

Mussel Monitoring Conference transcript

Key: Q= question, A= Answer, C= comment, CPM= cross- polarized light microscopy

Wednesday 8:30

Introduction:

Curt Brown- Director of research and development- Reclamation and development
The two top priorities in his office- climate change and invasive mussels. Both of these will greatly affect water issues. One of our main priorities needs to be developing tests to detect zebra/ quaggas. There will always be multiple methods, and we need to understand the strengths and weaknesses of the different methods. When we first try to detect mussels, we will want to use a highly sensitive test.
Curt: Asks how many put money into detection 5 years ago?- a few raised hands.
Key issues are fitting the technologies and the protocols to each individual case and area.

Marc- Through this conference we should be much better prepared in the west than we were in the northeast. -Explains the outline of the meeting and the format that will take place. We will hear examples from people in different regions in different states. On Thursday we hope to be able to make a draft outline of what needs to be implemented this year. In addition, we will touch on goals for future years. However, the focus needs to be on what we do NOW in 2009.

Objective I- Current Early Detection Best Practices,

- **Detection of juvenile settlers and adults-Current Best Practices (Vicki Milano)**

Methods and approaches for detecting juveniles and adults. (Not larvae)
(Works for CO division of wildlife)

We began rudimentary surveying in CO. Began with one technician, went out throughout the state and sampled. First finding of a zebra mussel was last January. We sampled over 100 waters. This report will focus on findings since last January. We needed to determine which waters were most at risk. Made a ranking of waters from 1-4 that determined risk. 1 is the highest, 4 the lowest. Found that 44 waters were very high or high. There were several considerations made in the rankings. (Get spreadsheet data) The best season to do the plankton tow samplings was generally in the summer, dependent on water temperatures. Four additional technicians were hired and they were assigned regions. The next step was to coordinate with the fisheries biologists- who provided boats. Each technician designed a map, and a schedule. If working in a state park, managers were notified and given details of their sampling. Local managers were also provided with data

sheets. Substrate deployment was in late spring/early summer. A length of rope was extended with a substrate attached. GPS position was recorded on substrate data sheets. To date, no adults have been found on any of the substrates. There were two adults found on a substrate in pueblo, but they were compromised by fungus, so we can't be sure they were zebra or quagga mussels. They were firmly attached with bisset threads. This year we plan to use flower pots instead of the plastic grids or PVC pipes. The grids and pipes have degraded. In the high and very high risk waters, substrates checked every two weeks.

Q- Was the pueblo lake a high risk? A- No risk assessment had been made yet. Now it's very high

We didn't have too much trouble with people messing with the substrates, tried to put them in inconspicuous areas. Vertical Plankton Tows- performed at substrate sites, 3-4 times per season, every 2 weeks for highly suspect waters. At BOR managed waters, two plankton tows were done, one for us, one for Denise at BOR. This will change, now we are going to take two aliquots from the same tow instead of doing two separate tows. Water Chemistry- 4 hydrolabs were purchased. One reading was done at the deepest part of the lake; all the basic tests were done. Shoreline Surveys- walked the shore examining rocks and wood to look for zebra or quagga mussels in 1-3ft of water. GPS- coordinates were recorded at all substrates, tows and starting points for shoreline survey.

FlowCam- an imaging system where the water flows through and pictures are taken, provides good baseline data, can do the work of numerous people. Info: Harry Nelson, Fluid Imaging Technologies. Vicki's people will be purchasing one this year. Each tech was given a microscope and did CP microscopy out in the field. Now, we plan do this in the lab instead. There a lot of Asian clams in the state, and this makes things more difficult since it can be difficult to tell the difference. Suspects were sent to John Wood at PICES for PCR confirmation. No positive results yet, fortunately.

Q- Are out of state boaters factored into risk assessment? A- not yet, but now they will be inspected.

Q- Water quality included in risk assessment? A- will be in the future, but wasn't originally

Q- How does CO compare to other states? A- can't comment. Larry- I will talk about it later. Vicki- followed Denise's guidelines used at Lake Mead

Q- What is flowcam used for? A- Flowcam will take the place of CP microscopy.

Q- Sounds like other states have gone through this. There's no info coming from the east that you used to make your studies? A- we didn't get info from the east, but mostly copied Denise's work.

Both 1 CP microscopy and 1 PCR used as confirmation now, instead of 1 CP microscopy and 2 PCR confirmations.

There are four temps working and myself. We may have difficulties because of hiring freezes. There will be two at my lab doing PCR and CPM (full time.)

Comment: I've used a lot of thick clay tiles, rather than flower pots. Still had a lot of breakage. Check out "saltile." Unfinished clay tiles, just drill a hole through it. 75 cents each.

Comment: I'm from California- We did a lot of work looking at rope, pots, there is a report online

C- Sandra- In most places in the east, the monitoring came too late. At Lake George however, early detection was found. Plankton tows, scuba surveys, substrates, many of these similar approaches were used. When adults were found, we were able to reduce the numbers greatly because of early detection.

C- Braided nylon with knots tied in it works well. Go to home depot and ask for the broken cement blocks.

