Biol Invasions (2019) 21:377–389
https://doi.org/10.1007/s10530-018-1862-5

ORIGINAL PAPER

Using eDNA, sediment subfossils, and zooplankton nets to detect invasive spiny water flea (*Bythotrephes longimanus*)

Jake R. Walsh · Michael J. Spear · Thomas P. Shannon · Patrick J. Krysan · M. Jake Vander Zanden

Received: 26 January 2018 / Accepted: 30 August 2018 / Published online: 25 October 2018

Abstract In light of the ongoing spread and adverse impacts of invasive species, there is an urgent need to develop more effective monitoring and management strategies. Such efforts are constrained by our limited capacity to efficiently detect invasive species. Here, we present the case of *Bythotrephes longimanus* (spiny water flea) invasion into Wisconsin lakes. Detecting *Bythotrephes* has proven to be challenging due to its capacity to persist at low densities and its highly seasonal population dynamics. We use *Bythotrephes* to explore detection using three monitoring methods: zooplankton net tows, environmental DNA (eDNA), and sampling of *Bythotrephes* tail spine subfossils in sediments. Detection probabilities were highly seasonal for both the net tow and eDNA sampling methods—though detections occurred one to two weeks earlier in net tows—and seasonal targeting substantially improved detection by both methods. Conversely, *Bythotrephes* spine subfossils were found in all 10 lakes with confirmed *Bythotrephes* populations and in all five samples taken from each lake, except for a single lake where four of the five samples had subfossils. This method was insensitive to seasonally varying population densities as sediments integrate over variation in population densities. In this case, detection and abundance estimation were well covered by sediments and zooplankton nets, respectively, and eDNA provided little additional benefit to surveillance. Our work highlights the importance of choosing methods that address both species life history and monitoring objectives when designing surveillance programs.

Keywords Invasive species · Detection · *Bythotrephes* · Lake sediment · eDNA

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10530-018-1862-5) contains supplementary material, which is available to authorized users.

J. R. Walsh (✉) · M. J. Spear · T. P. Shannon · M. J. Vander Zanden
Center for Limnology, University of Wisconsin – Madison, 680 N Park St., Madison, WI 53706, USA
e-mail: jransom.walsh@gmail.com

P. J. Krysan
Genome Center of Wisconsin and Department of Horticulture, University of Wisconsin – Madison, 575 Linden Dr., Madison, WI 53706, USA

Introduction

Invasive species are an issue of global concern, as a result of their accelerating spread coupled with their adverse ecological, economic, and human health impacts (Lodge et al. 2006; Simberloff 2011). At the heart of efforts to improve management and minimize impacts of invasive species is the fundamental issue of detection. Effective management requires basic information such as which sites are occupied by an invasive

species (Leung and Delaney 2006). Moreover, early detection of invasive species populations is widely recognized as a precursor for successful invasive species eradication and control (Hulme 2006; Simberloff 2014).

While invasive species detection may be a manageable task when considering a single site (i.e., lake), we are often concerned about invasive species on landscapes comprised of many sites. Limited monitoring resources generally prevent sampling of all possible sites (Leung and Delaney 2006). Moreover, even if an invasive species is present, it may not be detected if the species has seasonal dynamics, occurs at low densities, or if sampling methods are not well-suited for invasive species detection. This is particularly true in cases where monitoring is optimized for assessing overall community structure or estimating invasive species abundance rather than detecting a specific invasive species (Magurran and McGill 2011). For example, Harvey et al. (2009) found that zooplankton sampling protocols designed to assess community structure would often fail to detect the invasive fishhook waterflea (*Cercopagis pengoi*) in Lake Ontario. Delaney and Leung (2010) found that a 'total area search' sampling approach dramatically increased the probability of detecting an invasive coastal marine crab over use of quadrat sampling. The timing of sampling is also an important factor for species with seasonal migrations, behavior, or life-histories (e.g., O'Connell et al. 2006; Vine et al. 2009). Therefore, understanding which detection methods are robust to challenges associated with species detection (e.g., low densities, seasonality) will be critical for designing effective surveillance programs for aquatic invasive species.

This study uses the recent invasion of spiny water flea, *Bythotrephes longimanus*, in inland lakes of the Laurentian Great Lakes region as a model to further explore these challenges. *Bythotrephes* is an invasive predatory zooplankter that invaded the Laurentian Great Lakes in the early 1980s and has subsequently spread to hundreds of inland lakes throughout the Laurentian Great Lakes region (Yan et al. 2011). Notable ecological and economic impacts have been documented in invaded lakes with long-term monitoring programs (e.g., Harp Lake, ON, Canada—Yan and Pawson 1997a, Lake Mendota, WI, USA—Walsh et al. 2016a). Detection of *Bythotrephes* well after its initial establishment has been reported in Island Lake, MN, USA, (Branstrator et al. 2017) and Lake Mendota, WI, USA (Walsh et al. 2016b), and is likely due to *Bythotrephes* ability to persist at low densities (Drake 2004; Wittmann et al. 2011), its highly seasonal population dynamics that can vary by lake (e.g., Young et al. 2011), and its patchy distribution in lakes (e.g., swarming or diel vertical migration; Young and Yan 2008). In the case of Lake Mendota, sediment coring data indicated that *Bythotrephes* was present at low densities for at least a decade prior to the initial discovery and outbreak in 2009 (the year of a favorable, cool summer) despite three decades of intensive biweekly zooplankton net sampling (Walsh et al. 2016b). The undetected, low-density *Bythotrephes* populations of both Lake Mendota and Island Lake increased to high, impactful densities (Walsh et al. 2016b; Branstrator et al. 2017), demonstrating the potential for invasive species populations to undergo abrupt shifts.

In this study, we compile the results of our *Bythotrephes* sampling in 10 north temperate lakes from 2010 to 2016. Over this period, we used three different sampling approaches to monitor and detect *Bythotrephes* populations: zooplankton net tows, sediment sampling, and environmental DNA (eDNA). Zooplankton net tows are considered the traditional method for sampling an invasive zooplankter and are commonly used to estimate water column density. However, since *Bythotrephes* are known to have strong seasonal dynamics (Brown and Branstrator 2011; Walsh et al. 2016b) and single zooplankton tows only provide a snapshot of something that is likely to be temporally variable, zooplankton net sampling may fail to detect species. In the past several years, eDNA methods have emerged as promising tools for detecting aquatic invasive species (Ficetola et al. 2008; Jerde et al. 2011), and even estimating invasive species abundance (Takahara et al. 2012). The approach has not been widely applied to detection of invasive zooplankton, and macro invertebrates have proven to be challenging to detect by eDNA (Deiner and Altermatt 2014; Tréguier et al. 2014; Mächler et al. 2014; Roussel et al. 2015). Some have speculated that exoskeletons might limit shedding of some invertebrates' DNA into the environment (Tréguier et al. 2014), though regular molting could provide pulses of shed cells (Deiner and Altermatt 2014). Additional work is necessary to understand when eDNA is a useful tool for detection of invertebrates (Roussel et al.

