LOCKHEED MARTIN CORP

Moderator: Courtney Chambers
April 24, 2012
1:00 pm CT

Courtney Chambers: All right. Good afternoon everyone, thank you for joining us today. We're going to go ahead and get started at this time. My name is Courtney Chambers and I work at the ERDC Environmental Laboratory and Technology Transfer for Ecosystem Restoration and I'd like to welcome you today to a webinar about eDNA by Rick Lance and Heather Farrington both from the ERDC Environmental Laboratory.

This series of Web meetings on ecosystem restoration topics by ERDC and the Ecosystem Restoration Planning Center of Expertise is designed to address a variety of topics including training, lessons learned, research and development and emerging issues. The Web meetings are recorded and all the files are posted under the environment gateway under the Learning tab. And as we get into the meeting I'll post the link to where these webinars can be found in the chat feature which is in the lower right-hand corner of your screen so watch for that link that you can save and access at a later date.

The Learning Exchange Notification System is what we use to send initial e-mail notifications to everyone signed up for webinar announcements and those go out two weeks prior to a webinar from the Ecosystem Gateway e-mail address. In this e-mail we encourage you to register for the webinar which enables you to add it to your Outlook calendar then you'll also be sent a reminder the day before the presentation.

Okay just a few more notes before we begin today's session. We are going to have questions and answers for the last 15 minutes of the presentation and you can ask your questions verbally at that time after taking your phone off of

mute or again use the chat feature in the lower right-hand corner. One quick note about that, if you look at that feature it gives you the opportunity to select who you're sending the message to so if it's a question for everyone and our presenters, make sure that it says send to everyone. If you have a technical question regarding the webinar itself or how things are working, you're welcome to send it directly to me. And then we will cover as many questions as time allows but if we run out of time we can certainly follow up by e-mail with Kurt or Heather. Okay?

Please remember to keep your phones on mute while you're listening and please do not put us on hold with background music. In order to have a more comprehensive list of attendees I do ask that if you're calling in as a group you would take just a few minutes to write the names of your attendees in the chat box or simply the number of participants that you're calling in with and then send that message to me through that chat feature if you don't mind. And you can see an example of that on this introductory screen. It's the blue bullet there. That just gives us an idea of the attendance of each of our Web meetings.

Okay now I'd like to give you today's speakers on eDNA Dr. Rick Lance is a team leader for the conservation and ecological genetics and he is currently co-leading a team for eDNA monitoring of invasive Asian carp in the Chicago area as well as leading efforts to develop eDNA tools for endangered North American sturgeon and invasive zebra and quagga mussels. During his years at the Environmental Laboratory Dr. Lance has worked on a number of different projects including genetic studies of zebra mussels, endangered songbirds, endangered bats, native grasses, agave, fleabane, nectar-dwelling yeast and cave crickets. He has also continued to conduct research on bat ecology and led two recent pollination ecology projects. Currently though

much of Dr. Lance's work has focused on developing new eDNA capabilities for districts and other natural resource stakeholders.

Our other presenter that is going to be helping him today is Dr. Heather Farrington who is currently a working member of the eDNA team monitoring Asian carp in the Chicago area and is developing eDNA markers for endangered North American sturgeon. Heather Farrington joined the U.S. Army Corps of Engineers Environmental Lab in 2011 after completing her PhD last spring for her dissertation work on evolutionary and population genetics in Darwin finches of the Galapagos Islands.

More information about both of our speakers can be found in their bios posted on the Learning Exchange with the rest of today's meeting documents and again watch for that link in your chat box. We're very thankful to these presenters for their willingness to share with us today. So at this time I'll give you the presenter rights and we can begin.

Rick we're not hearing you. Are you on mute?

Rick Lance: Yes I'm on mute.

Courtney Chambers: Okay.

Rick Lance: If you can't hear me now still please let me know because I'm using the speaker phone. I'll use the handheld one if I'm not being heard well.

Courtney Chambers: It sounds good right now, thank you.

Rick Lance: Okay. So welcome to our webinar today. I want to thank everyone for taking the time to listen to our presentation. Today we're going to talk about a new

use for eDNA for DNA sampling and providing the needed data under some challenging conditions.