C- Substrates I'm using are plastic nursery trays. Drill a hole in the middle. 12 by 12. Put three in a row with corrugated pipe in the middle.

- **Microscopy- and PCR-Based detection and enumeration of Dreissenid larvae detection in the plankton-History and a primer (Marc Frischer)**

My charge today is to give an overview of the Cross Polarized Light Microscopy (CPM) and the Polymerase Chain Reaction (PCR) detection technologies and go over the strengths and weaknesses of each. There is a lot of frustration because often they disagree. This can be quite challenging. The process: first you collect plankton tows, next you may need to prepare the sample or concentrate it. Then, processing. Finally, analysis. We are focusing here on the veliger stage of the life cycle, and that's what we are looking for in these plankton tows, which are mostly full of algae and other plankton individuals. If the veligers are very rare, a lot of water has to be sampled. Under a basic microscope, the sample is a mess, with lots and lots of gunk. In 1995, Ladd Johnson described the use of CPM in his *Hydrobiologia* publication. It's a dissecting stereo microscope that has two polarizing filters that sandwich the sample. This makes it possible to identify a birefringent individual by the x mark. You can modify regular microscope and fit it with the filters fairly inexpensively.

Q- what's the magnification A- 6X at the objective

Typical protocol: samples collected, filtered between 100- 1000 L, the concentrated about a 1000 fold. Further concentration may take place by settling or centrifugation. Then they are enumerated by CPM. Report the Detection and quantity of larvae.

Challenge of CPMs: Can be very labor intensive, and tedious. Can take a special kind of person. If it takes a person 2 hours to count a sample, it will take the same amount of time each time. Tech needs to be well trained and experienced. Samples will look differently at different times of year; tech needs to be familiar with these changes. Error sources: can get false positives eg. Ostracods, sediment particles, technician may "want" to see a veliger, especially if they have been looking through a lot of samples. The X mark on the veliger is very distinct. False Negatives: Larvae can be obscured in dense samples, sideways orientation of

larvae may make it difficult to identify, not enough sample, poor preservation- the shells may dissolve. Preservation may obscure diagnostic features. One of the main challenges is the amount of time it takes to do CPM.

PCR

Allows for *in vivo* amplification of specific genes. These assays are targeted to unique sequences in their genes. Amplification specificity is determined by the sequence of synthetic small pieces of DNA (primers)

Protocol: Sample Collection, sample concentration, processing, and finally, analysis. It's possible to make PCR quantitative, but for now its only absence or presence. Samples are from March of 07, and show dreissena specific and universal eukaryotic. In this assay they are both in the 18S gene. Challenges: theoretically this can be done in a much timelier manner; many samples can be done in parallel. However, one needs a good lab set up and trained technicians
Error Sources: False positives: lab contamination, non-specific amplification
False negatives: empty shells, PCR inhibition through metals, humics etc., assay failure- template quantity, reagents etc. Promising technologies include the Qiagen BioRobot 8000 which can do 96 wells in a single format, and the *In situ* "GenoSensor" which can be deployed in the field to detect algae bloom species by PCR and RT-PCR

Q- Cost for each test using PCR? A- Hasn't really been quantified. John Wood- 27 dollars per sample when looking for fungus.

CPM and PCR: Pro's and Cons: CPM- not that much infrastructure is required, easily visible and satisfactory. Quite established and is quantitative. However, very labor intensive

PCR- objective that is a specific band on a gel. Scalable and automated. Very widely used diagnostic approach, used in hospitals frequently. Cons- requires a lot of infrastructure, expensive, not standardized yet. Nothings perfect.

Q- has anybody looked at gauging the centrifuge based on veliger weight and densities? A- some, not enough. A lot of work still needs to be done

Q- are we going towards dual conformation? To me it seems CPM is very good method and sure conformation. Why do we need PCR? A- I feel there's enough chance that the veligers can be misidentified that PCR is necessary.

Q- John Darling- In CPM can you tell the difference between Quagga's and Zebra's? A- Its very hard to tell. PCR CAN tell the difference.

Q- Marc Dahlberg- whats your QA/QC in PCR? A- Positive and negative controls, 4 total types of controls.

Q- Tim USGS- Can you do PCR to identify the sperm and eggs? A- don't catch them in plankton tows

Q- What metals cause the false negatives/positives? A- the cations, anything that binds cations can be a strong inhibitor of PCR. There are a lot of heavy metals, lead, cadmium.

Selenium? Probably not.

- **Microscopic detection of zebra and quagga mussel larvae in plankton samples, best practices, advantages, disadvantages, and technical hurdles (Denise Hosler and Steve Wells)**

Denise:

State of the art CPM: 3 methods- the core of engineer's method, the reclamation method (working with the veligers), Dr. Well's method.

There's a lot of variability with sampling. We have a developed flow path that a sample takes through the laboratory. We use a 63/64 micron net. The sample is preserved, no live samples; sample is logged so it can be tracked.