2015; de Souza et al. 2016). Finally, *Bythotrephes* tail spines are conspicuous, resistant to degradation, and accumulate in lake sediments (Beranek 2012; Walsh et al. 2016b; Branstrator et al. 2017). Since lake sediments integrate in-lake processes over time, sediment sampling is likely to provide a temporally stable signal, which could have important advantages for determining species presence and abundance.

For invasive species monitoring programs to be effective, we need to understand the different sampling approaches as well as their idiosyncrasies and utility for addressing specific objectives. What does a species' population seasonality mean for how we choose to sample? How are sampling effort, population density, and the probability of detection related? Here, we use invasive *Bythotrephes* populations in Wisconsin and Michigan (USA) as an opportunity to compare three sampling approaches. First, we compare zooplankton nets and sediment subfossils across ten lakes with established *Bythotrephes* populations. We then use a case study of *Bythotrephes* detection in Lake Mendota—where it reaches high abundances but is absent in the water column for the first half of the open-water season—to compare detection by zooplankton nets and eDNA. In addition to providing information to improve the detection of our study species, our comparative study yields insights into the promise and limitations of different methods in addressing different surveillance challenges (e.g., population seasonality) and objectives (e.g., detection or abundance estimation) that will help guide future surveillance efforts.

Methods

Comparing zooplankton nets and sediment subfossil sampling

We surveyed 10 north temperate lakes with verified reports of *Bythotrephes* using zooplankton net and sediment subfossil sampling (Table 1). These lakes were spread over two lake districts: four lakes from the Yahara River watershed in Dane County WI, hereafter "southern lakes" (Lake Mendota, Lake Monona, Lake Waubesa, and Lake Kegonsa), and 6 lakes from Northern Wisconsin (Forest, Vilas, and Iron Counties; Gile Flowage, Stormy Lake, Butternut Lake, Star Lake, and Trout Lake) and Upper Michigan (Lake Gogebic in Gogebic and Ontonagon Counties), hereafter "northern lakes".

Vertical zooplankton net tows are a common method used for zooplankton monitoring programs and we used this method to estimate *Bythotrephes* abundance in the water column (e.g., US-EPA 2016). We lowered a conical net of 500-µm mesh measuring 50-cm in diameter to 1 m above the sediment at the deepest location on each lake and then raised the net to capture zooplankton in the water column at a rate of $\sim$ 0.3 m/s. In lakes with oxidized hypolimnia (generally, the northern lakes here), it is possible that *Bythotrephes* diel vertical migration could influence its probability of detection and population density estimates as all tows were taken during the day and individuals could be located or clustered at depths within 1 m of the lake bottom. While migration is often dampened in this species due to trade-offs between predator avoidance and optimal habitat (Young and Yan 2008), this could influence our results in lakes where *Bythotrephes* migrates vertically. Zooplankton net sampling data from Stormy Lake, the Gile Flowage, and Lake Gogebic were provided by an external source using the same collection methods (Carol Warden, Wisconsin Department of Natural Resources and University of Wisconsin - Madison Trout Lake Station). Samples were processed in their entirety to estimate *Bythotrephes* densities in net tows. To estimate *Bythotrephes* mean annual population density in each lake with long-term monitoring and multiple samples taken within years (Lake Mendota, Lake Monona, Lake Waubesa, Lake Kegonsa, Gile Flowage, Stormy Lake, Trout Lake, and Lake Gogebic), we calculated the long-term average July–November density across the three tows taken each sampling date (individuals m^{-3}). Generally, sampling began in early May and was conducted fortnightly through early December, except in Lake Gogebic where sampling was conducted monthly. For analysis, we observe detection over the entire year, over a typical field season ("summer"; June–August), and over a field season targeted toward peak *Bythotrephes* abundance ("fall"; August–November). We calculated the probability of detecting *Bythotrephes* using a zooplankton net as the proportion of net tow samples containing at least one *Bythotrephes*. Since samples were processed in their entirety, any tow with at least one individual was considered a presence. Two lakes, Butternut Lake and Star Lake,

Table 1 Summary of 10 study lakes and detection method results

Lake (County)	Year of detection	Net detection probability (N)	Ekman detection probability (N)	Ekman density, subfossils per Ekman (S.D.)
Butternut Lake (Forest)	2014	0 (1)	1.0 (5)	720 (390)
Gile Flowage (Iron)	2003	0.87 (96)	1.0 (5)	1300 (240)
Lake Kegonsa (Dane)	2009	0.30 (33)	1.0 (5)	530 (230)
Lake Mendota (Dane)	2009	0.71 (1008)	1.0 (9)	12,000 (6200)
Lake Monona (Dane)	2009	0.22 (66)	1.0 (6)	900 (470)
Lake Waubesa (Dane)	2009	0.14 (42)	1.0 (5)	260 (220)
Star Lake (Vilas)	2013	1 (1)	1.0 (5)	190 (230)
Stormy Lake (Vilas)	2007	0.79 (204)	0.8 (5)	50 (50)
Trout Lake (Vilas)	2014	0.67 (103)	1.0 (5)	200 (330)
Lake Gogebic (Gogebic & Ontonagon, MI)	mid-1990s	0.77 (27)	1.0 (5)	2500 (810)

Detection probabilities are included with sample sizes (N) in parentheses. Densities of tail spine subfossil fragments in Ekman sediment grabs are included with the standard deviation (S.D.) over all grabs within a lake

were sampled only once with a zooplankton net (Table 1), though we include these lakes in the study as they were sampled multiple times using an Ekman grab. To obtain a single detection probability for each method across the ten study lakes, total gear detection probabilities were calculated as the average of mean lake detection probabilities (Table 1).

We sampled the sediments of each lake using five standard Ekman grab samples ($\sim$ 3.7 L sediment, the Ekman is a cube roughly 15.2 cm on an edge; note that we sampled Lake Mendota 9 times and Lake Monona 6 times; Table 1). Upon retrieval, we scraped and preserved the top 1 cm of the sediment sample to compare differences in tail spine densities in more recently laid sediment with sediment in the remaining Ekman grab. We then filtered the rest of the Ekman sample in the field through a 500-μm mesh. In the laboratory, we filtered both the top 1 cm and the remaining Ekman samples through a 500-μm mesh for additional sample cleaning. We identified and counted *Bythotrephes* tail spine fragments using a dissecting microscope and methods outlined in Beranek (2012). We counted and recorded each subfossil fragment (i.e., spine fragments), assuming each would constitute evidence of *Bythotrephes* presence in a lake. However, we do note that tail spine fragmentation might vary by lake depending on factors such as sediment conditions and fish digestion, potentially obscuring our comparisons of total subfossils across lakes. The top 1 cm samples were processed in their entirety, while the remaining sediment from each Ekman grab was sub-sampled using five, 5-mL subsamples with a Hensen–Stempel pipette in a homogenized beaker of suspended sample (often 400 mL total volume, $\sim$ 1% of the whole sample, but diluted to 800 mL for samples with many subfossils). The probability of detecting *Bythotrephes* in each lake is the proportion of sediment samples containing at least 1 tail spine collected in the top 1 cm layer or the five, 5-mL sub-samples.

