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Research Article

The ability of scent detection canines to detect the presence of quagga mussel (*Dreissena rostriformis bugensis*) veligers

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Abstract

The discovery of quagga mussels (*Dreissena rostriformis bugensis*) in Lake Mead in 2007 was the catalyst for the California Department of Fish and Wildlife to train their canines to detect adult quagga mussels by scent. The use of specialized detection dogs has increased the effectiveness of watercraft inspections and helped prevent further infestations by this invasive species. Since these canines are currently being utilized to detect adult and juvenile quagga mussels, we investigated if canines can detect the veliger larvae stage, as the transportation of larva via watercraft remains a potential pathway of introduction. Although the canines were already imprinted for adult quagga mussels, in which an odor is associated with reward, they required further training to reliably detect veliger larvae populations. Over the course of a 3-day study the canines' detection rates became more sensitive as familiarity with the veliger odor training progressed. For the lowest concentration blind trial (31 veligers per jar), all canines used in the experiment were able to correctly identify samples with veligers larvae after training.

Key words: invasive species, invertebrates, quagga mussel, canine, scent, conservation, detection

Introduction

Invasive quagga mussels (*Dreissena rostriformis bugensis)* (Andrusov, 1897) and other dreissenid mussels are detrimental to both the ecosystem and economy (LaBounty and Roefer 2007). The economic cost due to the invasion of zebra mussels *(Dreissena polymorpha*) and quagga mussels in the Eastern US was estimated to be as high as one billion dollars a year (Pimentel et al. 2005), and has increased as mussels invaded westward. Economic costs associated with infestations of water intake systems by these mussels are estimated to range from $100 million to $1 billion per year in North America (Pimentel et al. 2005; Bidwell 2010), with overall estimated costs in the United States ranging from $1 billion to $5 billion per year (Aldridge et al. 2006). Dreissenid mussels are difficult, if not impossible, to eradicate once introduced to a new environment due to a lack of natural predators, as well as other biological and environmental factors. As early as 2007, the California Department of Fish and Wildlife began to search for means of detecting dreissenid mussels on watercraft to prevent infestations (Volkoff et al. 2015). The Law Enforcement Division added the detection of dreissenid mussels to their already successful canine program.

With highly developed olfactory senses, canines can provide invaluable assistance in detecting scents that our senses cannot because they are up to 100,000 times more alert to smells than humans are. Canines were first used in conservation efforts in the 1990s. Recent applications have expanded both the scope and sophistication of canine contributions, particularly through scent detection and discrimination work (Browne et al. 2006). When compared to humans visually searching, canines have 2 to 4 times greater detection rates (Homan et al. 2001; Smith et al. 2001). Conservation canines are now being trained to recover carcasses, locate invasive and endangered species, detect animal scent trails,

and identify occupied burrows (Homan et al. 2001; Reed et al. 2011; Reindl-Thompson et al. 2006; Smith et al. 2001; Smith et al. 2003).

The California Department of Fish and Wildlife to date has had nineteen positive indications on watercraft where the presence of quagga mussels was detected by dogs and confirmed by humans (Shimek, personal communication, 15 June 2013). Mussel Dogs® currently performs inspections and education at Lake Sonoma, Lake Mendocino and Modesto Reservoir in California. During these events Mussel Dogs® had not yet had an incident where adult mussels were present on a boat under inspection (DeShon, personal communication, 14 October 2013). Since adult mussels are rare, determining if they can be detected during different stages of development is important. Through this experience it became imperative to determine if canines can detect mussel larvae in raw water contained by boats under inspection, since humans are unable to detect them due to their microscopic size (Johnson and Carlton 1996). Quagga mussels undergo metamorphosis from veliger to juveniles, but it is not until the juvenile stage they can be physically seen. This is the first time canines have been evaluated for their ability to detect mussel veligers (larvae) (Gerstenberger et al. 2011).

