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DNA barcoding vs. morphological identification of larval fish and embryos in Lake Huron: Advantages to a molecular approach

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ABSTRACT

The Great Lakes provide habitat to over 160 species of freshwater fish, many of which are ecologically and economically important. Concern for management and conservation of declining fish populations makes it important that accurate identification techniques are used for environmental monitoring programs. DNA barcoding may be an effective alternative to morphological identification for industrial monitoring programs of larval and embryonic fish, but comparisons of the two approaches with species from the Great Lakes are limited. It may be particularly important to examine this issue in the Great Lakes because a relatively young group of post-glacial fish species are present which may be difficult to resolve using morphology or genetics. Six hundred and fifty seven larval fish were identified from Lake Huron (Ontario, Canada), using morphology and DNA barcoding. DNA barcoding was used to identify 103 embryos that morphology could not identify. Morphological identification and DNA barcoding had a percent similarity of 76.9%, 96.6% and 96.6% at the species, genus, and family levels, respectively. Thirty-seven specimens were damaged and unidentifiable using morphology; 35 of these were successfully identified using DNA barcoding. However, 23 other specimens produced no PCR product for barcoding using 2 different primer sets. Discrepancies between morphology and DNA barcoding were driven by two major factors: inability of cytochrome oxidase I to resolve members of the genus *Coregonus* and limited resolution of morphological features for *Catostomus*. Both methods have pros and cons; however, DNA barcoding is more cost-effective and efficient for industrial monitoring programs.

Introduction

Industrial water usage, such as once-through cooling, may impact aquatic species across all life stages. Once-through cooling systems bring in lake water which is pumped into condenser units for cooling, then is released as a warmed surface effluent back into the lake. The effluents may contain trace amounts of chemical or radiological contaminants, exposing fish in the near-shore area, and the intake process may result in the impingement and entrainment of aquatic organisms (Bruce Power, 2005). Environmental monitoring programs monitor fish that are impinged at intake points and attempt to identify the species and numbers affected (Ko et al., 2013; Moura et al., 2008; Richardson et al., 2007; Valdez-Moreno et al., 2010). However, many of the individuals captured by water intakes are in the larval stage, and this stage can be very difficult to identify based on morphology. Several species share very similar traits at the larval stage, which makes accurate identification difficult without highly specialized training (Ko et al., 2013). In addition, in some cases the larval stage may appear quite different among members of the same species based on relative age ((Strauss & Bond, 1990; Teletchea, 2009). Difficulties with identification can be compounded when larval fish specimens are damaged while travelling through the cooling system (Strauss & Bond, 1990; Teletchea, 2009). Furthermore, morphological identification is generally unable to identify fish in the embryo stage due to the lack of morphological indicators (Strauss & Bond, 1990; Teletchea, 2009). As a result, any attempt at morphological identification requires highly trained taxonomists; and even with this training, it is recommended that larval fish only be identified to the family level (Ko et al., 2013). However, ecological studies and environmental monitoring often require information at the genus or species level, so better resolution is required.

It has been shown that the mitochondrial cytochrome oxidase I (COI) gene can serve as the universal genetic barcode to identify many different organisms (Hubert et al., 2008). Over the last decade, DNA barcoding has become a well-established technique used in a variety of different settings, from identifying fish market substitution

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(Barbuto et al., 2010; Galimberti et al., 2013; Pinto et al., 2013; Wong & Hanner, 2008) to species monitoring and conservation (Ardura et al., 2010; Hajibabae et al., 2007; Valdez-Moreno et al., 2012; Ward et al., 2008). Ko et al. (2013) examined DNA barcoding to evaluate the accuracy of traditional morphological identification of Taiwan larval fishes by five different larval fish taxonomists. Marine larval fish were collected in the northern, southern and north-western seawaters of Taiwan over a two-year period yielding a sample size of 100 specimens per year. From the 100 samples, 12 samples failed PCR and could not be identified; the remaining 88 were DNA barcoded to 87 families, 79 genera and 69 species. Each of the five taxonomists from separate laboratories then identified the species to the three taxonomic categories. Ko et al. (2013) found that the consistency of identification between the five laboratories was very low: ~80.1% for family level, ~41.1% for genus and ~13.5% for species level. Consequently, Ko et al. (Ko et al., 2013) proposed that morphological identification of larval fish should be more conservative and that DNA barcoding is a useful technique to verify the accuracy of larval identification between different taxonomists. However, few studies have addressed similar issues in other systems with different fish communities.

The Great Lakes provide habitat to over 160 species of freshwater fish. These fish are not only ecologically important for their surrounding ecosystem, but generate revenue in excess of 8 billion dollars annually (Krantzberg, 2006). Many of these freshwater fish are post-glacial, recently diverged, and exhibit interspecies COI haplotype sharing (April et al., 2011; Hubert et al., 2008; Kochzius et al., 2010; Renaut et al., 2009; Sajdak & Phillips, 1995). For example, the genus *Coregonus* contains over 30 freshwater species, many of which have both ecological and economical importance in the Great Lakes (Schlei et al., 2008). Furthermore, several of these species are listed as at risk of extinction, including shortnose cisco (*C. reighardi*), Great Lakes kiyi (*C. kiyi*) and shortjaw cisco (*C. zenithicus*) (Ontario, 2017). The *Coregonus* genus has had a relatively recent evolutionary divergence making it very difficult to identify species morphologically, especially when in their larval stages (Schlei et al., 2008). Coregonid larvae are morphologically similar and may also be more genetically alike than the marine species identified by Ko et al. (2013). Thus, validation of molecular approaches for larval freshwater species in the Great Lakes is critical.