Comparing zooplankton net and eDNA sampling in Lake Mendota

We compared the probability of detection using zooplankton nets and eDNA in Lake Mendota in 2016. We surveyed Lake Mendota weekly from May to September 2016, collecting a zooplankton net tow and a single surface water sample for eDNA at seven sites on each sampling trip. This paired sampling approach allowed for a 1:1 cross-comparison of zooplankton net and eDNA detection rates.

Bythotrephes is more abundant in Lake Mendota than in any lake surveyed here (annual peak densities > 150 individuals m^{-3}; mean densities in all lakes in Fig. S1), which makes Lake Mendota an ideal system to test detection by eDNA. For each sampling trip in 2016, we collected a single 0.5 L surface water sample in polypropylene containers at seven sites across Lake Mendota weekly (for seven total

samples). We sampled at the surface of Lake Mendota for *Bythotrephes* because vertical depth profiles indicate the species is found most often in the top 5 m of the lake, particularly during periods of hypolimnetic anoxia (Walsh *unpublished data*, Spear *unpublished data*). As *Bythotrephes* capacity for diel vertical migration can vary by lake, this should be considered when designing lake sampling surveys using eDNA. We stored samples in coolers for 1–4 h before vacuum-filtering water samples onto 0.7 µm pore size, 47 mm diameter glass microfiber filters (cat. no. 1825047, Whatman). Following overnight incubation at room temperature (ca. 25 °C) in 900 µL of Longmire's cell lysis buffer in 2 mL snap-top microcentrifuge tubes (Renshaw et al. 2015), we performed a phenol:chloroform:isoamyl (25:24:1) extraction and precipitated DNA from the aqueous phase with 500 µL of ice cold isopropanol and 250 µL of room temperature 5 M NaCl. We washed the resulting DNA pellets twice in 70% ethanol and allowed them to air dry for 20 min, then rehydrated them in 200 µL of a buffer solution of 10 mM Tris–HCl and 1 mM Na-EDTA. This extraction protocol is adapted from Renshaw et al. (2015). Extracted samples were stored at − 20 °C until amplification (1–30 days).

We performed Real-Time qPCR on extracted DNA using a BIO-RAD C1000 Thermal Cycler equipped with a CFX96 Real-Time System (Bio-Rad, Hercules, CA). Reactions used Ex Taq HS DNA Polymerase Hot-Start Version (TaKaRa Bio, USA) and a PCR primer pair specific for *Bythotrephes longimanus* (Forward: 5′-GCAGGAACTGGCTGAACA-3′, Reverse: 5′-AATAATAAAAGGAGGGCTGTAATACC-3′, amplicon length: 227 bp) targeting the mitochondrial cytochrome oxidase I (COI) region. This primer pair, designed in this study, was validated using DNA extracted from *Bythotrephes longimanus* tissue harvested from Lake Mendota. The specific sequences targeted by the primers were chosen because they had several mismatches with homologous regions of *Daphnia spp.* Specificity for the primers for spiny water flea was validated by Sanger sequencing of eDNA templates amplified from Lake Mendota water samples. PCR reactions contained 1X ExTaq PCR buffer (TaKaRa Bio, USA), 200 nM of each PCR primer, 200 µM of each dNTP, and 0.2X EvaGreen dye for real-time PCR detection (cat. no. 3100, Biotium, Fremont, CA). The following PCR profile was used: 55 cycles, T_d: 10 s at 96 °C, T_a: 30 s at 58 °C, T_e: 30 s at 68 °C. We performed four amplification reactions for each sample. A positive result in any of those reactions was interpreted as a positive result for that sample. Melt-curve analysis of the PCR products was performed at the end of each PCR to discriminate target amplicons from primer-dimers and other non-target products. Positive results were validated by matching the melt curve profiles to those obtained using tissue-extracted positive controls, also run in quadruplicate during each PCR. Positive products were also spot-checked for validity using Sanger sequencing and compared to the COI sequences of *Bythotrephes longimanus* available in GenBank. Samples were considered negative if no product amplified or if the product did not match the melt-curve of the positive controls. Negative control samples (Milli-Q Ultrapure water) were included at the collection, filtration, extraction, and amplification steps of the protocol. For collection control, one 0.5 L sample bottle was filled with Milli-Q water in the laboratory, taken into the field, uncapped, and lowered toward the water before being stored and filtered with the other sample bottles. For filtration control, 0.5 L of Milli-Q water was filtered alongside that date's samples. For extraction and amplification control, Milli-Q water was used in place of equivalent sample volume. We treated all equipment and surfaces with a 10% bleach solution followed by rinsing with clean water prior to each usage. Extracted DNA samples have been archived at − 20 °C. We report the probability of detection via eDNA in two ways. First, we consider a positive amplification (at least one of four replicate reactions) of any of the total seven samples on a given sampling trip as a positive detection for that entire trip (the probability of detection in a seven-sample survey). Most detection efforts using eDNA are conducted as surveys. However, we were interested in scaling single-sample probabilities across different sampling scenarios as well as directly comparing eDNA and zooplankton net methods (a single water sample for eDNA and a single net tow require similar sampling effort). For these purposes, we defined eDNA detection probability in a single water sample as the proportion positive amplifications (at least one of four replicate reactions) out of the total seven samples collected from Lake Mendota on a given sampling trip.

We used this cross-comparison survey to model the relationship between *Bythotrephes* population density

and detection from each method using generalized linear models ("glm"; R Core Team 2016) with binomial errors and a logit link function, regressing *Bythotrephes* presence and absence in each net or eDNA sample on $\log_{10}$ transformed population density. Here, the probability of detecting *Bythotrephes* in a sediment sample was always one since we have never failed to detect *Bythotrephes* using an Ekman grab in Lake Mendota.

Results

Zooplankton net and sediment subfossil sampling

The probability of detecting *Bythotrephes* using vertical zooplankton net tows varied from 0.14 (Lake Waubesa; 0.03 in summer, 0.25 in fall) to 0.87 (Gile Flowage; 0.94 in summer, 0.89 in fall) in lakes with more than one zooplankton tow over the study period (mean for all lakes = 0.56; Fig. 1, Table 1). Furthermore, the probability of detection using zooplankton net tows was highly seasonal (Fig. 1). *Bythotrephes* was nearly twice as likely to be detected in fall (P[detection] = 0.87) than in summer (P[detection] = 0.46) using net tows. This discrepancy was larger in southern lakes (0.82–0.20, respectively) relative to the northern lakes surveyed here (0.90–0.71, respectively). Similarly, *Bythotrephes* was reliably detected in net tows two months earlier in the northern lakes (0.87 in July in the northern lakes compared to 0.90 in September in the southern lakes). Targeting *Bythotrephes* monitoring to fall months (August–November) improved detection probabilities in all lakes (Fig. 1).

We detected *Bythotrephes* tail spines in all sediment samples except one of five samples from Stormy Lake (overall probability of detection, "P[detection]" = 0.8; Table 1). Mean tail spine fragment density in sediment samples ranged from 50 subfossils per sample in Stormy Lake to 12,000 subfossils per sample in Lake Mendota. Subfossil density was higher in the top 1 cm of sediment samples compared to density in the entire sediment sample in all lakes except Lake Kegonsa. The top 1 cm of the sediment sample ($\sim$ 6% by sediment volume) contained as many as 77% of the subfossils in the entire sediment sample for Stormy Lake and as few as 4.6% of the subfossils in Lake Kegonsa.

