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From molecules to management: Adopting DNA-based methods for monitoring biological invasions in aquatic environments ☆

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ABSTRACT

Recent technological advances have driven rapid development of DNA-based methods designed to facilitate detection and monitoring of invasive species in aquatic environments. These tools promise to improve on traditional monitoring approaches by enhancing detection sensitivity, reducing analytical turnaround times and monitoring costs, and increasing specificity of target identifications. However, despite the promise of DNA-based monitoring methods, the adoption of these tools in decision-making frameworks remains challenging. Here, rather than explore technical aspects of method development, we examine impediments to effective translation of those methods into management contexts. In addition to surveying current use of DNA-based tools for aquatic invasive species monitoring, we explore potential sources of uncertainty associated with molecular technologies and possibilities for limiting that uncertainty and effectively communicating its implications for decision-making. We pay particular attention to the recent adoption of DNA-based methods for detection of invasive Asian carp species in the United States Great Lakes region, as this example illustrates many of the challenges associated with applying molecular tools to achieve desired management outcomes. Our goal is to provide a useful assessment of the obstacles associated with integrating DNA-based methods into aquatic invasive species management, and to offer recommendations for future efforts aimed at overcoming those obstacles.

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1. Introduction

Aquatic invasive species (AIS) have long been recognized as significant stressors to marine and freshwater habitats (Carlton and Ruiz, 2005; Rilov and Crooks, 2009; Strayer, 2010). AIS not only drive ecological changes that threaten ecosystem integrity and native biodiversity (Ricciardi, 2007), but in the most dramatic cases, may also have substantial negative impacts on human well-being through loss of ecosystem services and imposition of opportunity costs associated with mitigation and control strategies (Pimentel et al., 1999; Bossenbroek et al., 2009; Pejchar and Mooney, 2009). While myriad options exist to reduce such impacts through management intervention, recent bioeconomic analyses support the conclusion that prevention of AIS can often be more cost-effective than post-establishment control (Leung et al., 2002; Lodge et al., 2006; Finnoff et al., 2007). These insights are reinforced by examples of successful eradication of incipient invasions detected at early stages of establishment (Anderson, 2005; Wimbush et al., 2009). Accordingly, early detection of incursions through rigorous monitoring programs is now commonly recommended as a priority strategy for AIS management (Lodge et al., 2006; Vander Zanden et al., 2010).

Unfortunately, early detection of AIS can pose formidable technical challenges (Hayes et al., 2005). Most obviously, effective early detection demands the ability to recognize the presence of AIS at extremely low population densities. Moreover, identifications in aquatic systems may have to occur under difficult conditions (e.g. visual surveillance by snorkel or SCUBA in low visibility waters), or may be necessary for life stages not amenable to identification by traditional methods (e.g. planktonic larvae). Recognition of these difficulties has led to calls for novel detection methods capable of overcoming some of these challenges. In particular, a great deal of recent research has focused on the development of molecular detection methods, especially those based on the detection of target species' DNA in environmental samples (reviewed in Mountfort and Hayden, 2006; Darling and Blum, 2007; Bott et al., 2010). Such approaches

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promise a number of potential benefits over traditional methods, including increased sensitivity and specificity as well as greater throughput and cost effectiveness (Box 1).

Despite the demonstrated utility of molecular tools for monitoring aquatic environments in other contexts (e.g. monitoring for wildlife, veterinary, and human pathogens; Cunningham, 2002; Ayers et al., 2005; Bowers et al., 2006; United States Environmental Protection Agency, 2010), various impediments stand in the way of broad adoption of DNA-based methods for early AIS detection. Of course, the technical development of useful DNA-based tools still presents numerous obstacles, particularly as applications grow more complex (see Darling and Blum, 2007; Bott et al., 2010 and references therein). But as molecular methods begin to be deployed in real-world AIS management contexts, novel challenges have emerged. As we detail below, it has become clear that there still exists considerable concern regarding the potential sources of uncertainty associated with DNA-based detection methods. Understanding and communicating such uncertainty has proven difficult in some cases, especially when management decisions must be made in politically charged situations. These observations suggest the possibility that technological advances in method development already may be outpacing frank discussion of how to actually utilize DNA evidence to inform management decisions. In order to effectively deploy DNA-based AIS monitoring tools as they become available, it is critically important that multiple stake-holders—method developers, resource managers, policy-makers, and public users of affected aquatic resources—participate in informed, transparent discussions of the benefits and limitations of various tools for early AIS detection. The aim of this paper is to contribute to such discussion by critically examining the current usage of DNA-based methods in decision-making contexts and exploring ways to facilitate the effective future integration of molecular tools with AIS management needs.

Box 1–Why DNA-based detection?

DNA-based detection methods promise a number of advances over traditional methods, which are predominantly based on visual identification of specimens using diagnostic morphological criteria. One clear advantage is the ability of DNA-based approaches to identify individuals that cannot be identified by traditional means. For instance, morphological identification keys are typically based on recognizable adult features, and often do not enable identification of sub-adult forms—forms which may be critically important in the establishment or spread of invasive populations (Besansky et al., 2003). In addition, for many taxa, morphological identifications are possible only at limited taxonomic resolution, in some cases restricted to the genus, family, or ''morphospecies'' level (Caesar et al., 2006). Logistical considerations also frequently favor DNA-based approaches. Traditional identifications often require a level of taxonomic expertise that may be expensive or difficult to access rapidly, leading to high per sample costs and prolonged turnaround times (Lawton et al., 1998; Mallet and Willmott, 2003). In contrast, many DNA-based methods rely on common molecular tools accessible to most laboratories. Although the initial investment associated with molecular method development may be high, subsequent employment of DNA-based methods can be executed with relatively high throughput and at low cost per sample. DNA-based methods are also expected to deliver substantial sensitivity benefits over traditional methods in cases of environmental monitoring (e.g. Jerde et al., in press). This promises to dramatically reduce the likelihood of false negative error.

2. DNA-based methods for confirming specimen identity

A substantial body of literature already exists on the current and emerging technologies supporting DNA-based AIS monitoring, and in recent years there has been a proliferation of studies describing the development of molecular detection tools potentially useful in management contexts (Mountfort and Hayden, 2006; Darling and Blum, 2007; Bott et al., 2010). But the actual application of such tools has lagged significantly behind technical advances, and as yet few examples exist of DNA-based methods that have directly influenced management decisions. In part, this may be attributed to the fact that substantial technical hurdles still exist for many applications. Molecular methods for AIS monitoring can range from relatively straightforward applications (e.g. confirmation of specimen identity or detection of target species in simple environmental samples) to the extraordinarily complicated (e.g. complete enumeration of species identity and abundance in a complex sample), and at the time of this writing availability of tools for deployment in the field has been limited principally to the former (Darling and Blum, 2007).

In particular, a number of recent cases have illustrated the utility of DNA-based methods for confirmation of specimen identity in support of visual surveillance efforts. This approach, conceptually akin to DNA barcoding (Hebert et al., 2003a), has become more widely accepted as managers recognize the limitations of morphological identification in the context of AIS early detection. For instance, in the spring of 2010 microscopic inspection of cultured oysters being imported from Denmark to the Netherlands revealed the presence of egg capsules morphologically similar to the invasive Japanese oyster drill *Ocinebrellus inornatus*. Given the difficulties associated with species-level identifications from egg capsules, molecular evidence was sought to confirm these preliminary identifications. When DNA sequence from the mitochondrial cytochrome c oxidase subunit I gene (COI, a standard for animal barcoding) indicated unambiguous matches to *O. inornatus* reference sequences, the oyster shipment was quickly quarantined and direction was given to quarantine future shellfish imports from that region of Denmark (Gittenberger, 2010; Gittenberger et al., 2010). Similarly, on Prince Edward Island, Canada, DNA evidence has been adopted as a standard tool to confirm identifications of potentially invasive tunicates associated with shellfish transfers between sites (Sarah Stewart-Clark, personal communication). Not only do DNA-based methods in these cases provide a level of certainty in specimen identification difficult to achieve through traditional morphological approaches, the time savings afforded by molecular confirmations can be critical to effective management. The possibility of confirming AIS detection in hours to days instead of weeks to months allows managers to act quickly, thus minimizing the risk of AIS spread.