- 1) Core of Engineers method: Interested in detecting veligers when they were rare. With the core method we were amplifying the methods way high. This is a combination of standard method. Veligers are allowed to settle, 98 percent of all veligers found in the first 15 mL. We use a Petri dish to dilute out junk. One of the aids in identifying veligers is the ability to take it back and forth from CPM to regular microscopes. With this method, it simplified the calculation. It's interesting that in 07 our numbers were much lower than now. Sometimes positive by microscopy but neg by PCR. Both times the adults were found later. Pueblo reservoir- early detection through CPM, but no adults yet. FlowCam is working fairly well.

Steve Wells (Portland State University)

CPM Methods:

Net opening radius is important. On average we collect 2000 L per tow. We focus on areas we think they will be at. Don't buy denatured ethanol. Not sure why regular ethanol works better, but there are many possible reasons. It may be difficult to get a hold of. Very flammable, so be careful with transport. There has to be Hasmat. Limited, but use FedEx or USUPS. Problem- if you don't collect veliger, its not good. You need to collect large amounts of samples, or you may miss them. Large sample size in necessary. Use statistics to figure out how many quadrats you need to do to detect a rare species. Increase the number of tows whenever possible to find rare species. Smaller quadrats, but more tows. Use more tows per sampling session. More tows total are needed, and they need to be spaced out.

Anaylsis: In 2007 we went through the entire 120 mL sample. Very time intensive. By going through entire sample you reduce false negative likelihood. Now we subsample, which decreases costs. A dense matrix will interfere with visibility. Can dilute the sample with tapwater, will help with very dense samples. Data Reporting and Cross- validation: Difficulties in detecting corbicula veligers- looks like a D shaped Dreissenid veliger, but its much larger, the ranges don't overlap however. Size is used to decide between the two. Another problem, in

some cases you'll have a dorsal/ventral view, you could misidentify it. Ostracods have appendages that are very distinct. Basically, if both have maltese crosses, go back and look at size, appendages and other morphological features. Digital photos are taken of everything so they can be sent out for outside opinions. When a positive veliger is found, it's isolated and sent to microscope techs or PCR.

- **PCR detection of zebra and quagga mussel larvae in plankton samples; best practices; advantages, disadvantages, and technical hurdles (John Wood) Work done at PISCES**

PCR is rapid implement, and can be scaled. 3hrs for 96 samples. Main advantage is species specificity; and there is a very large signal amplification. Our PCR is generally 45 cycles. We make a big deal out of leaking or cross contaminating samples. The sample prep is the main stumbling block. 1 L of sample is still a lot. PCR assays are 3 different steps. 1) DNA extraction- via silica membrane spin columns, 2) PCR reaction "normal" endpoint PCR , not real time. 3) Agarose gel electrophoresis to detect

Current ZM and QM PCR protocols: 18S and 28S . Four main protocols which are listed on powerpoint. To get the 1 L sample into a micro size, we centrifuge or settle and take the bottom 50 mL. Centrifuge again and take the bottom 500 microliters.-this is because this is the most you can put on a DNA spin column. DNA is extracted and put into 200 microliters of buffer. This actually tests only 1/100 of original 10 L sample. When there's not very many veliger's per sample, this scaling down can cause problems. When sample is dirty, it is even more difficult, and only a 1-5 ml sub-sample can be taken. With the dirty ones you're only getting 1/1000 of the original. When a veliger is found through CPM, and its taken off the slide and put into a microfuge tube, it can get lost in the transfer. Then we get a false PCR reading. Non- specific reactions will cause background bands. One of the ways of resolving the PCR ambiguities is to optimize PCR assay for maximum specificity- work on the reaction parameters, do tests for cross reaction species. If there are questions or ambiguities we'll retest the sample multiple times in the same PCR reaction. We may test the sample in PCR reactions with different targets -ITS vs. COX1.

Determine the DNA sequence of the questionable band.-however this ramps up time and cost. Most of the assays, other than marks, have been developed in the last year or so. We are talking about developing a plasmid. We want to improve the primers for the quagga mussels. Improving DNA extraction procedures is still the big hurdle. (Outlined in his powerpoint) New technology would no longer require spinning things through the membrane-this would do 24 samples at a time automatically. We want to explore options for density gradients. If we could make anit-veliger antibodies then we could attach them to the magnetic beads.

Of the three steps, DNA extraction is the one that needs the most work, while gel electrophoresis is quite standardized

Q- Are there lessons from other water sampling that we can learn from A- yes, whirling disease samples, but these are from clear trout streams.

Doing a lower tech DNA extraction is not a feasible option, lots of problems. The spin columns are much better, even though they are not built for large samples

The problems in the tow nets are sediment and algae, particulates, color.

Q-Bob Pitman- how can we clean up dirty samples? A- We will discuss that later.

- **Comparison of PCR and Microscopy. Results to date of analysis of western waters and round robin double blind comparisons. (Sandra Nierzwicki-Bauer)**

We've heard a lot about the good, bad and uncertain in these two methods.

Rationale- there are discrepancies in CPM and PCR results. In the 372 samples we tested 70.7 percent% of the time two methods matched, in 29.3 percent there were discrepancies. The structure of the experiment was to have 3 phases.

