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June 16, 2012

TO: Members of the National Invasive Species Council (NISC)

SUBJECT: Recommendations to NISC from the ISAC Meeting held May 22-24, 2012

During the May 22-24, 2012 meeting held in Portland, Oregon, the Invasive Species Advisory Committee (ISAC) approved two white papers, *Invasive Species and E-Commerce*, and *Validation of PCR-Based Assays and Laboratory Accreditation for Environmental Detection of Aquatic Invasive Species*. The papers include the following agreed upon recommendations:

Invasive Species and E-commerce

We conclude that relevant federal agencies need to adjust existing regulations and enforcement practices to better mitigate the risks of trade and transport of invasive species through e-commerce. We offer the following recommendations to enhance our collective ability to engage in e-commerce without promoting the introduction or spread of invasive and potentially invasive species.

1. ***Department of Interior, U.S. Fish and Wildlife Service (DOI/FWS) and U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA/APHIS):*** Expedite listing processes for the national importation of injurious wildlife and other animals and noxious plants under the Lacey Act, the Plant Protection Act and the Animal Health Protection Act to better assess and address emerging invasive species threats, including those associated with e-commerce.

2. ***DOI/FWS****:* Incorporate all species-specific data submitted with Form 3-177 declarations for wildlife imports into the Law Enforcement Management Information System (LEMIS) or another accessible database.

3. ***Department of Homeland Security (DHS):*** Expand cooperation with the U.S. Postal Service to monitor and increase the capability to interdict international mail containing potentially invasive species and encourage the U.S. Postal Service to expedite requirements for advance electronic manifests associated with packages sent through international mail similar to current practice for international express mail and consignments.

4. ***USDA/APHIS:*** Expand the scope of webcrawlers and related enforcement and monitoring activities used by the Smuggling Interdiction and Trade Compliance unit to include a broader array of invasive plants and plant pests, and enhance cooperation with U.S. Fish and Wildlife Service (DOI) to address injurious wildlife.

5. ***USDA Agricultural Research Service:*** Support development of and capacity for an Internet clearinghouse of federal and state-listed invasive species such as injurious wildlife, other animals and noxious weeds and of relevant regulations. Such a resource could be located at the National Agricultural Library's Invasive Species Information Center or another appropriate website and should include relevant agency contact information and a general reporting form that allows the public to report suspected violations.

6. ***DOI/FWS, USDA/APHIS, Department of Commerce, National Oceanic and Atmospheric Administration (DOC/NOAA) and other relevant agencies:*** Provide a reference catalog or database of taxonomic resources that commercial interests can use to verify the taxonomic identity of organisms in trade.

7. ***Department of State and Office of the US Trade Representative:*** Given that a significant portion of e-commerce entities is based outside the U.S., explore further cooperative and legal measures with foreign trading partners and relevant international institutions and other bodies to address the illegal import of invasive species into the U.S.

8. ***DOI/FWS, USDA/APHIS and DOC/NOAA:*** Promote outreach to individuals and businesses involved in the sale and exchange of species over the Internet to reduce intentional and unintentional sales or purchases of species listed as invasive in the U.S. or particular states.

Validation of PCR-Based Assays and Laboratory Accreditation for Environmental Detection of Aquatic Invasive Species (AIS)

To encourage the development of a validation/accreditation system for AIS environmental DNA (eDNA) detection methodologies and laboratories ISAC recommends that the NISC member Departments and Agencies and their partners consider adoption of the following recommendations:

9. Encourage and develop funding for the National Academy of Sciences to undertake a review of the reliability and effectiveness of Polymerase Chain Reaction (PCR) and other DNA-based applications for detecting AIS, focusing on establishment of appropriate validation processes and a framework and standards for this new and potentially invaluable tool in the early detection, eradication, prevention and control of AIS.

10. Establish and fund an ongoing independent performance testing program for laboratories utilizing DNA-based AIS detection methodologies such as that recently undertaken for evaluating laboratory performance in PCR detection of dreissenid mussel larvae (Frischer et al. 2011). Testing results should be made public so that managers may make informed decisions about the accuracy and reliability of a laboratory's performance when including an eDNA component in an AIS monitoring and early detection system.

11. Utilize lessons learned in establishing a laboratory performance testing system to fully develop a validation/accreditation program(s) for other invasive species eDNA methodologies and laboratories.

Potential Introduction of Invasive Weeds through Highway Construction

In addition to approving the E-commerce and PCR white papers, ISAC addressed the issue of soil and gravel movement during highway projects as a potential pathway for the spread of invasive weeds, and made the following recommendation:

Background: Fifty (50) State Departments of Transportation (DOTs) manage 17 million acres of roadsides or rights-of-way (ROWs) across the nation. The Federal Highway Administration (FHWA) provides funding for new construction/upgrade projects on interstate, state, county and municipality highways. These are called Federal-Aid Highways and they are maintained by state and local agencies after completion.

New projects, considered "road improvements" disturb soils. The movement of soils and gravel during highway work, and/or disturbance of competitive vegetation, increase the opportunity for invasive plant/noxious weed seed introduction and release. Best management practices to prevent or reduce invasions are necessary. On completion of construction, all maintenance responsibility transfers to the state and local agencies. The cost of prevention, as well as the cost of vegetation management after construction of these disturbed sites continues to stress local and state budgets at the same time they diminish.