New Zealand and Australia have been especially progressive in their adoption of barcoding-like approaches to support management decisions. In late 2007, routine defouling of an oil rig transferred from Australia to New Zealand revealed the presence of a suspected marine invasive, the brown mussel, *Perna perna*. Preliminary morphological identification was confirmed by COI sequence analysis, prompting seafloor dredging to remove the defouled material (Smith et al., 2008). Similar applications of DNA sequencing for confirmation of specimen identity have been employed in a number of AIS surveillance protocols throughout New Zealand (Smith et al., 2003, 2007; D'Archino et al., 2007). Most recently, in early 2010, visual report of a suspected incursion by the invasive marine tunicate *Didemnum vexillum* in Twofold Bay (New South Wales, Australia) triggered immediate management response, including delimitation surveys and implementation of a quarantine order pending genetic confirmation of

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identification. This rapid management response was called down when sequence analysis conclusively excluded *D. vexillum* and other potentially invasive tunicates from the numerous samples collected (Whan, 2010).

Wide ranging acceptance of DNA-based methods in such management roles is likely driven by several factors. First, these tools are technically straightforward and based on DNA sequence data capable of providing unambiguous identification with resolution at or below the species level (Hebert et al., 2003b). Second, growing awareness of DNA barcoding and its role as a supplement to morphological taxonomy has led to broad recognition of the difficulties associated with traditional identifications for certain organisms, including immature life stages and individuals belonging to morphologically cryptic taxa (Valentini et al., 2009). Thus, it is becoming increasingly clear to managers that DNA-based tools offer a service simply unavailable via more familiar techniques. Perhaps most importantly, however, the confirmatory nature of these approaches allows managers to relax concerns regarding the possibility of false positive results and their costly consequences (see below).

3. Pushing the limits of detection: DNA-based detections of target species in environmental samples

Unlike barcoding approaches, the use of DNA-based methods for detection of AIS propagules in environmental samples (e.g. plankton tows, benthic sediment cores, ballast water samples, etc.) is an innovation that has yet to achieve widespread acceptance in management contexts. This is despite the rapidly growing availability of molecular probes designed specifically for target AIS (Bott et al., 2010). The technical challenges associated with detecting target species in complex samples are significantly greater than those associated with individual identification of specimens via barcoding, and monitoring programs employing them are thus more prone to error (Darling and Blum, 2007). This may explain why managers have been slow to adopt targeted detection tools in decision-making frameworks where positive detections have direct impact on management responses. One example of such adoption is associated with the arrival of dreissenid mussels (the zebra mussel *Dreissena polymorpha* and the quagga mussel *Dreissena bugensis*) in the western United States (Hickey, 2010), which has provoked intense response including calls for heightened monitoring of at-risk water bodies. In most cases, these monitoring programs have adopted molecular approaches based on polymerase chain reaction (PCR) amplification of *Dreissena* DNA from environmental samples (Brown et al., 2009). The State of Utah Division of Wildlife Resources (UDWR), for instance, employs PCR as a cornerstone management practice, and requires positive DNA-based detection in order to classify a water body as ''detected''—a classification indicating confirmed presence of veligers in the water body and triggering interdiction of departing watercraft to enforce compliance with decontamination standards (Utah Division of Wildlife Resources, 2010). Other states have adopted similar protocols.

While molecular methods for dreissenid monitoring face technical challenges different from those associated with the specimen identification approaches described above, they are nevertheless employed in a similar confirmatory role. DNA-based detections are not accepted as sufficient evidence to designate confident detection of the presence of target AIS, but instead are employed as one among multiple screening protocols including traditional microscopic inspections of environmental samples. But what happens if DNA-based techniques are the only available tool for detection of target AIS at the desired level of sensitivity? Since one of the most decisive advantages of DNA-based methods over traditional monitoring is enhanced sensitivity, monitoring programs that rely solely or primarily on DNA-based techniques for front-line detections represent the inevitable future outcome of continued development and deployment of molecular monitoring tools. And while adoption of DNA-based methods in confirmatory or supportive monitoring roles has become more broadly accepted, decision-making based predominantly or entirely on molecular evidence is still fraught with challenges. No example better illustrates this than DNA-based monitoring for invasive Asian carp in the Great Lakes region of North America.

Asian carp were introduced into North America in the 1970s as a measure for cleaning aquaculture facilities, and they escaped from containment ponds in Arkansas into the Mississippi River basin shortly thereafter (Chick and Pegg, 2001; Kolar et al., 2007). Upon release into the wild, bighead (*Hypophthalmichthys nobilis*) and silver (*Hypophthalmichthys molitrix*) carp spread northward and by the mid-1990s were highly abundant in the Illinois River (Irons et al., 2007), which links directly to the Great Lakes via the 45 km long Chicago Sanitary and Shipping Canal (CSSC). Early concern over the potential threat posed by Asian carp to the Great Lakes has grown more pronounced as the range of these species has continued to expand (Chick and Pegg, 2001; Chick, 2002; Rothlisberger et al., 2010).

In September 2008, the U.S. Army Corps of Engineers (USACE) entered into an agreement with researchers at the Center for Aquatic Conservation at the University of Notre Dame (UND) to complete a risk assessment of AIS associated with the CSSC. While the overarching goals of this project were broad, the research aimed in part to address concerns regarding Asian carp expansion into the Great Lakes. That possibility remained despite the presence of electric dispersal barriers on the CSSC, which were designed to effect partial ecological separation between the Mississippi drainage and the Great Lakes (Brammeier et al., 2008; United States Army Corps of Engineers, 2010). In addition, given known limitations of traditional detection methods (primarily netting and electrofishing) at low population densities, UND researchers were charged with developing novel methods for monitoring the advance of the invasion front (Kolar et al., 2007; Peabody, 2010). The workgroup at UND, in association with The Nature Conservancy (TNC), performed studies to test the utility of environmental DNA (eDNA) detection for Asian carp monitoring (Jerde et al., 2010; Jerde et al., in press). This approach adopts standard molecular techniques (DNA extraction, species specific PCR, electrophoresis) to detect target species DNA present in material such as skin cells, mucus secretions, and feces released into the aquatic environment, allowing inference of target species presence in tested water bodies (Ficetola et al., 2008). From 2009 to 2010, approximately 2000 water samples were taken from the CSSC by the UND–TNC workgroup and were tested for the presence of bighead and silver carp DNA. Results of these studies found DNA from both of these species throughout the CSSC, and in one case as far north as Lake Michigan (Jerde et al., 2010; Jerde et al., in press).

The alarming discovery of Asian carp DNA north of the putative invasion front—and, more troublingly, north of the electric dispersal barriers—immediately elevated concerns. A number of groups argued for full separation of the Great Lakes and the Mississippi River basin and pushed for closure of the hydrological locks that lead directly into Lake Michigan (Brammeier, 2010; Hansen, 2010). This was met with strong and vocal resistance from local waterway operators who routinely use the canal (e.g. Dahlman, 2010). Ultimately, the call for physical separation of the Great Lakes from the Illinois River was echoed in a request by the State of Michigan to reopen a U.S. Supreme Court case from 1922 challenging Illinois' right to connect the two systems in the first place. The request, and the

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associated preliminary injunctions to force lock closure, was twice rejected by the Court (Egan, 2010). More recently, a lawsuit was filed in the US Northern District Court of Illinois seeking immediate action to prevent Asian carp entry into the Great Lakes, including plans for physical separation (Hall, 2010a). Although the suit is still pending, preliminary motions filed in that case to force permanent closure of the Chicago locks have been rejected (Hall, 2010b). Regardless of the ultimate outcome of these legal actions, DNA-based detections of Asian carp have already helped to trigger a variety of short- and long-term management responses, ranging from intensified monitoring to plans for physical blockage of known bypasses around the electric barriers (Asian Carp Regional Coordinating Committee, 2010).

The debate over the potential Asian carp invasion of the Great Lakes has drawn input from powerful stakeholders throughout the region. The City of Chicago and local Chicago business interests have cited the economic importance of maintaining open links between the Mississippi River basin and the Great Lakes (Schwieterman, 2010), while environmental groups and regional stakeholders concerned for the viability of the $7 billion Great Lakes sport fishing industry have urged rapid action to prevent further advance of Asian carp (Hansen, 2010). Caught in the middle has been the scientific evidence perceived to have initiated the dispute. The result has been intense and highly politicized scrutiny of the DNA-based methods adopted in the Asian carp monitoring program.

Criticism of eDNA monitoring has focused on a number of issues, including the inability of traditional methods to confirm eDNA detections, the possibility of alternative pathways for Asian carp DNA movement throughout the system, and the perception that the method under question had not been fully vetted prior to adoption of results in decision-making contexts (Dahlman, 2010; Frede, 2010; Frede and Denzler, 2010). Most notable, perhaps, have been concerns regarding the inability of managers to confirm eDNA detections with standard monitoring approaches. Not only has the demand to "show us the carp" been voiced by opponents of costly management actions (Dahlman, 2010), but official responses by federal management agencies have implicitly endorsed such views by claiming that eDNA evidence is insufficient to conclude the presence of Asian carp in tested waterways (United States Army Corps of Engineers, 2010). In fact, despite widespread recognition of the poor sensitivity of traditional monitoring—a difficulty that was meant to be overcome by the development of DNA-based methods—vast resources have been expended in attempts to capture carp upstream of the electric dispersal barrier, and the repeated failure of these efforts has been perceived by some stakeholders as strong evidence against the validity of eDNA detections (Egan, 2010b).

