Journal of Great Lakes Research xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Great Lakes Research

journal homepage: www.elsevier.com/locate/jglr

Range expansion and molecular confirmation of the Asian fish tapeworm in the lower Great Lakes and St. Lawrence River with notes on infections in baitfish

David J. Marcogliese [a,*], Andrée D. Gendron [a], Jonathon J.H. Forest [b], Wenxiang Li [a,1], Kellyanne Boyce [a,2], Fouad El-Shehabi [a], D. Andrew R. Drake [c], N.E. Mandrak [c], Jim Sherry [d], J. Daniel McLaughlin [b]

[a] *Aquatic Contaminants Research Division, Water Science and Technology Directorate, Science and Technology Branch, Environment and Climate Change Canada, St. Lawrence Centre, 105 McGill, 7th floor, Montreal, QC H2Y 2E7, Canada*

[b] *Department of Biology, Concordia University, 71741 Sherbrooke St. W., Montreal, QC H4B 1R6, Canada*

[c] *Department of Biological Sciences, University of Toronto Scarborough, 1265 Military Trail, Toronto, ON M1C 1A4, Canada*

[d] *Aquatic Contaminants Research Division, Water Science and Technology Directorate, Science and Technology Branch, Environment and Climate Change Canada, Canadian Centre for Inland Waters, 867 Lakeshore Rd., Burlington, ON L7S 1A1, Canada*

ARTICLE INFO

Article history:
Received 8 March 2016
Accepted 23 May 2016
Available online xxxx

Communicated by Stephen Charles Riley

Index words:
Freshwater fish
Cestode parasite
Invasive species
Schyzocotyle acheilognathi
Bait-fishes

ABSTRACT

The Asian fish tapeworm, *Schyzocotyle* (formerly *Bothriocephalus*) *acheilognathi*, was surveyed in fishes from the lower Great Lakes, surrounding waters, and St. Lawrence River between 2009 and 2015. Sequences of the internal transcribed spacer region (ITS1, 5.8S, ITS-2) and the barcode gene cytochrome oxidase 1 (CO1) are provided, confirming identification. Emerald shiners (*Notropis atherinoides*) collected in 2009–10 from the St. Clair River, the Detroit River, Lake Erie, the Niagara River, and Lake Ontario were infected at most localities with prevalence ranging from 1.9 to 20.0%. Infection intensity was negatively correlated with condition in these emerald shiners. Museum specimens of emerald shiners collected in 2007 from Lake Erie and vicinity also were infected at a prevalence of 5.0%. The parasite was observed for the first time in the St. Lawrence River at Beauharnois, QC, in a bluntnose minnow (*Pimephales notatus*) in June 2012. Subsequent infections in the St. Lawrence River were observed in two bluntnose minnows from Beauharnois in September 2012 and in emerald shiners from Beauharnois (prevalence = 12.0%) in June 2013, at Îles de la Paix, QC (prevalence = 6.7%) and Îles de Boucherville, QC (prevalence = 2.8%) in September 2013, and Îlet Vert, QC (prevalence = 1.8%) east of Montreal in September 2015. Samples of six other fish species from the Great Lakes, eight from surrounding small lakes and rivers, and eight species from the St. Lawrence River were uninfected. Of six species collected from six bait retailers along the north shore of Lake Erie and Lake Ontario, emerald shiners were infected at prevalences of 4.0 and 20.0% at two facilities out of six, indicating that infected baitfish have occurred in the wild at some harvest sites with sufficient prevalence to be transferred throughout the commercial supply chain. Results demonstrate that the Asian fish tapeworm has spread rapidly across the lower Great Lakes since its first observation in the Detroit River in 2002 and appears to be spreading eastward from its recent initial capture location in the St. Lawrence River.

Introduction

The Asian fish tapeworm, *Schyzocotyle acheilognathi*, is an invasive parasite that originated in China but is now found on every continent in the world with the exception of Antarctica (Choudhury and Cole, 2012; Scholz et al., 2012). Recently, this parasite, formerly known as *Bothriocephalus acheilognathi*, was assigned to the genus *Schyzocotyle* based on its relatively unique scolex shape (Brabec et al., 2015). The parasite is a true generalist, infecting a wide variety of fish hosts, primarily but not confined to cyprinids, which contributes to its invasiveness (Dove and Fletcher, 2000; Choudhury and Cole, 2012; Scholz et al., 2012). Another factor enhancing its invasiveness is its relatively simple two-host life cycle (Bauer and Hoffman, 1976; Scholz et al., 2012). Eggs are shed from the definitive host fish and then hatch into free-swimming coracidia. These are ingested by cyclopoid copepods, the only intermediate host required in the life cycle, which are then eaten by fishes to complete the cycle. At this larval stage, the parasite is a known generalist, infecting numerous widely distributed cyclopoid species (Marcogliese and Esch, 1989). Listed as "Pathogen of Regional Concern" by the US Fish and Wildlife Service (http://www.fws.gov/wildfishsurvey/regionalpath.htm), the parasite is considered