12. ISAC recommends the FHWA/USDOT require the inventory and control of invasive and noxious terrestrial and aquatic vegetation at all stages of construction/upgrade projects, beginning in FY14. Inventory and control should include but not be limited to the following preventative actions:

    - Inventory and control of existing corridor vegetation before project begins
    - Specify weed-free gravel, soils and mulches on each project
    - Clean construction/maintenance equipment before, during and after project
    - Construction funding be accountable for the costs associated with invasive and noxious vegetation monitoring/control following the project until a stable, sustainable plant community is established, not to exceed 5 years following the project completion

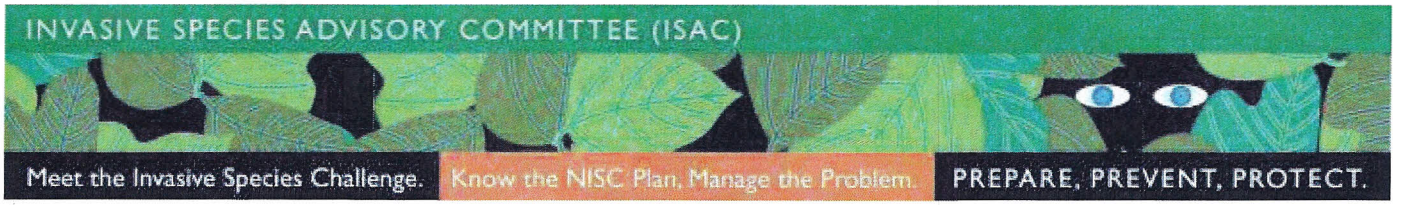
ISAC recommends that NISC adopt both white papers, the full versions of which are attached.

Sincerely,

E. Ann Gibbs
Chair, Invasive Species Advisory Committee
Maine Department of Agriculture

Attachments:

1. ISAC White Paper: *Invasive Species and E-Commerce,* dated May 24, 2012
2. ISAC White Paper: *Validation of PCR Based Assays and Laboratory Accreditation for Environmental Detection of Aquatic Invasive Species*, dated May 24, 2012
3. NISC Distribution List



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Invasive Species and E-commerce

Approved by ISAC on May 24, 2012

Issue

Internet commerce (hereafter e-commerce[1]) is a growing and vital part of the U.S. economy. Total e-commerce sales in the U.S. for 2011 totaled $194 billion, an increase of 16% over 2010. From 2002 to 2011, the proportion of reported e-commerce sales in the U.S. grew from about 1.4% to 5.5% of total retail sales (U.S. Census Bureau News 2012). Globally, e-commerce is expected to increase at a rate of 13.5% annually, amounting to $1.4 trillion in yearly sales by 2015 (Enright 2011). A portion of this activity includes the sale and trade of living organisms. Unfortunately, such organisms and other organisms that they may carry can be invasive species, defined by U.S. Executive Order 131121 as "alien [non-native] species whose introduction does or is likely to cause economic or environmental harm or harm to human health." Order 131121 mandates that Federal agencies work to ensure that they do not promote e-commerce in invasive species, because the order states that these agencies should..."not authorize, fund, or carry out actions that are likely to cause or promote the introduction or spread of invasive species...."

A number of government entities have jurisdiction over aspects of e-commerce including particular types of organisms (U.S. Department of Agriculture, Animal and Plant Health Inspection Service [USDA, APHIS] – plants, livestock and their products; Department of Interior [DOI], U.S. Fish and Wildlife Service [USFWS] – wildlife); shipping services (Department of Homeland Security [DHS], U.S. Postal Service [USPS]); imports and interstate trade (Federal government) and intrastate trade (state governments). However, e-commerce as a sector is evolving and expanding in volume at a rate that may exceed these various capacities to address the associated risks of introduction and spread of invasive species.

Action

This briefing paper, adopted by the U.S. Invasive Species Advisory Committee (ISAC), provides:

- Background information on the linkages between invasive species and e-commerce, and
- Recommendations to strengthen action by the Federal government to address the invasive species risks posed by e-commerce.

[1] E-commerce refers to "the buying and selling of products or services over electronic systems such as the Internet and other computer networks... [and] also includes the entire online process of developing, marketing, selling, delivering, servicing and paying for products and services." While typically associated with the worldwide web, e-commerce can also incorporate technologies such as e-mail, mobile devices, and telephones. http://en.wikipedia.org/wiki/Electronic_commerce (downloaded December 30, 2011).

Background

Scientific analyses and informal reviews of commercial websites and specific niche markets in the U.S. reveal a wide range of invasive species for sale, including many species regulated by state and federal laws. Identifying and managing the risks associated with e-commerce is particularly challenging because the Internet simply serves as a mechanism for processing commercial and non-commercial transactions between groups and individuals. Unlike other vectors of introduction of invasive species, e-commerce is not a physical means of moving organisms.

An analysis of the full role of the Internet in the spread of invasive species needs to consider the ranges and amounts of:

- Sectors and species traded: for example, pet and aquarium species, horticultural and agricultural species (plants, cuttings, seeds, soils), live food and bait, scientific and educational supplies, firewood and other biofuel stocks, and herbal or medicinal products;[2]
- Internet tools for the sale or trade of organisms or products that may be pathways for other organisms: commercial websites; auction sites such as eBay; classified ad websites such as Craigslist; online forums such as those hosted by Google Groups, Yahoo Groups, Facebook, Google+, and specialist groups; and other online social networking and communication tools;
- Actors in supply chains: importers, domestic breeders, resale entities, box stores and large-scale retailers, small businesses, brick and mortar stores, e-tailers, interest groups such as 4H Clubs, collectors and specialist groups interested in particular species, and the general public; and
- Shipping agents and routes: shippers can include public entities such as the U.S. Postal Service and private companies such as FedEx and DHL; routes of regulatory significance include imports into the U.S. and interstate and intrastate trade.

The scale and diversity of e-commerce present regulatory difficulties. Individuals and companies that sell through e-commerce may not be legally registered businesses and frequently do not disclose their specific location of operation. They frequently fail to acquire the appropriate licenses and permits, or to use appropriate labeling for packages. Sellers that are out of state or out of the country may undermine local efforts with cooperative retailers to limit the sale of invasive species. Sellers can use the relatively high level of anonymity associated with the Internet to skirt accountability and avoid identification, regulation, and prosecution. Shipping agents may not necessarily know they are transporting potentially harmful organisms, or that they are transporting live organisms at all.