These criticisms have persisted despite observations supporting the validity of the eDNA method, including the fact that in several cases Asian carp have been captured or otherwise observed in areas where only eDNA detections (and not traditional methods) predicted their presence (Hood, 2010; Jerde et al., 2010; Lodge, 2010; Jerde et al., in press). In recognition of the high profile implications of the eDNA approach, and in an attempt to allay concerns regarding method validity, UND–TNC provided weekly and monthly reports to USACE prior to submission of those results for peer-reviewed publication (Lodge, 2010; Mahon et al., 2010). In addition, two independent external audits of the UND–TNC laboratory (one led by the U.S. Environmental Protection Agency and one sponsored by USACE) have been invited since the initiation of the monitoring program. A report based on one of these audits identified sufficient quality control measures to support confidence in the eDNA method as a reliable indicator of Asian carp DNA presence in sampled waters, although limitations on the scope of the audit prevented assessment of interpretations regarding the relationship between eDNA presence and the distribution of the target species (Blume et al., 2010).

The application of DNA-based tools in Asian carp monitoring illustrates several important general points. First, in politically charged management scenarios any monitoring tool employed for AIS detection is certain to come under intense scrutiny, and in some cases may even face legal challenge. Second, situations in which novel DNA-based methods provide dramatic improvements in sensitivity over established monitoring approaches raise the difficult issue of how to ensure confidence in DNA evidence when effective confirmation in field settings may be impossible. This inevitably raises critical questions regarding the sufficiency of DNA evidence for triggering management responses. Finally, unlike traditional monitoring methods (particularly those involving visual surveillance), and despite broad acceptance of DNA evidence in other contexts (even legal ones, e.g. Saks and Koehler, 2005), DNA-based AIS monitoring is likely to invite heightened concern regarding the potential for errors with costly management implications.

4. Understanding sources of error

Multiple sources of error exist for all detection technologies. Specifically, any AIS monitoring protocol is susceptible to both false positive and false negative detections, which are respectively equivalent to type I and type II errors assuming the null hypothesis that AIS are not present in the system being tested. There are various possible sources of both types of error, and as illustrated in Fig. 1, it is possible to distinguish between errors attributable specifically to the DNA-based method being employed ("method" errors) and errors that arise during the monitoring process despite the effectiveness of that method ("process" errors). This distinction is critical not only for understanding inherent sources of error, but also for effectively communicating the limitations of monitoring protocols.

Most existing DNA-based methods for AIS monitoring rely on PCR to amplify target DNA present in samples at very low concentrations. The high sensitivity of PCR, though it enables detection of extremely rare targets, also makes it prone to false positives—a problem that has been recognized since the very early days of the method's popularity (Kwok and Higuchi, 1989). In particular, utmost caution must be taken to prevent contamination, as even very small amounts of contaminating target DNA can result in positive detections. Of more general concern to DNA-based detection methods is the possibility that assays might pick up "lookalike" non-targets. All DNA-based detection tools are based on the idea that relatively short stretches of the target genome can be used to uniquely distinguish it from non-targets (Box 2). However, DNA probes designed to recognize target templates can cross-react with non-targets if the similarity between target and non-target sequences is sufficiently high (Raut and Kapadnis, 2007; van Pelt-Verkuil et al., 2008). In the case of PCR-based methods, false positive detections resulting from such errors can sometimes be corrected by sequencing resulting amplicons. This option may not always be available, so ensuring method specificity through careful probe design is critically important.

Unfortunately, this does not exhaust the possible sources of type I error. False positive detections can also arise when target DNA is present in a water body despite the absence of viable individuals of the target AIS. Much concern over this source of error has been directed at the inability of DNA-based methods to distinguish between living and dead organisms (Mountfort and Hayden, 2006). Since only living individuals pose threats, it may

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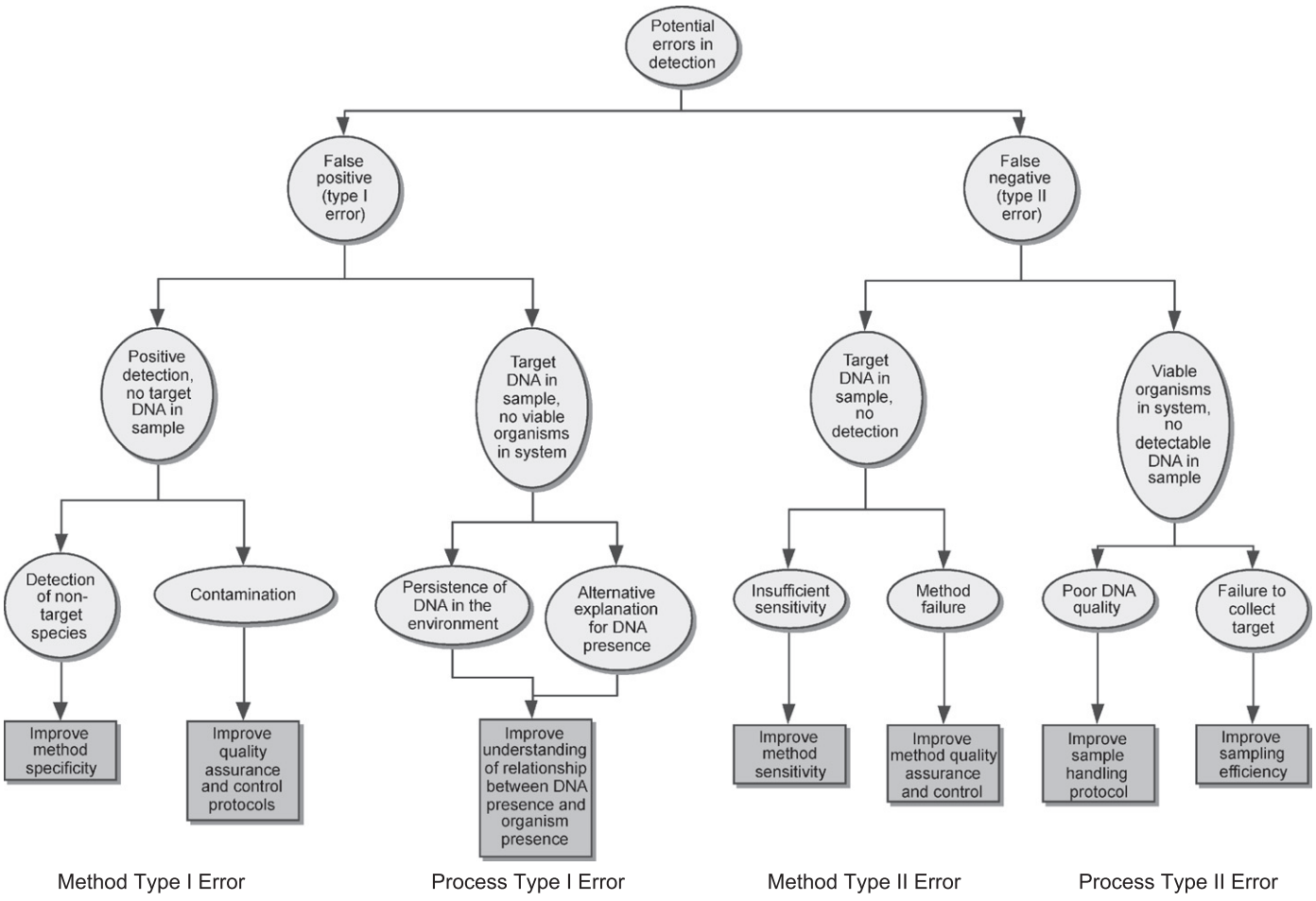

Fig. 1. Potential sources of error in DNA-based AIS monitoring protocols. Errors can be attributed specifically to the detection method employed (''method'' error) or to the overall monitoring process (''process'' error) including, for instance, sampling design, and sample handling.

prove important to avoid detection of DNA that persists in the environment subsequent to an organism's death. Additional possible sources of type I error have been highlighted by the adoption of DNA-based methods in Asian carp monitoring. Since sampling in this case does not actually involve capture of individual fish, critics have noted that there may be explanations for the presence of carp DNA in a water body that do not require the presence of viable fish—or indeed the presence of fish at all. Lodge (2010) notes a number of such proposed potential mechanisms for the spread of Asian carp DNA, including sewage effluent from humans or excrement from birds that had eaten carp, discard of carp carcasses into the canal, or transport and release of eDNA-laden water by barges. Challenges to monitoring programs facing such uncertainties are virtually assured, although inferences based on patterns of DNA-based detections may be bolstered by strong sampling design (Blume et al., 2010; Jerde et al. in press). It should be noted that while this source of error seems particularly problematic in the case of eDNA detections, in principle all DNA-based methods are subject to similar considerations. The problem is that there are still substantial gaps in knowledge regarding the relationship between presence of target DNA and presence of viable target individuals. While the latter may often prove to be the most parsimonious explanation for the former, future research to close this knowledge gap will be an important step in reducing uncertainty associated with DNA-based monitoring efforts.

