DNA-based identification of invasive alien species in relation to Canadian federal policy and law, and the basis of rapid-response management

Vernon G. Thomas, Robert H. Hanner, and Alex V. Borisenko

Abstract: Managing invasive alien species in Canada requires reliable taxonomic identification as the basis of rapid-response management. This can be challenging, especially when organisms are small and lack morphological diagnostic features. DNA-based techniques, such as DNA barcoding, offer a reliable, rapid, and inexpensive toolkit for taxonomic identification of individual or bulk samples, forensic remains, and even environmental DNA. Well suited for this requirement, they could be more broadly deployed and incorporated into the operating policy and practices of Canadian federal departments and should be authorized under these agencies’ articles of law. These include Fisheries and Oceans Canada, Canadian Food Inspection Agency, Transport Canada, Environment Canada, Parks Canada, and Health Canada. These efforts should be harmonized with the appropriate provisions of provincial jurisdictions, for example, the Ontario Invasive Species Act. This approach necessitates that a network of accredited, certified laboratories exists, and that updated DNA reference libraries are readily accessible. Harmonizing this approach is vital among Canadian federal agencies, and between the federal and provincial levels of government. Canadian policy and law must also be harmonized with that of the USA when detecting, and responding to, invasive species in contiguous lands and waters. Creating capacity in legislation for use of DNA-based identifications brings the authority to fund, train, deploy, and certify staff, and to refine further developments in this molecular technology.

Key words: DNA barcoding, molecular identification, exotic species, Canadian legislation, policy harmonization.

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Introduction

Invasive alien species (IAS)\(^2\) of animals and plants have invaded the terrestrial, freshwater, and marine environments of Canada and the USA, and they have become established and exert their deleterious effects upon native species communities, agriculture, forestry, fisheries, and industrial infrastructures (Pimentel 2005; Pimentel et al. 2007; Simberloff et al. 2005). IAS pose major destabilizing threats to various ecosystems (especially freshwater and forest) (Langor and Sweeney 2009), threats that are likely to increase because of human-caused habitat transformation (Marvier et al. 2004), climate change (Stachowicz et al. 2002), increased global trade (Jenkins 1996), and greater human mobility (Mack et al. 2000). A decade ago, 16 IAS were known to have cost Canadian agriculture, forestry, fisheries industry, and transportation USD13.3–34.5 billion/year (Environment Canada 2004), quite apart from the ecological costs of their competitive impacts on native biodiversity (Langor and Sweeney 2009).

Management of nuisance IAS requires an early, rapid, and reliable identification, but traditional morphological approaches are often limited by available expertise, time, specimen fragmentation, morphological similarity of species, or the specimens being in early and indistinct larval, egg, or seed stages. In this respect, DNA sequencing technology has helped overcome these limitations and has been used successfully to identify reliably species from all Kingdoms (Darling and Blum 2007). DNA barcoding (Hebert et al. 2003) provides a standardized approach and a universal operational framework for applications of this technology. This approach is minimalistic, making it relatively fast, inexpensive, and reproducible (Frézal and Leblois 2008; Hebert et al. 2003). It has been used to distinguish morphologically similar species reliably, and to segregate species in bulk environmental samples (Stein et al. 2014). Established standard DNA barcoding protocols (http://ccdb.ca/resources/) already exceed the minimum standards of International Sanitary and Phytosanitary Measures (ISPM No. 27) for pest identification (FAO 2006), which outline broad provisions for tracking the molecular methods used. This technology has been used successfully to identify Canadian freshwater fish (Hubert et al. 2008), and the different species in commercial fish products (Wong and Hanner 2008; Yancy et al. 2008). The facility with which identifications are made predisposes this technology’s use in situations where large numbers of containers and samples must be inspected, but low costs are imposed on the importation process. Moreover, while morphological identification of unambiguous specimens may be faster than sequencing when experts are available to undertake this task, expert opinion is not always accurate (Ko et al. 2013) and DNA-based approaches may thus be needed to substantiate an expert identification in a legal challenge.