The project team includes myself, Dr. Heather Farrington, then Eliecer Navarro, Xin Guan, Matthew Carr are technicians that work on these projects with us.

Okay so the challenges that we're interested in addressing is basically how to detect, monitor, maybe even survey species that are difficult to observe either because of rarity or because of their habits. And this really applies to aquatic species especially aquatic species that are rare either because they're endangered or maybe because at an invasion front like the Asian carp where there's still not very many in the area but they're potentially increasing in numbers.

So one way to get at this problem of trying to track and detect these species that are otherwise elusive or really difficult to detect is to use eDNA, environmental DNA and what environmental DNA is basically is just DNA that's left behind in the environment by that species, by individuals of that species. And it has some uses for species that are not so difficult to detect as well. It just kind of depends on what you want to know and sort of the environmental context. And we're basically working on developing a number of - expanding those capabilities and refining the capabilities that we do have.

So again environmental DNA or eDNA is basically biological materials that contain DNA that have been shed into an aquatic system in this case and it can be detected from filtrates of those water samples or just from some way of getting at the material in those water samples. And eDNA sources typically include things like scales, other cells that are secreted with the urine and the

feces, blood or other tissues from injured fish or maybe even reproductive cells that are broadcast into the water.

Now the ability to detect eDNA is based on the polymerase chain reaction or PCR. What PCR does is it basically takes a small amount of DNA and then makes billions of copies of select sites on chromosomes or simply sequences of interest. So for that reaction to take place we look at sort of the upper left. If you start with a piece of double-stranded DNA you heat it up in what's called a PCR thermal cycler. You heat it up to 95 degrees Celsius, you switch that double strand into single-stranded DNA. Once those are split into single strands you then cool that reaction down to about 55 degrees or so Celsius and allow primers to attach to that DNA.

Well primers are kind of the key. These are short pieces of DNA that should be specific to the species that you're interested in and attach to the piece of DNA within that species that you're interested in the marker. So once these attach to the DNA then you heat your reaction back up to about 72 degrees Celsius and then an enzyme called a DNA polymerase comes along and makes a copy at the site where those two primers attach.

The polymerase comes along and copies and you kind of see it on the bottom left there where we have two primers, one on each strand and then a big orange polymerase that copies in a particular direction. So you can basically go from two pieces of DNA to four pieces of DNA.

And if you go to the bottom right there you can see as you do that a number of times if you have say 30 or 40 cycles of this heating and cooling process you get an exponential increase in the amount of DNA. You go from two to four to eight to 16 and 32 and so forth from essentially a very small number of the

particular piece of DNA that you're interested in to billions of copies you can then work with and detect and those sorts of things.

And so these markers that are supposed to be species specific and target specific are really the key to having a good eDNA marker because if they're not species specific that also means that the primers may land on other species that you're not interesting in and result in false positives in the reaction or just an inability to detect if the species you want is actually there.

So an appropriate eDNA marker ideally would include basically the primers and the protocol would always be accurate, it would always detect individuals of target species and would not cross react or the terminology we use is cross amplify with nontarget species. So if for instance we were looking for a marker that would tell us if silver carp were in the system it wouldn't also react if it encountered DNA from say grass carp or common carp.

We'd like for it to be sensitive so when we're looking at eDNA, eDNA is typically, you know, it's been shed by - it's part of a dead cell or an injured cell, there's usually not very much of it, it's partly degraded so we want to have assay that's sensitive to small amounts of DNA. Of course we want it to be robust, we want to detect the DNA in the water sort of irregardless (sic) of whatever else is in the water because sometimes you can have compounds in the water say tannins from degrading leaves or bio salts from animal fecal material or from sewage that can basically disrupt your PCR and cause it not to work or not to work as well. So we want to have an assay that's really robust when you're basically taking samples from who knows what kind of water.