A range of scholarly work has addressed various aspects and sectors of e-commerce and other forms of trade in invasive or potentially invasive species. For instance, the Global Invasive Species Program (GISP, no date) provides a broad overview of the issues, Peters *et al.* (2006) examine the horticultural trade in Minnesota, Kay and Hoyle (2001) cover aquatic weeds sold through the Internet and mail, and *Stam et al.* (2006) and Walters *et al.* (2006) focus on the sale of species of *Caulerpa* in Florida. A number of reports have tried to quantify the volume of species being imported into the U.S., including Romagosa (2011) and Defenders of Wildlife (2007) for wildlife, and Smith *et al.* (2008) for fish. Recent studies from other countries include a broad overview of the issues in New Zealand (Derraik and Phillips 2010), an examination of the Internet pet trade in the U.K. (Parrott and Roy 2009), an analysis of the role of e-commerce in the spread of introduced freshwater aquarium fish in Brazil (Barroso de Magalhães and Jacobi 2010), and reports by the International Fund for Animal Welfare (IFAW 2005, 2008) on trade in endangered species and their parts through the Internet.

[2] This list will likely keep growing with the use of new species, end-uses, and pathways.

Live animal imports into the U.S.

From 1999-2009, over 2.8 billion live animals were legally imported into the U.S., the vast majority of which (about 88%) were ornamental fish (Romagosa 2011). Despite the fact that scientific and common names are required and submitted on Form 3-177 or attachments to the form (see Appendix 1), one study found that the USFWS Law Enforcement Management Information System (LEMIS) recorded the full taxonomic data for only 3.8% of all shipments (i.e., those species listed under the Convention on International Trade in Endangered Species) (Smith *et al.* 2008). Most species also entered without extensive scrutiny of their potential to harm the environment, agriculture, or human health in the U.S. Surveys of aquatic species sold in the Great Lakes region through the Internet and other sources found a significant percentage of known invasive species available for sale, misidentification of species, and high levels of live invertebrates hitchhiking on plants (Keller and Lodge 2007).

A significant portion of this volume in traded organisms can be associated with e-commerce. Experts estimate that there are at least 4,000 businesses and 15,000 individuals advertising reptiles over the Internet. Numbers of e-commercial traders of horticultural species are difficult to estimate, but conservative guesses place them in the tens of thousands (ISAC 2011).

We focus on those invasive and potentially invasive species that are formally regulated by Federal or state governments and thus restricted from trade and transport, such as those listed as noxious weeds or injurious wildlife. Regulation of these species can include prohibiting or otherwise restricting import into the U.S., forbidding movement between states, and prohibiting intrastate trade and other actions controlled by states. In some cases, a species can be regulated, not because it is itself invasive, but because it can carry pests, pathogens, or parasites directly, in packaging, or during conveyance. We divide our analysis into four areas:

1. Issues specific to e-commerce such as composition of the e-retail industry, Internet-related regulations, and on-line vendor and consumer awareness;
2. Relevant issues more broadly associated with commerce such as international and interstate regulations on trade, postal and courier services, species identification, and hitchhikers;
3. Control mechanisms such as web surveillance, outreach and education; and
4. Recommendations to NISC member agencies.

Issues specific to e-commerce

The Internet has unquestionably revolutionized how individuals and businesses communicate and make transactions, removing former geographical barriers and obstacles to communication. With regard to the movement and trade of invasive species, three areas are particularly notable: 1) increased diversity of commerce, 2) decreased ability of governmental authorities to implement and enforce regulations, and 3) the increasing role of the Internet in public awareness and education.

Increased diversity: The Internet has vastly expanded the range of people and businesses engaged in the movement and sale of plants and animals. For example, while a combination of large and small "brick and mortar" stores once held sway in the pet and aquarium trade, individual hobbyists, collectors, breeders, wholesalers and others can now easily engage in the sale of species. Some set up Internet-based businesses that cut out middlemen, maintain a low-cost infrastructure, access a broad range of potential buyers, process sales over the Internet, and use postal or express delivery services to send purchased merchandise. Traditional retailers have likewise diversified by adding Internet and mail sales to their businesses. This model has expanded the geographic reach of the market, facilitating transactions across the country and around the world.

The array of mechanisms for making transactions is also highly diverse, including standard retail websites, auction sites, local business and want ads, portals that facilitate communication between buyers and sellers, and specialty chat forums and user groups. Social media such as Facebook, Twitter, and Foursquare are further changing the landscape, particularly through informal retail arrangements. A shift to person-to-person transactions will likely continue, raising significant questions about whether and how e-commerce can be regulated.

Kudzu (*Pueraria montana* var. *lobata*)

Pueraria montana (Lour.) Merr. var. *lobata* (Willd.) Maesen & S. Almeida, introduced to North America from Asia and commonly known as kudzu, is a high-profile, invasive vine. In the southeastern U.S., kudzu is very widespread and forms famously dense blankets over whole trees. Despite this, multiple listings (e.g., "20 Seeds Pueraria lobata Kudzu Seeds") on eBay offer seeds for sale (eBay 2012), and search engines readily find on-line businesses selling seed, such as B & T World Seeds (B & T World Seeds 2012).

Suppliers are motivated in part by Internet sites that host questions such as:

> "Where can I buy Kudzu plants/seeds? Hi Everyone, I would like to buy Kudzu plants/seeds, but everywhere I look, I only see Kudzu destroying products for sale. No plants. I am quite aware of the rapid growth and the capabilities of this invasive species, so please don't tell me why not to buy it. I need it for observation purposes in a closed environment. Thanks, Batman" (Yahoo, no date)

Specialty cultivars of kudzu can also be found on-line:

> "*Pueraria lobata* 'Sherman's Revenge' (Sherman's Revenge Variegated Kudzu) For the gardener who has everything or as the perfect gag gift for a garden party, yes, it's variegated kudzu. Originally discovered in Japan, it was named by plantsman Barry Yinger, who's never met a variegated plant he didn't like. This fast-growing deciduous vine...rumors of several feet per hour when established are probably true...is covered with lovely trifoliate light green leaves edged in white. The vines are adorned with small clusters of Nu-grape soda smelling flowers in late summer. If you're going to have a weed, it might as well be variegated. Not recommended for states where it has been banned, and because there are so many, we won't ship out of state." (Plant Delights Nursery 2012)

Regulations and enforcement: The Internet has facilitated an increase in sales of organisms by individuals, not just by lowering overhead and transaction costs, but also by helping sellers circumvent state and federal regulatory requirements. For example, brick and mortar stores are frequently required by states or countries to apply and pay for licenses that allow them to move and sell species, and for permits to breed or import species into the country. Individuals sellers are often unaware of these requirements or may deliberately circumvent them by being located in another jurisdiction. Such sellers are often hard to trace; it may be difficult to hold them accountable; and efforts by enforcement agencies to pursue them may be time-consuming and expensive. The Internet has also made it easier to exchange information on how to avoid regulations, such as by falsifying documents or using transshipments, transfers between more than one shipper.