Canada is obliged to adopt measures to deal with IAS under Article 8 of the UN Convention on Biological Diversity, specifically the Aichi Biodiversity Target, No. 9 (https://www.cbd.int/sp/targets/). There is a general international agreement on strategy(ies) to deal with IAS (Simberloff 2009), requiring first, prevention of entry, followed by rapid-response—defined as a systematic effort to eradicate, or contain, invasive species while infestations are still localized (NISC 2008)—to eliminate nuisance IAS, and finally, containment to reduce spread of established IAS. This applies to IAS that may impact the health of both ecosystems and humans (Ondrejicka et al. 2014). However, there has been little emphasis on how to achieve the rapid identification of specimens upon which these strategies depend. For example, a report (Advisory Work Group Report 2009) to the Canada–US Great Lakes Water Quality Agreement of the International Joint Commission provides no mention of how identification will be made as the precursor to rapid-response management (RRM). The state of Maryland’s strategy (Smits and Moser 2009) to deal with IAS places reliance on a team of experts for species identification. Similarly, the 2012 Ontario Invasive Species Strategic Plan (OMNR 2012) envisages a team of experts created to identify any potential IAS. Neither strategy mentions DNA-based identification, such as DNA barcoding, even though it complements traditional taxonomic methods (Hajibabaei et al. 2007). DNA-based methods, if used as a component of regular monitoring, could help detect invasive species early, and provide authorities with the basis of (RRM) to contain and eradicate deleterious IAS. They could also aid in monitoring the efficacy of mitigation and remediation measures following an IAS outbreak.

While the use of DNA-based methods in IAS identification has already been proposed by Armstrong and Ball (2005), Darling and Blum (2007), Dawney et al. (2007), and Scheffer et al. (2006), the idea of incorporating such technology into regulatory policy and law has received little attention (Allendorf and Lundquist 2003; Floyd et al. 2010; Shine et al. 2000), although it is mentioned explicitly by FAO (2006). Unlike the USA, with which Canada shares common land, marine, and freshwater borders, Canada lacks a single federal IAS prevention act (Vásárhelyi and Thomas 2003; Thomas et al. 2009) and has only a national strategy which identifies the problem and priorities for action (Environment Canada 2004). However, this does not provide for specific procedures or protocols with respect to taxonomic identifications and has no regulative power. Ontario also has a strategy

\(^2\)The nomenclature used in this paper agrees with that adopted by Environment Canada (https://www.ec.gc.ca/eee-ias/) and the Convention on Biological Diversity (https://www.cbd.int/invasive/).
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Both the federal and provincial levels. Provision for this analytical tool could be incorporated into the regulations of Canadian laws dealing with IAS at the national level is critical. Legislation can authorize measures to prevent, detect, respond to, and manage IAS. Unfortunately, there has been little effort to realize these regulatory goals in the past decade. Fisheries and Oceans Canada admits that the current regulatory framework to address aquatic IAS consists of “a patchwork of regulations under federal or provincial legislation …”, and that “there is little consistency across this patchwork of legislation with regard to prohibited species, regulatory language or requirements. Furthermore, the current invasive species regulatory framework is limited by significant gaps in the management of the introduction and spread of aquatic invasive species” (Government of Canada 2014). The absence of a fast, reliable, and standardized method to identify such invasive species is one of the significant gaps.

Capacity building to deploy DNA-based identification at the national level is critical. Legislation can authorize the funding of programs to train personnel to conduct DNA barcoding, and their deployment in government facilities. This approach enables using universal reference DNA libraries, adopting uniform analytical protocols and developing universally applicable approaches for regulatory purposes.

The above interpretation of DNA barcoding is inclusive of a suite of technical approaches, aside from “traditional” Sanger sequencing (e.g., deep sequencing, qPCR). As well, it is not exclusive of the use of additional markers, provided that their use is thoroughly substantiated and agreed upon as an accepted standard by a broad community of practitioners (e.g., CBOL Plant Working Group 2009).

Why incorporate DNA-based identification into law?

Given the ability of DNA sequence information to provide rapid, reliable, and reproducible identification results, it could be assumed that this approach would quickly become the leading, default, method to identify specimens, so the need to incorporate provisions into regulations might appear unnecessary. However, deploying DNA-based methods at a national–international level assumes that legal provisions to use them already exist. Accordingly, the national invasive species strategy for Canada (Environment Canada 2004) has stated that legislative and regulatory provisions will be developed, including amendment of existing law, to strengthen measures to prevent, detect, respond to, and manage IAS. Unfortunately, there has been little effort to realize these regulatory goals in the past decade. Fisheries and Oceans Canada admits that the current regulatory framework to address aquatic IAS consists of “a patchwork of regulations under federal or provincial legislation …”, and that “there is little consistency across this patchwork of legislation with regard to prohibited species, regulatory language or requirements. Furthermore, the current invasive species regulatory framework is limited by significant gaps in the management of the introduction and spread of aquatic invasive species” (Government of Canada 2014). The absence of a fast, reliable, and standardized method to identify such invasive species is one of the significant gaps.

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Table 1. Canadian federal agencies, their jurisdiction, and principal legislation used to deal with invasive alien species.

