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Research Article

Aquaculture as a possible vector in the spread of *Posthodiplostomum centrarchi* (Hoffman, 1958) (Digenea: Diplostomidae) in Europe

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Abstract

This study presents findings on metacercariae of *Posthodiplostomum centrarchi* (Digenea: Diplostomidae) in largemouth black bass, *Micropterus salmoides* (Actinopterygii: Centrarchidae), transported from France to Ukraine for aquacultural purposes. The parasite was co-introduced with the fish host with a prevalence of 100% and an infection intensity range of 14–90 (mean 52.1 ± 28.5). The metacercariae were morphologically and genetically identical to those recently described from largemouth black bass in Portugal. Fish translocation for aquacultural purposes is discussed as a source of disease transmission.

Key words: Centrarchidae, co-introduction, Diplostomidae, pond fisheries, Dnieper River basin

Introduction

With increasing international fish trade and globalisation, the risk of cross border transmission of infectious agents has increased, leading to increased risk to human health and significant implications for international trade (Ababouch 2006). Aquaculture, as one source of intentional species introduction, has been implicated in the transmission of infectious diseases, the transmission of bacterial and parasitic diseases to wild fish and increased susceptibility of aquaculture-raised fish to wild fish diseases (Bauer and Hoffman 1976; Naylor and Burke 2005). One of the best documented examples is the transmission of the monogenean parasite *Gyrodactylus salaris* Malmberg, 1957 from Baltic *onto naïve* Norwegian Atlantic salmon, *Salmo salar* L., 1958, which led to the destruction of a number of wild populations (Bakke et al. 2007). More often, however, species co-introduced into a new range together with aquacultural species, act as a source numerous specific parasites that cannot infect native hosts (Goedknegt et al. 2016). In European fresh waters, for example, the specific monogenean *Gyrodactylus proterorhini* Ergens, 1964 (Mierzejewska et al. 2011) was co-introduced with introduced Ponto-Caspian gobiids (Gobiidae: Neogobiinae), and these are unable to survive on local hosts (Ondračková 2016).

Propagule pressure, which includes an estimate of the absolute number of individuals involved in any one release event and the number of discrete release events (Blackburn and Duncan 2001), is an important trigger for an introduced species' success in new habitats (Lockwood et al. 2005). Artificial fish stocking and intentional introduction for fisheries and aquaculture purposes increase the chances of successful introduction as it usually requires a large number of stocked individuals (Welcomme 1988) as large and diverse founder populations increase the probability of parasite co-introduction (MacLeod et al. 2010). In addition, aquaculture can also represent an important vector of invasive species (unintentionally co-introduced "hitchhikers"), such hitchhikers becoming competitors, predators or a source of pathogens and parasites to indigenous wildlife populations (Naylor et al. 2001).

Table 1. DNA sites and primers used for sequencing.

Region	Primer	Primer sequence	Annealing temperature °C	Reference
28S	LSU5	TAGGTCGACCCGCTGAAYTTAAGCA	57	Littlewood 1994
	ECD2	CCTTGGTCCGTGTTTCAAGACGGG		Littlewood et al. 1997
ITS1	BD1	GTCGTAACAAGGTTTCCGTA	57	Luton et al. 1992
	4S	TCTAGATGCGTTCGAARTGTCGATG		Bowles et al. 1993
ITS2	3S	GGTACCGGTGGATCACGTGGCTAGTG	57	Bowles et al. 1993
	ITS2.2	CCTGGTTAGTTTCTTTTCCTCCGC		Cribb et al. 1998

