Supplemental Materials

Table S1. Total Data by Month: Column 1—Locations and sample collection months; Columns 2–9—qPCR success rate by species showing total trials and triplicates by locality and month; Column 10—Metabarcoding data, OTUs and Reads for each sample collected. Codes in the table: XX—AIS not detected; Q—Positive for qPCR results at that location; M—Positive metabarcoding result at that location; Blue Letter—AIS was previously found at that location; Red Letter—New detection record of the AIS at that location; Green Fill—Positive qPCR result; Orange Fill—Positive metabarcoding result; Blue Fill—Positive for both types of assays.

1	2	3	4	5	6	7	8	9	10
	qPCR Data								Metabarcoding
	H. anomala		*D. lumholtzi*		*C. pengoi*		*E. ishnus*		Data
Locaiton/Month	**Total trials**	**Triplicates**	**Total trials**	**Triplicates**	**Total trials**	**Triplicates**	**Total trials**	**Triplicates**	**OTUs/Reads**
1. Presque Isle, PA, USA	Q		XX		Q		M & Q		
May	0/12	0/4	0/12	0/4	0/12	0/4	0/12	0/4	12/59,179
June	1/12	1/4	0/12	0/4	0/12	0/4	1/12	1/4	24/103,882
July	0/12	0/4	0/12	0/4	1/12	1/4	5/12	3/4	25/110,851
August	1/12	1/4	0/12	0/4	0/12	0/4	5/12	3/4	63/80,117
September	4/12	2/4	0/12	0/4	0/12	0/4	3/12 MB	1/4	69/55,886
Total PI qPCR success data	6/60	4/20	0/60	0/20	1/60	1/20	14/60	8/20	
2. Vermillion River, OH, USA	Q		Q		Q		Q		
June	2/12	1/4	0/12	0/4	0/12	0/4	4/12	2/4	28/86,633
July	1/12	1/4	0/12	0/4	0/12	0/4	5/12	3/4	48/57,735
August	1/12	1/4	3/12	2/4	2/12	1/4	0/12	0/4	22/116,741
September	3/12	1/4	0/12	0/4	2 /12	2/4	6/12	3/4	No data
Total VR qPCR success data	7/48	4/16	3/48	2/16	4/48	3/16	15 /48	8/16	
3. Huron River, OH, USA	Q		XX		Q		M & Q		
June	0/12	0/4	0/12	0/4	0/12	0/4	0/12	0/4	32/60,146
July	0/12	0/4	0/12	0/4	0/12	0/4	1/12	1/4	22/116,129
August	2/12	1/4	0/12	0/4	0/12	0/4	1/12	1/4	84/66,983
September	1/12	1/4	0/12	0/4	1/12	1/4	0/12 MB	0/4	51/134,766
Total HR qPCR success data	3/48	2/16	0/48	0/16	1/48	1/16	2/48	2/16	
4. Port Clinton, OH, USA	Q		XX		Q		M & Q		
May	0/12	0/4	0/12	0/4	0/12	0/4	0/12	0/4	No data
June	0/12	0/4	0/12	0/4	0/12	0/4	0/12	0/4	58/52,997
July	0/12	0/4	0/12	0/4	1/12	1/4	6/12 MB	3/4	46/38,382
August	0/12	0/4	0/12	0/4	0/12	0/4	0/12	0/4	36/106,648
September	5/12	2/4	0/12	0/4	1/12	1/4	1/12	1/4	27/110,277
Total PC qPCR success data	5/60	2/20	0/60	0/20	2/60	2/20	7 of 60	4/20	
5. Maumee Bay, OH, USA	Q		Q		XX		Q		
June	1/12	1/4	0/12	0/4	0/12	0/4	0/12	0/4	24/6,247
July	0/12	0/4	3/12	2/4	0/12	0/4	0/12	0/4	2/1,211
August	0/12	0/4	0/12	0/4	0/12	0/4	0/12	0/4	32/105,314
September	3/12	1/4	0/12	0/4	0/12	0/4	1/12	1/4	No data
Total MB qPCR success data	4/48	2/16	3/48	0/16	0/48	0/16	1/48	1/16	
6. Erieau, ON, Canada	Q		XX		Q		M & Q		
May	0/12	0/4	0/12	0/4	0/12	0/4	0/12	0/4	5/37,061
June	0/12	0/4	0/12	0/4	0/12	0/4	1/12	1/4	42/91,117
July	1/12	1/4	0/12	0/4	0/12	0/4	5/12	3/4	9/103,551
August	0/12	0/4	0/12	0/4	0/12	0/4	3/12 MB	1/4	108/67,058
September	2/12	1/4	0/12	0/4	1/12	1/4	3/12	1/4	No data
Total ER qPCR success data	3/60	2/20	0/60	0/20	1/60	1/20	12/60	6/20	
7. Long Point, ON, Canada	Q		XX		Q		Q		
May	0/12	0/4	0/12	0/4	0/12	0/4	0/12	0/4	12/4,4680
June	1/12	1/4	0/12	0/4	0/12	0/4	0/12	0/4	23/62,323
July	0/12	0/4	0/12	0/4	0/12	0/4	2/12	1/4	39/115,588

August	1/12	1/4	0/12	0/4	1/12	1/4	1/12	1/4	13/90,577
September	1/12	1/4	0/12	0/4	0/12	0/4	1/12	1/4	51/78,970
Total LP qPCR success data	3/60	3/20	0/60	0/20	1/60	1/20	4/60	3/20	
8. Port Colburn, ON, Canada	Q		Q		Q		Q		
May	0/12	0/4	0/12	0/4	0/12	0/4	0/12	0/4	23/167,187
June	1/12	1/4	2/12	2/4	0/12	0/4	1/12	1/4	3/84,881
July	3/12	1/4	0/12	0/4	1/12	1/4	2/12	1/4	29/117,793
August	1/12	1/4	0/12	0/4	1/12	1/4	2/12	1/4	41/123,948
September	3/12	1/4	0/12	0/4	1/12	1/4	4/12	2/4	102/57,090
Total PCol qPCR success data	8/60	2/20	2/60	2/20	3/60	3/20	9/60	5/20	