This study was designed to determine the viability of training canines to detect the presence of veligers in lake water known to contain quagga mussels. We predicted that canines that are already trained to detect adult and juvenile quagga mussels can be trained to detect quagga veligers. If true, the results will establish the benefit of adding the veliger stage of quagga mussels to the repertoire of the Mussel Detection Canine Programs currently being utilized as an effective detection and prevention tool from future infestation in lakes.

Methods

Canines utilized

Four canines were selected to participate in the study due to their ongoing exposure to adult and juvenile quagga mussels. Canines are trained (Table 1) to produce a final response indicated by a passive alert (sitting, staring, or lying down) when they detect the odor of dreissenid mussels, at which point the canine receives a toy reward. Canine 1, a 5-year-old male chocolate Labrador Retriever, was trained and certified exclusively to detect zebra and quagga mussels. Canine 2 (4-year-old male black Belgian Malinois), canine 3 (7.5-year-old male German Shepherd), and canine 4 (5-year-old male German Shepherd) (Figure 1) were trained to detect quagga mussels, abalone, deer, bear and lobster.

Table 1. Recommendations for selection, training and evaluation of canines for use to detect dreissenid mussels (modified from Smith et al. 2003).

Training Basics
Initial training: -evaluate response of the canine to a particular toy -select a canine with a strong desire to possess the toy -condition the canine to associate the scent of dreissenid mussels with the toy -train canine to indicate by sitting when detecting odor of dreissenid mussels -train canine to ignore odor from other sources
Field training: -evaluate canine performance in numerous situations and environments -select dog with high motivation and consistent performance
Controlled training: -evaluate ability of the canine to stay motivated with repetitive tasks and scenarios -expose canine to set-ups that contain odor and non-odor designs -select canine that performs well in both designs
Field and controlled training: -expose canines to varying concentrations of dreissenid mussels -establish training and maintenance training schedules -require experienced trainers and handlers -require annual team certifications

Materials

Quagga mussel veligers were collected from the Boulder Basin, Lake Mead, Nevada-Arizona, USA (36º1′50.69″N; 114º46′12.95″W) at 12 meters deep using a 64 μm pore size plankton net (Gerstenberger et al. 2011). A plankton net was utilized and lowered 6–11 meters per tow from a dock in the marina. A Nylon bag (500 μm mesh size) was used to filter large organisms such as daphnia and copepods in the field. The least developed veligers (i.e. trochophore) are usually longer than 100 μm and wider than 85 μm (Misamore et al. 2015). Therefore, a separate nylon bag (50 μm mesh size) was used to collect control water (non-veliger water). To determine veliger concentrations, wet slides were prepared utilizing ethanol to kill the veligers in a small portion of each freshly collected plankton sample (N = 3 for freshly collected lake plankton sample) and then a count was taken using a microscope equipped with cross-polarizing light (Olympus Stereo Zoom, model SZ4045ESD), the veligers were readily identifiable because they appeared as water droplets containing "rainbow drops of color" as a result of calcium in the shell, which refracted the colors in the water (Johnson and Carlton 1996; Gerstenberger et al. 2011). The veliger larvae populations were carefully counted to determine the exact number present in the

Figure 1. Water sample jars (A), testing buckets, experimental canines and handlers (B) (Photo by W.H. Wong and D. Farmer).

veliger larvae water for an accurate reflection of the capabilities of the canines of determining their presence or absence. After the veligers in a small portion of the plankton sample was quantified under the microscope, certain volume of lake water with certain amount of veligers were placed in one of five standard pint-sized glass mason jars (Figure 1A). Each jar contained approximately 360–425 mL of 50 µm mesh-filtered Lake Mead water (Mean = 401 mgL, SD = 18.9 mL). The positive sample jar contained both 50 µm mesh-filtered Lake Mead water and certain volume of 500 µm mesh-filtered plankton sample with veligers while there were only 50 µm mesh-filtered lake water for the negative (i.e. the control) sample jars. The Boulder Basin of Lake Mead is oligotrophic and clear. The water sampling site is nearby a long-term water quality monitoring station, CR346.4, where the Chlorophyll *a* concentration is usually < 1 mg/m^3 and water clarity is averaged at around 8 m (Wong et al. 2011). Therefore, there were generally no visible suspended particles in the water which would impact the processing between positive and negative samples.