The largemouth black bass, *Micropterus salmoides* (Lacepède, 1802) (Actinopterygii: Centrarchidae), is a commercial fish species with a natural range over much of North America, stretching from the Great Lakes basin in Canada (Ontario Province, Quebec) to northern Mexico (Slastenenko 1958; Page and Burr 1991). Owing to its popularity as a game-fish, it has been introduced into numerous countries around the world, including North, Central and South America, Europe, Asia and Africa (Britton et al. 2010). In Europe, it has been introduced as an aquacultural species, especially in the Iberian Peninsula, France and Italy (Ribeiro and Leunda 2012). In Ukraine, the fish has been present since the 19^{th} century, though only as a small local population in the north-western Shatsk Lakes (Kvach and Kutsokon 2017). Co-introduced monogeneans have previously been recorded on largemouth black bass in Great Britain and South Africa (Maitland and Price 1969; Truter et al. 2017), and a recent study in Portugal recorded the larval digenean (metacercariae) *Posthodiplostomum centrarchi* (Hoffman, 1958) (Kvach et al. 2017), a parasite naturally originating from the host's native range. This same parasite has also been documented in separated European populations of another centrarchid fish, the pumpkinseed *Lepomis gibbosus* L., 1758 (Kvach et al. 2017; Stoyanov et al. 2017; Ondračková et al. 2018). The metacercariae of this parasite are an agent of "white grub disease", known from a wide range of fish species (Hoffman 1958).

The aim of the present study was to assess whether this co-introduced parasite is present in largemouth black bass presently being stocked as an aquacultural species in Ukraine.

Material and methods

In 2016, 2+ largemouth black bass were transported from fish farms in Nancy and Le Mans, France, to the Osokorky Ponds (50º21′11″N 30º36′2″E) in the City of Kiev, Ukraine, for aquacultural purposes. The fish were held in the ponds over 2016–2017, with no other fish species present.

In autumn 2017, the now 3+ fish were harvested and 13 specimens submitted for parasitological analysis at the Ichthyopathology Laboratory of the Institute of Fisheries, Kiev. The fish were measured (in cm) and weighed (in g) before being frozen prior to further analysis (frozen fish have been shown to be an acceptable alternative method for parasitological studies when analysis of fresh fish is not possible; Kvach et al. 2018a). Prevalence (P, %), mean intensity (MI) and intensity range (IR) of infection were calculated in accordance to Bush et al. (1997). Metacercariae were isolated from the cysts, preserved in 70% ethanol and then stained and mounted in Canada balsam, following Georgiev et al. (1986). A subsample of the metacercariae were preserved in 96% ethanol for further molecular study. The balsam slides of 20 metacercariae were subjected to morphometric analysis under a Olympus BX50 light microscope, with identification features measured according to Kvach et al. (2017).

In order to confirm species identification, molecular analysis of ITS1-5,8S rDNA-ITS2-28S rDNA was performed. DNA was extracted from each metacercaria using the JetQuick kit (Genomed, Germany), the genomic region being amplified using the KAPA2G Robust HotStart PCR Kit (Kapabiosystems, USA; see Table 1 for primers and annealing temperatures). Sanger sequencing of the PCR products was performed commercially at GATC Biotech (Germany) and the sequences edited and aligned using Geneious v. 9.0.5 software (Kearse et al. 2012). The sequences were then compared with those from the largemouth black bass introduced into Portugal, NCBI Nos: MF170992, MF170993, MF170994 and MF170995 (Kvach et al. 2017) and from native largemouth black bass, denoted as *Posthodiplostomum* sp. 8, NCBI No: HM064962 (Locke et al. 2010).

Results

The mean total length of the fish examined in this study was 31.3 ± 2.8 (range = 27–35), mean standard length 27.2 ± 2.6 (22–30) and mean total weight 335.3 ± 37.5 (240–420). All fish examined were infected with white-grub disease (P = 100%). The dissection