<table>
<thead>
<tr>
<th>Federal Department</th>
<th>Jurisdiction</th>
<th>Principal Legislation</th>
</tr>
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<tbody>
<tr>
<td>Fisheries and Oceans Canada</td>
<td>Atlantic, Pacific, and Arctic coastal regions and Great Lakes boundary waters</td>
<td>The Fisheries Act</td>
</tr>
<tr>
<td>Health Canada though the</td>
<td>National import/export regulations, quarantine/inspection, animal/plant health, food security</td>
<td>Canadian Food Inspection Agency Act, Health of Animals Act, and Plant Protection Act</td>
</tr>
<tr>
<td>Canadian Food Inspection Agency</td>
<td>National biodiversity obligations</td>
<td>Canadian Environmental Protection Act: Canadian Endangered Species Protection Act, The Parks Act, Canada Border Services Agency Act, plus all other laws the Agency is authorized to enforce</td>
</tr>
<tr>
<td>Transport Canada</td>
<td>Trans-oceanic and other shipping and land transportation of goods into Canada</td>
<td>Canada Shipping Act</td>
</tr>
<tr>
<td>Environment and Climate Change</td>
<td>National biodiversity obligations</td>
<td>Department of Agriculture and Agri-Food Canada Act and collaboration with other Departments’ acts</td>
</tr>
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<td>Parks Canada</td>
<td>National Parks</td>
<td></td>
</tr>
<tr>
<td>Canada Border Services Agency</td>
<td>All entry points into Canada</td>
<td></td>
</tr>
<tr>
<td>Agriculture and Agri-Food Canada</td>
<td>National agricultural policy, reference libraries for species of agricultural importance</td>
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analytical facilities operating across the country, especially at major ports, airports, and land crossing points. Identification of a species relies upon a reference database in which the specimen’s DNA barcode profile is contained and linked to a taxonomically authenticated collection voucher specimen. That database requires continuous monitoring and updating to contain specimens of national and international origin (Ekrem et al. 2007), representing species of regulatory importance, as well as those with which they may be diagnostically confounded. This also requires the allocation of targeted funding. Similarly, the electronic database requires hosting, so that it can be accessed immediately. Such established facilities could be used cooperatively by a number of different federal departments, and by agreement, with provincial agencies. Newly arrived IAS are normally found in or at a given province, and not across the entire country, so provincial buy-in to federal capacity building is essential. In the absence of a national Canadian IAS act, the harmonization of the analytical protocols among different federal departments is critical, as is the harmonization of the same process between federal and provincial levels of government, should any province decide to use the same technology to identify IAS. Notably, a substantial network of public and private laboratories already exists, as does the capacity to certify them (ISO 17025 accreditation). However, there are no published protocols from any regulatory laboratories in Canada, as none is currently required under law.

The auditing of any analytical facility’s practice standards is important if its results are to be used in subsequent risk assessment and RRM, and, possibly, quarantine, eradication, and litigation. This is easier to achieve if there is a single legal protocol written into Canadian federal law, applicable throughout the country, e.g., similar to the ANSI standard for culture collections (American National Standards Institute 2015). There must also be provision for molecular identification technology to change as new analytical advances are made. Then updating of the methodology in all government facilities can be assured if the enabling legislation is in place.

Federal and provincial agencies that could use DNA barcoding and legislation

In the absence of a single, Canadian, invasive species control act, responsibility for dealing with IAS falls to several departments. Those federal agencies, their jurisdiction, and potential involvement with IAS are shown in Table 1. Not all departments are likely to be involved equally with IAS management. Fisheries and Oceans Canada, because of its mandate to manage coastal and boundary waters, plays a major role when dealing with aquatic IAS. Health Canada, through the Canadian Food Inspection Agency, plays a major role on dealing with IAS of plants and animals dispersing via land and impacting agriculture, horticulture, and food production issues. Transport Canada has responsibility for IAS that might arrive via shipping ballast water, or via land transportation. Canada’s national and international obligations to the UN Biodiversity Treaty (Convention on Biological Diversity (CBD)) are dealt with by Environment Canada. Responsibility to manage IAS that could affect the ecological integrity of protected national parks’ ecosystems comes under Parks Canada, especially in view of there being several terrestrial and marine national parks that lie on the Canada–US border. The Canada Border Services Agency may encounter diverse IAS at any of the entry points into Canada. Health Canada is involved with IAS because of the introduction and spread of disease vectors, parasites, and other disease agents into the country, either as a consequence of range expansion or the inadvertent introduction via human travel.