All trial samples contained various non-veliger organisms (rotifers, copepods and daphnia). Non-veliger organisms in control and veliger positive samples established that the dogs were differentiating the veliger odor from other odors present in lake water in general. Using natural lake water without veligers as the control is more realistic than using purified lake water. At the beginning of the experiment (i.e. before 50% dilution), the veliger jars contained veligers (Mean = 20.2 individuals/mL, standard deviation = 12.9) and rotifers and small daphnia and copepods (Mean = 12.0 individuals/mL, standard deviation = 7.3). The control sample had no veligers (Mean = 0, standard deviation = 0) but with a few rotifers (Mean = 3.6 individuals/mL, standard deviation = 0.55). The experimental jars are shown in Figure 1A. Equipment for the study included five five-gallon commercially made plastic buckets including snap on lids with three-inch diameter circular holes and a wooden shelf inside to place samples on (Figure 1B). Water sample jars were placed on shelf directly beneath hole and approximately one inch below the bucket lid. The wooden shelf was supported by PVC pipe of varying lengths secured by a round piece of wood with a metal plate at the base. Height of the shelf could be adjusted with different PVC lengths.

Ten five-gallon clean bucket systems were prepared during the study in case of accidental water contamination during the trials. Buckets containing

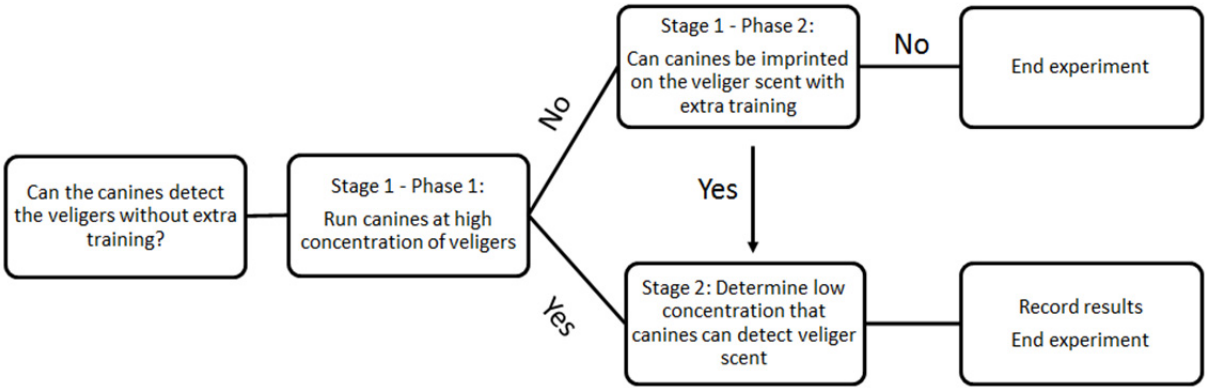

Figure 2. Outline of the experiment.

spills were quickly and entirely replaced with a new bucket system on several occasions, which alleviated lengthy downtime and the chance of contamination during the trials.

Procedure

Five buckets were used in each trial run with one bucket containing a veliger larvae population sample and four buckets containing control water samples. During the entire course, each trial run from each canine was random (i.e., the canine would select any number(s) of the buckets since it is not a forced choice procedure). The study was conducted in a room under ambient temperature. Initially the buckets were set up free standing in a circle in the center of the room with approximately three feet between buckets. Two cement bricks were placed into the base of each bucket on top of the existing housing and each sample jar was secured with Velcro to the wood base. Lids were added to the mason jars with holes poked through the center to allow odor to escape the jar, yet limit spillage. The tops of the buckets were wiped down with clean tissues between each run to reduce the presence of canine odor contamination.