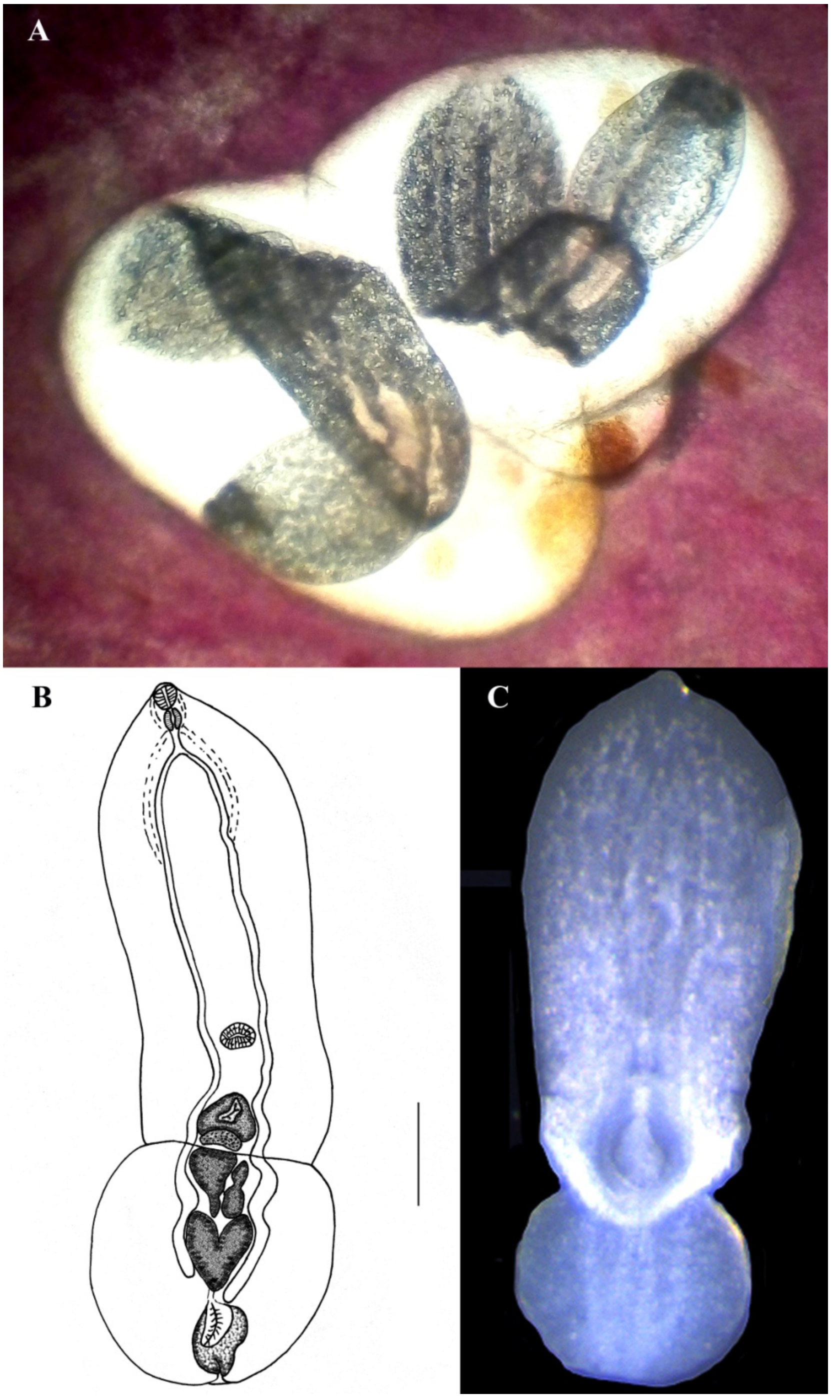

Figure 1. A) *Posthodiplostomum centrarchi* ex *Micropterus salmoides* from Ukraine. White-grub cysts in kidney tissue, B) – illustration of metacercariae (photograph of N. Matvienko); C) – live metacercaria isolated from a cyst (photograph of Y. Kvach). In all cases the scale bar = 200 µm.

of fish discovered the presence of ascites liquid in peritoneal cavity, yellowish colour of liver, kidney hydremia. Gut walls of infected fish were inflamed.

White (occasionally yellow) encysted metacercariae (Figure 1A) were located in the liver (MI = 29.4 ± 16.5; IR = 10–67), spleen (MI = 13.2 ± 6.2; IR = 4–25) and kidney (MI = 17.6 ± 12.8; IR = 1–30), with all infested organs together having an MI of 52.1 ± 28.5 and an IR of 14–90.

The metacercariae were identified as *P. centrarchi* based on morphological and metric characters: body 1100–1850 (mean 1353) µm long, divided into two parts (forebody and hindbody) separated by a constriction (Figure 1B, C). Forebody leaf-like, elongated and spoon shaped, ventral surface covered with small spines. Round or oval hindbody smaller, 34 to 79% of forebody length. Ventral sucker always larger than oral sucker, oral/ventral sucker ratio varying from 35 to 80%. Holdfast organ well-developed, triangular, round or oval in shape, located near the constriction at the posterior part of forebody, covered with small spines.