Those who want to be responsible may find it hard to find out what the relevant regulations are. There is no one comprehensive listing or guide to federal and state regulations on the transport and sale of plants, animals, and materials that could be a pathway for the transport of invasive species. Many states lack a standard means for communicating with non-registered businesses that work over the Internet. Policy-makers are still debating whether only in-state sellers should be licensed, how to design the process for licensing, and how to enforce regulations, all difficult issues. Many would argue that current policies have not kept up with the age of the Internet, resulting in an increasingly unregulated sector of trade in invasive and potentially invasive species. The current situation thus favors largely unregulated, virtual sellers, puts conventional stores at a significant competitive disadvantage, and increases the risks of the introduction and spread of invasive species.

Illegal Importation of Freshwater Ornamental Fish

Based on a search of websites, including news articles published on Practical Fishkeeping, there appear to be a number of ways to illegally import prohibited, freshwater, ornamental fish.

One avenue of illegal importation seems to center on websites such as Aquabid.com, where buyers bid on fish offered by a wide variety of sellers worldwide. As on eBay, fish are sold and shipped to the highest bidder. Some sellers on this website offered fish that were illegal to import into the U.K. and indicated that they would send them to buyers in the U.K. via ground postal service without the import license, health documentation, or notification of the Fish Health Inspectorate required by U.K. law. In some cases, fish were sent without documentation via EMS Express Mail, a service for documents and merchandise run by postal operators of the Universal Postal Union. Another apparent route for illegal importation of ornamental fish was for the seller to ship fish to a trans-shipper in a nearby country where the fish were not banned; buyers in the country where the fish were illegal then arranged with the trans-shipper to have the fish delivered to them.

A third means of illegal importation was to falsely declare the contents of a shipment on a custom declaration in the hope of getting the shipment past customs and wildlife inspectors. This may also involve shipping ornamental fish that are prohibited in one country first to a nearby country that does not prohibit them. They are then smuggled into the prohibiting country via ground transport to avoid the more rigorous inspection of international air shipments of live animals.

It is likely that these routes are being used to illegally import prohibited species into the U.S.

Education and public awareness: Perhaps the greatest commercial virtue of the Internet is that it allows individuals to readily find information, albeit sometimes unreliable, about products and sales. Individuals can locate sellers, details of the features and care of species, and information about how to circumvent rules or smuggle banned species. The Internet can also be a powerful tool for educating consumers. A number of targeted efforts in stores and at trade shows, such as Habitattitude™ and Be PlantWise, have helped educate those involved in conventional, face-to-face transactions. Such efforts are increasing their presence on the web, and there is a need to develop more effective methods to harness the power of the Internet to inform those involved in on-line transactions.

Issues more broadly related to commerce

While the Internet is facilitating a surge in the sale and trade of organisms, it cannot actually serve as a means for the physical movement of species. E-commerce is thus related to a number of other broader areas, including international and interstate commerce, postal and delivery services, taxonomy and species identification, and hitchhikers.

International and interstate commerce: Official federal and state lists of invasive species apply equally to electronic and non-electronic commerce. At the federal level, provisions of the Lacey Act on injurious wildlife allow USFWS to regulate the importation and interstate transport of animal species including wild mammals, wild birds, amphibians, reptiles, fishes, crustaceans, and mollusks that may prove harmful to humans, agriculture, horticulture, forestry, wildlife, or resources for wildlife in the U.S. Importers of wildlife are required to submit USFWS Form 3-177 (Appendix 1: Live Wildlife Import Declarations – Form 3-177). Similarly, the Plant Protection Act of 2000 (PPA) provides for the listing of noxious weeds, broadly defined as any plant or plant product harmful to crops, livestock, poultry, conditions for agriculture, irrigation, navigation, natural resources, public health, or the environment. Under this act, APHIS also regulates the importation and interstate movement of plant pests such as insects and pathogens and the commodities that may carry them. These regulations are named for the sections of the Code of Federal Regulations (CFR) in which they appear. For example, "Q37" applies to plants for planting and "Q56" applies to fruits and vegetables. Both the PPA and the Lacey Act work in tandem with the commerce clause of the U.S. Constitution to allow the federal government to regulate trade of potentially harmful species into the U.S. and across state borders.

State governments can similarly regulate the transport, sale, and possession of invasive species within states and many have developed legislation and regulations similar to the Lacey Act and Plant Protection Act that list prohibited species.

Such regulations are also becoming more frequent at the levels of the county and municipality, creating an increasingly complex regulatory system. As noted above, there is no single, regularly updated resource that includes all of this regulatory information and requirements to assist sellers trying to abide by regulations.