Of course, similar observations can be made regarding false negatives (Fig. 1). Target DNA present in a sample may not be detected due to insufficient sensitivity or simple failure of the method to perform as expected. Alternatively, the presence of viable organisms in a water body does not guarantee the presence of target DNA; ineffective sample preservation may lead to degradation of DNA prior to application of molecular methods, or sampling inefficiency may prevent the capture of rare targets (Hayes et al., 2005).

These considerations suggest that there are four critical points at which error can be introduced into any DNA-based monitoring program, and thus four points where effort should be directed to limit opportunities for error:

1. *Molecular assay design.* Researchers tasked with developing DNA-based detection tools doubtless recognize the importance of ensuring the sensitivity and specificity of those tools. Nevertheless, the potential for public scrutiny of these methods when deployed in management situations demands extraordinary standards for assay development (Box 3). These standards may not be fully met by the typical expectations of peer-reviewed publication. Guidelines do exist in other contexts for the development and validation of DNA-based methods for environmental monitoring (e.g. Parshionikar et al., 2009). One significant problem is that rigorous method development may be outside the common expertise of many laboratories (often academic) involved in generating molecular tools for AIS monitoring. Solutions to this problem may include greater involvement of government or private sector laboratories, greater availability of funding for academic laboratories

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Box 2–A primer on probes (and primers).

DNA-based detection methods rely on the idea that relatively short stretches of a species' genome can be used to target that species and exclude all others. The design of DNA probes capable of specifically recognizing the presence of target species DNA requires identification of these short, diagnostic stretches of the target genome. Detection technologies then exploit the complementary nature of DNA to physically bind probes to exposed target DNA templates. Binding of complementary single-stranded DNA molecules is a stochastic process governed by a number of factors, including the concentration of probe and template, the degree of complementarity between the two, and various reaction conditions that influence the thermodynamics of the binding reaction. Fig. 3 illustrates how the degree of complementarity between probe and template defines the target group, using examples of probes designed to detect invasive crabs of the genus *Carcinus* (Darling and Tepolt, 2008). In the presence of fully complementary target template, the binding of probe to the target sequence results in a stable double-stranded DNA complex (Fig. 3A). The binding reaction becomes less favorable as complementarity is reduced; in the case of very closely related templates, binding may occur (Fig. 3B), but when template sequence is highly divergent from that of the probe, binding will not occur under the conditions of the assay (Fig. 3C).

Different detection methodologies exploit this binding reaction in different ways. For instance, in the case of methods based on the polymerase chain reaction (PCR), two different probes (commonly referred to as PCR ''primers'') are designed to bind to regions flanking a genomic target, typically a stretch of DNA ranging from several dozen to several hundred nucleotides. Successful primer binding allows exponential replication, or ''amplification,'' of this short template fragment; once the fragment has been amplified sufficiently its presence can be easily detected by a variety of methods. Other technologies link the binding of probes directly to the production of some detectable signal, for instance initiation of a chemical reaction or an electrical signal upon probe binding.

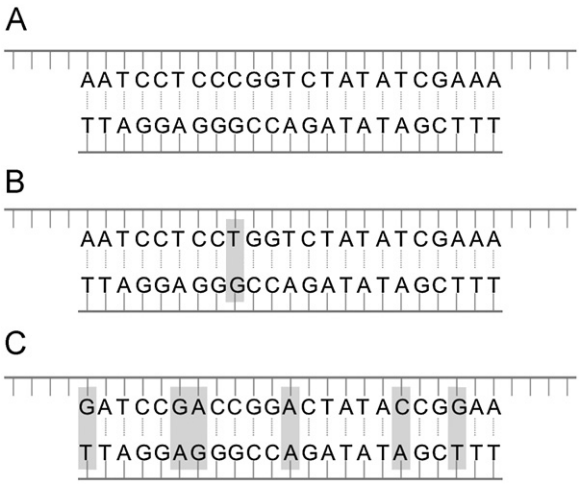

Fig. 3. Complementarity between probes designed for the invasive crab genus *Carcinus* (bottom DNA strand in each panel) and various potential templates (top strand). (A) Target template (*Carcinus maenas*); full complementarity with probe results in binding and positive detection. (B) Target template (*Carcinus aestuarii*, sister species); nearly full complementarity with probe. Despite single mismatch (shaded), probe binds to this template under assay conditions, resulting in positive detection. The sister species is thus included in the target group. (C) Non-target template (*Callinectes sapidus*, same family as *Carcinus*); multiple mismatches (shaded) result in failure of probe binding, thus excluding this species from the target group.

to pursue rigorous method validation testing, and improved options for public dissemination of the results of such testing.

2. *Laboratory quality control.* If molecular tools are to be adopted for routine AIS surveillance, decision-makers must be assured that errors resulting from faulty laboratory practices have been minimized to the greatest extent possible. Many managers may be familiar with requirements for accreditation or certification of laboratories employing molecular tests for other environmental protection issues (e.g. waterborne veterinary or human pathogens); similar mandates are ultimately to be expected in the case of AIS monitoring methods. Again, this will likely impose quality control criteria unfamiliar to many of the laboratories developing methods for AIS detection. DNA-based monitoring programs thus may sometimes require transitions from laboratories responsible for method development to those responsible for routine method application, making assay reproducibility a critically important consideration. Quality control measures must be extended to sample preservation and handling, as well, since appropriate assurances of sample quality will prove critically important to the avoidance of false negatives.
3. *Sampling design.* The effectiveness of a surveillance effort depends on numerous factors, including not only the sensitivity of the detection method employed but also the density of the target species and the spatiotemporal distribution of sampling effort (Hayes et al., 2005). Effective sampling schemes may prove crucially important to inferences regarding presence or absence of target AIS. Repeated positive detections or particular spatiotemporal patterns of positive detections may quell fears of false positives even in the absence of non-molecular confirmatory evidence (Blume et al., 2010). Understanding the implications of sampling design may also inform expectations regarding the likelihood of various sources of error. For instance, the so-called "false positive paradox" suggests that even highly specific DNA-based methods (i.e. those with extremely low false positive rates) could give misleading results when the expected incidence of targets in sampled waters is near or below the false positive rate (Madison, 2007). This suggests that complete false positive avoidance will be particularly difficult in situations where targets are expected to be rare—a condition that may describe many front-line AIS monitoring situations.
4. *Uncertainty in the relationship between presence of target DNA and presence of viable target organisms.* Understanding the fate of DNA in environmental samples is a critical research goal for DNA-based environmental monitoring (Matsui et al., 2001). Studies have revealed that the residence time of detectable target DNA can vary widely depending on environmental conditions (Hofreiter et al., 2003; Willerslev et al., 2007). In some cases this may mean that DNA persists in the environment despite the absence of viable target organisms (Ficetola et al., 2008). Although there may be methods capable of limiting false positive results due to detection of DNA from inviable individuals (e.g. Brescia et al., 2009), these have not yet been widely implemented or accepted. Alternatively, methods that rely on detection of more labile RNA templates (e.g. qPCR approaches; Mountfort and Hayden, 2006; Bott et al., 2010) may prove valuable in ensuring that positive molecular detections indicate very recent presence of viable target individuals.

Adequately accounting for error associated with molecular AIS monitoring programs will demand concerted effort to address as many of these critical points as possible. For example, the development of Asian carp eDNA detection methods has required implementation of multiple strategies to account for and reduce potential error. To limit uncertainty associated with probe design, researchers have utilized DNA sequencing to confirm identity of

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Box 3–Moving toward standards for molecular assay design.

The development of any DNA-based AIS detection tool obviously will be associated with particular challenges that guide appropriate assay design and testing. However, there are a number of general considerations that can help to define standard best practices.

