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Research Article

Advancing early detection technologies for *Dreissena polymorpha* (Pallas, 1771): comparing mussel detection in environmental samples with environmental DNA (eDNA) and detection canines

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Abstract

Early detection and rapid response represent cornerstones of effective management of biological invasions, and development of methods that increase the sensitivity and efficiency of species detection directly benefit such efforts. We compared environmental DNA (eDNA) and canine scent detection of *Dreissena polymorpha* (Pallas, 1771), the Eurasian zebra mussel, in lakes of central Texas, USA. Environmental DNA analysis has become routinely incorporated as a component of *D. polymorpha* management programs; however, canine scent detection has typically been limited to inspection for adult mussels on watercraft. Thus, our work represents the first attempt to evaluate detection of *D. polymorpha* veligers (i.e., free-swimming larval stages) and other microscopic traces in environmental samples with canine scent detection. The specific objectives of this study were to: 1) evaluate whether canines can detect *D. polymorpha* in environmental samples; 2) quantify and compare limits of detection of canine scent detection and eDNA analysis; and 3) assess the performance of detection technologies through blind screening of ten lakes. In order of objective, our major findings include: 1) canines can be trained to detect *D. polymorpha* veligers in water samples; 2) eDNA detection is 2–100x more sensitive than canine scent detection; and 3) canine scent detection and eDNA both appear to outperform microscopy for *D. polymorpha* detection in environmental water samples. However, more work is needed to understand the conditions dictating when canine scent detection or eDNA outperforms the other.

Key words: zebra mussels, early detection and rapid response (EDRR), eDNA detection, olfactometer, qPCR, scent detection by canines

Introduction

The first detection of Eurasian *Dreissena polymorpha* (Pallas, 1771; zebra mussels) in North America occurred in the Laurentian Great Lakes in 1988 (Hebert et al. 1989). Since then, *D. polymorpha* and the closely related *D. bugensis* (Andrusov, 1897; quagga mussels) have spread rapidly across the United States and Canada, invading lentic and lotic inland waters via both natural and human-mediated dispersal (Bossenbroek et al. 2001; Sieracki et al. 2014). High fecundity of *D. polymorpha* in combination with the production of

free-swimming larvae (i.e., veligers) facilitate dispersal via water currents and as byssally attached hitchhikers on recreational boats and other equipment (Johnson and Carlton 1996; Ram and McMahon 1996; Keller et al. 2007). The impacts of *D. polymorpha* invasion have been ecologically and economically devastating. Voracious filter feeders, *D. polymorpha* impose bottom-up disturbances on local food webs by reducing available water nutrients (Nakano and Strayer 2014) and alter food webs via reduction of food availability for native species (Holland 1993; Fahnenstiel et al. 1995; Miehls et al. 2009). Additionally, *D. polymorpha* act as potent ecosystem engineers that can increase water clarity by filter feeding and manipulate physical habitat structure by establishing dense colonies attached to all available hard substrates, including native mussels, crayfish, and other hard-bodied organisms. Notably, *D. polymorpha* also impact human wellbeing, as prevention and removal efforts for recreational equipment, power plants, municipal water facilities, dams, and other infrastructure cost over US$ 1 billion annually in the United States alone (Pimentel et al. 2005; Aldridge et al. 2006).

Thus, many incentives exist to promote effective prevention and management efforts for nonnative *D. polymorpha*. Early detection and rapid response (EDRR) represent cornerstones of effective invasive species management programs (Lodge et al. 2016), so improving methods for *D. polymorpha* detection represents a critical research and management goal. Traditional, visual methods of *D. polymorpha* detection include identifying adult mussels via SCUBA surveys as well as using cross-polarized light microscopy to detect veligers in plankton samples (Johnson 1995; Alix et al. 2016; Peñarrubia et al. 2016). Unfortunately, these methods can be labor intensive, time consuming, and expensive. Two emerging detection methods that may overcome previous limitations include canine scent detection and environmental DNA (eDNA) analysis.

Mussel detection canines are already frequently stationed at boat launches to inspect watercraft for the presence of *D. polymorpha* and *D. bugensis* adults prior to entry into uninvaded waterbodies. Canines have also been deployed in combination with other mussel surveillance methods such as the use of submerged settling plates (Lucy 2006). Scent detection canines have proven to be more accurate and sensitive in detection than standard environmental surveying technologies of multiple target organisms including tortoises and various carnivores (Cablk and Heaton 2006; Long et al. 2007a, b; Cablk et al. 2008), but their ability to detect traces of *D. polymorpha* in environmental samples remains relatively unexplored (but see DeShon et al. 2016).

Analysis of eDNA, genetic material collected and extracted from bulk environmental samples such as sediment, water, or air (Barnes and Turner 2016), represents another emerging method for rapid and sensitive detection of *D. polymorpha*. Analysis of eDNA has been applied broadly to

the detection of invasive fish (Robson et al. 2016), amphibians (Secondi et al. 2016), and invertebrates, including *D. polymorpha* (Egan et al. 2013; Goldberg et al. 2013; Larson et al. 2017). Demonstrated sensitivity and repeatability have led to the emergence of eDNA analysis as an integral component of many *D. polymorpha* surveillance programs (reviewed by Feist and Lance 2021).

Therefore, we aimed to evaluate and compare canine scent detection and eDNA analysis for detection of *D. polymorpha* in environmental samples. Specifically, our study included three objectives that challenged canine and eDNA detection sensitivity limits under laboratory and real-world conditions: 1) evaluate whether canines can detect *D. polymorpha* veligers in environmental samples under controlled laboratory conditions, 2) quantify and compare limits of detection of canine scent detection and eDNA analysis, and 3) assess the performance of canine scent detection and eDNA analysis through blind screening of ten lakes that are either infested with *D. polymorpha* or are negative to *D. polymorpha*.

Materials and methods

Study site

This work occurred in multiple human-constructed reservoirs in Texas, USA. Since its first detection in 2009 in Lake Texoma on the Texas-Oklahoma border, *D. polymorpha* has spread to waters across six river basins in the state and is predicted to continue to spread, especially in the northern and eastern regions of the state (Barnes and Patiño 2020). The state management agency Texas Parks and Wildlife Department (TPWD) maintains a *D. polymorpha* invasion status categorization system (https://tpwd.texas.gov/huntwild/wild/species/exotic/zebramusselmap.phtml) wherein lakes can be identified as "infested" (i.e., the site has a confirmed reproductive population; N = 31 in Texas as of this writing) or "positive" (i.e., adult mussels or veligers have been detected on more than one occasion, but there is no evidence of reproduction; N = 5). All other waterbodies are considered "unreported".

Biosafety measures

Throughout all research activities, we applied strict biosafety measures to prevent laboratory contamination and spread of *D. polymorpha* via contaminated equipment. Specifically, after each use, plankton nets were soaked in vinegar for one hour, followed by 10% bleach for ten minutes, then copiously rinsed with tap water and air-dried completely overnight. Other equipment (i.e., bottles, petri dishes, forceps) was decontaminated by soaking in 10% bleach for ten minutes, rinsed, and dried completely between uses.

Table 1. Canine collaborators.