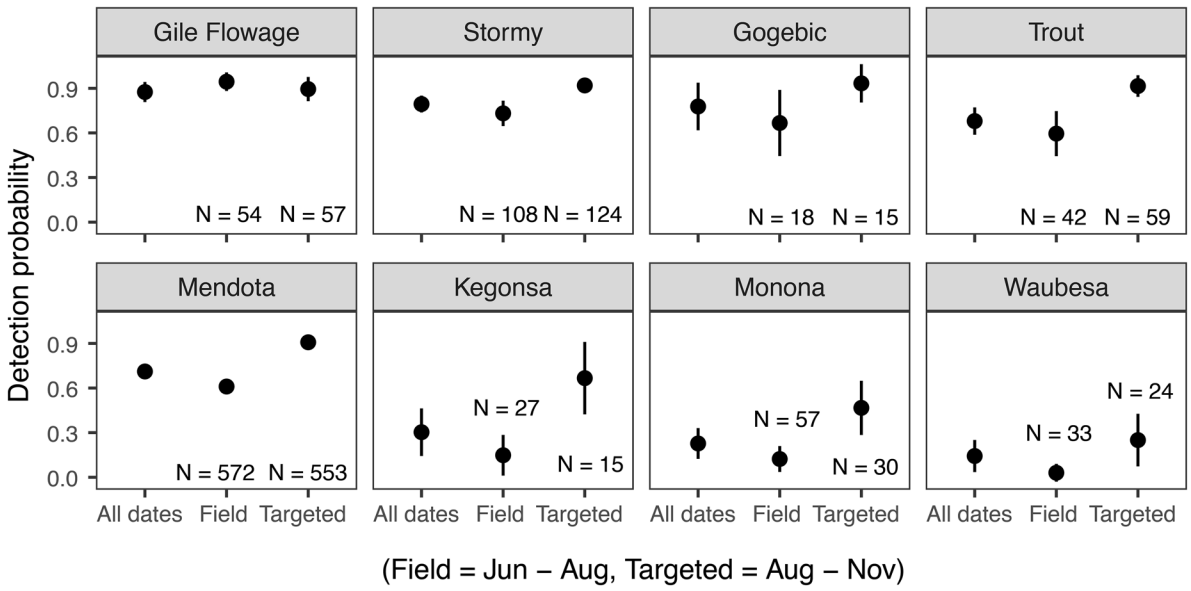

Fig. 1 Long-term net detection probability over all lakes with known populations of *Bythotrephes* (northern lakes top panels, southern lakes in bottom panels) for all sample dates, sample dates collected during a typical field season in summer, and sample dates during a targeted effort during fall peak abundance. Lines represent 95% confidence intervals (N = sample size)

Comparing environmental DNA to zooplankton nets in Lake Mendota

We concurrently collected water samples for eDNA and performed zooplankton net tows in Lake Mendota in 2016. Only one sampling date (17 August) exhibited false-positive eDNA detection (the filtration negative control). We failed to detect *Bythotrephes* using eDNA from May through mid-July in Lake Mendota in 2016 (Fig. 2a). *Bythotrephes* was detected earlier in zooplankton net tows (27 June) than eDNA samples (18 July) and was detected in net tows but not by eDNA across three trips from 27 June through 12 July (when densities increased from 0.78 to 30 individuals m^{-3}). Detection probability by eDNA increased with net-estimated water column densities, though with a lag of 1–2 weeks. eDNA produced lower detection probabilities than did net sampling throughout 2016 (Fig. 2a). The average probability of detection by a positive eDNA amplification in a seven-sample survey over the entire sampling season was 0.60 (0.16 single-sample probability).

The probability of detection using eDNA and zooplankton nets increased rapidly with *Bythotrephes* population density, but was higher overall for zooplankton net sampling (logit P[detection] = 3.5 ± 0.95 s.e. * $\log_{10}$ density − 0.62 ± 0.75 s.e.) than eDNA (logit P[detection] = 2.7 ± 1.1 s.e. * $\log_{10}$ density − 4.7 ± 1.6 s.e.), even when considering all seven spatial eDNA samples as one sampling effort (Fig. 2b; using the fitted formula to calculate the probability of detection in seven rather than one eDNA water sample). *Bythotrephes* was detected in all sediment samples from Lake Mendota. *Bythotrephes* was reliably detected using a zooplankton net by early July (P[net detection] = 0.95 ± 0.03 s.e. at 10 ind/m^3).

Overall comparison in Lake Mendota

Mean detection probabilities were higher for samples of lake sediments than both zooplankton nets and eDNA sampling of the water column (Fig. 3a). Over all lakes, the mean probability of detection was 0.89 for sediment samples and 0.49 for zooplankton nets. In Lake Mendota, the mean probability of detection was 1.0 for sediment samples, 0.71 for a single zooplankton net sample, and 0.60 for eDNA as a seven-sample survey (Fig. 3a).

Discussion

Programs aimed at minimizing invasive species spread and adverse impacts typically involve a combination of strategies: prevention, early detection, control, and eradication (Hulme 2006). Detection of

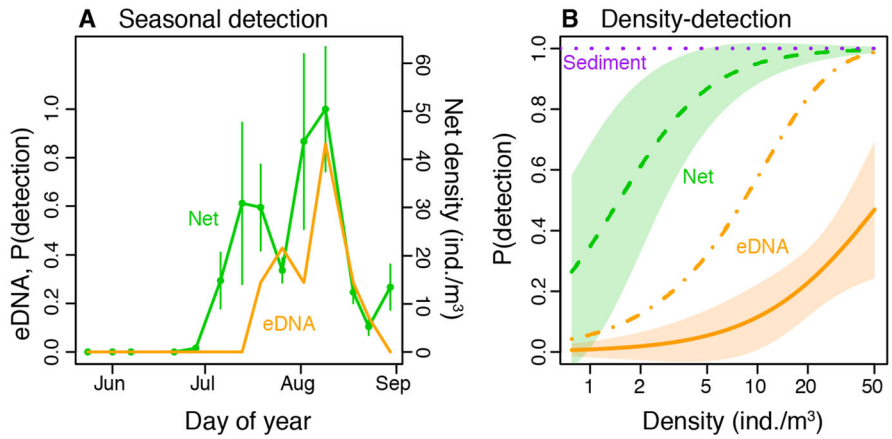

Fig. 2 **a** Comparison of the probability of detection by eDNA (orange) and zooplankton net density (± s.d.; green) in the cross comparison from Lake Mendota in 2016. Note that the probability of detection using a zooplankton net increases rapidly to one between 1 and 10 individuals m^{-3} (e.g., between late June and early July), which occurs each year as the *Bythotrephes* population in Lake Mendota is exceptionally abundant. **b** The relationships between population density and detection for each method for each sampling method in Lake Mendota (the relationship between density and detection in lake sediments is plotted with a purple dotted line, net detection with a green dashed line, eDNA single sample detection in an orange solid line, and eDNA seven sample detection in an orange dotted and dashed line)

