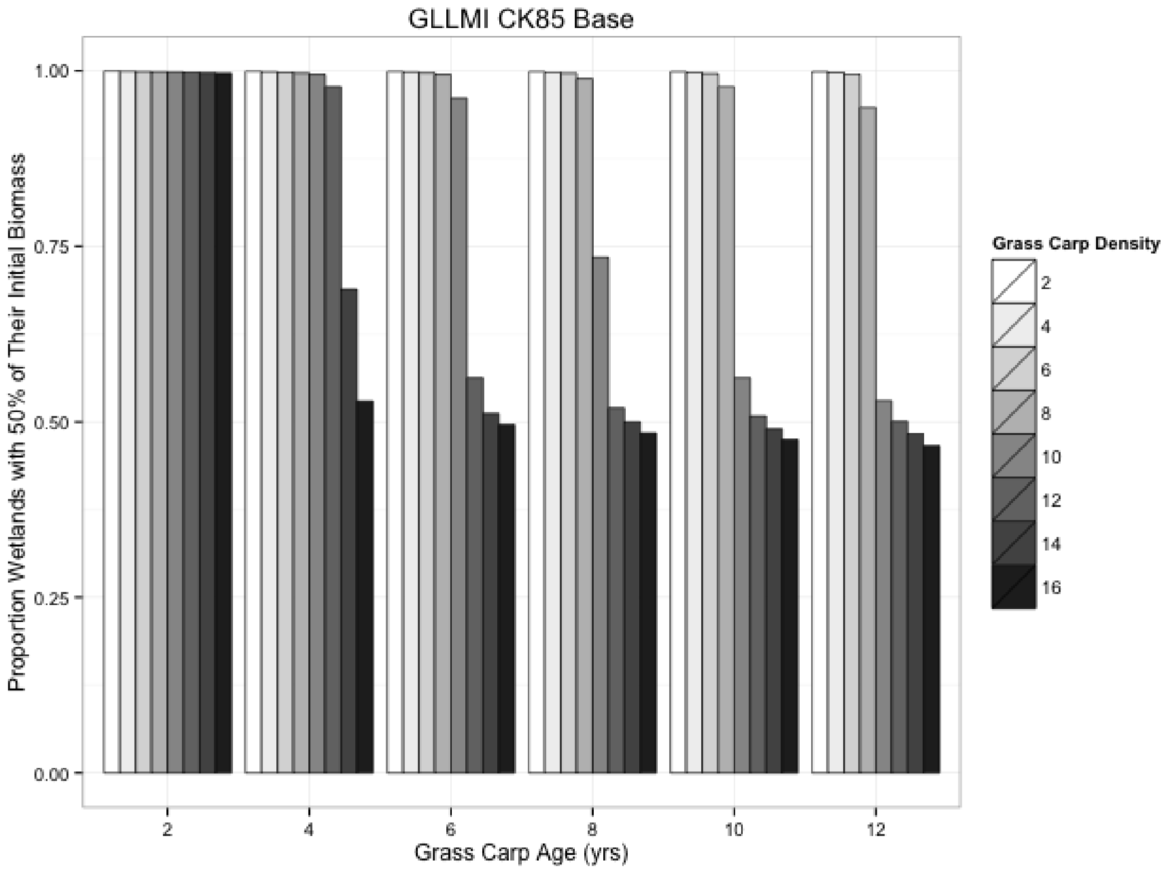


Figure 33. Example of site-level changes in biomass under the various Grass Carp densities and ages. The results for the GLLMI inventory using CK85 Base are presented and are representative of the general trend among all models for both inventories. See Gertzen et al. (2017) for further information.

Gertzen et al. (2017) does not address the effects of Grass Carp on the alga *Cladophora*, or include any effect that consumption of *Cladophora*, instead of wetland macrophytes might have on predictions of vegetation changes in the Great Lakes. Also, the Gertzen et al. (2017) study does not take into account the preference of Grass Carp for certain foods over others. Knowledge of the spatial distribution of individual vegetation species in the Great Lakes is lacking. Common submerged macrophyte species in the Great Lakes basin include *Ceratophyllum demersum, Chara* spp., *Elodea canadensis, Najas flexilis, Potamogeton richardsonii, Potamogeton* spp., and *Vallisneria americana* (Minns et al. 1993, Cvetkovic and Chow-Fraser 2011, Environment Canada 2004). *V. americana*, in particular, was negatively affected by pollution in the 1970s and began recovering by the late 1990s; it is an important vegetation species for waterfowl and fishes in the Great Lakes basin (Schloesser and Manny 2007). Several non-native macrophyte species are also common in the Great Lakes (e.g., *Myriophyllum spicatum, Najas minor,* and *Potamogeton crispus;* Trebitz and Taylor 2007). Grass Carp has been found to consume all of these species to some extent (Shireman and Smith 1983, Chilton and Muoneke 1992, Cudmore and Mandrak 2004, Jones et al. 2017a). Further, Grass Carp may consume wild rice *Zizania palustris,* which has a similar morphology (long and thin) preferred by Grass Carp; a plant in the Great Lakes that is of conservation, rehabilitation and cultural value (US EPA 2012).

Grass Carp is also known to consume terrestrial vegetation (Kilgen and Smitherman1971; Terrell and Fox 1974) by digging into banks and uprooting riparian vegetation (D. Chapman, USGS, pers. comm.). This method of feeding damages banks and may cause erosion and increased turbidity.