The Canadian invasive species strategy (Environment Canada 2004) identified early detection and rapid response as desired components of an IAS management
process, although there is no clear indication of how this will be achieved, or by which agency(ies). However, the strategy has identified federal regulative reform as the basis of future management capacity.

We do not intend to indicate how the existing different legislation and regulations used by each federal department and the province of Ontario could be amended to incorporate provisions for the use of DNA-based approaches for the rapid identification of newly detected IAS. However, the Fisheries Act will be examined as an example of how such provision could be accomplished, and used as a potential template for other federal legislation and regulation. The Fisheries Act is also chosen because it integrates concerns about IAS across the entire nation, and also because it relates to IAS matters impacting the Canada–US boundary waters of the Laurentian Great Lakes (Vásárhelyi and Thomas 2003; Thomas et al. 2009). This addresses the need to have “unifying policy” across the different jurisdictions of North America involved directly with aquatic IAS management (Perrault and Muffet 2002; Simberloff et al. 2005). This point of “unifying policy” and regulation across jurisdictions is also emphasized in the 2014 Fisheries and Oceans Canada Impact Analysis Statement (Government of Canada 2014).

Creating provision to use DNA barcoding in the Fisheries Act

The Regulations of this Act were amended in 2014 to authorize the use of deleterious chemicals to eradicate aquatic IAS in Canadian waters in the context of RRM (Government of Canada 2014). While this is a progressive amendment of the Regulations, the Regulations still do not stipulate how species identification would be achieved. The following proposed amendment of the Regulations could resolve this issue:

“Identification of a potential invasive species will be accomplished using standardized, appropriate, DNA-based identification, in combination with traditional morphological taxonomic methods, using a validated reference. This identification technology will be used as the basis of subsequent risk assessment and any rapid-response management plans. Use of this DNA-based identification may be applied as environmental DNA analysis as part of ongoing environmental monitoring of invasive species presence in Canadian waters”.

This wording is meant to permit the use of DNA-based identification methods as deemed appropriate in specific cases (e.g., mitochondrial introgression) and would allow the latest developments in DNA-based identification to be used without the need for revision of the regulation.

Such amendment of the Fisheries Act would be consistent with Canada’s obligations under the 1910 Boundary Waters Treaty Act. The Advisory Work Group Report (2009) to the International Joint Commission—bilateral advisory body to the governments of Canada and the USA on boundary water quality issues—indicated the need for RRM to deal with aquatic IAS as part of the Great Lakes Quality Agreement, especially in light of potential invasions from aquaculture escapees (Thomas et al. 2009). This report did not indicate how rapid identification might be achieved as a precursor to RRM. Including provisions to use DNA-based identification in an amended Agreement, although not legally binding, could be a strong precedent to herald similar amendment of the Fisheries Act. Another advantage of such an amendment of the Great Lakes Water Quality Agreement is that it could promote complementary change in US federal regulations used to deal with aquatic IAS, such as the Non-indigenous Aquatic Nuisance Prevention and Control Act, and the National Invasive Species Act. An essential component of unifying policy between Canada and the USA is that a common approach be used for identification involving both the protocol and forensic reference library. By having this provision identified in law, there is a bilateral obligation to endorse, support, use, and share this protocol, especially in its use for risk assessment and RRM involving both nations’ marine and inland waters. The Canadian Biodiversity Information Facility (CBIF) has provisions for harmonizing the application of standard taxonomic names across Canada, the USA, and Mexico through the Integrated Taxonomic Information System (ITIS; http://www.itis.gov/).

Discussion

The number of terrestrial and aquatic IAS that have entered Canada during the past 30 and more years (e.g., fig. 3 in Ricciardi 2001), and exerted their heavy impact on the national economy and biodiversity, attests to the need for the development of progressive IAS control regulation, as suggested in the 2004 Canadian IAS strategy (Environment Canada 2004). The 2014 amendment of the federal Fisheries Act and the 2015 passing of Bill 37, An Act respecting Invasive Species, by the Government of Ontario represent a good start. However, the amendments to the Fisheries Act do not apply to IAS management by other federal departments and their agencies. Both pieces of regulation are still limited by the absence of defined methods for the rapid and reliable identification of IAS, which is the essential precursor to appropriate risk assessment and RRM (Holland 2000). DNA-based approaches can, already, provide this critical capacity. For example, DNA barcoding can identify specimens to the species level across a large range of aquatic invertebrate and vertebrate IAS, which are among the species of most current concern to Canada (Government of Canada 2014).