Assay specificity. Various statistical methods facilitate *in silico* testing of species-specific DNA-based probes (e.g. Apte and Singh, 2007; Qu et al., 2009), and these constitute an important initial step in assay design. Such algorithms aid in recognition of diagnostic mismatches between target and non-target templates, and help to determine whether those mismatches are likely to result in differential binding under defined reaction conditions. Services such as short oligonucleotide BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) allow researchers to query probes against massive sequence databases, and should be utilized to determine whether there might be unanticipated and unwanted targets. Subsequent *in vitro* testing of probe specificity allows optimization of reaction conditions to ensure that differential binding between targets and non-targets is robust. In the case of PCR-based methods, sequencing of amplicons should be standard practice to ensure specificity. In assay development stages, we recommend sequencing of all positive detections; during assay deployment, sequencing should be conducted randomly on some fraction of all positive detections, and on all positive detections that occur in high priority sampling areas. Perhaps the greatest challenge for researchers will be determining the appropriate range of non-targets that must be tested to sufficiently assess assay specificity. We propose that *in vitro* probe assessment should consist minimally of tests against all non-target congeners of the target species, as well as all non-targets from the same family that may occur in the environments likely to be tested. In addition, we recommend that probes be tested against samples from known un-invaded environments with biological community composition similar to the environments being tested.

Assay sensitivity. Generally speaking, *in vitro* sensitivity testing consists of spiking samples with known quantities of target template or known numbers of target propagules. Unfortunately, such tests may be inadequate for usefully estimating detection limits if they are conducted in small volumes. For instance, determining that an assay can detect a single target species larva in a 100 μl sample may indicate a detection limit lower than one larva, but only in a very restricted sense. Much more useful than these method detection limits are process detection limits; for instance, can the same assay detect a single larva in a typical sample volume? We strongly recommend that claims of assay sensitivity be tested against such ''realistic'' scenarios, and that detection limits for target AIS be reported in units of number of propagules per unit volume or DNA template concentration. In addition, it is critical to assess the effects on sensitivity of the presence of non-targets. These effects can also manifest at both the method and the process level: does the presence of a known amount of non-target DNA reduce sensitivity by impacting probe binding efficiency? Does the presence of a known amount of non-target biomass reduce sensitivity by impacting the efficiency of target capture? Testing of spiked samples that include known quantities of non-target DNA or biomass should address these questions. Finally, methods should be employed to determine whether or not environmental factors likely to be encountered in field sampling are capable of inhibiting probe binding or PCR amplification, and steps should be taken as necessary to minimize the impacts of such inhibitors on assay sensitivity.

Utilization of appropriate controls. Positive controls are employed to ensure that assays are performing as expected, while negative controls alert researchers to possible contamination or specificity issues. Each is crucially important, and during assay development both should be employed at all critical process steps (including molecular detection, DNA extraction, and sample processing) to allow researchers to effectively recognize potential for error and troubleshoot accordingly. During assay deployment, we recommend that both method controls (blanks and spiked samples input into the molecular workflow) and process controls (blanks and spiked samples input into the sample processing workflow) be employed frequently to allow assessment of assay failure at both levels.

Determination of false positive and false negative rates. Descriptions of assay development should explicitly report rates of false positive and false negative detections. Unfortunately, the number of tests reported in existing literature is often insufficient to assess error rates in a way that is informative to potential end-users of the technology. For instance, one of our own publications (Darling and Tepolt, 2008) reports on results of only 45 true positive and 25 true negative tests; only 18 of those tests were conducted to assess detection of targets in mixed environmental samples. While no false positive or negative detections occurred in that study, explicitly recognizing error rates could better aid managers in assessing not only the frequency of error, but the degree to which the assay has been subjected to rigorous testing.

Defining ''positive'' detections. Determination of whether or not a test result is ''positive'' is often not straightforward. This is particularly true of standard PCR-based methods, where positive detections are judged based on visual inspection of amplified fragments on agarose gels, a scenario prone to subjective interpretation. We strongly recommend that assay development include standard definition of positive detections in a way that minimizes potential for observer subjectivity and bias. For instance, standard PCR results could be assessed by image densitometry of agarose gels, and cut-offs for positive detections defined based on variation observed in positive control reactions. Similar thresholds must be defined for other DNA-based methods (e.g. qPCR) to reduce or eliminate researcher-to-researcher variation in the assessment of results.

Repeatability and reproducibility. Repeatability is the agreement between results of assays conducted on the same sample under the same conditions and typically within a short time frame. For instance, multiple PCRs could be conducted in a single assay on the same processed sample in order to assess method repeatability. Reproducibility, on the other hand, is the agreement between results of assays conducted on the same sample under variable conditions (different reagent batches, instruments, or technicians) and sometimes over long time frames. Both repeatability and reproducibility should be demonstrated at the intra-laboratory level for all DNA-based detection assays. Ideally, assessment of reproducibility should extend also to the inter-laboratory level, and for effective broad deployment of molecular methods this will ultimately be the appropriate standard.

positive eDNA detections in 5% of all positive samples and for all samples testing positive in regions where Asian carp DNA had not been previously detected. In addition, both assay design and process quality control have been assessed in detail by independent teams of experts (e.g. Blume et al., 2010). Finally, sampling design has been critical to rendering the overall molecular monitoring program robust to unavoidable uncertainties. Inferences from the spatial distribution and temporal repeatability of positive detections, along with results of sampling from waters where the target species is known to be absent, increase confidence in the overall pattern of detection and reduce the plausibility of alternative explanations for the presence of target species' DNA (Jerde et al., in press). Such approaches not only limit error, but also allow managers to effectively weigh the

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strength of evidence and make appropriate decisions even when uncertainty and the possibility of error cannot be eliminated entirely. Additional future research—in particular, experimental examination of the sources and environmental fates of carp DNA released from living fish—would further cement confidence in the eDNA detection approach.

5. Fear of the false positive

Management of uncertainty is not solely a scientific issue; it is also an issue of public policy. It should come as no great surprise that decision-makers tasked with environmental protection seek to avoid error, and often are particularly averse to false positive detections. In public health contexts this aversion often stems from concern for negative public reactions—specifically, unnecessary "panics" stirred by announcement of a detected human pathogen when, in fact, that pathogen is not present (Committee on Human Biomonitoring for Environmental Toxicants, 2006). In the case of AIS monitoring and surveillance, managers are likely more concerned to prevent unwarranted expenditures of resources and undue inconvenience to users of public waters. Mobilization of second-tier monitoring responses, interdiction of watercraft, and closures of water bodies to incoming and outgoing traffic all have costs, both financial and political, that resource managers are keen to avoid unless absolutely necessary. Thus, AIS monitoring programs generally seek to minimize the likelihood of false positive detections, either through adoption of methods with low inherent false positive rates (e.g. morphological identification of captured individual organisms) or through implementation of monitoring protocols with multiple screening filters. *Dreissena* monitoring programs adopted by UDWR offer a case in point: waters are typically only listed as "detected" after identification of veligers by microscopy (in some cases both light microscopy and scanning electron microscopy) and positive detections from two independent PCR tests based on different markers, with sequence confirmation sought for all positive PCR amplifications (Hosler, in press; Utah Division of Wildlife Resources, 2010). The acceptance of molecular tools to inform management decisions in this context may in fact be driven largely by the perception that they reduce the likelihood of false positives.

Unfortunately, these approaches do not reduce error *per se*; rather, they allocate error away from false positives and toward false negatives (Committee on Human Biomonitoring for Environmental Toxicants, 2006). Generally, analytical methods with very low false positive rates (e.g. physical capture and identification of an individual) tend to have relatively high false negative rates. Adoption of multiple screening filters (i.e. employment of confirmatory detection) will reduce the likelihood of a false positive. However, since each filter has some non-zero probability of a false negative, the overall likelihood of a false negative result will be amplified. Additionally, the time needed to perform multiple layers of screening may hinder the ability of management agencies to act rapidly on pertinent information.

To some degree, this implicit bias of monitoring protocols toward false negatives, and recognition of the associated risks, is reflected in managers' willingness to initiate actions in response even to preliminary detections. For instance, UDWR's tiered categorization for waters appears to recognize the value of unconfirmed detections (i.e. "inconclusive" tests), and identifies appropriate management attitudes toward such detections (Utah Division of Wildlife Resources, 2010). The case of Australia's Twofold Bay is particularly illustrative in this regard: initial orders for quarantine of the Bay were based on preliminary morphological findings indicating the possible presence of a highly invasive tunicate, *D. vexillum*; this quarantine was later ended when DNA-based methods showed the initial detection to be in error (Whan, 2010).

There are two important points to be drawn from these examples. First, DNA-based methods are not the only surveillance techniques with potential for high false positive rates. A number of studies have now shown that despite best efforts taxonomic identifications based on morphological criteria can be error-prone, particularly for many aquatic invertebrate taxa (Geller et al., 2010). Second, managers may be willing to base expensive management responses on detection methodologies that potentially return false positives when (1) the costs of a false negative are high, and (2) there exist means of rapidly correcting for false positives. This may go a long way toward explaining the broad adoption of DNA-based methods to confirm uncertain specimen identifications based on morphology. In the case of response to *D. vexillum* in Twofold Bay, not only were government officials eager to avoid the first Australian incursion of this high-risk species, but the availability of a tool for unambiguous molecular identification of specimens allowed managers to react quickly, knowing that the possibility existed for rapid subsequent recognition of error.