Governmental roles and responsibilities in e-commerce of invasive species		
Agency	**Authority**	**Coverage**
USDA/APHIS	Plant Protection Act of 2000: 7 CFR 360 Noxious Weed Regulations	Federally listed noxious weeds
USDA/APHIS	Plant Protection Act of 2000: 7 CFR 319.37 (Q37) Plants for Planting 7 CFR 319.56 (Q56) Fruit and Vegetables	Imported plants, fruits and vegetables that may be invasive or serve as hosts for other invasive pests
USDA/APHIS	Animal Health Protection Act of 2002: 9 CFR 122 Organisms and Vectors 9 CFR 121 Select Agents and Toxins	Animals and animal products that may carry livestock diseases , and permits for moving various pathogens and their vectors
DOI/FWS	Lacey Act: 18 USC 42-43 Importation of Injurious Species (50 CFR 16), 16 USC 3371-3378 Prohibited Acts related to Unlawful Taking of Fish or Wildlife (50 CFR 10-15)	Federally listed injurious wildlife and prohibitions against the import, export, transport, sale, purchase, receipt, or acquisition of fish or wildlife in violation of U.S., state, or foreign law
DHS/CBP	Homeland Security Act of 2002: 6 USC 201-239 Directorate of Border and Transportation Security (7 CFR 319 & 330; 9CFR 94-96)	Border quarantine and inspection stations
DHS/CBP	Homeland Security Act of 2002: 6 USC 201-239 Directorate of Border and Transportation Security (7 CFR 319 & 330; 9 CFR 94-96)	Express delivery services
USPS	Postal Reorganization Act of 1970: 39 CFR 20 International Mail and 39 CFR 111, 121, and 122 Domestic Mail	Postal services
Federal government	U.S. Constitution	Imports and interstate trade
State governments	U.S. Constitution	Intrastate trade

Postal and express delivery services: Since the Internet is often used for transactions across significant distances, purchased specimens are generally sent by mail or express delivery services such as those of USPS, DHL, FedEx, and UPS. Such services have their own set of regulations concerning the shipment of species. All packages sent from abroad require a manifest that lists their contents and may be subject to non-intrusive inspection, such as with dogs or X-rays. Manifests for express delivery consignments must be submitted electronically, which allows for advanced targeting through a range of risk screening measures. DHS Customs and Border Protection (CBP) thus has some idea of what to expect prior to delivery of a package sent by express consignment. In contrast, packages send by international mail are currently exempt from the requirement for electronic manifests,

Appendix 1: Live Wildlife Import Declarations – Form 3-177[3]

As a general rule, all live wildlife[4] imported into the United States[5] for any purpose must be imported through a "designated port."[6] Under certain limited circumstances, arrangements may be made to use a port other than a designated port. In any event, such shipments must be declared on import and inspected by FWS or a designated alternative, such as CBP.

With limited exceptions, all live wildlife imports must be declared on a FWS Declaration Form, Form 3-177, as a pre-condition to inspection and clearance before any imported live animals may be released to an importer. Form 3-177 calls for submission of detailed information on the contents of the shipment. The importer must provide, among other information:

- Purpose code (i.e., personal, zoo, commercial, educational, circus, pet)
- Scientific and common names of each species in the shipment
- Quantity of specimens by species in the shipment
- Country of origin
- Transportation code (i.e., mail, air cargo, personal accompanying baggage)
- CITES Permit number, if applicable
- Wildlife source code (i.e., wild, captive bred, ranched)
- Total value in U.S. dollars
- Indication if venomous
- FWS License number if applicable

There are limited exceptions when such declarations are not required. For example, imports of live shellfish and fishery products imported for human or animal consumption, or fish taken for recreational purposes in Canada or México do not require the filing of Form 3-177. However, exceptions are not available if a permit is required under Part 16 (Injurious Wildlife), Part 17 (Endangered species), Part 18 (Marine mammals), Part 21 (Migratory birds), Part 22 (Eagle permits) or Part 23 (CITES). If the shipment is considered a "commercial" shipment[7] of live animals, the importer or exporter may have to obtain an import or export license under 50 CFR Part 14.91.

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[3] The regulations contain a variety of exceptions or conditions that apply to wildlife products (dead, preserved, dried, etc.), museum specimens, personal baggage, and household effects, etc.

[4] "Wildlife" includes "any wild animal, whether alive or dead, including without limitation any wild mammal, bird, reptile, amphibian, fish, mollusk, crustacean, arthropod, coelenterate, or other invertebrate, whether or not bred, hatched, or born in captivity, and including any part, product, egg, or offspring thereof." (50 CFR Section 10.12) Domesticated animals (50 CFR Part 14.4) are exempt unless specimens are from a wild population.

[5] Import means any wildlife introduced or brought into or landed on any place under the jurisdiction of the U.S. For imports see 50 CFR Parts 14.61 – 14.62; for exports see 50 CFR Parts 14.63 – 14.64.

[6] There are 18 designated ports, listed in 50 CFR Part 14.12. If special permits are not required, as under ESA or CITES, imports may also be cleared at certain border ports with Canada and Mexico or special ports in Alaska, Puerto Rico, the Virgin Islands, and Guam, provided completed Form 3-177 Declarations are submitted.

[7] "Commercial" means offering for sale or resale, purchase, take barter or transfer for gain or profit. There is a presumption that eight or more specimens constitute commercial use and a declaration is required.

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Validation of PCR-Based Assays and Laboratory Accreditation for Environmental Detection of Aquatic Invasive Species

Approved by ISAC on May 25, 2012

"Validation is the bridge between research and regulatory decisions!"
(Anything else is jumping across the abyss of unknowns to any possible conclusion!)

This white paper provides:

a) Background information on the use, accuracy and reliability of PCR-based assays such as environmentally sampled DNA (eDNA) for early detection of aquatic invasive species (AIS) and;

b) Recommendations for establishing a system for validating assays and accrediting laboratories that report on the presence or absence of AIS.

This white paper was developed by the members of ISAC and discusses the need for developing validation requirements for Polymerase Chain Reaction (PCR) and other DNA-based molecular assays that are increasingly being used to detect AIS. It does not provide a simplified checklist for evaluation of their ability to detect AIS. Rather, it is intended to demonstrate the need for a required and regulated framework to validate these molecular assays. A regulated framework for validation would greatly increase confidence in the utility of DNA-based assays and better enable decision-makers and managers regarding AIS detection, prevention, monitoring and control.

Issues

Aquatic invasive species can have major environmental, economic, and in some cases human health impacts. The National Invasive Species Council's (NISC) bureaus and agencies have a responsibility to make the most appropriate decisions possible and take timely action. However, traditional visual methods for the early detection and identification of invasive species are difficult and time-consuming to conduct in aquatic systems and maybe inadequate to support effective and timely actions. Delays, data gaps, and inaccurate information can be costly and allow an invasive species to become too well established and widespread to apply effective rapid response and eradication plans.