Here we compare the morphological identification of larval fish and embryos collected from the water intake system at a large nuclear power facility on Lake Huron, with DNA barcoding of the same specimens (Graham et al., 2016; Thome et al., 2016). Overall, our objective was to establish which method of identification was more accurate and cost effective for long-term environmental monitoring programs for large industrial operations using once-through cooling systems in the Great Lakes. We were also specifically interested in the accuracy of morphological identifications of lake whitefish (*C. clupeaformis*) and deepwater sculpin (*Myoxocephalus thompsonii*). Lake whitefish is ecologically and culturally important in Lake Huron, supporting a large commercial fishery and First Nations subsistence fishery (O'Neill, 2005; Overdyk et al., 2015; Schlei et al., 2008). Deepwater sculpin is listed as a species of special concern because of declining populations in the Laurentian Great Lakes (COSEWIC, 2015). Our work will help guide appropriate monitoring of industrial impacts on larval fish in the Great Lakes.

Methods

Sample collection

Bruce Power is a nuclear power plant located on the shores of Lake Huron in Tiverton, Ontario. The plant consists of eight CANDU pressurized heavy water reactors across two stations (four reactors in Bruce A and four reactors in Bruce B). Both stations rely on once-through cooling for steam condensation and have their own condenser cooling water circuit. Water is collected from Lake Huron via an intake tunnel located offshore on the lake bottom. Water travels through the intake tunnel into the forebay, which is located on shore adjacent to the Bruce A and B stations. From the forebay, water is pumped into the turbine hall for steam condensation and is subsequently released back into the lake via a surface discharge channel (Bruce Power, 2005). Fish larvae and embryo specimens were collected from March through December in 2013 and 2014 within the Bruce A forebay. Samples were collected using a round, 500 μm mesh plankton net that was 0.72 m in diameter and was lowered 3 m into the intake water for 5 to 140 min (median 36 min). Specimen collection occurred both during the day and at night. Between 2013 and 2014, there were 81 day and 80 night sampling efforts, with a minimum of three nets set per effort. Sampling was done during the day and night to avoid any bias due to nocturnal or diurnal behaviour of any specific species. Each collected specimen was given a unique identification number and larval fish were stored in 95% ethanol until morphological analysis. After morphological analysis, larval fish of the same species from the same collection time were stored together in 95% ethanol. Fish embryos from the same collections were stored together in 95% ethanol. All efforts resulted in the collection of 1740 larval fishes and 2831 fish embryos. Larval specimens were randomly chosen for both morphological identification and DNA barcoding so as to achieve a representative sample from all possible species. Fish embryos were randomly selected for DNA barcoding analysis.

Morphological identification

Samples were analyzed commercially (through an external contractor) by an expert ichthyologist who specializes in the identification of larval fishes from the Laurentian Great Lakes. Specimens were identified based on body shape, myomeres, pigmentation, meristic count, and fin characteristics (e.g., number, shape, relative position etc.). When possible, specimens were identified to the species level; otherwise, specimens were identified to the genus or family level. Results of morphologic identifications were recorded based on the unique identification number of each specimen. Fish eggs were not identified morphologically. The cost of the larval identifications was recorded for a comparison with DNA barcoding. Cost was calculated on a per-specimen basis in US Dollars (USD) and converted to Canadian Dollars (CAD).

Molecular identification

DNA was extracted from individual larval fish and embryos using spin column kits according to manufacturer guidelines (Qiagen DNEasy, Mississauga, ON; Norgen Biotech DNA extraction kit, Thorold, ON). When larval specimens were small (<12 mm in total length), the entire fish was used for DNA extraction; when larval specimens were larger, a portion of the body (up to 12 mm) was used for DNA extraction. DNA concentration from extractions was measured using a NanoDrop 1000 Spectrophotometer (Thermo Scientific) or Qubit fluorometric quantation (Life Technologies). All 260/280 and 260/230 ratios were within appropriate range of 1.8–2 for DNA analysis.

For all specimens, a 658 bp region of the COI mitochondrial genome was PCR-amplified using universal primers FishF1 (5′-TCA ACC AAC CAC AAA GAC ATT GCC AC-3′) and FishR1 (5′-TAG ACT TCT GGG TGG CCA AAG AAT CA-3′) (Ward et al., 2005). PCR reactions consisted of a total volume of 25 μL with components as follows: 1× PCR buffer; 2.5 mM of $MgCl_2$; 0.1 μM of each forward and reverse primers, 0.05 mM of each dNTPs, 0.31 units of *Taq* DNA polymerase, and 10 ng of template DNA. The thermal cycling regime consisted of: 2 min at 94 °C followed by 35 cycles of: 30 s at 94 °C, 40 s at 52 °C and 1 min at 72 °C. Final extension was for 10 min at 72 °C. PCR products were separated on a 1% agarose gel to verify the presence of a product in the target size range. Specimens that failed the initial PCR were run a second time using the universal fish primers FF2d (5′-TTC TCC ACC AAC CAC AAR GAY ATY GG-3′) and FR1d (5′-CAC CTC AGG GTG TCC GAA RAA YCA RAA-3′)

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