So the basic methodology that exists right now for taking eDNA samples is fairly simple. You go out in a boat or to the water, you take a bottle and

collect two liters of water from a number of sites in the area that you're interested in, you take that water back to the lab and you pour into a filter funnel like you see on the bottom right there. In that filter funnel there will be a little piece of filter paper with a 1.2 micron pour size and basically apply a vacuum to the bottom of that filter funnel and it pulls the water through and as the water passes through the filter it leaves behind the organic material within the DNA.

Then that DNA is then sent to ERDC in the case of the Asian carp and the sturgeon, it's doesn't have to be ERDC in all cases, and then that DNA is extracted using a commercial kit off that filter. Lets say for instance the ERDC lab or another lab that does the eDNA has that filter, that sample, we extract the DNA with a commercial kit and then we go for that polymerase chain reaction with those primers for that specific marker that we're interested in.

Once we finish that PCR reaction with that sample and we may do a number of replicates just for quality control and sometimes when you have really degraded samples or fairly diluted samples sometimes you have to run several replicates to actually detect the presence of that target -- so once we've completed the polymerase chain reactions we then run those on agarose gel. If the primers in the PCR have worked like we expect it to and if the target species' DNA was in the eDNA sample we expect to see those billions of copies of DNA form a distinct band in the gel at a certain size like a certain number of base case.

Say for instance for the silver carp that we do for the Asian carp work that's a 200 base pair PCR product that we expect to find, 200 ATCGTTCGs in a row for that particular DNA. So we expect to see a PCR product that matches that length.

Once we see something that looks like it's at that base pair length in that gel or something that's real close to it, we call that a confirmed positive and then we take that, we might do some additional PCR to make sure it's really there, make sure we didn't see any contaminate or something funny just didn't happen in the reaction and then once we feel that there's a fairly good chance that's actually the eDNA of the organism we're interested in, we take it to one additional verification step and that's DNA sequencing.

And here we actually look at the sequence of that little piece of DNA that we've generated and make sure that it matches what we expect that our target species DNA to look like. I think below here the image is actually a partial sequence from what we'd expect a silver carp sequence to look like so that's what we would expect to find when we sequence that DNA band. And if it doesn't match it then we know that something else has cropped up and we discard it.

And as we're running all of these steps we'll essentially be at the same time running a number of controls -- negative controls -- to make sure that we're not having any sort of contamination issues. One of the problems with an eDNA assay, it's really geared to be really sensitive to small amounts of DNA. It's also very sensitive or it quite readily detects also contaminating DNA. This is a problem that's always faced in the forensics world and also in the ancient DNA where they work with DNA from mammoths in permafrost or mummies and things. So it can be a real common problem and you have to have really sensitive assays and so we've taken a number of steps in our lab to prevent contamination and at the same time also to check for its occurrence.

So whenever we have a sequence that confirms that we're actually seeing the DNA that we expect to see and all the controls are clean then we have a confirmed positive for that particular species.

So where and how is eDNA currently being used? EDNA applications especially in aquatic systems really came to the forefront with the efforts to track the spread of Asian carp in and around the Corps' electrical barriers in the sanitation and ship canal and the associated waterways. The actual first use though in the literature of an eDNA type approach was a small European study looking for invasive bull frogs so that was the first study that we see then the carp.

And in the last few years, a couple years we've seen a number of other studies be published. One looked for an endangered tail frog and a giant salamander I think in the Rockies in the Western U.S., another one looking for various fish in Japan and then a recent one where they actually looked for a large number of aquatic and amphibious organisms in Europe. I know there's currently funded work to do endangered salamander work out west as well.

So it's a relatively new approach, eDNA for eDNA aquatic samples, eDNA assays of aquatic samples but it's something that's really picking up steam. A lot of work's being done and you can probably expect to hear about it quite frequently for various applications in the future. And really the range of capabilities for eDNA moving on to things like determining the sex of the organism in your sample to in some scenarios maybe actually estimating numbers of individuals or looking for a whole bunch of species at the same time. These are things that are probably already possible now in some scenarios and so you'll see these sort of capabilities broaden over the next little while.