The suggested wording to create provisions for DNA identification technology in amended regulations provided by the present paper could be incorporated into the regulations of the laws used by the different federal agencies listed in Table 1. It is consistent with the intent of the National Invasive Species Strategy for Canada.
The latter is particularly important when the expertise in a given geographical area are not familiar with the exotic species. Some invertebrates exhibit phenotypic plasticity which confounds the ease of traditional identification, but this problem can be obviated by DNA-based techniques (McGlasham et al. 2008). The delays and uncertainty in the identification of IAS are serious points to consider when inspections and quarantine hold up international shipping and transportation of goods. The potential litigation and associated costs of inspection, identification, and risk assessment attest to the importance of government-audited facilities, whose operational technology is supported under law.

The science of DNA-based identification is far ahead of regulations and enforcement, a gap we seek to bridge. At least one precedent for recognizing the use of DNA-based identification of species in law already exists. It is used as a standard method for fish species identification by the US Food and Drug Administration (Handy et al. 2011). While our paper has dealt with issues from a largely Canadian perspective, related international considerations apply to the USA and beyond. Under the Boundary Waters Treaty, and the subsequent Great Lakes Water Quality Agreement, Canada has a responsibility to develop a capacity to manage IAS in their contiguous waters (Vásárhelyi and Thomas 2003). These two nations have shown the ability to develop harmonized, complementary, regulations for dealing with marine IAS, when mandatory mid-ocean ballast water exchange regulations were developed (under the Canada Shipping Act and the US Nonindigenous Aquatic Nuisance Prevention and Control Act) for international shipping regardless of the Canadian or American destination port (https://www.tc.gc.ca/eng/marinesafety/oep-environment-ballastwater-menu-449.htm). Harmonized legal provisions for identification of specimens using DNA-based approaches would represent important progress, especially in view of the recent amendments of the Canadian Fisheries Act. Moreover, environmental DNA analysis (Ficetola et al. 2008) could become an important monitoring tool for detecting IAS recognized in both nations’ policy.

Recognition of the use of DNA-based identification in Canadian law would also represent a major commitment to Canada’s obligations under the CBD and the Aichi Biodiversity Target, No. 19. Furthermore this would create a model for other nations to incorporate a similar provision of their own, or developing IAS legislation. Paragraph 5(g) of Recommendation XIX/2 adopted by the 19th Subsidiary Body on Scientific, Technical and Institutional Advice to the UN Convention on Biological Diversity (UNEP 2015) encourages Parties to the CBD “To support the development, with the assistance, as appropriate, of the international barcode of life network, of DNA sequence-based technology (DNA barcoding) and associated DNA barcode reference libraries for priority taxonomic groups of organisms...”, thereby setting the international legal framework for incorporating DNA-based identification approaches into Canada’s national laws. Notably, the US Food and Drug Administration has
developed a protocol for building a reference standard sequence library for seafood identification (Deeds et al. 2014) that could be evaluated and potentially adopted by other jurisdictions.

Conclusions
The development and implementation of invasive alien species management strategies in Canada requires fast and reliable DNA-based identification tools, such as DNA barcoding. In order for these tools to be broadly deployed and integrated into a nation-wide biosurveillance effort, a conceptually novel operational framework should be deployed and supported by several important changes in legislation, policy, and governance. The starting point to such adoption would be an open dialogue with the civil service at both federal and provincial levels, and presentations to the appropriate parliamentary Standing Committees at both political levels. This approach would address both the science-policy and political aspects of invasive species management in Canada, while also giving it an international dimension.

1. DNA-based identification should be incorporated into Canadian federal law, as an accepted standard for validating taxonomic identity of invasive organisms and the basis of rapid response management.
2. It should be broadly adopted by key relevant federal agencies, such as DFO, CFIA, Transport Canada, Environment and Climate Change Canada, and Parks Canada, as part of their operating policies for monitoring and control of the spread of invasive species.
3. This move should garner support from provinces, territories, and other jurisdictions in adopting similar legislations and facilitating data sharing/exchange.
4. The deployment of a network of nationally/provincially accredited analytical laboratories should be mandated under the above legal provisions to facilitate rapid identifications on behalf of all federal and provincial government agencies, and a coordinated response to new invasions or range extensions.
5. The creation of a shared federally accredited and universally accessible DNA barcode reference dataset based on existing data platforms (such as BOLD or CANADENSYS) should be authorized under the respective articles of Canadian law for the above agencies.
6. Requirements/incentives need to be in place for the submission of raw digital DNA-based information from environmental surveillance activities (e.g., impact assessments) into a centralized data portal, to facilitate monitoring and detection of important invasive species.
7. DNA barcoding should become part of the agenda in trade negotiations between Canada and the United States, as well as other key trading partners (e.g., those with signed FTA’s), in an effort to harmonize regulatory frameworks at an international level.

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