The veliger larvae concentrations utilized were determined by initially creating a concentrated population to ensure an accurate imprint (defined as initial target odor discrimination training) of the veliger larvae signature odor. The methodology was to halve each veliger larvae population sample by 50% (dilution with veliger-free 50 µm mesh-filtered lake water) upon successful imprint down to the lowest detectable level by the canines. All the canines in the study were trained to search on command from their handler. Upon entering the room, the handler would instruct their canine to sniff the bucket lids and watch for the trained response indicating the canine had detected an odor that he was trained to recognize. The handler verbally acknowledged any alert to the proctor and waited for a positive or negative confirmation. If the alert was positive the handler provided a physical reward toy to the canine accompanied by verbal praise. Only one canine at a time ran each trial. Upon completion of each blind trial run, the handler and canine left the room and did not discuss the course with the other handlers. Run times varied between teams with approximately 10 minutes for each dog per trial.

The study consisted of two stages with the first stage imprinting dreissenid mussel odor on the canines, in high and reduced concentrations (Figure 2). The second stage of the study tested the accuracy of canines in detecting veligers in blind trials at a concentration consistent with what would be seen in the field.

STAGE 1: Training and Imprinting Phase One

Stage one started with a blind trial exposure to assess the ability of the canine to recognize veligers with no previous training. The handlers had no knowledge of which bucket contained the veliger sample prior to entering the room. Each canine was individually instructed to check the buckets upon verbal command by their handler, as well as physical direction using methodology consistent with their existing training (waving arm across buckets to provide general direction, tapping individual buckets for individual focus to each bucket, finger pointing to hole in center of bucket). The canines were run one at a time under careful observation by the

handler, with no other canine present. A proctor was present only to provide direction when an alert was called and record results.

The buckets were initially presented to the canine in a circular formation in the center of the room, in which one of the buckets contained the veliger larvae sample. This formation was changed to a straight line against a wall. Chairs were also added to this formation, with front edge of the chair slightly over the lids to limit spillage in the event the canine knocked over the bucket. Chairs were also added between the buckets to ensure even and ample spacing between the samples. The canines were then instructed by their handlers to sniff the bucket lids, while the handlers watched for any indication that they detected an odor they were trained to recognize. The air conditioning was turned off to prevent the movement of air across the bucket surfaces and to avoid contamination or scent confusion.

The veliger sample concentration was started at 2088 veligers/360 mL of water. As per protocol, the buckets containing water spills were replaced with clean bucket systems and jars.

STAGE 1: Training and Imprinting Phase Two

The handlers then imprinted the veliger larvae scent on their canines by verbally praising them and offering a reward when they correctly identified the veliger larvae bucket. During these drills, the buckets were periodically rearranged (both the veliger larvae bucket and the control buckets) to ensure the canines were discriminating odor and not marking bucket placement. Precaution was taken to ensure the canines were clearly discriminating veliger larvae odor to receive a reward.

After an evaluation of the blind trial and imprinting sessions, it was determined that additional training on varying veliger concentrations was needed before conducting more blind trial runs. Reinforcement runs were when a canine reached a bucket containing the veliger scent, the canine was immediately cued to sit and rewarded with his toy (Smith et al. 2003). The trainers and handlers felt it was necessary for the canines to correctly imprint the veliger larvae odor starting at a high concentration level and then evaluate that imprint with a 50% reduction to lower veliger larvae populations over the course of several trials to determine if the canines recognized the scent signature of the veliger larvae. The lowering density detection training focused on determining the lowest possible veliger concentration that the canines could find through scent detection, which was done through systematic sample concentration reduction.

Systematic sample concentration reduction began with 1000 veligers in 360 mL and was continually reduced by 50% for each consecutive run. The veliger sample in the sixth run contained 31 veliger larvae (1.5% of the original veliger concentration. The seventh run used only control samples with no veligers present. This insured the canines were clearly investigating each sample bucket presented to them—even when no positive bucket was available.

During the systematic sample concentration reduction, the runs were done as blind runs in the linear formation with the chairs against the wall, with one of the five buckets containing a positive sample, except in the last run in which 0 veligers were present. The configuration was changed during the seventh run to a circular formation. Results were recorded the same way as phase one.