Name	Sex (F/M)	Age (yrs)	Breed	Spayed/Neutered	Experience in Field (yrs)
Captain	M	8	Cocker spaniel/Golden retriever	Neutered	3
Dory	F	2	Black lab	Spayed	2
Edna	F	2	Chocolate lab	Spayed	0.5
Gilligan	M	4	Yellow lab mix	Neutered	2
Marlin	M	3	Black lab mix	Neutered	1
Moomba	F	3	Black lab/Husky	Spayed	1

Objective 1: Evaluate whether canines can detect D. polymorpha *veligers in environmental samples*

The purpose of this objective was to evaluate whether canines who have been trained in detection of adult *D. polymorpha* on watercraft can detect *D. polymorpha* veligers in environmental samples under controlled laboratory conditions. To train scent detection canines to identify *D. polymorpha* veligers, we collected and concentrated veligers from Canyon Lake Marina in "infested" Canyon Lake, Texas (29.900723 N; 98.234625 W) on 12–14 September 2021 using repeated vertical tows with 80-µm plankton nets. Following Johnson et al. (2019), we concentrated plankton via sequential filtration through 210-µm and 35-µm sieves and identified and enumerated veligers visually with cross-polarized light microscopy. Depending on the trial (see below), concentrated plankton were resuspended in bottled spring water or filtered lake water from uninvaded Calaveras and Braunig Lakes, also located in Texas. Resuspended plankton were stored in 50-mL conical vials at 4 °C for at least 12 h to ensure equilibration of the sample with the biological material.

All canine trials described in this study occurred at Chiron K9 in Somerset, Texas, and our test subjects consisted of six canines employed by a scent detection canine service provider called Mussel Dogs (Table 1). Canines were trained and tested using an automated, olfactometer-controlled 3-Alternative Forced Choice test procedure (Aviles-Rosa et al. 2021; Gallegos et al. 2022; Figure 1). Briefly, we used an olfactometer with the capacity to hold six sealed headspace vials, each containing a solid or liquid odorant source. Under computer-controlled activation, regulated airflow could be passed through a specified headspace vial, pushing odorant through a mixing manifold with clean air and delivered to a stainless-steel port where the canine can sample the odorant. Infrared beams measured canines' sampling times (i.e., time spent with nose in each port). Using reinforcement with a preferred toy, canines were trained to hold their nose in the port containing the target for a specified "hold time" while avoiding holding their nose within incorrect ports for the same length of time. Nose hold times were modified for each canine based on their proclivity to maintain a nose hold duration (see Results). After each trial, the olfactometer conducted a 25 s odor purge then pseudo-randomized the location for the next port (i.e., randomization was balanced across ports such that each port contained

Figure 1. Dog alerting to an olfactometer port. Three olfactometers are aligned on a table. The dog is indicating the center olfactometer port is presenting a target odor and is engaging in a nose hold response.

the target an approximately equivalent number of times). All training and testing occurred double-blinded via computer control such that handlers and observers did not know the correct location of the target odor.

During initial training, correct responses or "alerts" to the port containing the target odor (i.e., nose hold > criterion) were marked by the computer with an audible signal to a handler to deliver a reward. If the canine made a false alert (i.e., exceeding their hold criterion in a non-target port), a different audible signal sounded to terminate the trial. If the canine sampled all three ports and did not alert within the hold criterion, an "all-clear" response was scored. During initial training only, the program would wait for a correct response and ignore incorrect responses until the canine made a correct response, thereby allowing canines and handlers to learn from mistakes; however, the first response (i.e., the incorrect response) was still scored. This setting was not used for formal data collection (see below). This training progression for canine odor detection has been previously successfully used in our laboratory (Aviles-Rosa et al. 2021; DeChant and Hall 2021).

Table 2. Samples created for the Plankton Control Test.

Concentrated Plankton Source	*D. polymorpha* Status	Diluent Filtered Water Source	Sample Purpose
Canyon Lake	+	Canyon Lake	Target
Canyon Lake	+	Braunig Lake	Target
Lake Placid	+	Lake Placid	Target
Calaveras Lake	–	Calaveras Lake	Negative
Braunig Lake	–	Braunig Lake	Negative

In initial training, as many background odors as possible were eliminated to maximize any odor signature of *D. polymorpha*. Thus, detection targets consisted of a plankton sample resuspended in 5 mL spring water (~ 900 veligers/mL). Clean diluent spring water (5 mL) served as distractors/ alternative odor. Due to the three ports presented in olfactometer trials, dogs had a 1/3 chance of identifying the "correct" port via chance alone. Thus, canines were trained until they identified eight or more correct responses in a 10-trial session (corresponding to a binomial test where chance probability is 0.33, $p < 0.01$). Performance was confirmed on a second set of independently prepared target and distractor samples before advancing to the next detection session.

Next, canines were trained with target (~ 825 *D. polymorpha* veligers/mL) and distractor samples consisting of 50% spring water and 50% filtered water from Braunig Lake (i.e., where *D. polymorpha* is not known to occur) to increase complexity of "background" odors. As in previous clean water trials, canines were trained until they identified eight or more correct responses in a 10-trial session, and then performance was confirmed on a second set of independently prepared target and distractor samples before advancing to the next test.

Finally, to further increase background odor complexity as well as confirm that canines were responding to the presence of *D. polymorpha* veligers and associated odors, rather than simply an abundance of filtered plankton, we conducted a test in which plankton was identically concentrated from multiple lakes and reconstituted into filtered water sources, also from multiple lakes (Table 2). Overall, in these trials, three targets and two distractors were used; on each individual trial, a single target was presented in one port while the other two ports presented distractors, which could be the same or a different lake (Table 2).

Immediately following each test, canines were given a 10-trial control session. In this session, all samples were distractors (i.e., no *D. polymorpha* target sample was provided), but canines were trained/tested as previously described and samples were otherwise prepared in the same manner. If canines had learned to use an unintentional cue provided by the olfactometer or researchers, this would be revealed by canines performing above chance (i.e., 33% correct responses) even in the absence of a target odor; in contrast, if the canines were identifying only *D. polymorpha* odors, then canine performance would decline to chance levels.

To analyze canine performance in clean water and filtered lake water trials, we calculated mean accuracy (number correct response/total trials)

for all trials and control tests. Individual performance was considered above chance for a 10-trial session if seven or more responses were correct (binomial test p = 0.02). Overall group performance was compared to theoretical chance (one out of three, p = 0.33) using a one-sample Student's t-test. To evaluate the results of the concentrated plankton background trial, we calculated frequency of alerts to each odor type by scoring the number of trials a canine alerted to an odor relative to the number of trials the odor appeared. We applied a logistic mixed effect model to predict the probability of an alert by each odor type with a random intercept fit for each canine. Tukey post hoc tests enabled comparison between odors. We performed all analyses using R Version 4.1.1 and packages lme4, lmerTest, and emmeans (Bates et al. 2013; Kuznetsova et al. 2014; Lenth 2016).

Objective 2: Quantify and compare limits of detection of canine scent detection and eDNA analysis

Following identical collection methods to those used for Objective 1, we prepared a concentrated sample of 837 veligers/mL resuspended in filtered water from Lake Calaveras, Texas, which is not known to be invaded by *D. polymorpha*. From the initial sample, we prepared four serial dilutions of half-log steps (5 mL per sample, stored in amber vials). Three sets of dilutions were used for canine evaluation in the olfactometer (i.e., one for each olfactometer port). The fourth series was reserved for eDNA analysis.