3.2 BIRDS

Grass Carp has the potential to affect birds that nest or feed in Great Lakes wetlands by consuming submerged aquatic vegetation and competing for food with several bird species, as well as by altering wetland nesting habitat (Dibble and Kovalenko 2009). A literature review of the effects of Grass Carp stocking on the environment found variable effects on birds, with no effects on some species and negative (reduced mean biomass, abundance or concentration) effects on Hooded Merganser *Lophodytes culullatus*, Ruddy Duck *Oxyura jamaicensis,* and Ring-Necked-Duck *Aythya collaris* (Wittmann et al. 2014). Gertzen et al. (2017) evaluated the potential overlap between Grass Carp and 47 water birds along the Canadian portion of the Great Lakes. The list of 47 water birds was derived from a variety of sources, including: Meyer et al. (2006); Steen et al. (2006); and, expert opinion (Lyndsay Cartwright, TRCA; Ted Barney, Long Point Waterfowl; Jeff Krete, Ducks Unlimited Canada; Mark Gloutney, Ducks Unlimited Canada). Many more bird species migrate through and use the Great Lakes on occasion. A literature review was conducted to describe wetland bird nesting habitat requirements (wetland obligate, wetland facultative, and non-wetland), and types of food consumed (including the dominance of aquatic vegetation, aquatic insects and other aquatic invertebrates in their diets) for the 47 species included. The reliance on and importance of wetlands for these bird species was evaluated using expert judgment. High impact on 18 of these bird species is predicted, based on nesting habitat requirements, and the use of aquatic vegetation, aquatic insects, and other aquatic invertebrates as food sources. The species were classified as experiencing high potential impact if at least three of the four metrics were impacted; either all of the major food sources or two of the major food sources and their nesting habitat relied on wetlands. The remaining 29 species are predicted to experience moderate impact, as the initial bird list was already restricted to birds using Great Lakes wetland habitat for important portions of their life. The uncertainty remains about these impacts until a better understanding about whether or how quickly Grass Carp may reduce the density and diversity of macrophytes in Great Lakes wetlands, embayments, and nearshore areas, and until a better understanding of how wetland birds may adapt or use other habitats is known. A comparison of preferences of Grass Carp to requirement of birds based on macrophyte species is unknown and represents a knowledge gap.

3.3 FISHES

Grass Carp prefers shallow water habitat and areas with large amounts of aquatic vegetation. Adult Grass Carp are mostly herbivorous and would not compete directly with Great Lakes fish species for food because no native Great Lakes fishes are primarily macrophyte consumers. Larval and juvenile Grass Carp feed on rotifers, zooplankton, insect larvae, chironomids, cladocerans, copepods, crustaceans, and small fishes (Chilton and Muoneke 1992, Cudmore and Mandrak 2004) and directly compete for food with native fishes and their larvae (Dibble and Kovalenko 2009); however, by 4 to 6 weeks post-hatch, plants dominate the diet of juvenile Grass Carp (Chilton and Muoneke 1992, Opuszynski and Shireman 1995). Great Lakes fish species and life stages inhabiting coastal wetlands, nearshore littoral zones, and tributaries are more likely to be affected by Grass Carp than are pelagic species and life stages. It is likely that the potential effects of adult Grass Carp on the Great Lakes fish community would be indirect through Grass Carp effects on aquatic vegetation (Gertzen et al. 2017).

Submerged aquatic vegetation in the littoral zone and coastal wetlands in the Great Lakes provide important fish habitat and support a wide variety of resident and migrant fish species (Jude and Pappas 1992; Randall et al. 1996; Trebitz et al. 2009). More than 50% of the Great Lakes fish community uses aquatic vegetation for important life history needs such as spawning, refuge, and forage habitat (Gertzen et al. 2017). Fish species that use wetlands in the Great Lakes are estimated to make up half of the fish biomass (Trebitz et al. 2009). Distribution and abundance of fishes in littoral areas and coastal wetlands of the Great Lakes are significantly affected by type and abundance of aquatic macrophytes present (Randall et al. 2012, Cvetkovic et al. 2009). In a meta-analysis of studies on vegetation, structure and fish communities in lakes and rivers, Smokorowski and Pratt (2007) concluded that substantial decreases in structural habitat complexity are detrimental to fish diversity, simplify fish communities, and change species composition.

Published findings on the effects of Grass Carp introductions on fish communities were summarized in Cudmore and Mandrak (2004), who concluded that reported effects greatly varied and were often contradictory. Wittmann et al. (2014) reviewed approximately 200 papers for experimental evidence of Grass Carp effects on fish communities; however, it was determined that few studies (n = 2, 18 data points) used an appropriate statistical design and could be used in the study. A meta-analysis of the data also showed the effect of Grass Carp introduction on fishes was variable. Most studies focused only on the direct effect of Grass Carp on macrophyte removal, which was the goal of the Grass Carp introduction, although some do give secondary consideration to indirect effects on one or a few other taxa. In one study from the Karakum Canal (Turkmenistan), Grass Carp was found to harm local fish populations through the removal of submerged and floating macrophytes, which are typically used as spawning grounds by fishes and used by larvae and juveniles for foraging and growth during the first summer after hatching (Charyyev 1984 in Bogutskaya et al. 2017).

In support of this risk assessment, Gertzen et al. (2017) investigated how Grass Carp may affect Great Lakes fishes through its effect on aquatic vegetation by conducting a literature review of the habitat preferences and spawning needs of 136 fishes in the Great Lakes (including 18 non-native species) and how these overlap with Grass Carp habitat. The classification of Great Lakes fishes into potential effect categories was based on the Balon reproductive guild of the species, its preference for shallow-water habitat, and its affinity for vegetated habitat during spawning, YOY, and adult life stages. The potential harmful consequences of reductions in vegetation and wetland habitat due to Grass Carp on Great Lakes fishes is high for 33 fish species (at least four categories demonstrated high reliance on wetlands), moderate for 33 fish species (at least three categories demonstrated high reliance on wetlands), and low or unknown for 70 fish species (less than three categories demonstrated high reliance on wetlands or adequate habitat information not available). Of the 33 species classified as potentially experiencing high undesirable effects, 85% may experience consequences across all life stages, and the remaining species may experience consequences across at least two life stages. Fish species with low population numbers that rely on shallow, vegetated habitat may experience greater population-level effects, a loss of some populations, and a reduction in genetic diversity following Grass Carp introduction. See Gertzen et al. (2017) for a more detailed discussion.