Molecular assays based on PCR can amplify tiny amounts of DNA in water samples (i.e., environmental DNA or eDNA) and detect the presence of AIS at high levels of sensitivity and specificity (Blanchet 2012, Darling and Mahon 2011, Jerde et al. 2011). This approach is currently being used to detect Asian carp species and zebra and quagga mussel larvae in water systems. There is increasing interest in the development of additional PCR-based assays for these and other AIS.

These factors, coupled with the increasing availability of rapid molecular assay systems (kits) are greatly expanding the use of PCR-based technologies to detect AIS. Due to their relative sensitivity, the use of molecular assays is causing major paradigm shifts in the way that AIS are detected, monitored, and controlled.

The successful application of molecular technologies will increase the speed and number of samples that can be analyzed, making early AIS detection more likely and increasing the probability that AIS populations will be contained and eradicated. However, there are numerous concerns regarding the reliability of these assays which were originally developed for research applications rather than to inform regulatory and/or management decisions (e.g., Longshaw et. al. 2012). They have been conducted without appropriate validation of methodology or definition of minimum laboratory requirements. These concerns are especially important when molecular tests are the only means available/possible to detect AIS because "traditional" methods cannot be used. However, the consequences of trusting an assay that has not been validated could be far more damaging, destructive, and long-lasting in loss of agency credibility or harm to non-target species than the damage caused by the arrival and establishment of an invasive species. Due to their potential negative regulatory, economic, and ecological impacts, one may question why managers or agencies would attempt to make decisions regarding AIS based on results from assays that have not been validated and/or conducted by unaccredited laboratories.

Regardless of what assays are used, making authoritative public announcements and appropriate regulatory decisions requires a suitable number (statistically valid) of certifiable samples to be collected under strict protocols.

- The establishment of well regulated sample collection, sample custody, and analyses protocols will allow NISC agencies and their partners to provide authoritative public announcements and make appropriate regulatory decisions in order to avoid wasteful use of regulatory resources, unnecessary public confusion or unrest, national and international commercial damage, and legal remediation.

To ensure that decision makers can make appropriately informed decisions and most effectively use these powerful new techniques, they need to be assured that the information generated by assay results is reliable via high analytical specificity and diagnostic specificity for the target species in a tested water body. However, decision-makers often have little information concerning the accuracy or reliability of the various DNA detection methods being used or the performance quality of the various laboratories conducting them. In addition, commercial assay kits used by some laboratories are protected from public release of specific data concerning their contents and internal protocols that are considered confidential commercial business information. While a method may meet the needs for research applications and be published in a peer-reviewed journal, this does not equate to an assay being judged or accepted as validated for other applications. Decision-makers may initiate rapid response efforts based solely on eDNA evidence with little assurance of its quality or limitations. Currently, there is no formal process for approving sampling and testing protocols. Ultimately, this reduces the effectiveness of efforts to combat the introduction and spread of AIS.

- Although there is increasing use of PCR assays to detect AIS in aquatic systems and increasing reliance on them for making critical regulatory and management decisions, there is no formal organizational process for approving sampling and testing protocols and questions concerning their effectiveness remain.

Each of the AIS-detection assay/sampling protocol systems that are developed requires validation. They must be evaluated to ensure that the protocols used yield results that are: specific to the target organism (specific), can detect low concentrations of eDNA (sensitive), consistent over time (reproducible); provide results that are within acceptable limits of variation from replicate samples obtained from both within and among locations (precise); able to yield similar results under differing environmental and sample conditions (robust), and consistent with positive and negative control samples (accurate).

- A new assay needs to be evaluated against an established "gold-standard" or compared diligently to a long accepted methodology; validated for their specificity, sensitivity, precision, accuracy, robustness, and reproducibility; and, laboratories conducting the assays need to be accredited.

Moving from traditional visual identification methods to molecular detection assays involves complex paradigm shifts which have great importance for decision-makers. It is a shift from the identification of organisms at a specific location and time to the detection of the current and/or past presence of an organism. It is also a paradigm shift from direct detection (i.e., collecting a specimen) to indirect detection (i.e., collecting DNA shed from an organism).

- These paradigm shifts have enormous import for managers and require a correlation between "traditional" and "newer" approaches. Decision makers must have a clear understanding of the strengths and weakness of all the methods used.

The terms "validation" and "accreditation" have been defined by several quality assurance organizations and have been standardized domestically and internationally in support of trade and other agreements. However, these definitions have not been uniformly applied to the discussion of PCR-based assays for AIS detection in environmental samples ("validation" in this white paper is defined as: "the systemic and scientific evaluation of an assay to accurately define its usefulness, robustness, accuracy, specificity, sensitivity, and repeatability."). A lack of clear and consistent terminology has led to confusion and can hinder the progress of AIS detection or control efforts.

- The clear and consistent use of standard terminology is critical to avoiding confusion and understanding and effectively communicating the information used to make decisions.

No assay is 100% accurate and consistent. The utility of PCR-based AIS early detection methodologies for decision-makers would be greatly increased if decision-makers and the public had clear measures of the specificity, sensitivity, precision, and accuracy of reported results. Increased confidence in eDNA detection would allow regulators to make more informed decisions and take scientifically based actions at the earliest possible stage of invasion when rapid response and eradication efforts have the highest likelihood of success. It would also greatly augment public communication efforts. Similarly, independent performance testing, and eventually laboratory accreditation could direct decision-makers to high performing laboratories that consistently generate trustworthy results that can be tracked over time and among locations.

- The eventual outcome of evaluating laboratory performance would be the establishment of a national reference laboratory fully capable of meeting international requirements and standards.

Application of the concepts of assay validation and laboratory accreditation are urgently needed. For example, a lack of certainty and confusion regarding DNA-based detections has led some agencies to require, separate and independent verification of initial assay results before taking action (Darling and Mahon 2011). The degree of confidence that regulatory officials and private and public stakeholders have in the specificity, accuracy, and robustness of current eDNA assays for correctly informing AIS decisions could be greatly increased by establishing systems for performance testing, validation and accreditation to benchmark both methodological and laboratory performance.