So for Asian carp we've been involved with the Lakes and Rivers division, working with them on Asian carp detection for awhile now and that's really been our biggest effort to date. Asian carp if you're not familiar with them are two introduced species that had gotten into the Mississippi River watershed. There are great concerns about them outcompeting native feeder fish because the two carp species eat plankton so their potential to outcompete the feeder fish would then feed the game fish or the fish that are sought after by the fisheries trade. And so they've kind of spread and they kind of spread into towards Illinois and up towards the Great Lakes and there's a lot of concern they'll reach the Great Lakes and potentially cause ecological problems.

And so Corps basically put into place electrical barriers that are intended to stop the fish and what the Corps has done then in collaboration I guess with state and federal agencies, they go out and they take water samples from upstream and downstream of the barriers to check to see where the carp are and if they've gotten past the barrier somehow. And so then they take those samples and they send them to us to process.

Let's see. And the idea being that if they do detect, you know, if they consistently detect carp eDNA at a particular site upstream of the barrier, beyond the barrier then that would then trigger a response where they would drop netting and electroshocking, basically the works to try and capture, detect, capture and hopefully eradicate the fish if they were to somehow start gaining a foothold north of the barrier.

Along with just processing the samples for the Lakes and Rivers Division we've also become involved with the eDNA calibration study which is basically conducted in support of Asian carp. Basically the idea is the eDNA calibration study is a study to better understand the context of eDNA positive, there's several questions that could be addressed where areas of additional

information would improve our ability to interpret the data and make decisions based on eDNA results.

Some of these questions that would be helpful would include things like are there realistic alternative means by which eDNA could end up at a site upstream of a barrier besides the scenario where a fish has gotten past the barriers, we want to know how long the eDNA lasts in the system and how much eDNA does a fish generate. Additionally we're interested in developing ways to increase the throughput of the eDNA assay and to decrease the cost. All of these in some form or another are being addressed by this larger study, the eDNA calibration study.

So the eDNA calibration study or ECAL is a collaborative study with the Corps of Engineers, the US Geological Survey and the Fish Watchers. It's not actually funded by EPA, that's an error, it's funded by the Great Lakes Restoration Initiative and I think it's administered by the EPA but not actually funded by them. So some of our partners include of course the LRD, (Kelly Bearwalt) who's working for them out of I think she's actually assigned to the St. Paul district but is working essentially with LRD and with us on this project, our lab here in Vicksburg, Mississippi, USGS is a Columbia environmental research center in Columbia, Missouri, their upper Midwest environmental science center in La Crosse, Wisconsin and the Fish and Wildlife Services northeast fishery center in Lamar, Pennsylvania are the federal partners that are working together on this project.

And again the idea of ECAL is to develop better improved understanding of how the DNA behaves in the system, what are the alternatives for moving around the system and hopefully in the future some modeling to help us better understand how it actually moves with water flow and how to best use that data for decision making.

So the ECALs one of the things of course we're interested in is improving the throughput and the accuracy -- well not really accuracy because we have a fairly highly accurate approach now -- but just a way to get from point A to point B more quickly and cost effectively. Things like streamlining the sample collection process and developing faster DNA extraction methods and developing additional different types of marker systems and ways to analyze them.

We're hoping to be able to provide with some of the new markers at least some simpler broad estimates of population size, more information about when the sample entered the system, you know, taking into account what we'll find out about degradation rates, developing maybe some PCR markers, quantitative PCR also know as real-time PCR which is an approach that allows you to estimate the amount of DNA that you start with in a sample and in some cases that might be informative to the number of fish in the sample, not in every case but if you make certain assumptions in some scenarios it would be helpful on those lines.

And we might also look down the road into looking at next-generation DNA sequencing where you actually can take a single sample and generate hundreds of thousands of DNA sequences which is probably overkill right now relative to the needs for just detecting and monitoring Asian carp but we might find some use for it down the road. It's sort of the next wave of DNA capabilities.

So it's likely that there might be different ways for the DNA to move around in the system, our alternative vectors work. You know we've been thinking about in terms of when we get a positive where does it come from and how did it get there and so we kind of developed a framework for looking at it and

that framework included while the eDNA had to have an origin, a vector to move from where it was to where we found it, the receiving point where it ended up, release mode, a release point and then it's also affected by environmental factors that at some point we detect it and it's potentially been affected by all these things prior to our detection, some of them may be in the same thing, the receiving point and the release point for instance can be the same thing, the origin and the receiving point can be the same thing.