3.4 ABIOTIC FACTORS

Introduced Grass Carp populations have the potential to alter abiotic conditions in the Great Lakes. While data are limited and the effects at the Great Lakes scale are unknown, available research suggests that Grass Carp could affect factors such as conductivity, turbidity, nutrient cycling, primary production, and dissolved oxygen. Such effects would likely be mediated through Grass Carp's voracious consumption of vegetation (Sillis 1970), as many abiotic variables are closely linked to aquatic vegetation (Herdendorf 1987, Trebitz et al. 2007a). A meta-analysis of the effects of Grass Carp stocking on the environment found a significant cumulative effect of Grass Carp on the overall abiotic environment (Wittmann et al. 2014). Specifically, in areas where Grass Carp was present, water hardness, alkalinity, conductivity and salinity measurements increased significantly. Dissolved oxygen, nitrogen, phosphorus, and sediment metal concentrations increased slightly, whereas pH, and phytoplankton/chlorophyll *a* values decreased slightly (Wittmann et al. 2014).

High turbidity and sediment accumulation are concerns in the Great Lakes (Trebitz et al. 2007b). Increases in turbidity can diminish light penetration into the water and decrease the growth of submerged vegetation, reduce the ability of visual predators to forage, and hinder the successful development of early life stages of fishes (Lougheed et al. 1998, Trebitz et al. 2007b). Grass Carp may contribute to increases in turbidity through its observed behaviour of consuming terrestrial vegetation (Kilgen and Smitherman1971, Terrell and Fox 1974) by digging into banks and uprooting riparian vegetation (D. Chapman, USGS, pers. comm.). Such feeding behaviour may cause erosion to shore banks and increase turbidity in the adjacent waters.

Coastal wetlands play an important role in nutrient cycling and prevention of eutrophication (Mitsch 1992, Trebitz et al. 2007a, Sierszen et al. 2012). Wetland plants remove phosphorus and nitrogen from the water and store them in organic material and sediments (Sierszen et al. 2012). Great Lakes wetlands have already declined in size and quality due to anthropogenic activities (Herdendorf 1992) and if large populations of Grass Carp occur in the Great Lakes, they might further degrade vegetated wetlands resulting in the loss of ecosystem services including nutrient cycle control. Since the 1960s, the Great Lakes, and in particular Lake Erie, have experienced eutrophication and reductions in dissolved oxygen due to point and non-point source phosphorus inputs (Herdendorf 1992). Phosphorus management and the introduction of the filter-feeding Zebra *Dreissena polymorpha* and Quagga *D. rostriformis bugensis* mussels seem to have helped reduce the frequency of eutrophication events (Scavia et al. 2014); however, coastal wetlands continue to provide important nutrient sinks to help reduce eutrophication. The potential interaction between Grass Carp and dreissenid mussels in the Great Lakes represents a knowledge gap.

3.5 PATHOGENS/DISEASES

Wild Grass Carp already present in the waterbodies of the United States do not represent a risk of introducing non-native pathogens within their current range and represent no further threat to the Great Lakes than movements by any other wild cyprinids (Conover et al. 2007, A. Goodwin, USFWS, pers. comm.). For example, Grass Carp is known to host the Asian tapeworm *Bothriocephalus acheilognathi*s, a cestode parasite thought to be initially introduced into the U.S. in 1975 with imported Grass Carp from its native range in eastern Asia (Choudhury et al. 2006). However, this parasite is already known from the Great Lakes (Marcogliese 2008). However, additional importation of Grass Carp into the U.S. could introduce non-native pathogens with unknown potential consequences. The requirement of certified farm-raised Grass Carp by states (e.g., certified farms in Arkansas, New York State; A. Goodwin, USFWS, pers. comm.) to have a fish health certificate to indicate fish are free of diseases and important pathogens like VHS and SVC before they are stocked should minimize this threat. Although not a known current practice, additional importation of live Grass Carp into the United States from Asia could introduce non-native pathogens with unknown potential consequences.

3.6 SUMMARY OF MAGNITUDE OF ECOLOGICAL CONSEQUENCES

The likelihood of ecological consequences is considered at the lake-wide scale, although it is important to note that effects may be greater within localized wetlands if Grass Carp populations aggregate in these areas. Ecological consequence ratings were based on predicted decreases in submerged aquatic vegetation (SAV) area due to increasing Grass Carp densities (Table 3). Consequences of Grass Carp on other aspects of the biotic community (e.g., fishes, birds) and the abiotic environment (e.g., turbidity, nutrient cycling) are assumed to be indirectly related to the loss of SAV that, in turn, would result in ecosystem changes. The ecological consequences ratings do not take into account the possibility that *Cladophora* in offshore areas of a lake could sustain additional Grass Carp numbers and potentially increase ecological consequences across the whole lake. The probability of occurrence and introduction was also considered in the authors' rankings of the magnitude of ecological consequences.