Canines were evaluated using the same olfactometer system described in Objective 1 (Figure 2). We quantified detection sensitivity using the prepared serial dilutions and a two-down one-up descending staircase procedure (Leek 2001; DeChant and Hall 2021). In this procedure, if canines made two consecutive correct responses, the concentration of the odorant was decreased by a half-log step. If canines responded incorrectly, the concentration was increased by a half-log step. Testing continued until eight reversals in the direction of concentration change (up or down) occurred or a maximum of 40 trials. Due to olfactometer limitations (i.e., capacity for a maximum of six odors per run), only five dilution levels were trained at a time, with the final channel reserved for a negative control (i.e., filtered lake water without veligers added). If a canine successfully detected the five most concentrated dilutions, the canine would then re-start threshold assessment with a new range of dilutions beginning with the lowest concentration successfully detected. In trials using the six most diluted samples, the olfactometer procedure was changed to include only three dilution steps and three control samples to reduce the over-use of the odor from control vials and headspace of control lake water from dissipating across testing when canines required numerous trials to detect the sample at lower concentrations. Each canine completed the threshold assessment procedure twice. After every canine completed their first threshold assessment, a control test was conducted as previously described to verify that canines were not leveraging any unintentional cues. To analyze canine thresholds, the performances of

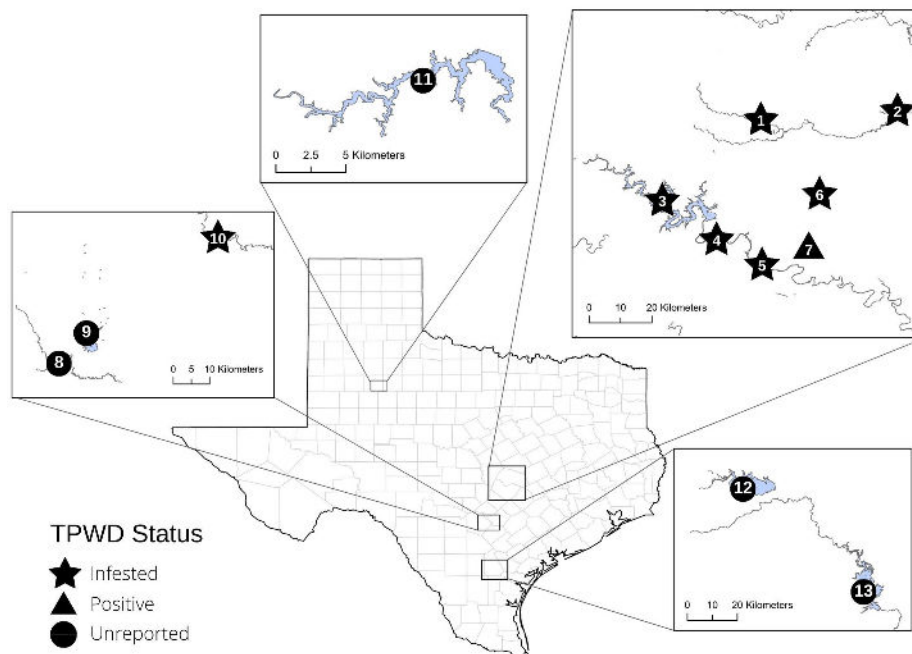

Figure 2. Study sites and their *D. polymorpha* status, according to TPWD. 1) Georgetown Lake, 2) Granger Lake, 3) Lake Travis, 4) Lake Austin, 5) Lady Bird Lake, 6) Lake Pflugerville, 7) Lake Walter E. Long, 8) Victor Braunig Lake, 9) Calaveras Lake, 10) Placid Lake, 11) Lake Alan Henry, 12) Choke Canyon Reservoir, and 13) Lake Corpus Christi.

each canine at each dilution was calculated. We fitted a probit psychophysical function for each canine using the quickpsy package where the guess rate was set at 0.33 (Linares and López-Moliner 2016), and we estimated 75% detection threshold and calculated 95% confidence intervals for each canine.

The fourth series of serial dilutions was reserved to quantify sensitivity of eDNA analysis. Samples from this dilution series were vacuum filtered using 1-µm polycarbonate track-etch 47-mm membrane disk filters (Whatman). We extracted eDNA from filters using a CTAB-chloroform procedure (Turner et al. 2014a), then quantified *D. polymorpha* DNA in each sample using a species-specific quantitative PCR (qPCR) assay targeting *D. polymorpha* CytB gene (Gingera et al. 2017). Each 20-uL qPCR reaction included 1 uM forward and reverse primers, 0.6 uM hydrolysis probe, 1x PerfeCTa qPCR ToughMix (Quantabio, Beverly, MA, USA), and 4 uL extracted genomic DNA. All samples were run in triplicate. We followed the methods of Klymus et al. (2020) to define eDNA limit of detection as the lowest concentration of veligers at which we achieve 95% detection and limit of quantification as the lowest concentration of veligers with a coefficient of variation below 35%.

Objective 3: Assess the performance of canine scent detection and eDNA analysis through blind screening of ten lakes

To compare the performance of eDNA and scent detection canines, we collected samples from ten novel Texas lakes with each lake being either infested with *D. polymorpha* or not recorded by TPWD to be infested with

them (Figure 2). Notably, test sites did not include Canyon Lake, Lake Calaveras, or Braunig Lake due to their use in earlier training. This test explicitly evaluated the generalizability of the canines' training to novel samples, which is an important step if dogs were to be used for real-world application (Edwards et al. 2017).

Collections occurred at public boat ramps or along lake shores between 28 September and 7 October 2021. Three liters of water concentrated via plankton net (as described in Objective 1) and three 500-mL surface water samples were collected per site. The three liters of netted water were further prepared for canine detection as described in Objective 1, while the three 500-mL surface water samples were directly filtered for eDNA analysis (see below). On each sampling trip, an additional 500-mL bottle of distilled water was placed in the cooler with the samples to serve as a negative control and subsequently treated as a real sample (i.e., "field blank").

Microscopy was performed following canine data collection on concentrated netted water samples after the canine trials to confirm results and provide a comparison of both methods (i.e., canine scent detection and eDNA analysis) with a traditional approach (i.e., microscopy). Specifically, we examined an aliquot of 1 mL from each sample using a Fien Optic FZ6T-TS Microscope at 4x magnification under cross-polarized light. For each lake, we scored *D. polymorpha* presence based on veliger presence as "detected" or "not detected."

Lakes were screened by canines in 10-trial sessions comprised of four trials with a known target (i.e., concentrated samples from Canyon Lake ~ 60 veligers/mL) and two known negative samples from Calaveras Lake. Correct responses to the Canyon Lake sample in these trials were reinforced. Each session also included two trials without positive samples presented (i.e., only presenting negative samples from Calaveras Lake), in which a correct "all-clear" response was rewarded. The remaining four trials were "unknown" in which one port presented a novel lake and the remaining ports presented negative Calaveras Lake water. In these four "probe" trials, neither an alert nor an all-clear were reinforced to avoid directly training a specific response and to assess dogs' spontaneous (untrained) response to novel samples, which is important if dogs were to be used as a screening tool (Edwards et al. 2017). The order of trials was randomized, but all canines screened the same lakes in the same order for logistic purposes relating to olfactometer setup. One canine, Gilligan, did not participate based on insufficient detection of known target samples.

After canines completed evaluation of Lakes Corpus Christi and Alan Henry, we began including these two as known negative samples based on historical data combined with negative results with canine scent detection and microscopy (see Results). Canines participated in two sessions of training (one for each lake) in which these two lake samples were included as negative distractors, and canines were reinforced for not alerting to them. After the training session, the lakes were incorporated as a "known negative" for the

remaining assessments. This additional training occurred to increase canine exposure to novel negative samples and provide them with additional feedback, which in turn enhances generalization across novel samples (Lubow 1974; Schrier and Brady 1987). For the remainder of unknown lakes, a 50% probability of a response was used as a simple differentiator for canines responding to a lake as "positive" or "negative".

Finally, we assessed all samples for the presence of *D. polymorpha* using eDNA analysis. We followed the same methods of vacuum filtration and DNA extraction as described in Objective 2. We used the same qPCR assay as well, except technical replicates were increased to six for each sample.

Results

Objective 1: Evaluate whether canines can detect D. polymorpha *in environmental samples*

Captain, Gilligan, and Marlin had previously been trained to "sit" as an alert response, whereas Dory, Edna, and Moomba were previously trained to "focus" at the odor source. This difference in prior training appeared to relate to differences in proclivity to maintain duration of a nose hold and ultimately the length of the nose hold selected for a response. Thus, canines Captain, Gilligan, and Marlin were trained with a 1.5 second hold time, while Dory, Edna, and Moomba were trained with a 3 second hold time. All canines exceeded the individual significance criterion (8 or more correct out of 10 trials; binomial test where chance probability is 0.33, $p < 0.01$) for clean water and filtered lake water background trials. This indicates each dog as an individual had a performance exceeding that expected by chance. However, no canine met this criterion for the all-distractor control tests (Figure 3). At the group level, canines exceeded *D. polymorpha* detection performance expected by chance in both trainings ($p < 0.001$) but not on the control tests ($p = 0.37$), confirming that canines were identifying the presence of *D. polymorpha* rather than responding to unintentional cues.