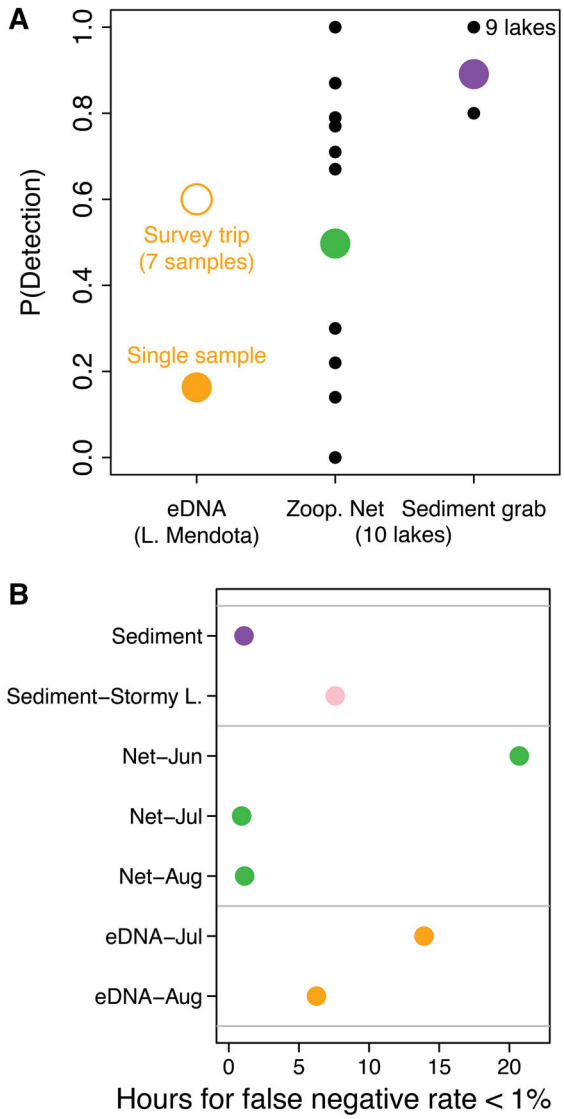

Fig. 3 **a** Summary of detection probability for the three methods. Large color-coded circles represent multi-lake mean detection probabilities for each method (eDNA in orange with a solid circle representing a single sample and an open circle representing seven samples in a multi-sample survey trip; zooplankton net in green; sediment grab in purple) and small solid black circles represent individual lake detection probabilities for net and sediment sampling. Note that the probability of detection using eDNA was calculated from Lake Mendota alone (in Lake Mendota P[detection, zooplankton net] = 0.71, P[detection, sediment] = 1.0). **b** Comparison of the three methods from the 2016 cross comparison in Lake Mendota in terms of the effort required to reduce false negative rates below 1% (color scheme as in A, see Supplementary Information for more details)

invasive species populations is a key part of their surveillance and is often at the heart of management efforts—it provides the basis for knowing at which locations a species occurs and informs subsequent management decisions (Bobeldyk et al. 2015; Leung and Delaney 2006). In many situations, we are dealing with a large number of potentially invasible sites. For example, the state of Wisconsin has ~ 15,000 inland lakes. Sampling all of them is cost-prohibitive and highlights the need for sampling methods and protocols that are maximally efficient for detecting populations. Moreover, detection of invasive species is particularly challenging in aquatic systems, which are often not amenable to direct observational sampling, and instead involves a grab bag of other sampling techniques. Other factors that make invasive species detection a challenge is that populations of invasive species sometimes occur and persist at low densities (e.g., Walsh et al. 2016b) and may exhibit seasonality that further reduces detection probability (e.g., Young et al. 2011). While we present a case of a single species (and a single lake in the case of eDNA comparison), our study highlights the value of strategically considering elements of invasive species' life histories (e.g., seasonality in population densities) that influence their detection. We found that the integrated record provided by sediment sampling or zooplankton net tows outperformed eDNA, and detection by nets and eDNA improved in seasonally-targeted scenarios. In the following paragraphs, we outline how understanding these differences among sampling methods as well as the life history of our study species can help inform the approach used to detect and monitor the species.

Detection by zooplankton nets

Bythotrephes monitoring has traditionally consisted of zooplankton net tows through the water column at a lake's deepest point. However, *Bythotrephes* population densities are highly seasonal (Brown and Branstrator 2011; Walsh et al. 2016b), giving a strong seasonal component to the probability of detection. We found that *Bythotrephes* was much more likely to be detected in late summer and fall than in early summer, which is consistent with other studies of *Bythotrephes* seasonal abundance (e.g., Berg and Garton 1988; Yan and Pawson 1997b; Brown and Branstrator 2011; Kerfoot et al. 2016). This discrepancy was much more extreme in the southern lakes

surveyed here where *Bythotrephes* densities were lower through early and mid-summer, likely due to high summer surface water temperatures (Walsh et al. 2016b). Most invasive species monitoring is conducted during summer months (e.g., June–August). Our sampling revealed that targeting sampling effort to late summer and fall months (August–November) increased detection probabilities in all lakes. Therefore, detecting species with seasonally variable abundance involves understanding the pattern of seasonality (e.g., abundant later in open water season), as well as potential regional variation in seasonality (e.g., abundant in fall in southern lakes versus summer and fall in northern lakes). Spatial variation in the seasonality of invasive species abundance is common and possibly related to climate and the phenology of species life history (e.g., Mwatawala et al. 2006; Nielsen et al. 2011; Churchill 2013), suggesting that more attention should paid to understanding variation in seasonality when designing surveillance programs.

Detection by lake sediments

Sediment sampling reliably detected *Bythotrephes* and we found evidence of *Bythotrephes* in all five sediment samples from 9 of the 10 lakes surveyed. The exception, Stormy Lake (4/5 detections), highlights several limitations of using lake sediments to detect and monitor invasive populations. Stormy Lake has a low density *Bythotrephes* population (5.7 individuals m^{-3}, mean July–November density; Fig. S1), which likely drives a low spine sedimentation rate. However, we processed a relatively small amount of sediment in sub-samples of the entire Ekman sediment grab. Processing additional sediment in the laboratory is a low-cost way to increase detectability by sediment sampling in low-density lakes (as in Stormy Lake; Fig. 3b). Stormy Lake's sediments are flocky (i.e., loose) and were challenging to core reliably with either gravity corers or Ekman grabs. We also found that sandy sediments (e.g., Butternut Lake) and sediments with a high density of coarse materials (e.g., wood, rocks, and leaves such as in the Gile Flowage) keep the Ekman grab from fully closing. Coarse material also obstructs processing under a dissecting microscope, though this was easily circumvented here by filtering through an additional coarse (> 1 mm) sieve and processing coarse material separate from fine material. Similarly, detecting *Bythotrephes* in lotic systems like rivers or reservoirs may prove more challenging as sediment export may flush tail spines before they are accumulated on lake or river bottoms. For example, *Bythotrephes* tail spines are not readily detectable in the sediment of the Fox River (Wisconsin; flows into Green Bay of Lake Michigan) even though they have been detected in the river water by zooplankton nets repeatedly (Bart T. De Stasio, *personal communication*). Targeting sampling to depositional habitats may improve detection in lotic systems. We also note that caddisfly larvae captured in sediment samples from Lake Gogebic systematically incorporate tail spines into their shell casings, possibly aggregating spines in habitats with caddisfly larvae. Spine detection in caddisfly casings represented the vast majority of spine detections in Lake Gogebic.