The ratings were evaluated separately for each lake based on average Grass Carp densities across the lake, the SAV area currently in each basin (Gertzen et al. 2017), and recommended stocking densities for controlling SAV (Lynch 2009). Currently, in each lake, Grass Carp densities are thought to be below thresholds required for a detectable impact. To estimate at what point in time Grass Carp densities might be large enough to have detectable impacts, the total number of Grass Carp required to exceed a consequence threshold was calculated as the product of the threshold densities (i.e., 5, 10, 15 Grass Carp per ha) and the current SAV area for each lake (Table 16). The number of years required to reach the threshold population sizes was calculated based on a population growth rate of 1.6 (C. Jerde, UNR, pers. comm.), assuming reproductive success in every second year and seeded with an initial population of 100 (lakes Superior, Huron, and Ontario) and 1,000 individuals (lakes Michigan and Erie) (Table 16). No density-dependent effects were incorporated into the population growth model although such effects are not likely to be relevant at early stages of invasion and, given the lack of information available to adequately understand Grass Carp population biology for each lake, the same model is applied to all lakes.

Table 16. For each Great Lake: the current SAV area, estimated population sizes for four impact thresholds (Pop. Size); and predicted number of years to threshold population sizes (Years to Impact) based on initial populations of 100 and 1000 Grass Carp individuals. Negligible consequences (no detectable SAV area changes) are expected at densities <5 Grass Carp per ha.

	LAKE	**Superior**			**Michigan**			**Huron**			**Erie**			**Ontario**		
	SAV Area (ha)	10166			13426			41668			27840			21720		
	Starting Pop.		**100**	1000		100	**1000**		**100**	1000		100	**1000**		**100**	1000
Ecological Consequence Rating	*No. per ha*	*Pop. Size*	*Years to Impact*		*Pop. Size*	*Years to Impact*		*Pop. Size*	*Years to Impact*		*Pop. Size*	*Years to Impact*		*Pop. Size*	*Years to Impact*	
Low	2	20,332			26,852			83,336			55,680			43,440		
	4	40,664			53,704			166,672			111,360			86,880		
Moderate	5	50,830	**15**	10	67,130	15	**10**	208,340	**18**	13	139,200	17	**11**	108,600	**16**	11
	6	60,996			80,556			250,008			167,040			130,320		
	8	81,328			107,408			333,344			222,720			173,760		
High	10	101,660	**16**	11	134,260	17	**12**	416,680	**19**	14	278,400	18	**13**	217,200	**18**	13
	12	121,992			161,112			500,016			334,080			260,640		
	14	142,324			187,964			583,352			389,760			304,080		
Extreme	15	152,490	**17**	12	201,390	18	**13**	625,020	**20**	15	417,600	19	**14**	325,800	**19**	14
	16	162,656			214,816			666,688			445,440			347,520		

Overall, the magnitude of ecological consequences of triploid Grass Carp in the Great Lakes was rated as negligible with moderate certainty based on current stocking and environmental conditions with no new additional prevention or management action. This rating was based on the following: current densities have had an undetectable effect; the low likelihood of influx of triploids over time because of distance from intensive and permitted stocking facilities; and, the limitation of consequences to an individual's lifespan (Table 17a). Even if a substantial number of triploid Grass Carp were released into the Great Lakes, threshold values for ecological consequences would not be passed and ecological consequences would remain negligible at the lake-wide scale. We expect similar ecological consequences on an individual basis for triploids and diploids; however, the increase in the magnitude of ecological consequences over time for diploids is linked to growing population sizes. Thus, for diploid Grass Carp, increasingly higher ratings were given for lakes Michigan, Huron, Erie, and Ontario over time, reaching extreme by 50 years (Table 17b). Ratings for the ecological consequences of diploid Grass Carp considered both the consequence thresholds as well as the current status of Grass Carp occurrence in and around the Great Lakes basin, as such Lake Superior remained negligible over time (Table 17b) given the low probability of introduction.

Table 17. Magnitude of lake-wide ecological consequences ratings and certainties for each lake for (A) triploid, and (B) diploid Grass Carp, 5, 10, 20, and 50 years from the risk assessment baseline (i.e., 2014). Ratings were based on consequence thresholds and the probability of occurrence or introduction. Consequence Rating (Rank): Negligible (N), Low (Lo), Moderate (M), High (H), Extreme (E) (see Table 3 for description of ecological consequence ratings and associated consequence thresholds). Certainty of data (Cert.): Very Low (VLo), Low (Lo), Moderate (M), High (H), Very High (VH) (see Table 2 for description of certainty of data categories).

A) TRIPLOID MAGNITUDE OF ECOLOGICAL CONSEQUENCE

	Superior		**Michigan**		**Huron**		**Erie**		**Ontario**	
Time step (yr)	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
5	N	M	N	M	N	M	N	M	N	M
10	N	M	N	M	N	M	N	M	N	M
20	N	M	N	M	N	M	N	M	N	M
50	N	M	N	M	N	M	N	M	N	M

B) DIPLOID MAGNITUDE OF ECOLOGICAL CONSEQUENCE

	Superior		**Michigan**		**Huron**		**Erie**		**Ontario**	
Time step (yr)	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**	**Rank**	**Cert**
5	N	Lo	N	Lo	N	Lo	N	Lo	N	Lo
10	N	Lo	N	Lo	N	Lo	Lo	Lo	N	Lo
20	N	Lo	M	Lo	Lo	Lo	M	Lo	Lo	Lo
50	N	Lo	E	Lo	E	Lo	E	Lo	E	Lo

4.0 OVERALL RISK ASSESSMENT

As noted in Section 1.2, the overall probability of occurrence and introduction (Section 2.5) and the magnitude of the ecological consequences (Section 3.6) were combined to obtain a final risk for triploid (Figure 34) and diploid (Figure 35) Grass Carp for each lake taking into account 5, 10, 20, and 50 year time periods from the risk assessment baseline (i.e., 2014).