In plankton background trials, canines demonstrated greater response rate to samples with *D. polymorpha* compared to the samples with concentrated plankton from lakes without *D. polymorpha* (all $p < 0.001$; Figure 4). Canines also showed lower alert rates for positive samples reconstituted into Canyon Lake water compared to positive samples reconstituted with water from *D. polymorpha*-negative Braunig Lake ($z = 4.14$, $p < 0.001$). However, no decrement was observed when veliger-containing plankton from a second infested lake, Lake Placid, were used.

Objective 2: Quantify and compare limits of detection of canine scent detection and eDNA analysis

Canine detection declined as the concentration of the veliger sample decreased toward each individual's threshold detection limits (Figure 5).

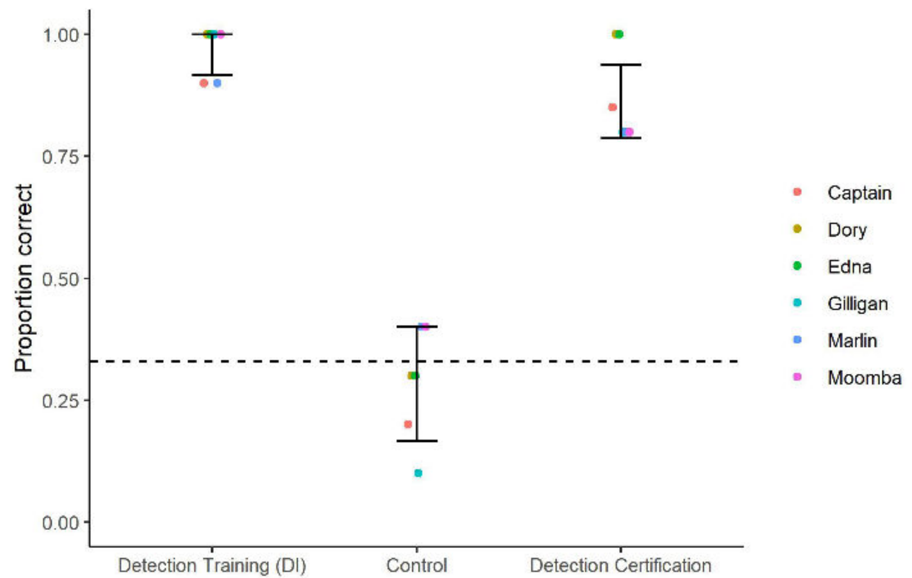

Figure 3. This graph shows each canines' detection accuracy (chance = 0.33; dashed line) on the detection training (veliger detection in DI water from DI distractors), the control test (all DI water) and the detection certification (veligers re-suspended in clean lake water from clean lake water). Error bars show the bootstrap estimated 95% confidence intervals.

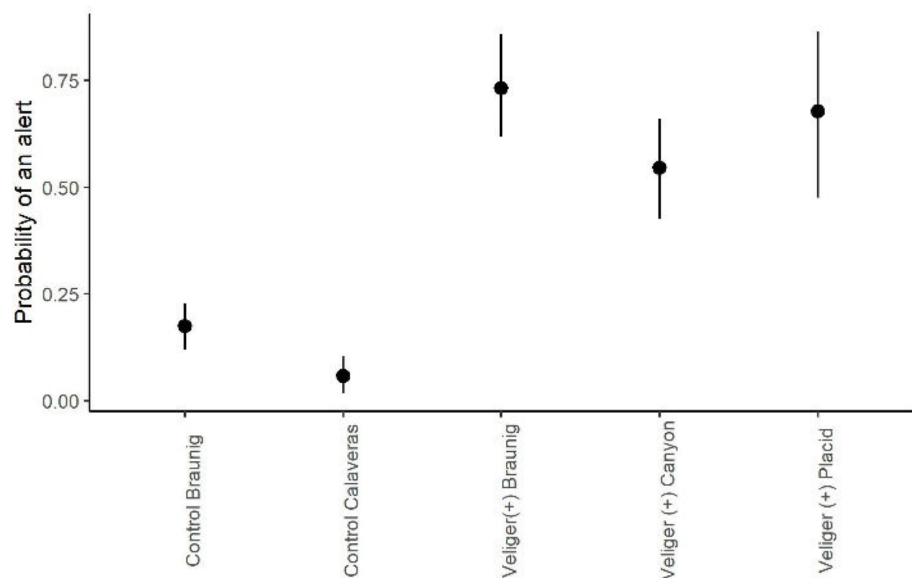

Figure 4. Responses to each odor during the Plankton Control Test. Points show the mean and error bars show the bootstrap estimated 95% confidence intervals.

Four of six canines showed detection limits within a 15–38% dilution of the concentrated veliger sample (~ 825 veligers/mL), whereas two other canines showed substantially lower detection limits ranging from 2% to 0.1% dilution of the veliger sample (Table 3), suggesting an overall detection limit range of 0.825–313 veliger/mL among the canines in our study. The all-distractor control test associated with this Objective yielded an overall accuracy rate of 40%, which did not differ from the likelihood of randomly selecting the correct olfactometer port by chance (i.e., 33%; binomial test $p = 0.27$), and confirmed that canines were not leveraging unintentional cues.

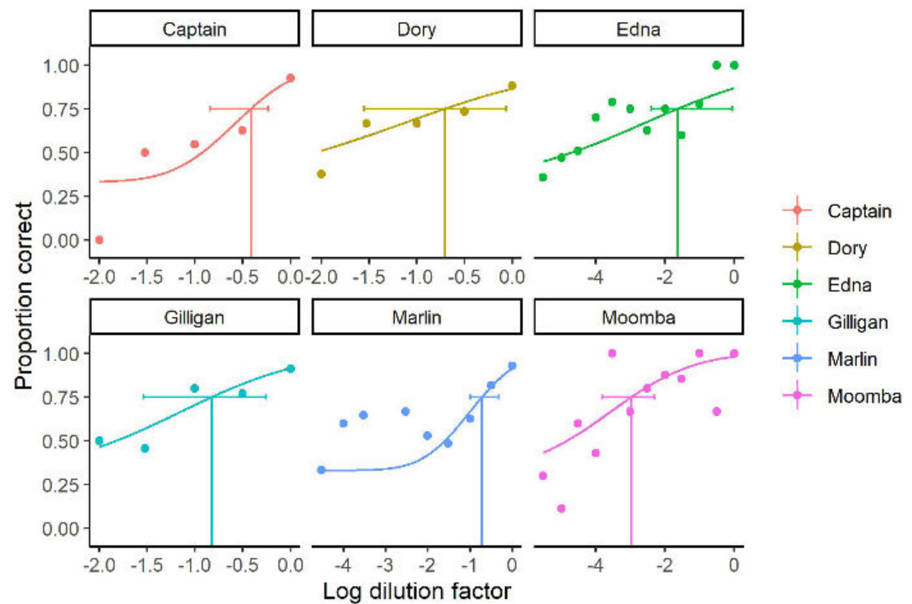

Figure 5. Performance of each canine across dilution levels of the concentrated veliger sample. Error bar shows the confidence interval for the 75% detection threshold.