STAGE 2: Lowest Concentration Blind Trial

Each team performed four separate blind runs utilizing a concentration of 31 veligers on day three. Five buckets were used in circular formation with chairs included for stability, with one containing the veliger sample and the remaining four containing control water samples. Each blind run had the buckets rearranged and fresh samples were exchanged with used samples.

One refresher training run was conducted with the veliger larvae population at 31 prior to the blind runs. Both the veliger larvae buckets and the control sample buckets were rotated frequently during the blind runs to ensure none of the canines would mark any of the bucket placements in the circle during the study.

Results

STAGE 1: Training and Imprinting Phase One

In the initial blind run with buckets in a circular configuration, only Canine 1 was able to correctly identify the bucket containing the veliger sample. Canine 4 was the second canine to run. Under this configuration, Canine 4's trial was not completed and results were inconclusive. This was due to the canine disrupting the buckets and spilling the water samples. It was determined a new configuration was necessary, so Canine 2 and 3 did not participate in this run (Table 3).

After increasing the concentration of veligers in the sample and rearranging the buckets in a linear formation against the wall, Canines 1, 3, and 4 were able to correctly identify the veliger sample. Canine 2 was unable to identify the veliger sample.

Table 2. Stage 1 result of all attempts by all canines in which handlers were aware of the contents of each sample, "yes" denotes a correct alert in response to samples containing veligers or a lack of an alert in the case of samples not containing veligers. "No" indicates a failure to correctly alert or not alert. Run 0 consisted of initial highest concentration imprint training. Runs 1 through 7 consisted of systematic sample concentration reduction runs.

Run Number	# of Veligers/ Jar	Attempt	Canine			
			Canine #1	Canine #2	Canine #3	Canine #4
0	2088 veligers	1	Yes	No	Yes	Yes
		2	Yes	No	Not Run	Not Run
1	1000 veligers	1	Yes	No[1]	No[1]	Yes
		2	Yes	No[1]	No[1]	Yes
		3	Yes	Yes	Yes	Yes
		4	Not Run	Yes	Yes	Not Run
2	500 veligers	1	Yes	Yes	Yes	Yes[2]
		2	Yes	Yes	Yes	Not Run[2]
3	250 veligers	1	Yes	Yes	Yes	Yes
		2	Yes	Yes	Yes	Yes
4	125 Veligers	1	Yes	Yes	Yes	Yes
		2	Yes	Yes	Yes	Yes
5	62 Veligers	1	Yes	Yes	Yes	Yes
		2	Yes	Yes	No	Yes
		3	Not Run	Not Run	Yes	Not Run
6	31 Veligers	1	Yes	Yes	Yes	Yes
		2	Yes	Yes	Yes	Yes
7	0 Veligers	1	Yes	Yes	Yes	Yes

[1]Reinforcement runs performed
[2]Canine #4 not run for attempt #2 due to spilling bucket in attempt #1

Canine 2 inaccurately identified two control buckets in his initial run (Table 2). A second run was done after the handler attempted to imprint the canine on the sample, in which Canine 2 was still unable to correctly identify the veliger sample.

During the systematic sample concentration reduction, Canines 1 and 4 alerted on all veliger samples correctly. In the first two run attempts, with concentrations at 1000 veligers, Canines 2 and 3 did not alert (Table 2). After reinforcement runs, Canine 2 alerted successfully in all subsequent runs as the veliger concentration decreased (Table 2) and Canine 3 alerted successfully in all runs except in run 5 (Table 2). As expected, the four canines did not alert on any of the five buckets in run 7, which contained only control samples (Table 2).

The reinforcement run used the same concentration of 1000 veligers as in run 1. Canines 1, 2, and 4 correctly identified the veliger sample with no inaccurate alerts (Table 3). Four more blind runs were run with buckets in linear formation (Table 3). All canines were showing signs of fatigue and boredom. The trials were terminated and the handlers met to discuss the situation. Buckets were changed to a circular formation for the next 4 runs, in which all canines exhibited multiple inaccurate alerts. Canine 1 was not able to complete the final 2 runs.