The only phase that's complete is phase 1. In phase II we would look at processing compared to sample complexity. In phase III we'd look at sampling issues, differences and getting them more standardized. The objective in phase II is to determine the variability in the analytical procedures and assess whether one is better than the other. Wanted to look at detection,

quantification and inter-lab variability. Experimental design: collected z veligers, quantified with microscopy. In lake champlain veligers have not been identified. No confirmed reports. Took known numbers of veligers and distributed them into clean lake water 40 mL samples. There were 12 replicate sets, 5 per set, with 5 different concentrations of veligers. (0- over 100) Each lab processed the entire sample volume and reported methods,

presence/absence detection and quantification if possible. There were 5 CPM and 5 PCR labs. PCR has a slightly more consistent rate of detection. For sample that had high numbers of veligers CPM was fairly quantitative.

Q- in the samples that had 1-5 veligers, did you go back and check those numbers and calculate a percent error? A- no, we kept one sample set though.

We can reach 100 percent accuracy with both methods, as 2 labs were able to. The goal is to have everyone do this. Conclusions: Both methods have analytical errors. Error rates are roughly comparable in both methods. Error rates are greatest for detection at low numbers of veligers. At best, quantitative data should be used in a semi-quantitative manner. Not all labs and methods are equivalent-there needs to be standardization for analytical methods. Future Studies- Increased replication of samples with low veliger counts. Different sample set variables. General recommendations: inter-laboratory calibration standards, lab certifications? Eg. That QA/QC is being carried out etc.

Sandra- in the future, we need to standardize data reports.

Marc- unfortunately, there was a lot of discrepancies, still, even with this simple study.

Q- is it possible that some of these veligers were just shells and there weren't enough tissue for the PCR? A- the samples were pretty fresh, we looked at them, we think they were pretty good

C- I'm concerned about using 25 percent ethanol

Q- Which methodology was more likely to correct due to clean water? A- Not sure, didn't really look at it that way.

Marc- The overview shows CPM is more accurate, but there's more variability between labs with PCR, which might be the cause of some of these variability's.

C- I don't think 25 percent is enough, because of all the other plankton and algae.

Q- How did the FlowCam do? A- It did very well

- Open Discussion. Immediate needs, best practice based on current methods. Future research needs.

** No open discussion due to time constraints**

Objective II- Current Western Early Detection and Monitoring Programs: Needs for a Comprehensive Regional Plan (Moderator Paul Heimowitz)

- Introduction by moderator. Setting the stage, issues and challenges of establishing a comprehensive standardized western early detection plan. Explanation of choice of "representative speakers (Paul Heimowitz) **Note Taker came in late and missed this section**

- **Southwest Region (Utah) Current detection and monitoring program. Needs for a comprehensive plan. (Larry Dalton)**

Utah has a plan that is currently in Washington DC being reviewed. This comprehensive plan has several parts, including media and education, decontamination and interdiction, etc. They looked at the east and crunched the numbers involved in maintenance, repair, and containment strategies if they do have infestation. In Utah there are lots of primary pipelines and canals while in the east there's not as many. If mussels get into these pipes, there's much less flow through the ditches, canals, pipes etc. This is a huge problem. Utah's legislature became very interested about learning these statistics. The legislature passed a bill with a lot of money for this issue.

Economic Impacts of dreissena: If boaters and anglers are unhappy with their experience due to ecosystem impacts on fish, they may not fish. Anglers spend a lot of money in Utah, and it can't afford to have them leave. In Utah they

bring down the water levels in reservoirs and lakes. Exposed, dying mussels are stinky and make the experience negative. In addition, sharp shells make the beach experience less enjoyable.

The name of the bill is Senate Bill 238- Aquatic Invasive Species Interdiction Act. It is important to get a cause development of Resoponse Plans and Approve.

UDWR's team receives 1.4 million annually which supports 47 employees.

There were 38 waters that were prioritized for risk. The first water that was found to be infested was a level 3 water, with 1 being the highest risk. That should tell you something.

Utah's protocol (get from powerpoint) for determining Dreissenid infestation.

Electric Lake in Utah has been deemed infested. 3 veligers were found through CPM. Two other labs deemed that there was a positive match for zebras. As soon as a water is deemed infested or suspicious the water body is put under containment. In these waters, the boats are cleaned off, hosed down with scalding water. The waters are drained for 7 days in the summer, 18 days in the spring or fall. **More details of containment plan are in the powerpoint.**

- **Columbia River Basin (Allen Pleus) Washington, Idaho, Montana, Oregon, Nevada etc.**

There are 70 sites that are being sampled, and a similar risk assessment was used for site choices and sampling frequency that others have used. We often see evidence on the boats themselves. They are asked where they came from. Every summer, temp employees go out and do interviews with boaters to ask about invasive of lots of types. They also physically inspect boats. They have a training and education program to target employees as well as the general public. Any boats at Washington state port of entry stations are inspected. They have signs displaying regulations for Aquatic Invasive species. Surveillance/Take downs: if a boater doesn't try to take off scum and vegetation off the boat, they are pulled over later. Rapid Response Plan: once it is agreed a body of water is contaminated, there's a plan- *Report can be viewed on their website.*

Allen Pleus

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C- Washington state would be interested in looking at the FlowCam. We don't have the money for a person to do CPM. Samples are sent to New York.