Unfortunately, if managers are unable to correct for such errors, they will be more inclined to postpone responses, resulting ultimately in heightened risk of negative AIS impacts. This conclusion highlights a potentially serious general problem for integration of DNA-based methods into AIS monitoring, a problem exemplified in the case of Asian carp in the CSSC. The fact that carp eDNA detections have already triggered significant management responses indicates a willingness among some stakeholders to act despite uncertainty, attesting to the serious risks associated with delaying action. However, the position of other stakeholders seems to indicate desire for a tiered monitoring approach in which initial eDNA detections require confirmation by alternate methods, such as capture or observation of fish, that are less prone to false positive error. The problem is that there exist no such alternatives with sufficient sensitivity to confirm positive eDNA detections at the putative invasion front, where targets are likely very rare. Given acknowledged limits to alternative detection methods (Kolar et al., 2007; Lodge, 2010; Peabody, 2010; Jerde et al., in press), false negative results of those methods are a very likely outcome even when personnel are able to target specific waters based on eDNA detection patterns. Generally speaking, adoption of confirmatory methods with expected false negative rates higher than those of first-tier methods would be a recipe for confusion. Management programs based on this principle are likely to be wasteful, expending valuable resources on first-tier monitoring that is not trusted and second-tier monitoring that is not effective. At worst, such an approach threatens to erode confidence in the utility of detection methods generally, ultimately limiting the ability to effectively martial valuable scientific information to guide management decisions.

This problem is not unique to the Asian carp situation. DNA-based detection methodologies will typically have substantially lower detection thresholds than traditional methods (Darling and Blum, 2007; Bott et al., 2010). In many cases, it will thus prove impossible to design tiered early detection protocols in which alternative methods can be used to confirm DNA-based detections in the field. In such situations, managers may have limited options for confirmation, such as resampling water bodies and considering additional positive DNA-based tests to be confirmatory, or waiting until targets are common enough to be effectively detected by other methods—which would allow additional time for establishment and spread of invasive populations. Such decisions must be guided not only by the known sensitivity limits of available detection methods, but also by the predicted risks associated with incursions of AIS at various population densities.

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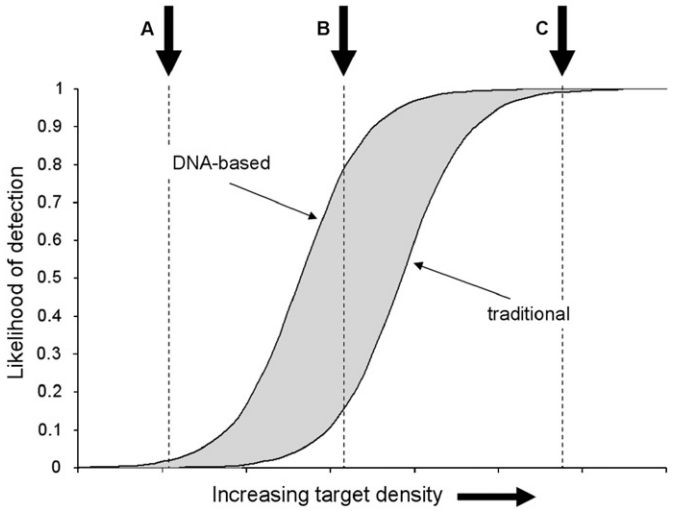

Fig. 2. Hypothetical model illustrating the expected relative sensitivity of DNA-based and traditional AIS detection methods. Likelihood of detection (*y* axis) increases with increasing target density (*x* axis), but at different rates for the different methods. The shaded area indicates target densities at which DNA-based methods are more sensitive than traditional methods. *A*, *B*, and *C* represent three acceptable risk levels potentially driving management decisions. In situation *A*, unacceptable risk is surpassed at very low target densities. In this case, neither detection method provides adequate sensitivity to detect targets at acceptable risk levels. For *C*, target densities must be very high before unacceptable levels of risk are reached. In this case, DNA-based methods offer little if any sensitivity benefit over alternative methods (although there may still be benefits in terms of analytical turnaround times or relative costs). In situation *B*, DNA-based detection methods are far more effective than traditional tools at the target densities conferring unacceptable levels of risk. In such cases, DNA-based methods may be required to trigger management even when no alternative is available to confirm positive detections. Note that scale on the *x* axis will vary with the particular methods under consideration; in some cases, differences in sensitivity between DNA-based and traditional methods may have to be measured on log scales.

Fig. 2 illustrates how the hypothetical relationship between expected detection thresholds and predicted invasion risk might influence decisions regarding the utility of various monitoring tools. For extremely damaging invasives, even very low population densities may represent high levels of risk to a system. In such cases, there should be a premium placed on detection sensitivity, and DNA-based methods may be the only tools capable of triggering management actions prior to assumption of unacceptable invasion risks. Most troubling for managers may be the fact that in such cases DNA-based methods simply will not be amenable to realistic (i.e. field-based) validation, and researchers may have to rely strictly on controlled experimental approaches to demonstrate the reliability of those methods. This not only places additional demands on the rigor of assay design and the importance of quality control measures, but in politicized management scenarios it also virtually ensures challenges to method validity.

Of course, managers are not only keen to avoid false positives, and there are certainly arguments that false negatives must be even more diligently guarded against (Lam and Gray, 2003). The ability to prevent or mitigate the negative ecological impacts of biological invasions is likely highest at early stages of invasion, declining rapidly once introduced populations have established and begin expanding (Touza et al., 2007). Further, recent studies have revealed the cost effectiveness of early detection and monitoring for invasive species, suggesting that expenditure of resources on prevention should reduce overall economic impacts of AIS (Leung et al., 2005). These observations would appear to argue for monitoring approaches designed primarily to avoid false negatives, even if those approaches incur opportunity costs associated with "unnecessary" management actions. In fact, this has been one of the most frequently voiced arguments for the adoption of DNA-based monitoring tools (Box 1). While reduction of false negative rates remains a goal for DNA-based method development, the fact that molecular tools are typically pursued to overcome insufficient sensitivity of traditional tools suggests that concern over false negatives is not a significant hurdle in the adoption of DNA-based monitoring approaches. Rather, recognition of the value of preventative management and the corresponding need to avoid false negatives would seem to encourage implementation of high sensitivity DNA-based detection methods. Nevertheless, the specter of potential increases in false positive rates associated with highly sensitive methods continues to delay their acceptance.

One significant problem is that even risk averse decision-makers are likely to prefer control to prevention. Finnoff et al. (2007) explain this rather counterintuitive result by recognizing the greater uncertainty associated with the productivity of prevention relative to control. Managers generally recognize, at least implicitly, that even the most strident prevention measures cannot fully eliminate the risk of invasion. Furthermore, prevention measures deployed in systems that would not have been invaded otherwise seem to be patently wasteful. The productivity of prevention thus depends not only on uncertainties associated with prevention technologies, but also on uncertainties associated with the assessment of initial invasion risks. In contrast, the payoffs associated with control efforts (i.e. removal of existing invaders) are more clearly and immediately measurable. Managers averse to risk and inclined to prefer more certain benefits per cost will thus generally favor portfolios of invasion management strategies heavily weighted toward control. Similarly, while managers may acknowledge that it is impossible to reduce the false negative detection rate to zero in any system receiving AIS propagules, it is conceivable to reduce the false positive rate to zero—most obviously by avoiding monitoring altogether. Reduction of false positive error thus presents a less uncertain proposition, one that may prove attractive to risk averse decision-makers and managers seeking to maximize the productivity of AIS management strategies.

But, as Finnoff et al. (2007) argue, this perspective can lead to greater likelihood of invasion and, ultimately, reduced overall social welfare. Decision-makers naturally seek to avoid public perception of wastefulness associated with responses to false positive detections. But they should be similarly inclined—arguably more inclined—to avoid public perception of wastefulness associated with controlling invasions that could have been prevented by more sensitive monitoring approaches. At the very least, it is incumbent upon those decision-makers to transparently communicate to the public how error is being allocated by the choices made in designing early detection and surveillance programs.

6. Conclusions

DNA-based methods have the potential to dramatically improve our capacity for AIS early detection and monitoring. Unfortunately, their application also has the potential to amplify the uncertainty already associated with AIS risk assessment (Sikder et al., 2006; Benke et al., 2010). The analysis presented here suggests that there are multiple opportunities to manage that uncertainty so that DNA evidence can effectively inform critical management decisions. On the one hand, design and implementation of molecular methods for AIS monitoring must be stringently controlled, and those responsible for development of such methods must welcome the scrutiny accompanying what amounts to a novel forensic application of molecular technology.