Overall risk for triploid Grass Carp was low (green) for all lakes for all time periods (Figure 34). The likelihood of occurrence was very likely for lakes Michigan and Erie for all time periods and it is noted that arrival is already considered to have occurred in these two lakes. Ranks for likelihood of occurrence increased for all other lakes over time. The magnitude of ecological consequences remained negligible for all lakes over all time periods, as triploids are functionally sterile for management purposes. Even if an influx of triploid Grass Carp to the Great Lakes basin was to occur (although not expected) it is not expected to surpass consequence thresholds for any of the lakes over any of the time periods, although localized impacts within certain areas of a lake may be significant.

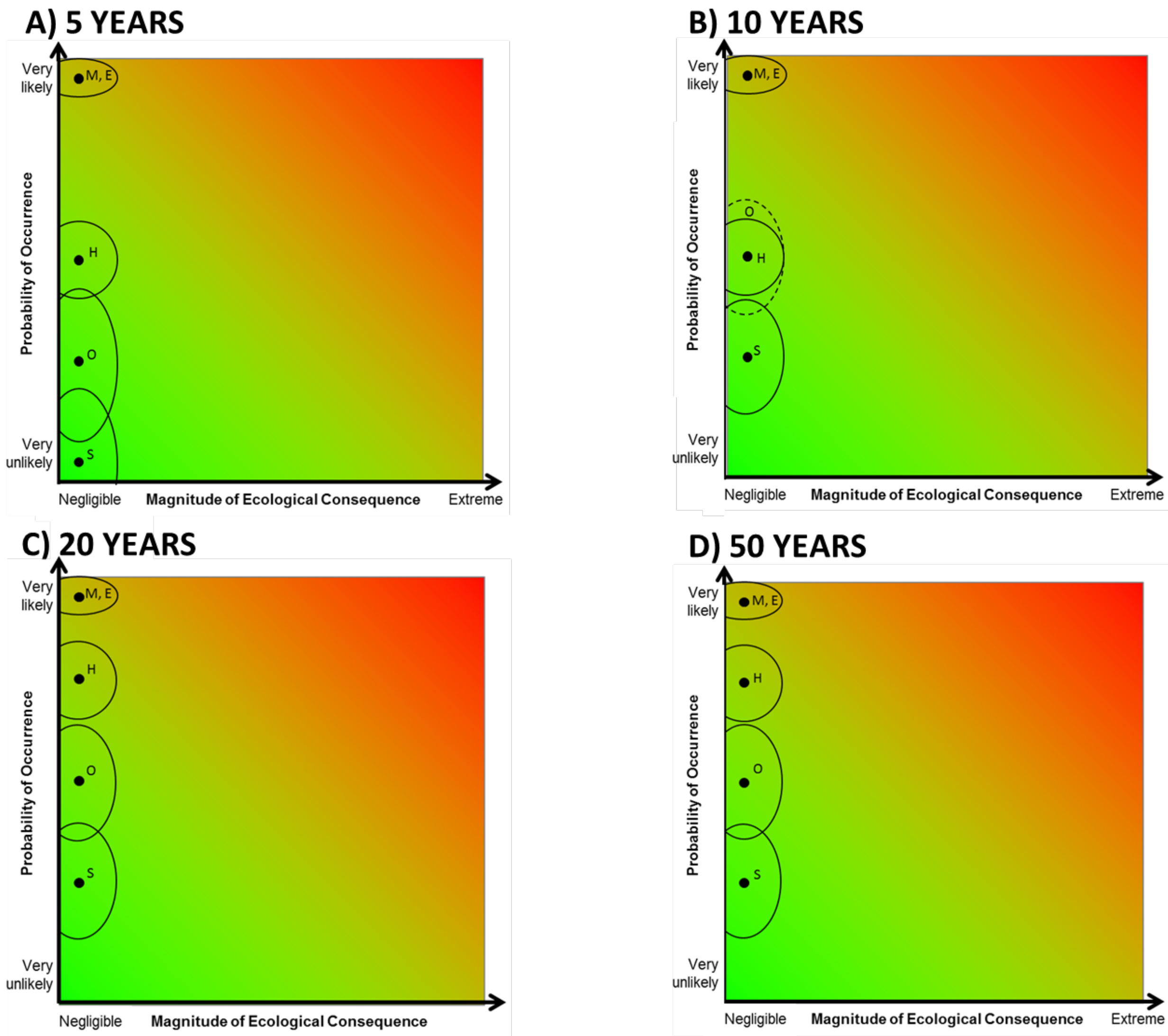


Figure 34. Probability of occurrence and magnitude of the ecological consequences for triploid Grass Carp over A) 5 years, B) 10 years, C) 20 years and D) 50 years from the baseline (i.e., 2014) as a graphic representation to communicate risk for triploid Grass Carp. S = Lake Superior, M = Lake Michigan, H = Lake Huron, E = Lake Erie, O = Lake Ontario; ellipses are representative of amount of certainty of data around ranks with broader ellipses representing greater uncertainty of data. Overall Risk: Green = Low Risk; Yellow = Medium Risk; Orange = High Risk; Red = Extreme Risk (Modified from Mandrak et al. 2012). Note: Grass Carp is considered to have already arrived to lakes Michigan and Erie.

Overall risk for diploid Grass Carp increases over time from low (green) to high and extreme (orange and red) for all lakes except Lake Superior which remains low (green) (Figure 35). The probability of introduction for lakes Michigan and Huron increases to very likely at 10 and 50 years, respectively, while lakes Erie and Ontario increase to high at 20 years and moderate by 50 years, respectively (Figure 35). The magnitude of ecological consequences increases from negligible to extreme by 50 years for lakes Michigan, Huron, Erie and Ontario (Figure 35), as they reach the estimated consequence density thresholds (Tables 3 and 16) and considering the current Grass Carp occurrences and probability of introduction. Lake Superior remains negligible over time given the low likelihood of introduction (Figure 35).