- **Metropolitan Water District of Southern California- current detection and monitoring program. (Ricardo De Leon)**

Quagga mussels first discovered in force in Jan. 6, 2007. Most detection methods were initially just diving. There were 1 or 2 mussels per sq meter. Trash

racks are completely closed shut with Zebra mussels. At Parker dam in Lake Havasu, there was huge population growth in a single year. Due to the warmer weather, growth can take place extremely quick. We have a huge number of mussels and the needle in the haystack is not an issue. It is necessary to have rapid response plan, which is funded, that can be implemented at any time. We established a vulnerability assessment based on area and where they like to colonize. We have quagga control studies that include several aspects, copper-based coating, standard monitoring plate used as a control. Trash racks that had the coating appeared to control their growth. Lots of research has been done on controlling adult populations. At Lake Mathews, they depleted oxygen below 70 ft and that killed all the mussels. Another area they are looking at is whether veligers are alive or not. They looked at vital dyes under microscope. Using UV fluorescence. Routine precautions for PCR: facilities, UV hoods, dedicated autoclaving materials etc. QA.QC.

Primer Selection- there are separate primers for zebra and quaggas. Standard PCR conditions. Optimization of primers.

- Round Robin Discussion

Q- Are there penalties for bad boaters? A- we can arrest you, take your boat , officers are skilled and if there is intent to violate the law there are more consequences. At one time there were 50 boats that were in violation. etc.

Q- Are the sites being monitored in the Columbia river basin plankton tows? A- Yes, plankton tows from dock side by volunteers.

Q- For Ricardo- Siphons on the canals? A- They dewater those siphons sometimes, chlorinate and this kills veligers

Q- Ever thought of generating revenue from boats? A- There is a fee for registration and we get 1.50 plus .50 and that's about 480,000 a year.

Q- Was there impacts on the fishery from the Oxygen deprivation in the CA sites? A-no, big lakes, and fish could swim away from low oxygen areas

Q- Larry, when do you switch to containment? A- anytime we have any indication of even a veliger, we start making the switch. We may slow down the containment if enough samples are clean over time

Rick- My TOC was around 1

Q- Anybody actively involving volunteer groups. A- CA sea grant is involving 4H

Coordinated Detection Monitoring opportunities in the West: -Field Monitoring

Larry- We are taking lots of vertical tows, but in shallow waters we're using a Honda trash pump, we can therefore avoid it from hitting the bottom. No horizontal tows. We are sampling the green and Colorado rivers, they are much easier to sample than stagnant bodies.

Q. What type of tow for the location? A- Reservoirs and lakes there are more options.

What is needed is standard protocols for sampling based on an area's parameters, and resources available. Need a risk assessment for the different types of habitats within a body of water. Need to examine where they might particularly begin to go to first. There is no correlation between veliger and adult locations. It would be useful to have specific factors that need to be examined in monitoring. If you want to go above and beyond, fine, but what particularly should be required- A systematic review of risk systems and studies on how well they perform. It would be good to do a controlled study where all water system were treated equally- but there are many complications to this, perhaps needs to be ranked on water body size.

Q- Is there a need for consistencies in sampling between different states? A- Because states have different policies, boat users don't understand the different requirements. There needs to be consistency to be efficient. There needs to be clear definitions of the words used in management eg "infestation." Sample collection and preservation needs to be standardized. Standardization is necessary, but there isn't enough research to give clear recommendations on what should be done, or what the best practices are.

C- There should be standardized protocols per type of water body.

C- Shouldn't there at least be a temporary standardization so we are able to compare results?

C- The PCR people could get together this afternoon and decide how much ethanol to put in preserved samples.

C- The preservation issue is key. However, logistically it might differ because of how far out the sampling sites are, how they can be kept cold. Etc.

Q- Is there a point that a sample is so dirty that its useless? A- not useless, but we can only get a smaller subsample. Some times of year are cleaner than others.

Q- What's the next level of sampling after there is a first hit on infestation? A- no clear view

Having any data on a water body is useful. You're not going to catch the first veliger. Its important to communicate to the managers how much money something will cost now and how much it will cost later if nothing is done now. They also need to know what probability you will have of detecting something if its there based on what resource you are given.

Decisions need to be made on substrates versus tows. When to use the different methods.

Perhaps- After one hit, sampling efforts need to stepped up to get a good idea of the level of infestation.

Zebra and Quagga mussels are a debilitating disease, and after even the first sign, efforts should be stepped up considerably at all levels

-Veliger Analysis

C- We must improve our turnaround time from sample collection to results. Sample collectors need to get enough resources to permanently build infrastructure so labs can hire people and materials. The problem is not going away, and the number of samples is only going to continue.