Table 3. Estimated 75% detection threshold and 95% confidence interval. Expressed in terms of the dilution factor of the concentrated veliger sample (3.42 mL of a counted 825 veliger/mL sample)

Canine	Threshold Estimate	95% CI Lower Limit	95% CI Upper Limit
Captain	0.386	0.171	0.646
Dory	0.198	0.050	2.045
Edna	0.023	0.003	0.421
Gilligan	0.150	0.019	0.345
Marlin	0.190	0.078	0.591
Moomba	0.001	0.000	0.007

In eDNA analysis, the positivity rate within samples (i.e., among three technical replicates) ranged from 33% to 100%. Following the methods of Klymus et al. (2020), we defined eDNA limit of quantification as 606 veliger/mL and limit of detection as 16.5 veliger/mL (though we note that at least one technical replicate amplified for standards as low as 0.026 veligers/mL). One of six technical replicates of extraction negative controls amplified, resulting in an estimated eDNA concentration of 0.000006 ng/μLDNA, far below the limit of detection of the eDNA assay, so it is unlikely that this contamination influenced inferences of this trial. As expected, no qPCR non-template controls amplified.

Objective 3: Assess the performance of canine scent detection and eDNA analysis through blind screening of ten lakes

Using microscopy, we identified *D. polymorpha* veligers in five out of the ten sites surveyed (Table 4). This traditional method was time consuming and physically straining, requiring over four hours at the microscope. Microscopy proved especially difficult with more turbid samples, which

Table 4. Summary of results from all detection methods utilized in Objective 4, published status of lakes by TPWD, and results from eDNA analysis. Canine detection results are based on ≥ 50% probability of detection.

Lake Name	Latitude	Longitude	Texas Parks and Wildlife Division	Microscopy	Canine Olfactometry Detection	eDNA Technology
Alan Henry	101.0828798	−33.0413504	Unreported	–	+	+
Austin	97.8515401	−30.3385372	Infested	+	+	+
Choke Canyon	98.3278964	−28.4913944	Unreported	–	+	–
Corpus Christi	97.9091393	−28.1364242	Unreported	–	–	–
Georgetown	97.7524935	−30.6756803	Infested	–	+	+
Granger	97.3589258	−30.6950187	Infested	–	–	–
Lady Bird	97.7467023	−30.2610202	Infested	+	+	+
Pflugerville	97.5721083	−30.4415265	Infested	+	+	+
Travis	98.0037452	−30.4415348	Infested	+	+	+
Walter E. Long	97.6016689	−30.2980307	Positive	+	+	+

could have caused lakes where veligers were present to be incorrectly classified as *D. polymorpha* negative. Certain macroinvertebrate larvae resemble *D. polymorpha* under cross-polarized light, which could also hinder correct determination of invasion status.

Canines demonstrated high discrimination (i.e., 80% correct) when blindly presented with samples on which they had been previously trained (Figure 6). Using a 50% detection criterion, canines indicated detection of *D. polymorpha* in Lakes Alan Henry, Austin, Choke Canyon, Georgetown, Ladybird, Pflugerville, Travis, Walter E Long. Canines did not detect *D. polymorpha* in Corpus Christi or Granger Lakes (Figure 6). Overall, canine results matched the results of eDNA analysis for nine of the ten lakes (Table 4). However, canine scent detection was the only method that identified Choke Canyon as *D. polymorpha* positive. Canines also detected *D. polymorpha* in Lake Alan Henry, though veligers were not observed in this lake using microscopy, and eDNA quantification suggested that veliger counts were below the sensitivity quantified in experimental Objective 2. In eDNA analysis of the same lakes, no extraction or qPCR negative controls amplified *D. polymorpha* DNA, as expected. Standard curves across multiple qPCR runs demonstrated $R^2 = 0.99 \pm 0.002$ (mean ± standard deviation) and efficiency = 88.8 ± 2.6%. *D. polymorpha* eDNA was detected in seven of the ten lakes (Tables 3, 4, 5). Notably, one eDNA-positive lake, Lake Alan Henry, is not known by the state management agency TPWD to be invaded by *D. polymorpha*. On the other hand, TPWD categorizes Lake Granger as infested, but eDNA was not detected in this experiment. Both results are consistent with conclusions in canine screening, though veligers were not observed via microscopy from either lake.

Discussion

Invasion science is a complex field requiring multidisciplinary approaches to maximize understanding (Lennox et al. 2015); likewise, effective management of biological invasions requires a diversity of approaches and tools. In the present study, we have quantified and compared the performance of several methods for detection of notorious invasive species *D. polymorpha*.

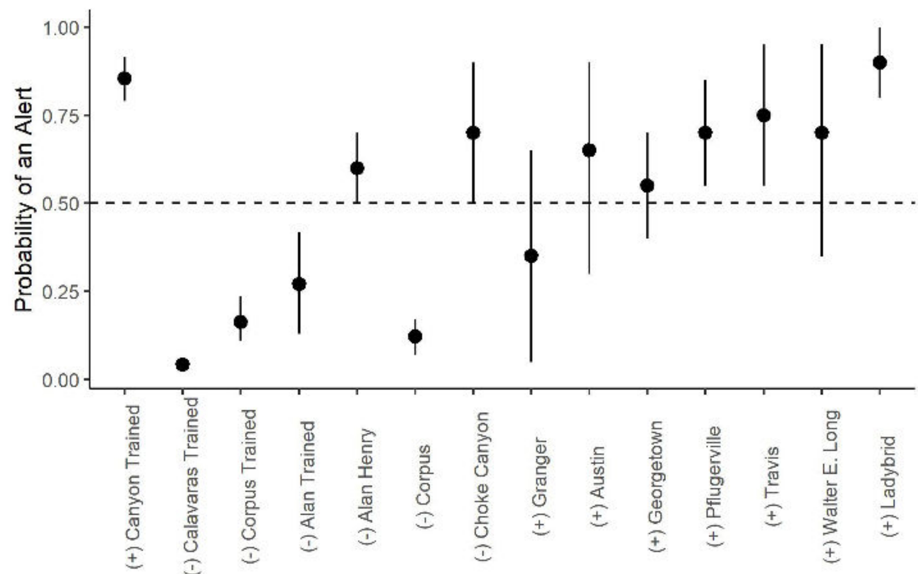

Figure 6. Canine responding to each unknown lake and the explicitly trained samples. Points show the mean and error bars show the 95% confidence intervals. Positive sign indicates presence of zebra mussels and negative sign indicate no recorded presence based on TPWD records.

Table 5. Summary of results from eDNA analysis of lakes with positive *D. polymorpha* detection.

Lake	Number of Samples Detected	Number of Wells Detected	Average Quantity (pg/mL)	Standard Deviation (pg/mL)
Alan Henry	1 of 3	1 of 18	0.034	9.25
Austin	3 of 3	18 of 18	12.977	0.21
Georgetown	3 of 3	18 of 18	9.598	0.73
Lady Bird	3 of 3	11 of 18	0.124	19.33
Pflugerville	2 of 3	12 of 18	1.130	17.44
Travis	3 of 3	18 of 18	19.219	0.26
Walter E. Long	3 of 3	18 of 18	0.595	0.46

Notably, ours is the first study to evaluate the performance of scent detection canines when detecting *D. polymorpha* in water samples, making the likely targets of detection to be veligers or their metabolic wastes or other associated biological debris. These "shed" materials also presumably represent the components of eDNA. In addition, veligers are the target of microscopy-based screening. This set up a relevant and useful opportunity for comparison of detection methods and subsequent consideration of how these methods might be combined to maximize early detection and rapid response efforts for managing *D. polymorpha* introduction and spread.