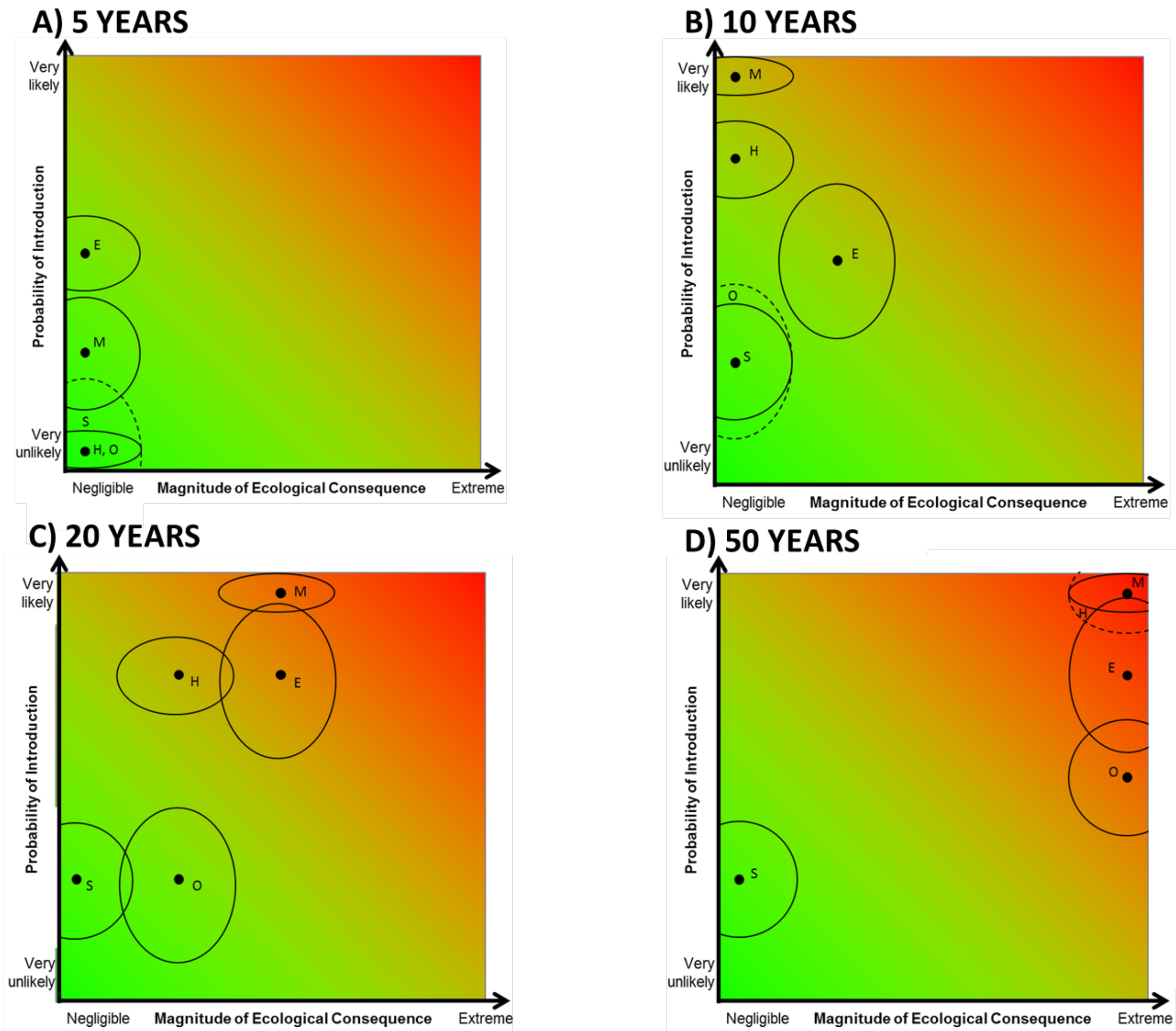


Figure 35. Probability of introduction and magnitude of the ecological consequences for diploid Grass Carp over A) 5 years, B) 10 years, C) 20 years and D) 50 years from the baseline (i.e., 2014) as a graphic representation to communicate risk for diploid Grass Carp. S = Lake Superior, M = Lake Michigan, H = Lake Huron, E = Lake Erie, O = Lake Ontario; ellipses are representative of amount of certainty of data around ranks with broader ellipses representing greater uncertainty of data. Overall Risk: Green = Low Risk; Yellow = Medium Risk; Orange = High Risk; Red = Extreme Risk (Modified from Mandrak et al. 2012). Note: Grass Carp is considered to have already arrived to lakes Michigan and Erie.

5.0 CONSIDERATIONS

Risk assessments are based on best information available at the time of the assessment, and should identify knowledge gaps and uncertainties. These knowledge gaps and uncertainties can be reduced through further research at any point in time following the assessment.

Knowledge gaps were identified by the authors and additions were made at the peer-review meeting. These knowledge gaps are:

- Different life stages of Grass Carp were not assessed specifically for each step in the assessment. Information and knowledge on younger life stages is lacking.