Asian carp species currently threaten the Great Lakes. The use of eDNA evidence indicating that Asian carp could be in Lake Michigan has been the subject of heated controversy (Jerde et al., 2011) and extensive review (see below). Currently, litigation is shackling Asian carp control because of a lack of convincing correlations between visual traditional methods (i.e., having captured fish at a specific

location and time) versus PCR detection of carp DNA in water samples. In early 2010, the Solicitor General informed the US Supreme Court, in part, that the use of a PCR eDNA assay for detecting invasive Asian Carp as:

> "the best information available.....the government has not rejected any option ..... compelled by the facts. ..... Nothing in federal law warrants second-guessing its expert judgment that the best information available today does not yet justify the dramatic steps Michigan demands."[1]

Again, in February 2010 testimony to the US House of Representatives stated:

> "Because eDNA is a new approach to assessing the presence of Asian carp and is being applied operationally before standard independent scientific review could occur, the Corps (U.S. Army Corps of Engineers) continues to collaborate with the University of Notre Dame to determine what eDNA does and does not tell us and continues to research how to improve the usefulness of this technology to inform management decisions."[2]

More recently in May 2011, a U.S. Army Corps of Engineers expert gave testimony in the U.S. Appeals Court stating:

> "Efforts to corroborate eDNA results with traditional methods of capturing fish have not been successful thus far."[3]

Perhaps the most compelling testimony that eDNA is an emerging technology and not validated is from the US Army Corps of Engineers before the U.S. Supreme Court dated January 2010:

> "Scientific research typically follows a process that includes a hypothesis regarding a topic, predictions about experimental or observational results based on the hypothesis, gathering of data, analysis of data, assessments of prediction accuracy, revision of the hypothesis, conclusions, and iterations if necessary. This process allows for revision and fine-tuning of hypotheses as predictions are tested and more information becomes available, and allows for an increasingly better understanding about the phenomenon or topic of interest. Hypotheses regarding the robustness and information content associated with positive eDNA detections are currently being formulated by Notre Dame (7e. above). In scientific research processes and terminology, this would involve further gathering and analysis of data to be used in testing predictions and assessing hypotheses regarding the inferential power of the eDNA method. This is a critical process in making sure that strong scientific conclusions are made and appropriate management actions undertaken." [4]

Could the "best information available" be devastatingly wrong if there is a deficiency of solid science (still in research mode) or a lack of validation of the assay or accreditation of the laboratory before it is

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[1] US Memorandum in Opposition, January 2010, to the US Supreme Court hearing Michigan's renewed petition for closure of the Chicago Area Waterway System to prevent Asian Carp species from entering the Lake Michigan from the Illinois River system.

[2] Statement of: Major General John Peabody, Commander, Great Lakes and Ohio River Division, U.S. Army Corps of Engineers, Before: Subcommittee on Water Resources and Environment Committee on Transportation and Infrastructure, United States House of Representatives on Asian Carp and the Great Lakes, February 9 2010

[3] Slater. U.S. Army Corps of Engineers, US Court of Appeals, 7 Circuit, May 5, 2011.

[4] Declaration of Dr. Elizabeth C. Fleming, Senior Executive Service, Director of the Environmental Laboratory, and Civil Works Business Area Lead at U.S. Army Engineer Research and Development Center. App. 30a http://www.supremecourt.gov/specmastrpt/us_appendix_to_renewed_opp.pdf

applied in a real life situation? Indeed, because of the regulatory, interstate commerce, and legal concerns regarding use of eDNA to detect the presence of Asian carp, the methodology and laboratory which developed it have undergone an extensive independent review process (Battelle Memorial Institute 2010, United States Environmental Protection Agency 2010, Asian Carp Regional Coordinating Committee 2012). The laboratory audit reviewed and reported on: 1) staff qualifications, training and quality assurance roles, 2) laboratory facilities, 3) field sampling practices, 4) eDNA methodology, 5) PCR methodology, and 6) quality assurance systems (United States Environmental Protection Agency 2010). This audit may be an initial step for future eDNA assay validation and laboratory accreditation.

- At the very least, laboratories using eDNA technology for early detection and monitoring of AIS should be offered the opportunity for independent performance testing as has been done for dreissenid mussel PCR detection (Frischer et al. 2011) with public access to performance results so that entities seeking the laboratories' services can be confident of their accuracy, reliability, and capacity to detect target species' DNA. The availability of such independent performance testing could be a step in the eventual development of comprehensive eDNA methodology validation and laboratory accreditation systems.

Recommendations

To encourage the development of a validation/accreditation system for AIS eDNA detection methodologies and laboratories, ISAC recommends that the NISC member Departments and Agencies and their partners consider adoption of the following recommendations.

- Encourage and develop funding for the National Academy of Sciences to undertake a review of the reliability and effectiveness of PCR and other DNA-based applications for detecting AIS, focusing on establishment of appropriate validation processes and a framework and standards for this new and potentially invaluable tool in the early detection, eradication, prevention and control of AIS.

- Establish and fund an ongoing independent performance testing program for laboratories utilizing DNA-based AIS detection methodologies such as that recently undertaken for evaluating laboratory performance in PCR detection of dreissenid mussel larvae (Frischer et al. 2011). Testing results should be made public so that managers may make informed decisions about the accuracy and reliability of a laboratory's performance when including an eDNA component in an AIS monitoring and early detection system.

- Utilize lessons learned in establishing a laboratory performance testing system to fully develop a validation/accreditation program(s) for other invasive species eDNA methodologies and laboratories.

Background

Molecular PCR-based assays amplify trace amounts of DNA by orders of magnitude. Using short highly specific segments of DNA called primers; these primers are a critical component of the assays that can detect the presence of target organisms' DNA in water samples. This approach has been used in attempting to monitor the spread of quagga mussels (*Dreissena rostriformis bugensis*) by detecting DNA from their larvae in plankton net tow samples in the western United States (Hosler 2011, Turner et al. 2011) and the free DNA of Asian carp (i.e., environmental DNA or eDNA) in water samples from the Chicago Area Waterway System (Jerde et al. 2010, 2011). A large proportion of DNA is "conserved" among species. Only a small amount is unique to a species. Isolating specific and stable primers to bind to "i.e., probe" a target DNA sequence is difficult. Primer selection and PCR protocols can profoundly alter the results obtained. Primers must be highly specific to the target species (Darling and Mahon 2011).