Scent detection canines have become common tools for the detection of adult *D. polymorpha* traveling as hitchhikers on recreational boats and other equipment, but we have now demonstrated that canines can also successfully identify *D. polymorpha* veligers in complex environmental samples. We observed canine lower detection limits ranging from 0.825–313 veligers/mL, depending on individual canine. Although these detection limits are generally eclipsed by the eDNA limit of detection established using the

same samples (i.e., 16.5 veligers/mL), the ability of canines to detect *D. polymorpha* scent within a presumed cacophony of background odors is impressive and portends a continued role for scent detection canines as an element of a comprehensive *D. polymorpha* early detection and rapid response campaign.

Our assessment represents one of the most rigorous tests to date of canine detection of any scent. Frequently, canine performance is only assessed on samples used in training with known outcomes by the experimenter (Edwards et al. 2017) with infrequent test of canines' ability to generalize to novel untrained samples (Elliker et al. 2014). Detection assessments often fail to use operationally relevant frequency of targets and non-targets. Our survey of actual lakes across Texas, where *D. polymorpha* spread is ongoing, represents a collection of targets of high management relevance. Additionally, the sample outcomes were unknown to the experimenters at the time of testing (but would eventually be revealed using two other established *D. polymorpha* detection methods).

Canine training occurred rapidly over two weeks based on study timelines and *D. polymorpha* reproductive seasons. Due to the novelty of training veliger detection, timing of reproductive cycles, and usage of our olfactometer system, more extended training phases were not feasible. A final consideration is that canines had a limited scope of training samples (i.e., lake sources). When using canine detection across novel samples, it is ideal to have as many positive and negative samples as possible (Hernstein et al. 1976; Schrier and Brady 1987; Bhatt et al. 1988; Essler et al. 2021). Overall, because threshold and detection sensitivity limits are related and known to change with increased training and experience (i.e., Yee and Wysocki 2001; Hall et al. 2016), the canine sensitivity limits observed in our study do not necessarily reflect the maximal achievable sensitivity limits, but rather a demonstration that relevant sensitivity limits may be achieved following initial training.

Variation between canines could be related to differences in individuals' proclivity for and experience with alerting via nose hold. Canines Gilligan, Captain, and Marlin had reduced criterion for a nose hold due to previous training for a "sit" alert response. This reduced nose hold time may be related to some of the variability in performance at lower concentrations because these canines did not have as much "nose in port" time sampling the odor. We cannot discern the degree to which this difference impacted our results at this time because the canines' previous training was not a controllable/manipulable variable in our study. Nonetheless, previous research has suggested that the type of response a canine makes can have performance effects (Essler et al. 2020). Thus, the type of response a canine is trained to make, and its impact on *D. polymorpha* or other invasive species detection would be a useful evaluation in future research.

Following quantification of the limit of detection of each method, we conducted "blind" assessment for *D. polymorpha* in ten Texas lakes using both eDNA analysis and canine scent detection in addition to traditional microscopy. It is encouraging that eDNA analysis, canine scent detection, and microscopy reached similar conclusions in seven of the ten lakes (two negatives and five positives for *D. polymorpha*). However, from a management perspective, a concerning result was the detection of *D. polymorpha* by both canine and eDNA methods in Lake Alan Henry, since this site is currently unreported by TPWD. The relatively low quantification of eDNA at this site suggests that Lake Alan Henry may be in an early—and perhaps mitigatable—stage of the invasion process. However, the low quantity of eDNA detected at Lake Alan Henry also casts doubt on canine detection based on sensitivity limits established earlier in our study. Furthermore, when the Alan Henry sample was used as trained negative (unfortunately, based on microscopy results, historical knowledge, and a need to provide canines more examples of negatives), canines readily learned to treat the sample as negative with minimal training, which would require substantial training if a strong odor signature was available. Nevertheless, the canine result, especially supported by eDNA data, suggests that Lake Alan Henry should be considered as a high-priority target for additional *D. polymorpha* monitoring.

In Choke Canyon Reservoir, which is not known to contain *D. polymorpha* by TPWD, microscopy and eDNA analysis did not detect the species. However, canines confidently (i.e., response rate approximately 80%) indicated detection of *D. polymorpha* in samples from this site. If this represents a false positive by canines, one explanation could be that some overlap occurred in the odor signature of Choke Canyon with that of the other samples where *D. polymorpha* truly were present. Alternatively, the odor profile of Choke Canyon samples may have been more novel than other samples, leading canines to respond errantly when encountering strikingly different stimuli. Therefore, this result may simply indicate that the canines required more *D. polymorpha* negative lakes in their training experience to refine their categorization of novel lakes, which was not possible within the scope of our work, given the few negative lakes within travel distance of the study site. Alternatively, as we suggested with results from Lake Alan Henry, positive results from Choke Canyon Lake could elevate the need for further surveys to preempt *D. polymorpha* establishment and spread.

A final lake in which our observations (i.e., failure to detect *D. polymorpha* with canine scent detection, eDNA analysis, or microscopy) conflicted with TPWD status was Granger Lake, which is labeled "infested" by the state agency. Given that all three detection tools were applied to replicate samples from a single access point on the lake, a parsimonious explanation could be that our negative result was driven by a heterogeneous spatial

distribution of *D. polymorpha*, its eDNA, and odors around the lake. Indeed, spatial heterogeneity of eDNA represents a well-documented challenge to eDNA analysis in aquatic systems (i.e., Takahara et al. 2012; Hunter et al. 2015; Bedwell and Goldberg 2020). Thus, increased sampling of Granger Lake could elucidate understanding of *D. polymorpha* status in the lake, increase knowledge about the transport and ecology of eDNA, and inform development of optimal eDNA sampling strategies.

Overall, our results reveal strengths and weaknesses of both eDNA analysis and canine scent detection, leading us to consider how multiple technologies may be combined to maximize effectiveness of early detection and rapid response efforts. We note that independent eDNA monitoring (i.e., by a management agency or private association) may be more cost effective than canine detection because after the initial expense of purchasing infrastructure such as a PCR system, upkeep is minimal and routine, especially compared to the daily housing, feeding, and other welfare activities (i.e., scheduled and illness-related veterinary visits) required for canine husbandry. Moreover, the ability to rapidly shift from one target to another depends only on the availability of published assays, which, in the case of high-profile invasive species, are increasingly abundant (i.e., Goldberg et al. 2013; Turner et al. 2014b; Secondi et al. 2016). Developing canine scent detection programs for new targets could re-purpose already in use programs, saving on start-up costs. Overall, the economic comparison between eDNA and canine-based detection methods likely comes down to the purpose of sampling.

For situations where results are needed rapidly, such as on-site monitoring of watercraft as they enter uninvaded lakes, canine detection represents the optimal method despite decreased sensitivity. Analysis of eDNA offers increased sensitivity at the cost of processing time. The two approaches may work most effectively in tandem, beginning with rapid triage across many points within a lake or many waterbodies across a landscape with canine scent detection to identify sites for slower but more sensitive eDNA analysis. Particularly for samples with ambiguous canine results or to confirm canine positive detections, eDNA analysis could follow. Although our study design assessed canines exclusively in a lab setting (i.e., water samples were collected and delivered to canines for testing), canines could reasonably be deployed for on-site surveillance. Based on observed canine sensitivity limits, it is unlikely that bringing the dog directly to a shoreline would yield a confident assessment of lake invasion status; however, a simple sample concentration step could enable dogs to rapidly process samples on location. Future evaluation of this possibility would be beneficial to optimize potential scent detection canine applications. Management and future research could leverage both eDNA analysis and canine scent detection to increase cost-effectiveness and maximize efforts to understand and manage invasions by *D. polymorpha* and other invaders.

Authors' contribution

MAB and NJH developed the research concept and study design. All authors participated in data collection. MAB and NJH analyzed and interpreted the data. DLD and BS trained and handled the canines. NJH provided ethics approval. MAB and NJH acquired funding. APW and KP wrote the original draft, and all authors reviewed and edited the manuscript.