C-We all need to build in-house resources for at least looking at CPM. Anyone with bio background can be taught how to do it. Each agency needs to look at doing the analysis in-house, instead of contracting out

C- PCR for use as a diagnostic tool has a rather long learning curve. It may be taught in highschool, but you need someone who does this on a regular basis and gets very good in order to have reliable results. *Differing opinions on this and the learning curve*

Q-How realistic is it for states to cooperate based on the current money and staffing? A- Probably one state won't be able to do PCR for another, budgets won't allow it.

C- A basic fish and wildlife service won't be able to get PCR equipment and staff.

All fish health monitoring sites have PCR capabilities. They might not be thrilled to do it, but they could be about to assist.

Marc- There needs to be a standard method for dealing with PCR detection in larvae.

-Part of the difficulty is communication, negative results are not published, and this information does not get out there.

C- Due to the political and media ramifications of calling something "infested" it is too risky to use anything but an official lab that is certified. If there is any doubt about the result, there can be consequences.

Marc- there needs to standards on the sensitivity and specificity of the assay.

Elizabeth- How is a lab certified? What does this mean? A- APHIS- A certification costs money, so labs may invest in things other than certification, like equipment

- **Similarities and differences between states-
Constraints/Priorities/Capabilities**
- **Open discussion regarding possible criteria and risk scale for a general (regional) recommendation for concluding that a water body is infested**

A lot of states require dual conformation. Many require first CPM, then PCR, or two independent PCR conformations. Washington is very proactive even though they have not found any in the water at this time. The upper managers often decide to skip funding this, until they find something, then they throw millions of dollars at it. In the east they spread like wildfire and there wasn't really a chance for early detection. Now there is a rapid spread of larvae but not that of adults.

Britton: What are your thoughts on a definition of "infestation" or established population?

A- If you wait till you've found adults, then its already too late. Larvae alone should be enough for an infestation definition.

If you pull out a tow and there are lots of veligers, this tells you something about a population. If you continue to find veligers, you must have adults at some area.

Dave- there should be a gradient, perhaps beginning with the term "detected" Perhaps the term "exposed" should be used when very few veligers are found.

Dave- We feel that PCR may be improved very quickly, and the time needed for results may be reduced dramatically in the future, huge differences.

Term 'exposed' should be applied if there's an id by one method but not confirmed by another.

-Maybe we need to consider that every water body is infested due to the statically low probability of finding a veliger.

Dave- from a management point of view, we can't call everything infested.

John Darling.- Language has political weight, we need to decide how these are used.

- Because there is very little resources and action given to something unless it's at a critical level, we should set the bar low.

Elizabeth- if you are pulling up veligers week after week, it's infested even if you can't find adults.

-After something is exposed, it should stay that way forever. However, there should be a set number of years without any positive results before something is pulled off a "watch" list.

-If you've only found a small number of veligers, you are going to be challenged on the authenticity of your findings and be asked for a lot of proof before you are give money.

-Larry- Key is pointing out to someone the monetary benefits of early detection and containment. Utah did not get the political backlash at all. Instead they got a pat on the back for helping out and getting a head start on this issue.

Zebra/Quagga is getting a lot of media attention, so people are starting to pay attention.

-Even if the individual states don't adapt these terms, the 100th meridian can at least have specific definitions which they (the states) can choose to implement.

-Anytime you find any positive result (CPM, boat, PCR etc.) there is potential infestation.

- Terms thrown around- a gradient of : negative, positive, infested. The term exposed may also need to be used.
- Theres also an exposed gradient to consider. Given the number of bodies of water that are connected, should something be labeled exposed based on boater surveys.

Terms need to be linked to **certain actions**

Definitions of Water Conditions:

- Not tested
- Undetected: no evidence has ever been found to suggest presence of species, continue with same level of monitoring
- Exposed: (indirect evidence) eg. Boater survey suggests pathway
- Suspect: (direct evidence) found by one method but not confirmed by another, or a direct find of contaminated boat/other in a water body.
- Detected: confirmed by PCR and microscopy multiple times
- Infested: signifies in situ reproduction

What are the triggers? Flow plans?

Comment on collection- In monitoring protocols we need to acknowledge that first findings have been found through substrate and outreach efforts, as well as veliger detection. If all the money is put into veliger, less will be spent on outreach, which is very important.

Day Two: Thursday

Introductions:

Curt Brown: We got kicked out of the room because the sec. of the interior had to give a presentation to all employees. Brief comment on yesterday- my background is risk analysis management, so we need to think hard about the action that can be taken at each stage. Monitoring is important, but the comment the person made about assuming everything is infested is in some ways valid. Need to think about the relationship between actions taken at different stages. We need to know how much analysis we need to do to make decisions. A manager will be informed about probabilities, so they may not support a lot of money for monitoring at a place with extremely low probabilities of exposure.