STAGE 2: Lowest Concentration Blind Trial

All canines were able to complete 4 blind runs attempting to detect a sample containing only 31 veligers. During these blind runs, all 4 canines were able to accurately alert on the correct sample indicating the veliger positive sample with no inaccurate alerts (Table 4).

Discussion

All of the canine teams in this study were pre-certified teams currently working in the field. The handler had to be able to recognize a positive indication by the canine.

STAGE 1: Training and Imprinting Phase One

Out of two canines, Canine 1 was the only dog to alert on the veliger sample. Canine 1 is the only canine in the study that is exclusively trained to detect quagga mussels and familiar with the bucket set-up. Canine 4 had a tendency to knock over the samples, creating unexpected problems which caused his run to be terminated; therefore, his results are inconclusive. Prior training, specifically on this type of bucket set-up, could have alleviated this problem.

Table 3. Stage 1, number of alert behaviors during all attempts in which handlers were unaware of the contents of each sample, during each attempt samples containing a defined density of veligers were paired with control samples containing no veligers (0 veligers). Initial circular arrangement run consisted of blind run to determine veliger recognition without prior training. **Handlers called all instances that they thought the canine was alerting.**

Arrangement of samples	# of veligers in the sample	Canine			
		Canine #1	Canine #2	Canine #3	Canine #4
Initial Circular Arrangement	0 veligers	0	Not Run	Not Run	Inconclusive
	574 veligers	1	Not Run	Not Run	Inconclusive
Standard Sample Arrangement	0 veligers	1	2	2	3
	1000 veligers	1	0	0	0
	0 veligers	1	0	1	0
	1000 veligers	1	1	1	1
	0 veligers	1	0	0	0
	500 veligers	1	1	1	1
	0 veligers	0	2	3	3
	250 veligers	1	0	0	0
	0 veligers	2	1	Not Run	Not Run
	125 veligers	0	1	Not Run	Not Run
Circular sample arrangement	0 veligers	1	0	1	1
	250 veligers	0	1	1	1
	0 veligers	1	2	0	0
	125 veligers	0[1]	0	1	1
Circular sample arrangement with chair augmentation	0 veligers	Not Run	0	0	0
	62 veligers	Not Run	1	1	1
	0 veligers	Not Run	2	1	0
	1000 veligers	Not Run	0	0	1

Handlers were not aware of the contents of each sample.
[1]Canine quit working; pulled for the day.

Table 4. Stage 2 result of all attempts to detect 31 veligers by all canines in which handlers were unaware of the contents of each sample, "Yes" denotes a correct alert in response to samples containing veligers, "No" indicates a correct response to not alert on 0 veliger samples.

Attempt	Veligers	Canine			
		Canine #1	Canine #2	Canine #3	Canine #4
1	0 veligers	No	No	No	No
	31 veligers	Yes	Yes	Yes	Yes
2	0 veligers	No	No	No	No
	31 veligers	Yes	Yes	Yes	Yes
3	0 veligers	No	No	No	No
	31 veligers	Yes	Yes	Yes	Yes
4	0 veligers	No	No	No	No
	31 veligers	Yes	Yes	Yes	Yes

As the trials continued, the canines learned not to scratch at the buckets and this problem was completely eliminated.

It was determined that Canine 2 was not scent discriminating the veliger sample at this time. In order to continue the study, it was decided to spend time training each canine on the varying veliger concentrations. When a canine reached a bucket containing the veligers, the canine was immediately cued to sit and rewarded with his toy (Smith et al. 2003). The trainers and handlers felt it was necessary for the canines to correctly imprint the veliger odor starting at a high concentration and then reevaluate that imprint with a 50% reduction of veligers. If a canine didn't recognize the 50% reduction, reinforcement runs were done to imprint at that level.

