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Study description

Assessing the performance of different sampling methods used for early detection monitoring (EDM) is a critical step in understanding the likelihood of detecting new non-indigenous species (NIS). However, EDM performance metrics based on detection probability estimates for surrogates such as established NIS or rare indigenous species may not accurately reflect survey effectiveness for newly-introduced NIS. In the St. Louis river estuary (SLRE) which includes the port of Duluth-Superior, the recent discovery of white bass (*Morone chrysops*) and gizzard shad (*Dorosoma cepedianum*) provided a unique opportunity to compare the detection of two new NIS using data from three different EDM survey approaches that were employed during these introductions.



White bass is considered native to all the Great Lakes except Lake Superior. However, they were historically present in the SLRE as reported in catch data from 1981-1988, but have not been observed since, despite significant annual monitoring. Their recent discovery represents a re-introduction.



Gizzard shad are found in all four lower Great Lakes but not Lake Superior. They are an important prey species as juveniles in some systems (adults reach 8-12 in.) and have been introduced both intentionally and unintentionally. To our knowledge they have not been previously reported in the SLRE.

EDM survey annual effort

- EDM survey approaches varied by targeted life-stage, media, and taxonomic method.
- Although annual effort varied within and amongst survey approaches, all surveys used a spatially-balanced probabilistic design to independently assess the fish community at a similar spatial scale.

Survey approach	Adult-Juvenile	Ichthyoplankton	eDNA
Sample media	Physical	Physical	Water
Taxonomic method	Morphological ¹	DNA-based ² (HTS ³)	DNA-based (HTS ³)
Survey year			
2009-2013	Annual (2009-2013) August: 20EF, 20FN, 20BT	2012 May-June: 24BS, 45TT, 37NN 2013 June-July: 20BS, 23TT, 23NN	No Survey
2014	August: 40EF, 20FN, 50BT	No Survey	No Survey
2015	August: 20EF, 20FN, 50BT	June-July: 21TT, 21LS, 21LT June: 20TT, 21TT, 21LT	No Survey
2016	May: 36BT August: 20EF, 20FN, 40BT October: 35 BT	June: 13TT, 13NN, 13LS July: 12TT, 12NN, 12LS	June: 120 WG October: 120 WG
2017	May: 36BT August: 20EF, 20FN, 38BT October: 35 BT	June: 25TT, 25NN, 25LS	No Survey
2018	May-June: 20EF, 20FN, 40BT August: 20EF, 20FN, 40BT October: 20EF, 20FN, 40BT	No Survey	August: 60 WG October: 60 WG

¹ID's were verified using DNA analysis of finclip samples (C01 genetic marker).

²morphological Q/A on 10% of ichthyoplankton samples; individual larvae ID's confirmed by DNA analysis (C01 genetic marker).

³High throughput sequencing (HTS); refers to DNA metabarcoding technique. Genetic markers: ichthyoplankton 16S; eDNA 12S and 16S. Gear abbreviations: EF = electrofishing, FN = fyke net, BT = bottom trawl, BS = beach seine (larval), TT = tucker trawl, NN = neuston net, LS = larval sled, LT = light trap, WG = water grab (eDNA)

Analytical approach

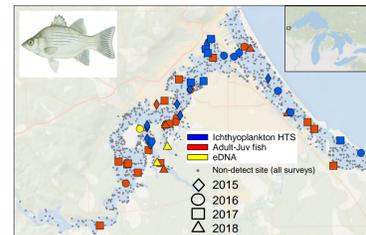
We used annual detection rates and spatial patterns to compare the relative detection effectiveness of each approach for both species.

Fish ages were estimated to determine cohort year and to calculate earliest possible year of introduction and lag time for initial detection.

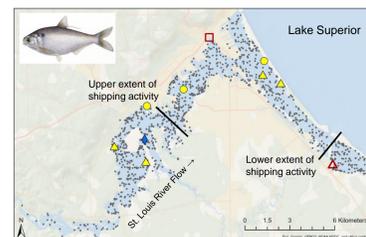
To evaluate effort prior to initial detection, probability curves were calculated for each survey approach (using data from detection years) to determine effort required for 95% detection probability.

Survey detection data

Annual detection site distribution



Widespread and consistent annual detection of white bass in physical surveys; low and spatially limited detection in eDNA surveys



Relatively low overall annual detection of gizzard shad; eDNA higher relative to physical surveys.

Gizzard shad detection locations widely scattered. Juvenile specimens (open symbols) found only in 2 extreme backwater locations and not as part of formal early detection monitoring (i.e., non-EDM). Eggs found in ichthyoplankton survey were upriver from shipping activity (seiche or other vector?)