Marc:

Issues for Lunch Meeting:

Highlights of yesterday: Standardized preservation and handling of plankton tows

- Improving turnaround time (2009)
- Standardizing inter-lab sensitivity and specificity (reports of detection limits)
- Organizing a technical workshop
- Definition of a Western Region

- **Review Task objective and outcome. To draft the early detection component of the 2009 comprehensive monitoring plan (Moderator Marc Frischer)**
- **Current status of Western invasion (distribution of confirmed observation of adults and larvae) (Elizabeth Brown)**

She shows a current map of infestation. Lake Mead was the beginning of the serious look at this problem. Jan. 6 quagga mussels were found on a cable entering the breakwater. Checked substrates and found additional samples. They have continued to find lots of adults in the Boulder Basin area. Thanks for outreach samples. Now it's in lots of parts of Nevada –CO River and aqueduct, mostly places from Lake Mead south. There are 28 quagga mussel locations in CA, the surprise was the zebra mussel in San Justo, adults were found in that reservoir. All the others were quaggas. They have increased sampling above San Justo, they found a veliger at one location but nothing else. IN AZ Lake Havasu and Lake Pleasant have found to have quagga mussels adults. Both lake marinas had confirmed findings. The Central Arizona Project (CAP) has found a few adults, but there are not oodles yet.

CO- we have 7 “positive” waters. All positive waters have found veligers only- Pueblo, Granby , Grand Lake, Shodow Mountain, Willow Creek, Rarryall Reservoir, Jumbo Reservoir. All these were in 2008. Some bodies have found both quagga and zebra some one or the other. At Pueblo we are consistently finding veligers but no adults.

Q-No adults found on substrates? A- have substrates, turned over rocks, none found

Utah- Main one is Electric Lake which has a confirmed veliger. There is a watch list of 8 waters but they have been confirmed by one test but not the other.

Q- In lake Granby they say it could be just someone dumping there water, but to me it seems that if four connected bodies have them it kind of disputes that it was just a dumping event

C- In Nevada we found an adult in Boulder City, we think its because it has a raw water connection to Lake mead- plumbing connecting issue. Observations- In Lake Mead and other areas we were focused on looking for adults, they didn't necessarily behave the way we expected them to. They had plenty of time to have adult colonies before we were really looked for them. We feel that we should have looked for veligers earlier.

Q- Not clear on the nature of the infestation on that lower part of the CO river- Near imperial dam we are only seeing scattered populations- why is this? Might provide clues on habitat specifications.

Q- When finding veligers what does that trigger? A- Same as UT , all boats inspected when leaving, containment mode, triggers large financial commitment,

also has increased monitoring. In pueblo we send in remote vehicle, divers, basically turning over every rock, increased monitoring at upstream and downstream sites. If we do have + in the future we'll already know what to do. Carlie Ronca has been instrumental in developing plans. We aren't using PCR at pueblo, because we know they are there. We are just counting now.

Chris Holdren- At early response is there too much reliance on PCR? Isn't CPM more sensitive right now? If multiple labs give + on CPM then isn't that enough and shouldn't we act sooner without PCR if we have qualified people on the CPM?

C- Support of that comment, CPM is faster and should hold its weight

Q- Source of contamination ? A- watercraft

C- On the nationwide map, thing that struck me was the locations with stars need more attention. Important to relay boat information, a lot more stars will be up there.

For Colorado there are only two stars. A- We've only found the two. The pueblo boat never made it to the water, eBay bought boat. We have an active plan to check boats. Lots of inspections, proposing 56 stations.

Dave- The number of stars is underrepresented; in CA there was 308 in the last two years. A single star can be more than one event at one area.

- **Conduct a discussion and develop as much of a consensus as possible on issues critical to drafting recommendation for a comprehensive early detection plan**

Marc: There seems to be consensus that there may be too many terms. We need to make long term and short term goals that are most needed

1. Definition of the "Western Region"

Dave: we had a 17 member planning team that decided this is important. 100th meridian is an informal group, how are the recommendations we make fit into the western regional panel. We don't have the WRP here in full, so we need to figure out how it fits in.

Elizabeth: The question is is everything west of 100th meridian the west, or do we go with the WRP definition which is the 19 western states? That includes HI and AK. AK is definitively interested in being included. Canada is also interested in being included, they aren't here today though. Mexico also interested.

C- Why couldn't we use the same recommendations as other parts of the country?

Dave- the southeast is also a large region that's not infested, not sure why that is.

Marc- We need to figure out which states and areas are going to be included and will agree to standardized various methods. That's why its important to decide what the west actually includes.

Dave- CO is already looking at UT and NV and what other states are doing, there's collaboration already. Shouldn't all 100th meridian states be included?

C- Figure out where the edge is going to be.

Mike Stone-WY- There's another organization, the Western Region of fish and wildlife and they are developing a plan for aquatic invasives. Sounds like there are three main organizations doing things.

Elizabeth- we should send recommendations made here to all 3 groups, and the chairs should get together to discuss it.

Dave- Need to include both groups in our planning.

Elizabeth- these are highly specialized groups

C- Do they take into account watershed connectivity? Dave: There is some, 100th meridian and Western Panel have overlapping members and areas.

Chris- We are missing the four downstream states that have water connectivity- Missouri, Iowa, Arkansas, and Louisiana.

Dave- Need to focus on the players who will actually implement the plan.

Marc- I hear two proposals, WP states, or WP states plus downstream other states.