- Given lack of measurement of total monitoring effort, the current status of Grass Carp is unknown.
- The extent to which biological and behavioural differences exist between triploid and diploid Grass Carp (e.g., mortality, growth, spawning behaviour, movement).
- Extent of trade of diploid and triploid Grass Carp.
- There is little knowledge on the extent of illegal trade.
- There is little knowledge on the possibility of intentional stocking for cultural or nefarious reasons.
- Information is lacking on the use of baitfish and bycatch in baitfish harvest, especially on the U.S. side of the basin.
- Specific information on the potential invasiveness of Grass Carp was not available for use in the ballast water movement model; values used represent generic scenarios that can be applied to reflect the establishment characteristics of a given species, so the results provide a sensitivity analysis using probability values.
- It is unknown whether Grass Carp would occur in areas of *Cladophora* abundance.
- There are a lack of data on the frequency of suitable spawning conditions in the Great Lakes basin.
- Information on cues to spawn is variable.
- Reproductive behavior is largely unknown, including how individuals find each other for spawning, and whether a critical number of individuals are required to initiate spawning behaviour.
- The potential for lentic spawning (i.e., where eggs fall to substrate) needs to be further investigated; while it has not been observed in its native range, this does not mean it cannot happen in the introduced range.
- The relationship between overwinter survival (L_{crit} and proportion survival) to thermal survival from environmental niche models is unknown.
- The effect of predation and competition and resource limitation on overwinter survival is not known.
- Whether reproductive movements would enhance spread, or perhaps limit spread because of the need to remain close to spawning rivers or due to aggregation of fish because of reproductive behaviour or response to reproductive pheromones.
- There is a lack of knowledge regarding individual movements given there is some variability with individual fish.
- No published studies have been undertaken to directly determine the extent of fish movement through the New York Canal System.
- No published studies have been undertaken to directly determine the extent of fish movement through the Trent-Severn Waterway.
- Understanding movement of fishes from Lake Erie to Lake Ontario through the Niagara River, by surviving the descent over Niagara Falls.
- The depth limits of Grass Carp in lake systems.
- In general, there is a lack of information on impacts from Grass Carp in the wild.

- A comparison of macrophyte species preferences of Grass Carp to macrophyte species requirements of birds.
- Species composition of macrophytes within SAV locations within the Great Lakes basin is not known.
- The potential influence of Grass Carp on zebra mussels is unknown.
- Further targeted research of the ecological changes associated with Grass Carp is needed, particularly with natural populations in temperate climates and lake systems.
- There is no information available to predict facilitated invasions of other species by Grass Carp and biotic interactions.
- Lack understanding of lake-specific potential population biology (age to reproduction, spawning temperature patterns, etc.) to inform population growth models for each lake.

Many of these knowledge gaps result in low certainty rankings due to the lack of data and the quality of data that are available. These key areas of uncertainty are:

- The extent of human-mediated release (i.e., bait, stocking and trade) into all lakes for both triploid and diploid Grass Carp where more information and data would strengthen the advice surrounding arrival from this potential entry route.
- The likelihood of establishment of diploid Grass Carp over time for lakes Superior, Huron and Ontario.
- The likelihood of spread of diploid Grass Carp to lakes over time.
- Magnitude of ecological consequences ratings for diploid Grass Carp in all lakes were given low certainty; further targeted research of the ecological changes associated with Grass Carp is needed, particularly with natural populations in temperate climates and lake systems.

Risk analysis is composed of risk assessment, risk management, and risk communication (Mandrak et al. 2012). This risk assessment should be helpful as a baseline measure of risk while future action or mitigation plans for Grass Carp are developed. This baseline measure can then be compared to an analysis of risk with potential future actions to identify change in risk. The results of the risk assessment will be communicated to the public, resource managers, and decision makers in both countries.

While risk of Bighead and Silver carps was previously assessed (Cudmore et al. 2012), Black Carp has not yet been assessed for the Great Lakes. Due to time and resource constraints, it was decided to focus solely on Grass Carp at this time. A binational risk assessment for Black Carp focusing on the Great Lakes, following a similar process for the bigheaded and Grass carps, will be conducted separately at a later date.

Aquatic invasions can be considered natural disasters (Ricciardi et al. 2011). The further into the invasion process (pre-arrival, arrival, survival, establishment, or spread), the more difficult and costly it is to halt or manage (Leung et al. 2002). Preventing the earlier stages of the invasion from occurring, such as arrival, is, therefore, the most feasible and effective management effort that can be taken (Mack et al. 2000, Leung et al. 2002). As time passes, Grass Carp continue on the invasion pathway within and towards the Great Lakes, with occurrences and evidence of reproduction within some of the Great Lakes increasing; thus, time to prevent Grass Carp from arriving to the other Great Lakes is running out. Therefore, activities that specifically target pre-arrival, such as some of those being implemented by the ACRCC in the U.S. and Canada, continue to be important (see ACRCC 2014b and ACRCC MRWG 2014 for complete descriptions). Likewise, given that the number of individuals entering an ecosystem (i.e., propagule pressure) is paramount to establishment (Lockwood et al. 2005) and that prompt removal of initial individuals detected from a system is key to effective control (Simberloff 2010), additional actions targeting arrival, survival, establishment, and spread provide further opportunities to interrupt the invasion process (on-going efforts in the U.S. and Canada are described in ACRCC 2014b and ACRCC MRWG 2014).

There is an expected time lag associated with seeing the full consequences of an established population of an invasive species, such as Grass Carp in the Great Lakes; however, this should not be interpreted that there is time to wait before acting. The opportunity to prevent these predicted consequences may not persist. Ongoing management actions on both sides of the border continue while additional management options exist and further research can be conducted, to interrupt the trajectory to minimize the risk predicted within this assessment. We can, with effective prevention and control actions, continue to delay when these consequences would occur, and the level of impact, if Grass Carp became established in the Great Lakes. This delay will provide time to conduct further research into eradication and control options, as well as minimize and postpone overall costs of high control and management efforts, and costs associated with impacts.

It needs to be noted that the scientific advice provided here is just that, advice, there are no recommendations made. It is recognized that managers and decision-makers take into account other types of information and use all information available to them to make decisions on directions, policies, or activities.