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Ethics and permits

Environmental samples were collected under the auspices of Texas Parks and Wildlife Exotic Species Research Permit No. RES 08 21-157. Vertebrate animal work was reviewed and approved by the Texas Tech Institutional Animal Care and Use Committee (AUP # 21013-03).

References

Aldridge DC, Elliott P, Moggridge, GD (2006) Microencapsulated BioBullets for the control of biofouling zebra mussels. *Environmental Science & Technology* 40: 975–979, https://doi.org/10.1021/es050614

Alix M, Knights R, Ormerod SJ (2016) Rapid colonisation of a newly formed lake by zebra mussels and factors affecting juvenile settlement. *Management of Biological Invasions* 7: 405–418, https://doi.org/10.3391/mbi.2016.7.4.10

Aviles-Rosa EO, Gallegos SF, Prada-Tiedemann PA, Hall NJ (2021) An automated canine line-up for detection dog research. *Frontiers in Veterinary Science* 8: 775381, https://doi.org/10.3389/fvets.2021.775381

Barnes MA, Patiño R (2020) Predicting suitable habitat for dreissenid mussel invasion in Texas based on climatic and lake physical characteristics. *Management of Biological Invasions* 11: 63–79, https://doi.org/10.3391/mbi.2020.11.1.05

Barnes MA, Turner CR (2016) The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics* 17: 1–17, https://doi.org/10.1007/s10592-015-0775-4

Bates D, Maechler M, Bolker B, Walker S (2013) lme4: Linear mixed-effects models using Eigen and S4 (1.0-5.) [R package]

Bedwell M E, Goldberg CS (2020) Spatial and temporal patterns of environmental DNA detection to inform sampling protocols in lentic and lotic systems. *Ecology and Evolution* 10: 1602–1612, https://doi.org/10.1002/ece3.6014

Bossenbroek JM, Kraft CE, Nekola JC (2001) Prediction of long-distance dispersal using gravity models: zebra mussel invasion of inland lakes. *Ecological Applications* 11: 1778–1788, https://doi.org/10.1890/1051-0761(2001)011[1778:POLDDU]2.0.CO;2

Bhatt RS, Wasserman EA, Reynolds WF, Knauss KS (1988) Conceptual behavior in pigeons: categorization of both familiar and novel examples from four classes of natural and artificial stimuli. *Journal of Experimental Psychology*: *Animal Behavior Processes* 14: 219, https://doi.org/10.1037/0097-7403.14.3.219

Cablk ME, Heaton JS (2006) Accuracy and reliability of dogs in surveying for desert tortoise (*Gopherus agassizii*). *Ecological Applications* 16: 1926–1935, https://doi.org/10.1890/1051-0761(2006)016[1926:AARODI]2.0.CO;2

Cablk ME, Sagebiel JC, Heaton JS, Valentin C (2008) Olfaction-based detection distance: a quantitative analysis of how far away dogs recognize tortoise odor and follow it to source. *Sensors* 8: 2208–2222, https://doi.org/10.3390/s8042208

DeChant MT, Hall NJ (2021) Training with varying odor concentrations: implications for odor detection thresholds in canines. *Animal Cognition* 24: 889–896, https://doi.org/10.1007/s10071-021-01484-6

DeShon DL, Wong WH, Farmer D, Jensen AJ (2016) The ability of scent detection canines to detect the presence of quagga mussel (*Dreissena rostriformis bugensis*) veligers. *Management of Biological Invasions* 7: 419–428, https://doi.org/10.3391/mbi.2016.7.4.11

Edwards TL, Browne CM, Schoon A, Cox C, Poling A (2017) Animal olfactory detection of human diseases: guidelines and systematic review. *Journal of Veterinary Behavior* 20: 59–73, https://doi.org/10.1016/j.jveb.2017.05.002

Egan SP, Barnes MA, Hwang CT, Mahon AR, Feder JL, Ruggiero ST, Tanner CE, Lodge DM (2013) Rapid invasive species detection by combining environmental DNA with light transmission spectroscopy. *Conservation Letters* 6: 402–409, https://doi.org/10.1111/conl.12017

Elliker KR, Sommerville BA, Broom DM, Neal DE, Armstrong S, Williams HC (2014) Key considerations for the experimental training and evaluation of cancer odour detection dogs: lessons learnt from a double-blind, controlled trial of prostate cancer detection. BMC *Urology* 14: 1–9, https://doi.org/10.1186/1471-2490-14-22

Essler JL, Wilson C, Verta AC, Feuer R, Otto CM (2020) Differences in the search behavior of cancer detection dogs trained to have either a sit or stand-stare final response. *Frontiers in Veterinary Science* 7: 118, https://doi.org/10.3389/fvets.2020.00118

Essler JL, Kane SA, Nolan P, Akaho EH, Berna AZ, DeAngelo A, Berk RA, Kaynaroglu P, Plymouth VL, Frank ID, Weiss SR (2021) Discrimination of SARS-CoV-2 infected patient samples by detection dogs: a proof of concept study. *PLoS ONE* 16: e0250158, https://doi.org/10.1371/journal.pone.0250158

Feist SM, Lance RF (2021) Advanced molecular-based surveillance of quagga and zebra mussels: A review of environmental DNA/RNA (eDNA/eRNA) studies and considerations for future directions. *NeoBiota* 66: 117, https://doi.org/10.3897/neobiota.66.60751

Fahnenstiel GL, Lang GA, Nalepa TF, Johengen TH (1995) Effects of zebra mussel (*Dreissena polymorpha*) colonization on water quality parameters in Saginaw Bay, Lake Huron. *Journal of Great Lakes Research* 21: 435–448, https://doi.org/10.1016/S0380-1330(95)71057-7

Gallegos SF, Aviles-Rosa EO, Hall NJ, Prada-Tiedemann PA (2022) Headspace sampling of smokeless powder odor in a dynamic airflow context. *Forensic Chemistry* 27: 100402, https://doi.org/10.1016/j.forc.2022.100402

Gingera T, Bajno R, Docker M, Reist J (2017) Environmental DNA as a detection tool for zebra mussels *Dreissena polymorpha* (Pallas, 1771) at the forefront of an invasion event in Lake Winnipeg, Manitoba, Canada. *Management of Biological Invasions* 8: 287–300, https://doi.org/10.3391/mbi.2017.8.3.03

Goldberg CS, Sepulveda A, Ray A, Baumgardt J, Waits LP (2013) Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). *Freshwater Science* 32: 792–800, https://doi.org/10.1899/13-046.1

Hall NJ, Smith DW, Wynne CD (2016) Effect of odorant pre-exposure on domestic dogs' sensitivity on an odorant detection task. *Applied Animal Behaviour Science* 178: 80–87, https://doi.org/10.1016/j.applanim.2016.02.003

Hebert PD, Muncaster BW, Mackie GL (1989) Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 46: 1587–1591, https://doi.org/10.1139/f89-202

Hernstein, R., D. H. Loveland, and C. Cable. 1976. Natural concepts in pigeons. *Journal of Experimental Psychology: Animal Behavior Processes* 2: 285–302, https://doi.org/10.1037//0097-7403.2.4.285

Holland RE (1993) Changes in planktonic diatoms and water transparency in Hatchery Bay, Bass Island area, western Lake Erie since the establishment of the zebra mussel. *Journal of Great Lakes Research* 19: 617–624, https://doi.org/10.1016/S0380-1330(93)71245-9

Hunter M E, Oyler-McCance S J, Dorazio RM, Fike JA, Smith BJ, Hunter CT, Reed RN, Hart KM (2015) Environmental DNA (eDNA) sampling improves occurrence and detection estimates of invasive *Burmese pythons*. *PLoS ONE* 10: e0121655, https://doi.org/10.1371/journal.pone.0121655

Johnson LE (1995) Enhanced early detection and enumeration of zebra mussel (*Dreissena* spp.) veligers using cross-polarized light microscopy. *Hydrobiologia* 312: 139–146, https://doi.org/10.1007/BF00020769