Age-cohort determinations (adult-juv and ichthyoplankton)

White bass	Survey Year			
	2015	2016	2017	2018
Larvae presence (age-0)	DNA	DNA	DNA	No Survey
# YOY (age-0, 30-80mm)	0	2	40	2
Adult total lengths (mm)	96, 336	112, 128, 258, 264, 326, 362	166, 325, 341, 352	162, 176, 182, 255, 352
Adult age (otolith or estimated*)	1+, 5+	NA*, 1+, 2+, 2+, 5+, 6+*	1+, 4+, 5+, 5+	1+, 1+, 1+, 2+, 7+
Cohorts present (in order)	2010, 2014, 2015	2010, 2011, 2014, 2015, 2016	2012, 2013, 2016, 2017	2011, 2016, 2017, 2018

Gizzard shad	Survey Year		
	2015	2017	2018
Stage or total length (mm)	eggs	not captured	85, 99**
Age estimate	age-0	age-0**	age-0**
Cohorts present	2015	2017	2018

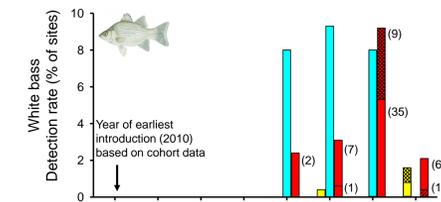
Multiple life stages captured for white bass in all survey years
2017 increased YOY (and detect. rate) suggests successful spawn

All cohorts from 2010 – 2018 represented for white bass

Current year cohorts only

YOY = young-of-year
*adult age estimated using length data (VonBertalanffy growth model).
**Non-EDM fish; age-0 estimate based on published regional length-age data.

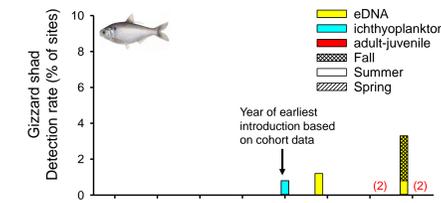
Annual effort and detection rates



White bass detected in all surveys beginning in 2015

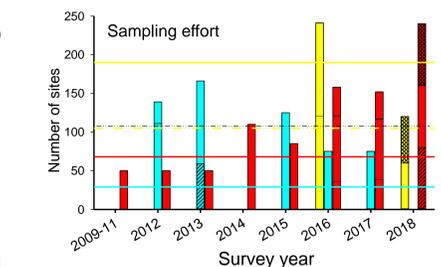
Detection rate consistently high in ichthyoplankton surveys

(#) = number of individuals captured in adult-juvenile surveys



Gizzard shad detection highest and most consistent in eDNA surveys

Gizzard shad not detected in adult-juv EDM, although 2 captured in both 2017 and 2018 non-EDM sampling (red#).



Horizontal lines indicate sampling effort required to achieve 95% detection probability. White bass = solid; gizzard shad = dashed (only eDNA had sufficient data).

eDNA effort required for 95% detection probability of white bass >> gizzard shad.

For white bass, effort for 95% detect. prob. highest for eDNA, lowest for ichthyoplankton.

Adult-juv effort was >90% detect probability in pre-detect years.

Findings

- White bass likely present up to 5 yrs prior to first detection; gizzard shad detected in first year of introduction?
- Physical surveys (adult-juv, ichthyoplankton) detected white bass with greater sensitivity than eDNA surveys
 - PCR or primer bias may contribute to low eDNA detection of white bass
- eDNA surveys produced multiple gizzard shad detections, despite lack of detection in physical surveys.
 - abundance too low for physical detection, or eDNA dispersed widely relative to abundance, or both
 - strict criteria for accepting eDNA "hit" provides high confidence in detections
- eDNA surveys detected gizzard shad with greater sensitivity compared to white bass

NIS and EDM implications

Both species appear to be at early stages of invasion, but present different challenges for EDM

- data supports a small but naturally reproducing white bass population (multiple cohorts and life stages present annually).
- gizzard shad detections may represent repeated novel introductions (restricted distribution, no fish >age-0 detected to date).

Both species could represent interlake transfer, since intra-basin ballast regulations not as strict as for transoceanic vessels

- further research using genetic markers could help reveal NIS origins and uncover high-risk pathways within the Great Lakes.

Our findings show interactions between EDM survey approach and species, and highlight potential risks and biases for NIS detection

- Morphologically based surveys can be less discerning for unfamiliar or cryptic species, however physical detection is needed to support DNA-based findings
- HTS improves sensitivity and accuracy of ichthyoplankton surveys, but may not detect some species due to poor DNA amplification or low biomass
- eDNA can increase survey power and sensitivity, but may differ amongst species, viability is uncertain, and life stage and abundance info is lacking

We conclude that using complementary sampling methods can balance strengths and weaknesses to provide more reliable EDM