Detection by environmental DNA

The probability of detecting *Bythotrephes* using eDNA lagged behind detection by traditional zooplankton net sampling in the 2016 cross-comparison in Lake Mendota (Fig. 2a). eDNA is highly sensitive for detection of other species (Rees et al. 2014). However, some species can be challenging to detect by eDNA (Roussel et al. 2015), and this highlights the need to improve understanding of when eDNA is a useful tool for species detection (de Souza et al. 2016). *Bythotrephes* life history as an aquatic invertebrate may obstruct detection using eDNA (sensu Tréguier et al. 2014; Mächler et al. 2014). Generally, the eDNA method should increase probability of detection by leveraging the "cloud" of genetic material shed by an organism (e.g., sloughed cells, gametes, and excrement). This dissipation of genetic material in the environment effectively increases the area or volume within which a single organism may be detected. However, with a chitinous exoskeleton and largely asexual reproduction, *Bythotrephes* might not shed and broadcast DNA like "messier" species for which eDNA has been successful, such as carp (Jerde et al. 2011; Takahara et al. 2012; Mahon et al. 2013), salamanders (Olson et al. 2012; Pilliod et al. 2014; Pierson et al. 2016), or otters (Thomsen et al. 2012; Kelly et al. 2014). In fact, single-species eDNA assays have underperformed traditional detection methods for other invertebrate species (Tréguier et al. 2014; Mächler et al. 2014), and examples of zooplankton detection using eDNA are limited in the literature

(Deiner and Altermatt 2014). Tréguier (2014) suspects low environmental extracellular DNA limits eDNA detection of crayfish. Low shedding of genetic material could also explain low eDNA detection rates of *Bythotrephes* as well as the time lag between zooplankton net detection and eDNA detection probabilities (Fig. 2). Upgrading from a single-species assay to a metabarcoding approach could increase eDNA detection sensitivity, as metabarcoding approaches to eDNA detection have outperformed traditional freshwater invertebrate sampling for some species (Gardham et al. 2014; Deiner et al. 2016). Restricting eDNA sampling to the surface water is worth considering when discussing performance of the assay. Though vertical profiles of Mendota indicate *Bythotrephes* is most prevalent in the top 5 m of the lake (Walsh *unpublished data*, Spear *unpublished data*), additional hypolimnetic water sampling might improve eDNA detection, and should be included in lakes where *Bythotrephes* exhibits DVM. However, hypolimnetic sampling might substantially increase collection time, as decontamination of at-depth sampling equipment would be required to consider multiple sampling sites independent.

Method cross-comparison and recommendations

To pair with our results regarding detection rates, we conducted a coarse analysis of the time investment in the field and laboratory using each method (see Supplementary Information for details and Fig. 3b for coarse estimates of the time investment required to reliably detect *Bythotrephes* using each method). Each method represented a wide range of sampling efficiencies in terms of the time investment required to reliably (false negative rate $< 1\%$) detect *Bythotrephes* (Fig. 3b). While sampling with a zooplankton net was the most time-efficient method (for example, collecting seven zooplankton net samples is comparable in effort to the seven-sample eDNA survey here, but would yield a detection probability of $1 - (1 - 0.71)^7 = 0.9998$), sampling with a zooplankton net was inefficient early in the summer. Sampling in late summer and fall addressed this challenge. Unlike zooplankton nets and eDNA, sediment sampling is insensitive to seasonality in *Bythotrephes* population abundance. However, challenges associated with sampling low-density lakes with loose and flocky sediments, such as Stormy Lake, could limit the efficiency of sediment sampling. For example, it would take roughly 7.5 h to sample and process enough sediment to reduce the false negative rate in Stormy Lake to less than 1% versus only 1 h in Lake Mendota. Therefore, sediment samples from lakes without *Bythotrephes* would require at least 7.5 h of effort to ensure that *Bythotrephes* is detected in lakes similar to Stormy Lake. Since most lakes are unlikely to contain *Bythotrephes*, sediment sampling may only be time-efficient at this detection rate and effort in lakes that are suspected to contain *Bythotrephes* (e.g., targeted risk assessment in the sediment sampling design; Rew et al. 2006). Finally, while eDNA was a relatively inefficient method here, we note that 1) eDNA can be scaled with larger in-laboratory capacity and 2) eDNA can be used to sample for multiple species (e.g., via a metabarcoding approach, which may be more effective for invertebrates; Gardham et al. 2014 and Deiner et al. 2016).

The life history characteristics that might limit eDNA detection are the same that make sediment sampling so effective for *Bythotrephes* detection. For example, *Bythotrephes* exoskeleton and tail spine are unlikely to shed eDNA but are passed intact through fish digestive tracts and are well-preserved and conspicuous in lake sediments (Beranek 2012; Walsh et al. 2016b; Branstrator et al. 2017). Similarly, *Bythotrephes* asexual reproduction does not release gametes into the water column to be detected as eDNA. However, asexual reproduction allows for rapid population growth (generation time $\sim$ 10 days in the laboratory at 21 °C; Kim and Yan 2013), depositing spines into the sediment. In these ways, understanding *Bythotrephes* life history can at least partially explain the counter-intuitive result that sampling using lake sediments and zooplankton nets could outperform eDNA assays, which are often sensitive and reliable (Rees et al. 2014).

While eDNA underperformed relative to other methods here, we provide several cases in which eDNA sampling could be more appropriate than either zooplankton net or sediment sampling. First, eDNA was reliable in late summer of the 2016 survey of Lake Mendota, suggesting that targeted eDNA sampling effort could still reliably detect *Bythotrephes*. As eDNA becomes more accessible and simpler to use with technological advances, it may be more efficient to survey using eDNA rather than zooplankton net samples. This is particularly true over many lakes or

samples as eDNA can be processed in parallel while zooplankton net and sediment samples must be processed in sequence. eDNA could also be used to detect a broader suite of nonnative species, capturing information on multiple species' occurrences using a single method rather than multiple specialized methods. Finally, DNA may preserve well enough in sediment samples or even in sedimented *Bythotrephes* resting eggs to explore using sediment samples as a secondary test for the presence of *Bythotrephes* DNA. Turner et al. (2015) found that fish eDNA was 8 – 1800 times more concentrated in lake sediments (per gram) than in water (per milliliter). Accumulation of DNA in sediment may help overcome challenges associated with low concentrations of *Bythotrephes* eDNA in water.