If you think about it there are just some different scenarios that you could assume reasonable. For instance let's say you had a live fish in Dresden Lock which is downstream of the barrier, well the silver carp jumps, we know that they jump and land upon barges on occasion. Well that barge may during its travels at some point be along the north shore of the Chicago River, we know the guys on the barges kick the fish off the barges at some point. Well somebody might kick that fish off the barge, that dead fish off the barge at that point at some river mile. If the water's cold and it's high flow, that DNA could potentially spread over a fairly good volume of water.

Alternatively if we had on that barge a gull could find that, you know, a gull or some other bird scavenger could find that dead fish, they could eat that fish, fly to Lake Calumet, poop out that DNA, that fish DNA into Lake Calumet let's say and if it was cold water with no flow we might see that particular DNA hit there for quite awhile. The same thing happened I know in the Calumet River for instance and if the water was cold and it was high flow that DNA spread - we wouldn't see as much if there was a carcass in the water but you would see a few points potentially spread out over a fairly short time.

So there's a lot of contextual things that we want to be able to take into account when we look at eDNA hits to help us understand just what the risks

are down the road and basically to be better informed beyond just the simple presence/absence type of data.

We had a workshop in Chicago not too long ago, it was last November. It's a bunch of experts in various skills.We talked about what those different factors could be. And we identified a bunch of them. There are carp sold for food in the fish markets in Chicago. And the blood and guts and heads and whatever potentially end up in storm sewers to be washed out of those storm sewers and we get a positive match from basically fish pieces parts but not actually from live fish.

The carp were being used to create liquid fish fertilizer. We've been testing that and found the DNA in the fertilizer. That was one thing that we were concerned with.

You know, of course other predators may eat the fish or carry the fish and then deposit DNA, you know, anywhere else, somewhere, you know, within their foraging ranges or their migratory pathways.

We know the barges can carry the fish around and other boats can carry the fish around. So there's just a large number of different ways that the - well especially a number of ways that the DNA can move around the system in addition to the scenario where, you know, fish surpasses the barrier.

You know, of course, you know, there are a lot of different ways that the DNA can actually get into the system from the fish whether it's a live fish swimming, a fish carcass that's sitting in the water or next to the water, or predator feces. And the DNA can actually, you know, get to the sediment and actually persist for quite a long time.

And there are number of environmental factors, temperature, oxygen content, water PH, the amount of UV light that DNA is exposed to. It can all affect the degradation rate of the DNA and our ability to detect it. So that's kind of an overview of what's been going on with the carp work.

Another thing that we've done is worked on invasive zebra muscles. And this is sort of a study project that (Matt Carr) and I worked on in 2010. You know, zebra muscles are a problem, you know, they're invasive. They clog up pipes, intake pipes at power plants and all kinds of structures they can attach to in the water.

They spread in these juvenile free floating stages. They kill native muscles. You know, they attach to that. They cause all sorts of problems. And, you know, hundreds of millions in dollars in cost over time.

So one thing is we are interested in is we want to, of course, can we detect zebra muscles via eDNA. And too, is there a way that maybe we can tell whether or not the DNA that we detect is associated with the free floating live or is it just dead cell material?

And then thirdly we also are interested in developing a new way to actually sample for eDNA.

So we did this little pilot study on Lake Pepin in Wisconsin and Minnesota I guess. Again we were looking at ways to detect zebra muscles, discriminate between live and dead cells. Hopefully - to do that we basically had a photo reactive dye that will reach through dead cell walls and attach to the DNA or they will just attach to DNA that is just free floating in the water, but can get through living cells.

And when that photo reactive dye attaches to the DNA it makes it so that you can no longer detect it with (PCR). So the idea was that if we went out and detect zebra muscle DNA. Then also took a companion sample that was exposed to this photo reactive dye and then we saw a little difference in the amount of DNA that we found that, you know, the most that we'd see that, especially if we locked the entire system. We would know if that DNA that we detect was dead cells.