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Confidence in the effectiveness of DNA-based tools and the laboratories that implement them will be best ensured through rigorous examination of assay design (e.g. through implementation of standards such as those recommended in Box 3) and independent assessment of quality control measures. In addition, sampling strategies must be designed in such a way that inferences regarding AIS presence can be rendered robust to the potential for errors, and future research should aim to better understand the relationships between DNA-based detections and the presence of target organisms.

On the other hand, decision-makers must recognize and transparently communicate tradeoffs in error allocation associated with monitoring programs. Pleas to eliminate the possibility of false positive error may entail acceptance of increased false negative errors, and such decisions should be publicly acknowledged. Similarly, employment of highly sensitive DNA-based methods will often necessitate acceptance of greater potential for false positive detections, particularly when those methods are the only ones sufficiently sensitive to detect invasions at critical thresholds (Hayes et al., 2005). While complete avoidance of false positives (and their associated costs) may be impossible in such situations, these decisions must be placed in the context of more complete cost-benefit assessments of AIS prevention, and must be balanced explicitly against the reduced risk of damaging invasions afforded by sensitive early detection.

Can DNA-based methods provide evidence that is sufficient to inform AIS management decisions? The short answer is "yes." After all, DNA evidence is now widely accepted in other decision-making contexts, even those that invite intense legal, political, and public scrutiny (Saks and Koehler, 2005). But much work needs to be done before DNA-based detections are widely adopted for AIS monitoring. As we have suggested here, this work is not limited to the technological advances driving development of new detection tools. It includes also additional research goals associated with understanding sources of uncertainty in DNA-based monitoring programs, as well as effective negotiation of the tradeoffs between various sources of potential error. Consideration of these issues should proceed apace with technological advance, so that implementation of newly available technologies can be immediately and effectively responsive to critical management needs.

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References

Anderson, L.W.J., 2005. California's reaction to *Caulerpa taxifolia*: a model for invasive species rapid response. Biological Invasions 7, 1003–1016.

Apte, A., Singh, S., 2007. AlleleID: a pathogen detection and identification system. Methods in Molecular Biology 402, 329–346.

Asian Carp Regional Coordinating Committee, 2010. Asian carp control strategy framework.

Ayers, K., et al., 2005. International accreditation of sandwich hybridisation assay format DNA probes for micro-algae. New Zealand Journal of Marine and Freshwater Research 39, 1225–1231.

Benke, K.K., et al., 2010. Risk assessment models for invasive species: uncertainty in rankings from multi-criteria analysis. Biological Invasions 13, 239–253.

Besansky, N.J., Severson, D.W., Ferdig, M.T., 2003. DNA barcoding of parasites and invertebrate disease vectors: what you don't know can hurt you. Trends Parasitology 19, 545–546.

Blume, L., et al., 2010. Laboratory audit report: Lodge laboratory, Department of Biological Sciences, University of Notre Dame.

Bossenbroek, J.M., et al., 2009. Advances in ecological and economic analysis of invasive species: dreissenid mussels as a case study. In: Keller, R.P. (Ed.), Bioeconomics of Invasive Species: Integrating Ecology, Economics, Policy, and Management. Oxford University Press, Oxford, pp. 244–265.

Bott, N.J., et al., 2010. Toward routine, DNA-based detection methods for marine pests. Biotechnology Advances 28, 706–714.

Bowers, H.A., et al., 2006. Detection of *Pfiesteria* spp. by PCR in surface sediments collected from Chesapeake Bay tributaries (Maryland). Harmful Algae 5, 342–351.

Brammeier, J., 2010. Permanent prevention of Asian carp in the Great Lakes. Report to the Subcommittee on Water Resources and Environment, Committee on Transportation and Infrastructure.

Brammeier, J., et al., 2008. Preliminary feasibility of ecological separation of the Mississippi River and the Great Lakes to prevent the transfer of aquatic invasive species. Report to the Great Lakes Fishery Commission.

Brescia, C.C., et al., 2009. *Cryptosporidium* propidium monoazide-PCR, a molecular biology-based technique for genotyping of viable *Cryptosporidium* oocysts. Applied and Environmental Microbiology 75, 6856–6863.

Brown, E., et al., 2009. Interagency *Dreissena* monitoring plan for western waters. Draft report to the 100th Meridian Initiative.

Caesar, R.M., Sorensson, M., Cognato, A.I., 2006. Integrating DNA data and traditional taxonomy to streamline biodiversity assessment: an example from edaphic beetles in the Klamath ecoregion, California, USA. Diversity and Distribution 12, 483–489.

Carlton, J.T., Ruiz, G.M., 2005. The magnitude and consequences of bioinvasions in marine ecosystems: implications for conservation biology. In: Norse, E.A., Crowder, L.B. (Eds.), Marine Conservation Biology: The Science of Maintaining The Sea's Biodiversity. Island Press, Washington, DC, pp. 123–148.

Chick, J.H., 2002. Illinois waterway barrier workshop. Report to the Great Lakes Protection Fund.

Chick, J.H., Pegg, M.A., 2001. Invasive carp in the Mississippi River Basin. Science 292, 2250–2251.

Committee on Human Biomonitoring for Environmental Toxicants, 2006. Human Biomonitoring for Environmental Chemicals. National Academies Press, Washington, DC.

Cunningham, C.O., 2002. Molecular diagnosis of fish and shellfish diseases: present status and potential use in disease control. Aquaculture 206, 19–55.

D'Archino, R., et al., 2007. Invasive marine red alga introduced to New Zealand waters: first record of *Grateloupia turuturu* (Halymeniaceae, Rhodophyta). New Zealand Journal of Marine and Freshwater Research 41, 35–42.

Darling, J.A., Blum, M.J., 2007. DNA-based methods for monitoring invasive species: a review and prospectus. Biological Invasions 9, 751–765.

Darling, J.A., Tepolt, C.K., 2008. Highly sensitive detection of green crab (*Carcinus maenas* and *Carcinus aestuarii*) larvae in mixed plankton samples using polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP). Aquatic Invasions 3, 141–152.

Ficetola, G.F., et al., 2008. Species detection using environmental DNA from water samples. Biology Letters 4, 423–425.

Finnoff, D., et al., 2007. Take a risk: preferring prevention over control of biological invaders. Ecological Economics 62, 216–222.

Geller, J.B., et al., 2010. Genetic perspectives on marine invasions. Annual Review of Marine Science 2, 367–393.

Gittenberger, A., 2010. Schelpdier import monitoring protocol. GiMaRIS report issued by Vereniging van Importeurs van Schelpdieren (in Dutch).

Gittenberger, A., et al., 2010. Inventarisatie van de soortendiversiteit in tarra van mosselen uit Ierland, Verenigd Koninkrijk en Denemarken, voorjaar 2010. GiMaRIS report issued by Vereniging van Importeurs van Schelpdieren (in Dutch).

Hansen, M., 2010. Asian carp and the Great Lakes. Statement before the United States House of Representatives Committee on Transportation and Infrastructure, Subcommittee on Water Resources and Environment, Washington, DC.

Hayes, K.R., et al., 2005. Sensitivity and cost considerations for the detection and eradication of marine pests in ports. Marine Pollution Bulletin 50, 823–834.

Hebert, P.D.N., et al., 2003a. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London Series B-Biological Sciences 270, 313–321.

Hebert, P.D.N., et al., 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London Series B-Biological Sciences 270, S96–S99.

Hickey, V., 2010. The quagga mussel crisis at Lake Mead National Recreation Area, Nevada (USA). Conservation Biology 24, 931–937.

Hofreiter, M., et al., 2003. Molecular caving. Current Biology 13, R693–R695.

Hosler, D.M. Early detection of dreissenid species: zebra/quagga mussels in water systems. Aquatic Invasions 6, in press, doi:10.3391/ai.2011.6.2.

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Irons, K.S., et al., 2007. Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, USA—is this evidence for competition and reduced fitness? Journal of Fish Biology 71, 258–273.

Jerde, C., et al. Sight unseen detection of environmental DNA for surveillance of aquatic organisms at low densities. Conservation Letters, in press, doi:10.1111/j.1755-263X.2010.00158.x.

Jerde, C.L., et al., 2010. Final report: aquatic invasive species risk assessment for the Chicago sanitary and ship canal. Report to the United Sates Army Corps of Engineers, Environmental Laboratories, Cooperative Environmental Studies Unit, Vicksburg, Mississippi.

Kolar, C.S., et al., 2007. Bigheaded Carps: A Biological Synopsis and Environmental Risk Assessment. American Fisheries Society, Bethesda, MD.