* Corresponding author. Tel.: +1 514 283 6499.
E-mail address: david.marcogliese@canada.ca (D.J. Marcogliese).
[1] Present address: State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, P. R. China.
[2] Present address: Science Department, South Devon College University Centre, Long Road, Paignton, TQ4 7EJ, United Kingdom.

http://dx.doi.org/10.1016/j.jglr.2016.05.008

Please cite this article as: Marcogliese, D.J., et al., Range expansion and molecular confirmation of the Asian fish tapeworm in the lower Great Lakes and St. Lawrence River with notes on infections in baitfish, J. Great Lakes Res. (2016), http://dx.doi.org/10.1016/j.jglr.2016.05.008

problematic, causing a variety of pathological problems and even mortality in fish hosts (reviewed by Scholz et al., 2012). Already a widespread problem in aquaculture (Choudhury and Cole, 2012), it has also become a potential conservation threat as it occurs in endangered and threatened fishes in the southern United States such as the humpback chub (*Gila cypha*) and the Topeka shiner (*Notropis topeka*) (Brouder and Hoffnagle, 1997; Choudhury et al., 2004; Bean et al., 2007; Koehle and Adelman, 2007). Lack of host specificity, particularly within Cyprinidae, raises concern that imperiled fishes in the Great Lakes–St. Lawrence River basin, such as endangered pugnose shiner (*Notropis anogenus*), endangered redside dace (*Clinostomus elongatus*), and threatened silver shiner (*Notropis photogenis*) could also be at risk of infection.

While the parasite is relatively widespread in the USA with records from over a dozen states (Choudhury et al., 2006), with the most recent records in emerald shiner (*Notropis atherinoides*), spottail shiner (*Notropis hudsonius*), mimic shiner (*Notropis volucellus*), and sand shiner (*Notropis stramineus*) from Lake Huron and Lake St. Clair, Michigan (Muzzall et al., 2016). However, there are few confirmed reports in Canadian waters. Confirmed infections include a single worm in a white bass (*Morone chrysops*) in Lake Winnipeg's southern basin (Choudhury et al., 2006), and another single infection in a bluntnose minnow (*Pimephales notatus*) from the Detroit River, which connects Lake Erie and Lake Huron in the Great Lakes (Marcogliese, 2008). Since that time, *S. acheilognathi* is reported to be widespread in minnows in the Lake Winnipeg drainage (see Bensley et al., 2011; Choudhury and Cole, 2012). Given uncertainty regarding the extent of this parasite in the Great Lakes–St. Lawrence River basin, the goal of this study is to examine the distribution and spread of *S. acheilognathi* in minnows across the lower Great Lakes since its initial discovery in those waters in the early 2000s, and its subsequent spread to the St. Lawrence River, which drains the Great Lakes into the Atlantic seaboard. Given the broad host specificity of this tapeworm, numerous other potential host species were examined from the Great Lakes–St. Lawrence River basin. Baitfish also were examined from angling retail facilities along the north shore of lakes Ontario and Erie to better understand transfer potential of the cestode via the live baitfish pathway. Lastly, the barcode portion of the cytochrome oxidase gene and the internal transcribed spacer region of ribosomal RNA of parasites were sequenced, to provide molecular corroboration of morphological species identification.