STAGE 1: Training and Imprinting Phase Two

Each handler knew the placement of the veliger larvae bucket prior to each training run to give them the ability to accurately reward their canine

immediately upon successful detection. This was an important part of the training process. Each canine checked all five buckets multiple times. When the canine alerted on the veliger sample, if he did not produce an alert on his own—he was instructed to alert (utilizing each natural alert of the canine such as sitting/staring/lying down) through verbal encouragement (sit command and/or verbal praise). Each was then rewarded for successful detection with verbal praise and a physical reward (a ball, tug or toy depending on the canine) to reinforce the veliger larvae scent picture. The number of times this exercise was conducted varied depending on each dog and his ability to understand the veliger larvae odor consistently.

The veliger larvae bucket and control buckets were moved multiple times during this training to insure the canines were differentiating the odor of the veliger larvae versus the placement of the buckets. Great care was taken at this stage to ensure the canines recognized they were being rewarded for their positive alert response only upon recognition of veliger larvae odor.

Within a three day period the canines recognized the veliger odor, after some reinforcement training with Canines 2 and 3 (Table 2), and were able to respond accurately as each concentration was reduced by 50%. It was determined that the canines could be trained to identify and alert on varying veliger concentrations.

After the sixth run (1.5% of original sample), it was determined that no further veliger population reductions were necessary for this study. At this low level of veligers (31 veligers per jar or 0.086 veligers/mL), given the relatively higher natural veliger mortality (Sprung 1987) and disturbance to the veligers during transportation (Choi et al. 2013), the risk for transported veligers to survive in receiving waters should be quite low. At 31veligers/Jar and higher, the canines were consistently exhibiting a clear understanding of the veliger larvae scent signature and accurately alerting.

During training runs, run 7 contained only control water samples; none of the four canines alerted on the buckets. This ensured the canines were clearly investigating each sample bucket presented to them—even when no positive buckets were included in the set-up.

An evaluation by the trainers and handlers revealed indicators that the dogs were frustrated from working the buckets repeatedly in the same room over the course of two days. This type of work was out of their normal scope of work. The difference in the training runs versus the blind trials was also evaluated. When the handlers knew the correct placement of the veliger bucket in a test run, they were able to quickly reward their canine upon a successful alert. During the blind trials, there was a time lapse between calling the alert and being rewarded. This seemed to occasionally cause confusion since the canines would sometimes move away from the veliger bucket to continue working. When that happened, it seemed to cause the handler to doubt the accuracy of the dog and they would not call the alert or would move the dog past that particular bucket without a sufficient presentation as the run continued. Blind trials do ensure the handler does not unconsciously cue the canine.

This confusion shows the importance of solidly training the dogs on odor before conducting blind runs, in this study and in the field. By solidly training the dogs on the veliger odor, the handler trusts the dog. In the field, the handler does not give the dog the normal reward on odors that are unconfirmed. The odor is also reinforced in the field by hiding known odors. As seen in the field, with proper rest, motivation, and training, the canine can work reliably all day. Once odor is imprinted, the canine should be able to recognize the odor. Additional study should be done to determine minimum amount of reinforcement necessary to retain this ability.

It was noted that all the canines needed to put their nose directly above the surface of the jars in order to ensure a good sniff of the water samples. If a canine was moving too quickly—he had to be redirected to stick his nose into the bucket lid prior to making the decision to move on or alert. This repetitive, slow and deliberate presentation seemed to cause frustration in some of the canines as the buckets were rechecked numerous times during the same run.

It was consistently observed that once the canines clearly recognized the veliger scent, they were able to differentiate the odor from the control samples. The handlers admitted that they felt frustrated with the testing procedures after running the course over the two day period. Their canines periodically displayed alerts on the correct veliger larvae buckets, but the handlers did not call the alerts due to their own feelings of frustration in reading the alerts of their canines after their canine exhibited signs of fatigue, frustration and/or excitement. Taking the mid-day break and rearranging the buckets into a circular formation helped get the canine teams focused on successfully detecting the veliger samples.