Molecular analysis confirmed that the metacercariae obtained in our study were identical to those recovered from the Portuguese largemouth bass (Kvach et al. 2017), and related to *Posthodiplostomum* sp. 8 recovered from the same species in Canada (Locke et al. 2010).

Discussion

In this study, we document a case of co-introduction of trematode metacercariae with non-native commercial fish introduced from France to Ukraine. Intentional fish introductions are known vectors of parasites spread in Ukraine. For example, the Asian tapeworm, *Khawia sinensis* Hsü, 1935, a specific parasite of the common carp, *Cyprinus carpio* L., 1758, has been introduced into many fish farms in the western part of Ukraine, (e.g. Kulakovskaya 1963), while the tapeworm *Schyzocotyle acheilognathi* (Yamaguti, 1934) was introduced into Ukraine with the Asian grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844) in the 1960s (Davydov et al. 2012). The Asian tapeworm is now widely distributed in other European countries (Oros et al. 2009), while *S. acheilognathi* has a worldwide distribution (e.g. Salgado-Maldonado et al. 2015). These cestodes are euryxenous parasites that can infect a range of native fish species from other taxonomic groups, including the big-scale sand-smelt, *Atherina boyeri* Risso, 1811, in Italy, Ukraine and Turkey (Giovinazzo et al. 2006; Çolak 2013; Kvach and Drobiniak 2017), and seven native fish species in Turkey (Öztürk and Özer 2014).

Unlike the cestodes mentioned above, *P. centrarchi* is a stenoxenous parasite, with its metacercarial stage only infecting centrarchid fish (Hoffman 1998). Two centrarchid species are known to have been introduced in Ukraine, the largemouth black bass and the pumpkinseed (Kvach and Kutsokon 2017). While presence of *P. centrarchi* has been confirmed in pumpkinseed from the Czech Republic, Slovakia and Bulgaria in the Danube basin (Kvach et al. 2017; Stoyanov et al. 2017), it has not yet been registered in Ukraine (Shumilo 1953; Kulakovskaya and Koval 1973; Rubtsova 2015a, b; Kvach et al. 2018b). Stenoxenous parasite introductions into Ukraine are known for the Chinese sleeper, *Perccottus glenii* Dybowski, 1877, however, the fish acting as a hitchhiker to commercially important fish; hence, aquaculture is probably one of the important spread vectors of this fish in Ukraine (Kutsokon 2017; Kvach and Kutsokon 2017). Two parasite species have been introduced into Ukraine with the Chinese sleeper, the Asian cestode, *Nippotaenia perccotti* (Akhmerov, 1941), and the monogenean, *Gyrodactylus perccotti* Ergens and Yukhimenko, 1973 (Kvach et al. 2016, 2018b). Unlike *P. centrarchi*, however, the host-specific parasites of the Chinese sleeper are introduced together with their host and only infect invasive host in the introduced range (Goedknegt et al. 2016).

Parasitic species can act as invasive species, as well as environmental factors affecting free living invasive species in recipient ecosystems (Machkevskyi et al. 2015). Herons (Ciconiiformes: Ardeidae) are the definitive host of *P. centrarchii*, while physid snails (Mollusca: Physidae) act as first intermediate hosts (Miller 1954). While the grey heron, *Ardea cinerea* L., 1758, is the definitive host in Europe (Stoyanov et al. 2017), the first intermediate host has yet to be confirmed. Theoretically, the widely introduced American physid snails, *Physella gyrina* (Say, 1821) and *Physella acuta* (Draparnaud, 1805), could be playing this role (Kvach et al. 2017 and references therein), particularly as *P. acuta* sensu lato has already been registered in the Middle Dnieper (Semenchenko et al. 2016). Consequently, herons preying on largemouth black bass in fish ponds could lead to further distribution of *P. centrarchi* in northern Ukraine, especially as one of the prospective intermediate hosts of *P. acuta* is the pumpkinseed, which is already found at a number of localities in the City of Kiev (Tsyba 2011; Afanasyev et al. 2017). As such, further distribution of white-grub disease in Ukraine appears plausible in the near future.

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