Johnson LE, Carlton JT (1996) Post-establishment spread in large-scale invasions: dispersal mechanisms of the zebra mussel *Dreissena polymorpha*. *Ecology* 77: 1686–1690, https://doi.org/10.2307/2265774

Keller RP, Drake JM, Lodge DM (2007) Fecundity as a basis for risk assessment of nonindigenous freshwater molluscs. *Conservation Biology* 21: 191–200, https://doi.org/10.1111/j.1523-1739.2006.00563.x

Klymus KK, Merkes CM, Allison MJ, Goldberg CS, Helbing CC, Hunter ME, Jackson CA, Lance RF, Mangan AM, Monroe EM, Piaggio AJ, Stokdyk JP, Wilson CC, Richter CA (2020) Reporting the limits of detection and quantification for environmental DNA assays. *Environmental DNA* 2: 271–282, https://doi.org/10.1002/edn3.29

Kuznetsova A, Brockhoff B, Christensen HB (2014) lmerTest: tests for random and fixed effects for linear mixed effects models (lmer objects of lme4 package) (2.0-3) [R package]

Larson ER, Renshaw MA, Gantz CA, Umek J, Chandra S, Lodge DM, Egan SP (2017) Environmental DNA (eDNA) detects the invasive crayfishes *Orconectes rusticus* and *Pacifastacus leniusculus* in large lakes of North America. *Hydrobiologia* 800: 173–185, https://doi.org/10.1007/s10750-017-3210-7

Leek MR (2001) Adaptive procedures in psychophysical research. *Perception & Psychophysics* 63: 1279–1292, https://doi.org/10.3758/BF03194543

Lenth R (2016) Least-Squares Means: the {R} Package {lsmeans}. *Journal of Statistical Software* 69:1–33, https://doi.org/10.18637/jss.v069.i01

Lennox R, Choi K, Harrison PM, Paterson JE, Peat TB, Ward TD, Cooke SJ (2015) Improving science-based invasive species management with physiological knowledge, concepts, and tools. *Biological Invasions* 17: 2213–2227, https://doi.org/10.1007/s10530-015-0884-5

Linares D, López-Moliner J (2016) quickpsy: An R package to fit psychometric functions for multiple groups. *The R Journal* 8(1): 122–131, https://doi.org/10.32614/RJ-2016-008

Lodge DM, Simonin PW. Burgiel SW, Keller RP, Bossenbroek JM, Jerde CL, Kramer AM, Rutherford ES, Barnes MA, Wattmann ME, Chadderton WL, Apriesnig JL, Beletsky D, Cooke RM, Drake JM, Egan SP, Finnoff DC, Gantz CA, Grey EK, Hoff MH, Howeth JG, Jensen RA, Larson ER, Mandrak NE, Mason DM, Martinez FA, Newcomb TJ, Rothlisberger JD, Tucker AJ, Warziniack TW, Zhang H (2016) Risk analysis and bioeconomics of invasive species to inform policy and management. *Annual Reviews of Environment and Resources* 41: 453–488, https://doi.org/10.1146/annurev-environ-110615-085532

Long RA, Donovan TM, Mackay P, Zielinski WJ, Buzas JS (2007a) Comparing scat detection dogs, cameras, and hair snares for surveying carnivores. *The Journal of Wildlife Management* 71: 2018–2025, https://doi.org/10.2193/2006-292

Long RA, Donovan TM, Mackay P, Zielinski WJ, Buzas JS (2007b) Effectiveness of scat detection dogs for detecting forest carnivores. *The Journal of Wildlife Management* 71: 2007–2017, https://doi.org/10.2193/2006-230

Lubow RE (1974) High-order concept formation in the pigeon. *Journal of the Experimental Analysis of Behavior* 21: 475–483, https://doi.org/10.1901/jeab.1974.21-475

Lucy F (2006) Early life stages of *Dreissena polymorpha* (zebra mussel): the importance of long-term datasets in invasion ecology. *Aquatic Invasions* 1: 171–182, https://doi.org/10.3391/ai.2006.1.3.12

Miehls ALJ, Mason DM, Frank KA, Krause AE, Peacor SD, Taylor WW (2009) Invasive species impacts on ecosystem structure and function: a comparison of Oneida Lake, New York, USA, before and after zebra mussel invasion. *Ecological Modelling* 220: 3194–3209, https://doi.org/10.1016/j.ecolmodel.2009.07.020

Nakano D, Strayer DL (2014) Biofouling animals in fresh water: biology, impacts, and ecosystem engineering. *Frontiers in Ecology and the Environment* 12: 167–175, https://doi.org/10.1890/130071

Peñarrubia L, Alcaraz C, biji de Vaate A, Sanz N, Pla C, Vidal O, Viñas J (2016) Validated methodology for quantifying infestation levels of dreissenid mussels in environmental DNA (eDNA) samples. *Scientific Reports* 6: 39067

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52: 273–288, https://doi.org/10.1016/j.ecolecon.2004.10.002

Ram JL, McMahon RF (1996) Introduction: the biology, ecology, and physiology of zebra mussels. *American Zoologist* 36: 239–243, https://doi.org/10.1093/icb/36.3.239

Robson HL, Noble TH, Saunders RJ, Robson SK, Burrows DW, Jerry DR (2016) Fine-tuning for the tropics: application of eDNA technology for invasive fish detection in tropical freshwater ecosystems. *Molecular Ecology Resources* 16: 922–932, https://doi.org/10.1111/1755-0998.12505

Schrier AM, Brady PM (1987) Categorization of natural stimuli by monkeys (*Macaca mulatta*): effects of stimulus set size and modification of exemplars. *Journal of Experimental Psychology: Animal Behavior Processes* 13: 136–143, https://doi.org/10.1037/0097-7403.13.2.136

Secondi J, Dejean T, Valentini A, Audebaud B, Miaud C (2016) Detection of a global aquatic invasive amphibian, *Xenopus laevis*, using environmental DNA. *Amphibia-Reptilia* 37: 131–136, https://doi.org/10.1163/15685381-00003036

Sieracki JL, Bossenbroek JM, Chadderton WL (2014) A spatial modeling approach to predicting the secondary spread of invasive species due to ballast water discharge. *PLoS ONE* 9: e114217, https://doi.org/10.1371/journal.pone.0114217

Takahara T, Minamoto T, Yamanaka H, Doi H, Kawabata Z (2012) Estimation of fish biomass using environmental DNA. *PLoS ONE* 7: e35868, https://doi.org/10.1371/journal.pone.0035868

Turner CR, Barnes MA, Xu CC, Jones SE, Jerde CL, Lodge DM (2014a) Particle size distribution and optimal capture of aqueous macrobial eDNA. *Methods in Ecology and Evolution* 5: 676–684, https://doi.org/10.1111/2041-210X.12206

Turner CR, Miller DJ, Coyne KJ, Corush J (2014b) Improved methods for capture, extraction, and quantitative assay of environmental DNA from Asian bigheaded carp (*Hypophthalmichthys* spp.). *PLoS ONE* 9: e114329, https://doi.org/10.1371/journal.pone.0114329

Yee KK, Wysocki CJ (2001) Odorant exposure increases olfactory sensitivity: olfactory epithelium is implicated. *Physiology & Behavior* 72: 705–711, https://doi.org/10.1016/S0031-9384(01)00428-0

Web sites and online databases

Johnson G, Duckworth J, Schmidt S (2019) Aquatic Invasive Species Early Detection and Monitoring Program: Laboratory Standard Operating Procedures Spring 2019. Montana Fish, Wildlife, and Parks. https://fwp.mt.gov/binaries/content/assets/fwp/conservation/ais/reports/2019-ais-laboratory-standard-operating-procedures.pdf (accessed 13 February 2022)