The need to rapidly detect AIS has led to the recent development of numerous PCR-based and other molecular detection assays for the analysis of environmental samples (Darling and Blum 2007, Li et al. 2011, Darling and Mann 2011, Mahon et al. 2011, Blanchet 2012). Numerous molecular assays have been published for detection of aquatic organisms including microbial pathogens (i.e., viruses, bacteria, protozoa and helminthes) (Toze 1999); bivalves (Claxton and Boulding 1998); fish (Jerde et al. 2011); and amphibians (frogs and salamanders) (Goldberg et al. 2011). However, the various molecular assays that have been used to detect a target organism's DNA in water samples obtained from the field (United States Environmental Protection Agency 2010) have not been standardized using validated assays conducted in accredited laboratories. Only one report of a laboratory performance evaluation that examined 11 laboratories' performance in identifying zebra mussel larva (veligers) DNA is available (Frischer et al. 2011). This "double-blind, round robin" evaluation using standardized low target organism density water samples found that:

- PCR techniques were the least reliable detection method. The traditional visual microscopic examination under polarized light was most reliable and accurate (75.8% versus 96.3% accuracy in determining presence/absence). This finding has led to legitimate concerns regarding the accuracy and reliability of eDNA for early AIS detection.

Of 11 laboratories tested, the most common error was failure to detect eDNA (i.e., false negative test result) in samples known to contain veliger DNA. There was also considerable variation (lack of precision) among laboratories. The average precision of more "experienced laboratories" as defined by the study was 86.9% while that for laboratories with less experience with these assays was considerably lower at 62.4% (Frischer et al. 2011). This round-robin conclusion will not be totally known until the information on the diagnostic sensitivity and specificity become known with assay validation.

As with all assays, a major concern is positive test results that do not reflect the true presence of the AIS at a location (i.e., false positives). This may be due to sample contamination, problems with the assay, DNA from dead target organisms, and/or only the target DNA and not the organism itself being present. DNA may last 14-25 days in the water column (Dejean et al. 2011) and can be carried by water currents far from the actual range of the target species. For example, it is not clear if Asian carp DNA in areas of the Chicago Area Waterway System was a "false positive" finding (Jerde et al. 2011). In addition, carp DNA could have been released into waterways from rinse water from fish markets and/or from ice melt-water used to store harvested carp (Asian Carp Regional Coordinating Committee 2012). There are several possible sources of target species DNA, such as 1) sewage discharge, 2) discarding remains of target species in slaughter and processing activities, 3) dead individuals captured elsewhere and transported by humans or wildlife and, 4) uptake of water from a target species habitat by boats or barges followed by discharge into an area outside the target species' range (Darling and Mahon 2011, Asian Carp Regional Coordinating Committee 2012).

Conversely, false interpretations may occur due to insufficient test sensitivity or because, even if target species is present, AIS DNA may not be in the water sample collected or the concentration is below the limits of detection of the assay (Darling and Mahon 2011).

- Four potential sources of error in eDNA testing are:
  1) lack of assay sensitivity and/or specificity,
  2) insufficient laboratory quality assurance, i.e., sample contamination, failure to follow protocols, and misinterpretation of results,
  3) ineffective sampling design and protocols to maximize potential for discovering the target species DNA, and
  4) lack of understanding of the relationship between a detection of a target species' DNA and actual target species presence, including DNA persistence and opportunities/vectors for its transport outside the range of the target species (Darling and Mahon 2011, Asian Carp Regional Coordinating Committee 2012).

Existing Validation Requirements for DNA-based Detection Assays. Currently, at least two federal agencies have some level of regulatory control regarding PCR assays developed and validated for marketing in the United States. The Federal Drug Administration (FDA) is responsible for enforcement of the Federal Food, Drug, and Cosmetic Act that covers *in vitro* diagnostic devices which are a subset of medical devices "intended for use in the diagnosis of disease and other conditions, including determination of the state of health, to cure, mitigate, treat, or prevent disease or its sequel." The Animal and Plant Health Inspection Service through the Center for Veterinary Biologics regulates the licensing and sale of diagnostic kits used in detecting animal diseases under the authority of the Virus Serum Toxin Act. Both agencies are involved in assuring that commercially available kits for running assays are safe, effective, reliable, and truthful in their label claims.

Existing Accreditation of Laboratories Offering DNA-based Detection Systems. Currently, there is no required independent or regulatory oversight of laboratories conducting and performing DNA-based AIS assays when using in-house primers, reagents, protocols, and technologies. There are numerous "quality" concerned organizations which orient their policies and philosophies towards globally standardized laboratory quality and analytical assay validation. These "quality" associations/organizations are voluntary. Membership brings recognition of a laboratory's effort to conform to quality standards in several areas important to reliable and reproducible laboratory operations and outcomes.

Generally "inspections" (i.e. audits, reviews, verifications, etc.) by quality organizations are conducted by a team of experts from member laboratories. Each team member can be specialized in some area of concern to the quality standards being verified. Typically areas reviewed, observed and audited are facilities, equipment, personnel qualifications, protocols, references, mechanisms of internal control and direction, etc.

There are areas of exceptional standards in some regulatory programs for the prevention, control, and eradication of animal disease where participation may need to meet mandatory standards for facilities, equipment, and personnel. Many protocols in these regulated laboratories are standardized in accordance with international trade agreements or other legally binding documents. Personnel must follow the various "Uniform Methods and Rules" used when testing for animal pathogens of commercial and economic significance. It appears that human and animal health is well on its way to utilizing reliable, validated assays for information regarding disease. There is also a system in place for plant health certification by way of testing for plant pathogens. These programs could serve as models for development of validation of protocols and accreditation of laboratories providing DNA-based AIS detection systems.

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