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7.0 APPENDICES

7.1 APPENDIX 1 – ACRONYMS

Acronym	Definition
ACRCC	Asian Carp Regional Coordinating Committee
ACRCC MRWG	Asian Carp Regional Coordinating Committee Monitoring and Response Workgroup
AIS	Aquatic Invasive Species
CAWS	Chicago Area Waterway System
BMP	Best Management Practices
CSAS	Canadian Science Advisory Secretariat
CSSC	Chicago Sanitary and Ship Canal
DFO	Department of Fisheries and Oceans
DNR	Department of Natural Resources
eDNA	Environmental DNA
ENM	Environmental Niche Models
GLANSIS	Great Lakes Aquatic Nonindigenous Species Information System
GLFC	Great Lakes Fishery Commission
GLIN	Great Lakes Information Network
GLLMI	Great Lakes Low Marsh Inventory
GLMRIS	Great Lakes and Mississippi River Interbasin Study
IDNR	Indiana Department of Natural Resources
ILDNR	Illinois Department of Natural Resources
MDNR	Michigan Department of Natural Resources
MICRA	Mississippi Interstate Cooperative Resource Association
MNDNR	Minnesota Department of Natural Resources
NAS	Nonindigenous Aquatic Species
NTGCICP	National Triploid Grass Carp Certification and Inspection Program
NYDEC	New York Department of Environmental Conservation

Acronym	Definition
ODNR	Ohio Department of Natural Resources
OMNRF	Ontario Ministry of Natural Resources and Forestry
PAFBC	Pennsylvania Fish and Boat Commission
SAV	Submerged Aquatic Vegetation
SOP	Standard Operating Procedures
SVC	Spring Viremia of Carp
USACE	U.S. Army Corps of Engineers
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey
VHS	Viral Hemorrhagic Septicemia
WDNR	Wisconsin Department of Natural Resources
YOY	Young-of-Year

7.2 APPENDIX 2 – GLOSSARY

Term	Definition
Arrival	The repeated detection of at least one Grass Carp in at least one part of the lake basin within any continuous five-year period for a given Great Lake. The likelihood of Grass Carp entering one Great Lake from another Great Lake is not assessed in the likelihood of Arrival section but rather in the likelihood of Spread section (Section 2.4), which assesses the movement of individuals or expanding populations to one or more of the Great Lakes (inter-lake movement).
Baitfish	A variety of small fishes, with species used dependent on local regulations, supply, and angler preference, used to attract large predatory fish.
Diploid	Fertile; having two sets of chromosomes.
Ecological consequences	The effect of a species on its abiotic and biotic environment. Specifically in this document, ecological consequences were assessed for vegetation, birds, fishes, abiotic factors as well as for pathogens/diseases.
eDNA	Genetic material found in bulk environmental samples (e.g., soil, water, air) without isolating the individual organisms or their parts; dissolved DNA and/or fragments of tissue containing DNA that remain suspended and detectable in the water column for extended periods, ranging from days to weeks. It is defined by the process used to collect it.
Establishment	The presence of a self-sustaining population, which is defined as occurring when individuals spawned within the Great Lakes basin, have subsequently successfully reproduced. The establishment of Grass Carp in the Great Lakes is dependent upon availability of suitable spawning and nursery habitats, enough individuals for positive population growth, stock size required for effective recruitment, and survival of early life stages (considers predation, food availability and overwinter survival).
Great Lakes basin	The connected Great Lakes basin is defined as the Great Lakes *and* its tributaries to the first impassable barrier (Figure 2); Lake St. Clair is considered to be part of the Lake Erie basin. The geographic scope of the basin for this risk assessment was based on Neeson et al. (2015) (Figure 2). Neeson et al. (2015) evaluated the probability of migratory fish passage through tributaries across the Great Lakes basin. For this risk assessment, tributaries were deemed impassible if the probability of fish passage was 0 (red areas on Figure 2) while tributaries with probability of fish passage greater than 0 (blue and yellow areas contiguous with the Great Lakes on Figure 2) were deemed passable. The Chicago Area Waterway System (CAWS) represents a unique set of conditions where the primary flow is away from, rather than towards, the lakes; therefore, we interpreted the extent of the study area for this risk assessment to end at the Chicago Lock and O'Brien Lock and Dam, and the mouths of the Calumet and Little Calumet Rivers.

Term	Definition
Human-mediated release	Introduction of a non-native species assisted or primarily driven by humans. This type of release can be for the purpose of e.g., vegetation control, sport opportunities or spiritual/ethical reasons.
Introduction	Probability of introduction for diploids considers the likelihood of arrival, survival, establishment, and spread.
Laker ballast	Lake water that is held in tanks and cargo holds of ships to increase stability and maneuverability during transit. Unlike the ballast water in freighters that originate outside of the Great Lakes-St. Lawrence river basin, ballast water in freighters that remain in the basin (known as “lakers”) is not treated for aquatic invasive species.
Pathway	The route between the source region of a non-native species and its location of release.
Spread	Movement of individuals or expanding populations into new areas within the basin, between lakes; but not into the basin, as this is arrival.
Stocking	The practice of raising fish in a hatchery and releasing them into a waterbody to supplement existing populations, or to create a population where none exists.
Survival	Individuals do not die upon arrival and adults live through winter months in the Great Lakes basin.
Triploid	Sterile Grass Carp that are produced in hatcheries by physically shocking the eggs immediately after fertilization either with temperature (hot or cold) or pressure. The resulting fish are triploid (3N) because each cell has an extra set of chromosomes.
Vector	Dispersal mechanisms that move non-native species from one region to another; the manner in which a species is carried along a pathway.