Materials and methods

Fish sampling and examination

Emerald shiner were collected with a beach seine (22.6 × 1.15 m; 3 mm mesh) deployed by hand from each of 14 localities in the St. Clair River, the Detroit River, Lake Erie, Lake Ontario, and Lake St. Francis (St. Lawrence River) in June 2009 and June 2010, with one site sampled in September 2009 (Fig. 1, Table 1). Spottail shiner also were collected at six localities in 2009 and one in 2010 at the same time (Table 1). Bluntnose Minnow, emerald shiner, and/or sand shiner were collected from five to nine localities in the St. Lawrence River in June 2012–15, and two and five localities in September 2013 and September 2015, respectively (Fig. 2, Table 1), using the same beach seine deployed by hand or partially from a boat. Additionally, eight other species of fish were collected from various localities in the St. Lawrence River in June 2014 (Table 2), eight species from inland lakes and rivers in Ontario

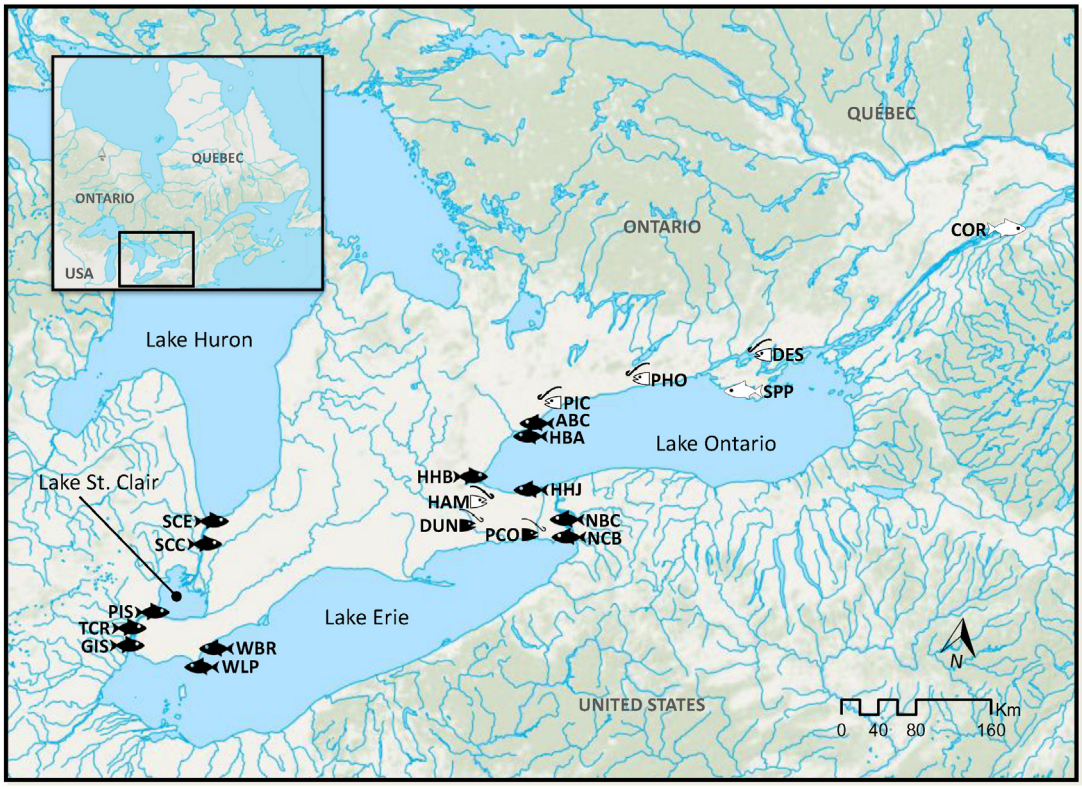

Fig. 1. Sampling localities for emerald shiner (*Notropis atherinoides*) and spottail shiner (*Notropis hudsonius*) in the Great Lakes, 2009–10, and retail shops where fishes were obtained in 2012 during a survey of the invasive Asian fish tapeworm, *Schyzocotyle* (=*Bothriocephalus*) *acheilognathi*. Closed symbols represent locations where *S. acheilognathi* was detected, and open symbols where it was not detected. From west to east, sampling localities illustrated by fish silhouettes are SCE = St.Clair–Pt Edward; SCC = St Clair River–Corunna; PIS = Peche Island; TCR = Turkey Creek; GIS = Grosse Île; WLP = Wheatley–Long Point; WBR = Wheatley Harbor–Branch; NCB = Niagara River–Crystal Beach; NBC = Niagara River, Black Creek; HHJ = Hamilton Harbor–Jordan; HHB = Hamilton Harbor–Bayfront; HBA = Humber Bay; ABC = Ashbridges–Bay Coatsworth Cut; SPP = Sandbanks Provincial Park; COR = Cornwall. From west to east, retail shops illustrated by fish and hook silhouettes are DUN = Dunnville; HAM = Hamilton; PCO = Port Colborne; PIC = Pickering; PHO = Port Hope; DES = Deseronto. Inset represents the location of the lower Great Lakes in northeastern North America.

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