But if some of that DNA persisted and we still saw (PCR) product we would know that they were probably intact cells in that sample, which in most cases would probably be the living. So that was something else that was a potential capability that we were interested in.

And then we also learned about this other method for sampling eDNA. In this case what we did is we took a seed cloth of 40 micron mesh cloth, attach it to the other PVC pipe, and then pump 10 liters of water across that seed cloth down that pipe.

This would eliminate the need to take things back to the lab and filter them through the filter funnels, which would save a lot of time and a lot of effort on the part of the people that have to do the filtering. So we also tested that approach relative to the approach of taking the two liter samples, putting them in the lab.

Again, the results of that one when we looked at the DNA bonding guide we saw some mixed results. But it did look like in some cases that we had actually eliminated the (PCR) signal, which would indicate that we had indeed had samples that were just entirely, you know, dead cellular material.

More studying needs to be done on that, but I think there's something that might actually be - there's something there that might actually, you know, at least be of some real help in detecting zebra muscles, which is what most of zebra muscle monitoring really looks for, you know, presence.

The seed sample approach that we tried worked a lot better than the standard approach. And we're doing some more tests on that. And it would I think really improve the efficiencies of sampling as far as, you know, the sampling filtering component scale.

Again, so as part of (ECAL) we in line to improve our sampling efficiency. We did some more tests that seed method versus the standard grab sample that we did. And we also looked at different volumes of material and different volumes of water, you know, in our samples. And here you see kind of the result of that. In red are the number of positive hits and blue is the number of total samples that we took. You see on the far left that it's just the grab samples.

We actually went to a site on the Illinois River where we knew the carp were somewhat present, but not really common. The graph samples, standard samples, really didn't detect anything. But the, you know, as we increased the amount of material that we put through the filter method we saw increasing numbers of positive hits.

We also looked at sampling different strata in the water. And we found that sampling the top of the water, at least for carp, worked best. And that, you know, again, the more water we put through the seed cloths, you know, it seemed to improve our detection probability. We only had, you know, like 15 samples for this. So it's a really small sample set. And we're going to repeat

these experiments later on this summer as part of the study with larger numbers of samples.

Let's see. Okay, so another area that we've been working in is developing eDNA capabilities for detecting and monitoring sturgeon. You know, sturgeon are a major issue for the Corp of Engineers. I think eight of nine of the North American sturgeon species are one way or another either entirely protected as threatened endangered species or have protected populations. And I know that they have concerns over sturgeon impact in a lot of dredging and habitat modification type projects from both coasts and also from the major river systems in North America.

And I think we're probably a really good candidate for, you know, having a real use for eDNA monitoring. You know, there's relatively rare locations where, you know, where they exist. You know, for instance there's been some interest were they think occur in some systems, but they have not been able to find them.

So a lot of times they're really rare. You know, they're bottom floaters and hard to observe. You know, they move around a lot, they move back and forth, you know, between ocean and the fresh water systems.

And so really, you know, hard to detect. And so we're thinking DNA is probably really good. It's a good application for eDNA capabilities and tools.

And we think that, you know, eDNA could be used to monitor their seasonal movements, identify habitat use, where they are, what types of habitats they're using, determine times when they're in, they're not in systems, you know, environmental windows.

And also again, you know, looking at areas where we might want to restock or apply some sort of conservation effort or ecological restoration to see if the fish are already there or not.

But again, you know, as we've talked about with the ECALs, you know, you have to take into context the environmental factors of where you're sampling and then come up with a study plan that takes those into account so that you , you know, it can help you interpret that data.

So well first thing we had to do to develop this sturgeon capability was to develop, you know, markers, eDNA markers. And we've been working with eight potential genetic markers for the sturgeon. These are all found in mitochondrial DNA. And all animals have nuclear DNA, which is the DNA in your nucleus. But we all also have these mitochondria that are little powerhouses of the cells. And in each of those mitochondria are a little circle of DNA. And it's really common in cells. And so there's lots and lots of it. And so it makes it easier to detect. So we've been working with mitochondrial DNA.