Kwok, S., Higuchi, R., 1989. Avoiding false positives with PCR. Nature 339, 237–238.

Lam, P.K.S., Gray, J.S., 2003. The use of biomarkers in environmental monitoring programmes. Marine Pollution Bulletin 46, 182–186.

Lawton, J.H., Bignell, D.E., Bolton, B., Bloemers, G.F., Eggleton, P., Hammond, P.M., Hodda, M., Holt, R.D., Larsen, T.B., Mawdsley, N.A., Stork, N.E., Srivastava, D.S., Watt, A.D., 1998. Biodiversity inventories, indicator taxa and effects of habitat modification in tropical forest. Nature 391, 72–76.

Leung, B., et al., 2005. Managing invasive species: rules of thumb for rapid assessment. Ecological Economics 55, 24–36.

Leung, B., et al., 2002. An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. Proceedings of the Royal Society of London Series B-Biological Sciences 269, 2407–2413.

Lodge, D.M., 2010. Asian carp and the Great Lakes. Statement before the United States House of Representatives Committee on Transportation and Infrastructure, Subcommittee on Water Resources and Environment, Washington, DC.

Lodge, D.M., et al., 2006. Biological invasions: recommendations for US policy and management. Ecological Applications 16, 2035–2054.

Madison, B.L., 2007. Mathematical proficiency for citizenship. In: Schoenfeld, A.H. (Ed.), Assessing Mathematical Proficiency. Cambridge University Press, Cambridge, pp. 113–124.

Mahon, A.R., et al., 2010. Environmental DNA monitoring and surveillance: standard operation procedures. Report to the United States Army Corps of Engineers, Environmental Laboratories, Cooperative Environmental Studies Unit, Vicksburg, Mississippi.

Mallet, J., Willmott, K., 2003. Taxonomy: renaissance or Tower of Babel? Trends in Ecology and Evolution 18, 57–59.

Matsui, K., et al., 2001. Estimation of the fate of dissolved DNA in thermally stratified lake water from the stability of exogenous plasmid DNA. Aquatic Microbial Ecology 26, 95–102.

Mountfort, D., Hayden, B., 2006. Techniques for detection and enumeration of marine biosecurity risk species and compliance testing.

Parshionikar, S., et al., 2009. Method Validation of U.S. Environmental Protection Agency Microbiological Methods of Analysis.

Peabody, J., 2010. Asian carp and the Great Lakes. Statement before the United States House of Representatives Committee on Transportation and Infrastructure, Subcommittee on Water Resources and Environment, Washington, DC.

Pejchar, L., Mooney, H.A., 2009. Invasive species, ecosystem services and human well-being. Trends in Ecology and Evolution 24, 497–504.

Pimentel, D., et al., 1999. Environmental and economic costs of nonindigenous species in the United States. Bioscience 50, 53–65.

Qu, W., et al., 2009. MFEprimer: multiple factor evaluation of the specificity of PCR primers. Bioinformatics 25, 276–278.

Raut, A.D., Kapadnis, B.P., 2007. Nonspecific PCR amplification of the 16S rRNA gene segment in different bacteria by use of primers specific for *Campylobacter*, *Arcobacter*, and *Helicobacter* spp. Journal of Clinical Microbiology 45, 1376–1377.

Ricciardi, A., 2007. Are modern biological invasions an unprecedented form of global change? Conservation Biology 21, 329–336.

Rilov, G., Crooks, J.A. (Eds.), 2009. Biological Invasions in Marine Ecosystems: Ecological, Management, and Geographic Perspectives. Springer-Verlag, Berlin.

Rothlisberger, J.D., et al., 2010. Future declines of the binational Laurentian Great Lakes fisheries: the importance of environmental and cultural change. Frontiers in Ecology and the Environment 8, 239–244.

Saks, M.J., Koehler, J.J., 2005. The coming paradigm shift in forensic identification science. Science 309, 892–895.

Schwieterman, J.P., 2010. An analysis of the economic effects of terminating operations at the Chicago river controlling works and O'Brien locks on the Chicago area waterway system.

Sikder, I.U., et al., 2006. Knowledge-based risk assessment under uncertainty for species invasion. Risk Analysis 26, 239–252.

Smith, P., et al., 2008. Identification of suspect mussels (*Perna*) in New Zealand. Report to the National Institute of Water and Atmospheric Research.

Smith, P.J., et al., 2007. First record of the Australian ascidian *Eudistoma elongatum* in northern New Zealand. New Zealand Journal of Marine and Freshwater Research 41, 347–355.

Smith, P.J., et al., 2003. DNA and morphological identification of an invasive swimming crab, *Charybdis japonica* (A. Milne-Edwards, 1861), in New Zealand waters. New Zealand Journal of Marine and Freshwater Research 37, 753–762.

Strayer, D.L., 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshwater Biology 55, 152–174.

Touza, J., et al., 2007. Economic analysis of invasive species policies. In: Nentwig, W. (Ed.), Biological Invasions. Springer, Berlin, pp. 353–366.

United States Army Corps of Engineers, 2010. Dispersal Barrier Efficacy Study: Interim IIIA—Fish Dispersal Deterrents, Illinois and Chicago Area Waterways Risk Reduction Study and Integrated Environmental Assessment.

United States Environmental Protection Agency, 2010. Method A: Enterococci in water by TaqMan quantitative polymerase chain reaction (qPCR) assay.

Valentini, A., et al., 2009. DNA barcoding for ecologists. Trends in Ecology and Evolution 24, 110–117.

Vander Zanden, M.J., et al., 2010. A pound of prevention, plus a pound of cure: early detection and eradication of invasive species in the Laurentian Great Lakes. Journal of Great Lakes Research 36, 199–205.

Willerslev, E., et al., 2007. Ancient biomolecules from deep ice cores reveal a forested Southern Greenland. Science 317, 111–114.

Wimbush, J., et al., 2009. Eradication of colonizing populations of zebra mussels (*Dreissena polymorpha*) by early detection and SCUBA removal: Lake George, NY. Aquatic Conservation-Marine and Freshwater Ecosystems 19, 703–713.

van Pelt-Verkuil, E., et al., 2008. Principles and Technical Aspects of PCR Amplification. Springer, Berlin.

Internet references

Dahlman, S., 2010. Chicago river businesses to corps of engineers: show us the carp! Marina City news, Chicago. 〈http://www.marinacityonline.com/news/carp0318.htm〉. Accessed October 14, 2010.

Egan, D., 2010. Symied by high court, states ponder options on Asian carp. Milwaukee Wisconsin Journal Sentinel (online), Milwaukee. 〈http://www.jsonline.com/news/wisconsin/92091904.html〉. Accessed October 14, 2010.

Egan, D., 2010b. Asian carp search turns up nothing. Milwaukee Wisconsin Journal Sentinel (online), Milwaukee. 〈http://www.jsonline.com/news/wisconsin/87629187.html〉. Accessed October 14, 2010.

Frede, L., 2010. Asian carp solution: use science, not scare tactics. The Hill, Washington DC. 〈http://thehill.com/blogs/congress-blog/energy-a-environment/108371-asian-carp-solution-use-science-not-scare-tactics〉. Accessed October 14, 2010.

Frede, L., Denzler, M., 2010. Human introduction confirmed in Asian carp discovery. Unock Our Jobs. 〈http://www.unlockourjobs.org/2010/08/05/press-release-human-introduction-confirmed-in-asian-carp-discovery/〉. Accessed October 14, 2010.

Hood, J., 2010. Asian carp creeps into Lake Calumet. Chicago Breaking News Center, Chicago. 〈http://www.chicagobreakingnews.com/2010/06/officials-asian-carp-found-in-chicago-waterway-system.html〉. Accessed October 14, 2010.

Hall, N., 2010a. Five states file new lawsuit against federal government to protect Great Lakes from Asian carp. Great Lakes Environmental Law Center. 〈http://www.greatlakeslaw.org/blog/asian-carp/〉. Accessed October 14, 2010.

Hall, N., 2010b. Another setback in the legal fight to keep Asian carp out of the Great Lakes. Great Lakes Environmental Law Center. 〈http://www.greatlakeslaw.org/blog/2010/12/another-setback-in-the-legal-fight-to-keep-asian-carp-out-of-the-great-lakes.html〉. Accessed January 13, 2011.

Utah Division of Wildlife Resources, 2010. Threats and prevention efforts in Utah. 〈http://wildlife.utah.gov/mussels/status_defined.php〉. Accessed October 14, 2010.

Whan, S., 2010. Tests clear Twofold Bay of exotic sea squirt invasion. 〈http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0007/334249/Tests-clear-Two fold-Bay-of-exotic-sea-squirt-invasion.pdf〉. Accessed October 14, 2010.

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