In this repetitive format the canines became frustrated with the trial runs, periodically displaying

frustration behavior. The following were noted in different canines at different times: barking, automatically alerting on the first and/or last buckets in the row, knocking random buckets over by pawing or biting at them, sitting on all the buckets one after the other unassociated with sniffing the buckets, refusal to sniff each bucket as it was presented. Not all of the canines demonstrated all of these behaviors. Future canines in similar experiments should be exposed to the trial configuration to associate this situation with being rewarded.

Despite all the frustration that existed during the blind trial exposure between both canines and handlers, the canines still displayed a clear understanding in discriminating the veliger samples at a low threshold of odor.

The consensus was it would be ineffective to continue the study under the existing conditions. In an attempt to give the canines a fresh presentation of the buckets and thereby stimulate their interest in searching in a slightly different format, the original concept of putting the buckets in a circle in the center of the room was utilized. This was also prompted by Canine 3's behavior of a tendency to alert on end buckets without checking them first. This time chairs were included in the circle for bucket stability. The canines and handlers were given a two hour break and the study was resumed as a blind trial with four more trial runs completed.

In the interest of determining the viability of canines to detect the veliger larvae, instead of repeating the previous two days trials by starting at a higher veliger larvae concentration and then systematically reducing the veliger concentration—the only sample to be utilized would be the lowest threshold veliger concentration previously detected (31 veligers). This number is significant from a practical standpoint since it represents a very low concentration that is likely to exist during most of the year in a naturally occurring lake environment. If the dogs can detect veligers at this low population (31 veligers), they would be able to provide a practical detection service during routine inspections. Thirty-one represents such a low concentration that it is highly unlikely the veligers would be capable of establishing a population; therefore, if canines can detect such a low veliger population (31 veligers), their chance of detecting higher populations is significantly better, thus ensuring even greater overall success in the field.

It was illustrated that canines can be successfully imprinted with the odor of quagga veligers at levels practical in the field. It has been reported the average boat leaving Lake Mead could carry 98 to 144 living veligers in its residual water (Choi et. al 2013). Veligers are present in Lake Mead throughout the year, and densities can be as high as 39 veligers/L (Gerstenberger et al. 2011). Further study should be done to determine the extent of the canines' ability to detect quagga veliger odor. It is also suggested that more trials per dog (> 4) over a longer time (> 10 minutes) to find the lowest veliger number in an experimental jar (i.e., < 31 veligers) that a canine can detect. Furthermore, a more complex experimental design with multiple jars as control (i.e., no veligers) and multiple jars as positive samples (i.e., with veligers). Finally, there were still a few other creatures (i.e., small daphnia and copepods) left in the testing jars which may provide potential bias to the result although their numbers were every low in the highly diluted low-veliger concentration trials. There is still a small chance that the dogs were not trained to detect veligers only. Therefore, the small non-veliger organisms should be isolated from the testing jars in future studies to avoid any potential bias in result interpretation. Furthermore, pilot studies mimic real operational search conditions in which the rate of positives would be much lower should be conducted to confirm the presence of veliger in situ.

Canines could provide effective augmentation to current inspection programs. Canine abilities could improve both the speed and effectiveness of inspections particularly where human inspectors would be impaired such as low light conditions or inaccessible reaches of the watercraft.

Handler and Trainer Discussion

The trainers and handlers agreed this study might have been more effective if the canines had the opportunity to train with the bucket system utilized in the trials prior to the study. This would have saved time spent in training and shortened the learning curve. If all of the canines had a familiarity with the buckets, there would have been more time available to challenge the canines with a greater variety of veliger concentration placements.

It is crucial to the success of a Veliger Detection Canine Program for the canines to receive ongoing maintenance training specifically on veligers to insure they retain the odor signature in their repertoire. This is no different than any other odor a trained canine being utilized in the field must undergo. The importance of using properly trained and tested detection canines and professionally trained handlers cannot be overstated (Reindl-Thompson et al. 2006).

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