We explored the relationship between *Bythotrephes* densities in the lake's water column and tail spine densities in lake sediments (see the Supplementary Information for details) and this analysis revealed a potential additional benefit of sampling lake sediments for *Bythotrephes*. While zooplankton net sampling provides the most precise measure of present-day, in-lake density, our results revealed that sediments might provide a coarse but low-effort estimate of average long-term (e.g., over decades), in-lake population densities. In particular, the top layers (e.g., 15 cm, the depth of an Ekman grab, corresponding to nearly 30 years in Lake Mendota; Walsh et al. 2016b) integrate both within and among year variation in *Bythotrephes* abundance (Fig. S2). While sediment sampling addresses the challenges associated with seasonal variation in *Bythotrephes* abundance, longer-term changes in abundance can affect in-lake density if estimated using sediment tail spine densities. While tail spine densities in lake sediments are a good predictor of long-term mean abundance in the water column here, this could vary by invasion history (e.g., lakes invaded by *Bythotrephes* for varying periods of time) and long-term variation in *Bythotrephes* abundance (e.g., population growth, decline, or collapse). Both of these sources of variation highlight the value of using discrete sediment core layers as a means to observe *Bythotrephes* invasion history in lakes without needing to invest in long-term monitoring using zooplankton net methods (e.g., Walsh et al. 2016b; Branstrator et al. 2017).

These three methods complement one another in regard to designing an aquatic invasive species surveillance program. For example, both eDNA and tail spines in sediment samples are not direct evidence of an established population and each would require additional sampling via zooplankton net for confirmation of establishment. As a result, we recommend using eDNA or sediment sampling to locate possible *Bythotrephes* presences that have gone undetected for some time. Such a search could be directed by *Bythotrephes* distribution modeling to locate lakes at the highest risk of invasion (Rew et al. 2006). Then, zooplankton nets can be used to confirm an established and active *Bythotrephes* population. Similarly, in the case of monitoring for early detection, only eDNA and zooplankton net sampling could be used effectively as the accumulation of sediment subfossils presumably would take some time. Furthermore, while we did not attempt to use eDNA quantitatively here, both eDNA and sediment sampling can be used semi-quantitatively (Doi et al. 2015) to help allocate additional zooplankton net monitoring effort after initial confirmation (e.g., in high eDNA or sediment subfossil density lakes).

In summary, all three methods have useful applications and our recommendations for using these methods depends on the systems being sampled. Also, while we do not have general information to make recommendations about eDNA sampling, we do note that targeting sampling to periods of high predicted *Bythotrephes* abundance was effective here (late summer and fall in Lake Mendota) and could have applications for early detection in cases where *Bythotrephes* reaches high densities quickly. In lakes with sediments that are amenable to sediment grabs, we recommend sampling for sediment subfossils, which can be conducted year-round. In lakes where sediment sampling is obstructed by substrate (e.g., sand or gravel) or the lack of accumulation in the sediments (e.g., reservoirs or rivers), we recommend seasonally targeted (late summer and fall), high-volume zooplankton net tows (e.g., oblique tows). We would also recommend this net approach in high risk lakes where early detection is a primary goal (e.g., suitable lakes near invaded lakes). In any case, our results suggest that sediment sampling would be a valuable addition to *Bythotrephes* monitoring programs and require relatively little additional information (e.g., system substrate and flow patterns) to determine whether the method would be effective such as in the ten cases here.

Acknowledgements We would like to thank Carol Warden for field and lab work conducted in Stormy Lake, Gile Flowage, and Lake Gogebic, the North Temperate Lakes Long Term Ecological Research program (NTL-LTER) field crew, including Pam Montz for field and lab work conducted in Trout Lake, and Ted Bier and Kirsten Rhude for field and lab work conducted in Lakes Mendota and Monona. We also thank Kassandra Ford, Marco Scarasso, Michaela Kromrey, Bridget Murphy, Sommer Kuhn, and Michael Josephson for their work in the lab and field. We thank Scott Van Egeren and Maureen Ferry for suggestions and advice in designing and applying this study. Finally, we thank multiple colleagues for comments and friendly review including Emily Stanley, Steve Carpenter, Tony Ives, and Randy Jackson. This work was funded by the Wisconsin Department of Natural Resources, the NSF NTL LTER Program (Grants DEB-0217533 and DEB-1440297), and the office of the UW-Madison Vice Chancellor for Research and Graduate Education.

Author Contribution JRW, MJS, TPS, PJK, and MJVZ designed research; JRW, MJS, and TPS performed research; JRW, MJS, TPS, PJK, and MJVZ analyzed data; JRW, MJS, TPS, PJK, and MJVZ wrote the paper.

References

Beranek A (2012) An assessment of the long-term phenology and impact of *Bythotrephes longimanus* in Island Lake Reservoir, Minnesota, using sediment records. University of Minnesota, Master's Thesis

Berg D, Garton D (1988) Seasonal abundance of the exotic predatory cladoceran, *Bythotrephes Cederstroemi*, in Western Lake Erie. J Great Lakes Res 14:479–488

Bobeldyk AM, Rüegg J, Lamberti GA (2015) Freshwater hotspots of biological invasion are a function of species–pathway interactions. Hydrobiologia 746:363–373

Branstrator DK, Beranek AE, Brown ME, Hembre LK, Engstrom DR (2017) Colonization dynamics of the invasive predatory cladoceran, *Bythotrephes longimanus*, inferred from sediment records. Limnol Oceanogr 62:1096–1110

Brown ME, Branstrator DK (2011) Patterns in the abundance, phenology, and hatching of the resting egg stage of the invasive zooplankter *Bythotrephes longimanus*: implications for establishment. Biol Invasions 13:2547–2559

Churchill C (2013) Spatio-temporal spawning and larval dynamics of a zebra mussel (*Dreissena polymorpha*) population in a North Texas Reservoir: implications for invasions in the southern United States. Aquat Invasions 8:389–406

de Souza LS, Godwin JC, Renshaw MA, Larson E (2016) Environmental DNA (eDNA) detection probability is influenced by seasonal activity of organisms. PLoS ONE 11:e0165273

Deiner K, Altermatt F (2014) Transport distance of invertebrate environmental DNA in a natural river. PLoS ONE 9:e88786

Deiner K, Fronhofer E, Machler E, Walser J, Altermatt F (2016) Environmental DNA reveals that rivers are conveyer belts of biodiversity information. Nat Commun 7:12544

Delaney DG, Leung B (2010) An empirical probability model of detecting species at low densities. Ecol Appl 20:1162–1172

Doi H, Uchii K, Takahara T, Matsuhashi S, Yamanaka H, Minamoto T (2015) Use of droplet digital PCR for estimation of fish abundance and biomass in environmental DNA surveys. PLoS ONE 10:e0122763

Drake JM (2004) Allee effects and the risk of biological invasion. Risk Anal 24(4):795–802

Ficetola GF, Miaud C, Pompanon F, Taberlet P (2008) Species detection using environmental DNA from water samples. Biol Lett 4:423–425

Gardham S, Hose G, Stephenson S, Chariton A (2014) DNA metabarcoding meets experimental ecotoxicology: advancing knowledge on the ecological effects of copper in freshwater ecosystems. Adv Ecol Res 51:79–104

Harvey CT, Qureshi SA, MacIsaac HJ (2009) Detection of a colonizing, aquatic, non-indigenous species. Divers Distrib 15:429–437

Hulme PE (2006) Beyond control: wider implications for the management of biological invasions. J Appl Ecol 43(5):835–847