There was a vote for the Western Region to include the 19 Western Panel States plus Canada and Mexico regions that wish to be included. Almost unanimous

C- its best not exclude anyone

Dave- We MUST focus on a particular group with similar interests and early detection issues.

Elizabeth- We are different than the east so it's appropriate to centralize our efforts because we are dealing with early detection and not containment for the most part

Dave- Minnesota is still fairly un-infested

Larry- UT is very much patterned off of Minnesota. They have a great website and plan and for 20 years they have held them off.

Marc- there should be open communication

Dave- we should actively seek information from the east, not just leave communication open.

Marc- If analysis infrastructure is put up, places like Minnesota and other un-infested states will use these areas as well

C- BC, Alberta and Saskatoon parts of Canada

Mexico- Northern Mexican states

2. State vs. basin organizational structure

Skipped this section- covered in western region discussion

3. Monitoring and Early Detection best practices:

Substrate sampling:

Vicki- 10ft intervals

C- Ongoing research at Mead trying to identify specific parameters of substrates, suggests that testing a substrate in an unexposed area won't tell you very much.

Lake Mead is working on substrate method research.

We should find out from UNVL – Sarah Muetting- what the results of their substrate research is.

C- If for 2009, we must be more specific,

Dave Robinson- Just about any substrate will work, maximize surface area and strength, edge habitat is important, easy to handle, lightweight.

Vicki- Prudent to specify that we use a heavy nylon rope with knots tied on it.

C- Put a piece of braided nylon rope in the water, intersecting surfaces, PVC on the end.

C- Even the rope by itself in the water, put some concrete on the end, no problem. Keep it simple.

Elizabeth- if you just put the rope in, it won't get buried

Marc- **So, it doesn't really matter what substrate method you use, its purpose is presence- absence**

C- is there a standardized frequency you need to be statistically robust?

Oregon- It's hard to know where to put them, we still don't know enough about them to know where to put the substrates. The substrate stuff needs to be involved in the outreach programs. Involve the public! Very important outreach tool. Post signs on how to preserve and where to take samples.

Substrate and plankton tows at same location?

-Its important to do sampling at high risk areas- boat dock, infrastructure, dams, outflows.

C- In rivers systems the veligers get mixed up right after they flow through dams at the mixing area, places with turbulence

C- At most dams you can get a sample right there, good place to get a sample.

If you're using static samplers, a good place is mixing velocity gradients.

-Mike WY- please give the starting states some guidance, if its simple its easier for us to get more done.

Keep it simple at first

Paul- when haven't talked about divers much- options for early detection, recruiting volunteers with certification, training protocols,

Scott CO- ask your police departments that have divers.

Send substrates to the BOTTOM- not just 100 ft, divers really need to know what they are looking for.

C- I see divers as inspections, not monitoring, divers very expensive

Veliger Sampling

1. Microscopy

Should be a rating system of confidence, not just relationship confidence because you know the lab person. Articulate the facts, required to have more than one

person or lab make the call before changing action plan. Any analysis method should be independently verified.

Elizabeth- Two CPM, or Two PCR is fine, basically if you have two well trained people who can confirm something that's enough.

Vicki- Our standard is you have to confirm something with two different kinds of tests.

Allen- I want to have a clear list and documentation of exactly how they came to the conclusion that they have a positive ID. And this should be sent back with the results so they can give this information to their managers. When the results are sent back, please send photographs of the confirmed sample, CP and un-CP photos, and photos of what are not veligers.

Action Item- Standardized educational materials for detection. Consolidate the materials.

John- How much are we willing to spend to learn more? We are not going to learn more about PCR and its potential unless we spend the money. Even if you think CPM is sufficient, we should continue to fund PCR research.

Detection by PCR first, does not give you the option of going back to CPM.

Marc- PCR doesn't use the whole sample

Vicki- when we announced detection, we are flooded with emails, asking how do you know for sure? For purposes of politics and the media, you need two different tests.

Marc- Lets make a list of all the separate proposals. For purposes of PRESENCE or ABSENCE, not quantification

1. One detection event verified with PCR and Microscopy

Vote: almost unanimous

2. One detection event that is verified by microscopy by 2 experts with multiple high quality photos

Vote: 24 yay, 14 nay- politically not possible

3. One detection event that is verified by PCR by two different assays

Note: no discriminatory effort between live or dead

Vote: 24 yes, 11 no

4. Two detection events (two independent samples) that are verified by 1 or 2 methods.

Note: Are multiple detections necessary to say there was a confirmed sampling. How many people think you need two confirmations to declare presence.

Vote: 2 yes, the rest no

Marc:

Proposal that we move the last item to the action items.

Larry: not a practical reality to have two detections because the turnaround time is too long in between analysis.

Recommendations for responses to first detections:

C- We must deal with this information from a standpoint of what specific actions are taken

John- At the risk of a complication, there's a difference between failure to confirm, and a negative.

Allen- If I have a detection confirmed by 2 experts or by 2 methods, I think the burden is on the two different events

-Recommendation to not discuss the specific details of the analytical methods at this point, but to convene a separate meeting for a technical workshop