Jerde CL, Mahon AR, Chadderton WL, Lodge DM (2011) "Sight-unseen" detection of rare aquatic species using environmental DNA. Conserv Lett 4:150–157

Kelly RP, Port JA, Yamahara KM, Crowder LB (2014) Using environmental DNA to census marine fishes in a large mesocosm. PLoS ONE 9:e86175

Kerfoot WC, Hobmeier MM, Yousef F, Lafrancois BM, Maki RP, Hirsch JK (2016) A plague of waterfleas (*Bythotrephes*): impacts on microcrustacean community structure, seasonal biomass, and secondary production in a large inland-lake complex. Biol Invasions 18:1121–1145

Kim N, Yan ND (2013) Food limitation impacts life history of the predatory cladoceran *Bythotrephes longimanus*, an invader to North America. Hydrobiologia 715:213–224

Leung B, Delaney DG (2006) Managing sparse data in biological invasions: a simulation study. Ecol Model 198:229–239

Lodge DM, Williams S, MacIsaac HJ, Hayes KR, Leung B, Reichard S, Mack RN, Moyle P, Smith M, Andow DA, Carlton JT, McMichael A (2006) Biological invasions: recommendations for U.S. policy and management. Ecol Appl 16(6):2035–2054

Mächler E, Deiner K, Steinmann P, Altermatt F (2014) Utility of environmental DNA for monitoring rare and indicator macroinvertebrate species. Freshw Sci 33:1174–1183

Magurran AE, McGill BJ (2011) Biological diversity: frontiers in measurement and assessment. Oxford University Press Inc., New York

Mahon AR, Jerde CL, Galaska M, Bergner JL, Chadderton WL, Lodge DM, Hunter ME, Nico LG (2013) Validation of eDNA surveillance sensitivity for detection of Asian carps in controlled and field experiments. PLoS ONE 8:e58316

Mwatawala MW, De Meyer M, Makundi RH, Maerere AP (2006) Seasonality and host utilization of the invasive fruit fly, *Bactrocera invadens* (Dipt., Tephritidae) in central Tanzania. J Appl Entomol 130:530–537

Nielsen AL, Hamilton GC, Shearer PW (2011) Seasonal phenology and monitoring of the non-native *Halyomorpha halys* (Hemiptera: Pentatomidae) in soybean. Environ Entomol 40:231–238

O'Connell AF, Talancy NW, Bailey LL, Sauer JR, Cook R, Gilbert AT (2006) Estimating site occupancy and detection probability parameters for meso- and large mammals in a coastal ecosystem. J Wildl Manag 70:1625–1633

Olson ZH, Briggler JT, Williams RN (2012) An eDNA approach to detect eastern hellbenders (*Cryptobranchus a. alleganiensis*) using samples of water. Wildl Res 39(7):629–636

Pierson TW, McKee AM, Spear SF, Maerz JC, Camp CD, Glenn TC (2016) Detection of an enigmatic Plethodontid salamander using environmental DNA. Copeia 104:78–82

Pilliod DS, Goldberg CS, Arkle RS, Waits LP (2014) Factors influencing detection of eDNA from a stream-dwelling amphibian. Mol Ecol Resour 14:109–116

R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing

Rees HC, Maddison BC, Middleditch DJ, Patmore JRM, Gough KC (2014) REVIEW: the detection of aquatic animal species using environmental DNA—a review of eDNA as a survey tool in ecology. J Appl Ecol 51:1450–1459

Renshaw MA, Olds BP, Jerde CL, McVeigh MM, Lodge DM (2015) The room temperature preservation of filtered environmental DNA samples and assimilation into a phenol–chloroform–isoamyl alcohol DNA extraction. Mol Ecol Resour 15:168–176

Rew LJ, Maxwell BD, Dougher FL, Aspinall R (2006) Searching for a needle in a Haystack: evaluating survey methods for non-indigenous plant species. Biol Invasions 8:523–539

Roussel J-M, Paillisson J-M, Tréguier A, Petit E (2015) The downside of eDNA as a survey tool in water bodies. J Appl Ecol 52:823–826

Simberloff D (2011) How common are invasion-induced ecosystem impacts? Biol Invasions 13:1255–1268

Simberloff D (2014) Biological invasions: What's worth fighting and what can be won? Ecol Eng 65:112–121

Takahara T, Minamoto T, Yamanaka H, Doi H, Kawabata Z (2012) Estimation of fish biomass using environmental DNA. PLoS ONE 7:e35868

Thomsen PF, Kielgast J, Iversen LL, Wiuf C, Rasmussen M, Gilbert MTP, Orlando L, Willerslev E (2012) Monitoring endangered freshwater biodiversity using environmental DNA. Mol Ecol 21:2565–2573

Tréguier A, Paillisson J-M, Dejean T, Valentini A, Schlaepfer MA, Roussel J-M (2014) Environmental DNA surveillance for invertebrate species: advantages and technical limitations to detect invasive crayfish *Procambarus clarkii* in freshwater ponds. J Appl Ecol 51(4):871–879

Turner CR, Uy KL, Everhart RC (2015) Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biol Cons 183:93–102

US-EPA (2016) United States Environmental Protection Agency: Zooplankton sampling methods

Vine SJ, Crowther MS, Lapidge SJ, Dickman CR, Mooney N, Piggott MP, English AW (2009) Comparison of methods to detect rare and cryptic species: a case study using the red fox (*Vulpes vulpes*). Wildl Res 36:436–446

Walsh JR, Carpenter SR, Vander Zanden MJ (2016a) Invasive species triggers a massive loss of ecosystem services through a trophic cascade. Proc Natl Acad Sci 113:4081–4085

Walsh JR, Munoz SE, Vander Zanden MJ (2016b) Outbreak of an undetected invasive species triggered by a climate anomaly. Ecosphere 7:e01628. https://doi.org/10.1002/ecs2.1628

Wittmann MJ, Lewis MA, Young JD, Yan ND (2011) Temperature-dependent Allee effects in a stage-structured model for *Bythotrephes* establishment. Biol Invasions 13:2477–2497

Yan N, Pawson TW (1997a) Changes in the crustacean zooplankton community of Harp Lake, Canada, following invasion by *Bythotrephes cederstroemi*. Freshw Biol 37:409–425

Yan ND, Pawson TW (1997b) Seasonal variation in the size and abundance of the invading *Bythotrephes* in Harp Lake, Ontario, Canada. Hydrobiologia 361:157–168

Yan ND, Leung B, Lewis MA, Peacor SD (2011) The spread, establishment and impacts of the spiny water flea, *Bythotrephes longimanus*, in temperate North America: a synopsis of the special issue. Biol Invasions 13:2423–2432

Young JD, Yan ND (2008) Modifications of the diel vertical migration of *Bythotrephes longimanus* by the cold-water zooplanktivore, Coregonus artedi. Freshw Biol 53(5):981–995

Young JD, Strecker AL, Yan ND (2011) Increased abundance of the non-indigenous zooplanktivore, *Bythotrephes longimanus*, is strongly correlated with greater spring prey availability in Canadian Shield lakes. Biol Invasions 13:2605–2619