So we've identified any potential markers for a sturgeon. And actually these markers detect all sturgeons. They don't detect their species. But can be used to detect just basically if there's a sturgeon in a sample.

We've tend to be successfull on seven of the nine North American sturgeon and now have samples from the other two that we haven't tested. We're testing those as we speak I think. So we should shortly have those tested on all the species. And so far they've worked just fine.

We also are basically testing them - you know, we can kind of test to kind of get an idea whether or not they're going to cross react with non-target species

based on just what you know about their DNA sequence. And there's a big database called (Gen Bank) that has DNA sequences for, you know, hundreds of thousands of species probably. So we can kind of check it that way.

But just kind of as another way to check it and just to kind of give us, you know, a better feel for how well they're going to work. We can test our markers against other DNA from other species to make sure they don't cross react. And these are the species that we've been testing our new markers with to make sure that they don't cross react.

And the real problem is (paddle) fish, right, because (paddle) fish are the closest relative of sturgeon. So we do have a few of our markers that will sometimes detect (paddle) fish, but if we ramp down the sensitivity a little bit we no longer have that cross reaction. So that's something - we're working just so that we can keep that sensitivity and not detect paddle fish, but that's sort of an ongoing thing.

And we are working on ways to drill down to detecting individual, you know, specific, sturgeon species as opposed to just detecting sturgeon in general. You know, as of right now with just the markers that we've developed for sturgeon as a whole there are a number of areas where, you know, and this is a very broad, general map. You know, but places where there's only I think sturgeon species are supposed to occur, based on what's known and what's been mapped.

And so for instance here for green and white sturgeon, you know, the number varies where you only have one species or the other. And so our markers worked perfectly well for detecting those species by themselves.

And species you were detecting plus you could use them wherever either of these species occur. And actually right now we're working on developing ways to discriminate between those two in areas where they do occur, but that's again ongoing.

And here's just some other maps. We won't go into detail. But just outlining some areas where the current markers that we've developed could be used to detect, you know, the sturgeon species not just because they're the only two, but the only sturgeon in that system. And here again just some more of the sturgeon. Right now we're also working on discriminating tools to discriminate between Alabama sturgeon as well, because we have the right kinds of samples and enough samples to do that.

And our hope was to develop, you know, ways to discriminate doing all these species. And there's really no timeline on how long it's going to take to develop each species specific toolset, because, you know, we have to go through a lot of testing.

We're not sure how long it will take to successfully develop these species specific ones. And so we've started with green and white and Alabama's, because we have the samples in hand, and because they're relatively, you know, different genetic - roughly speaking, considering that all the sturgeon are pretty closely related and have similar DNA. These are some of the ones that have more similarities than others. So that's kind of what we're working on right now with those sturgeon.

And right now what we're trying to do is we're developing these tools called (unintelligible). And basically you use the same marker that we've developed that detects all sturgeon. But the (unintelligible) should allow us to block the

detection of one or the other. And we're going through tests on those right now.

Okay, so this is the final slide. And so the idea again that we have for all these different applications for carp, for sturgeon, for muscles, you know, and really anything that you would be interested in, because everything has DNA.

And so this is largely applicable with any aquatic species. You know, to develop protocol that are relative, you know, that have high through put so that you get your results fairly quickly and that are fairly cheap.

You know, right now each carp samples probably costs about $175 to process between man hours and supplies. We hope for the sturgeon to be able to get that down to $60 over time, because we probably won't need to write as many replicates and things like that.

We're hoping to get that down to about $60 per sample when we're done optimizing the protocol. These protocols will be available for anybody to use. So it won't be like, you know, we're developing a process that can only be done here in ERDC. But, you know, it can be adopted by just about anybody that has the setup to do it in their labs. So it should be a protocol that will benefit not just us but, you know, all natural resource stakeholders down the road that are interested in these species.

And again ultimately the eDNA can be used to guide, if want to go out and sample with more standard approaches or more (unintelligible) approaches. It could be used to basically monitor the presence of species in the system, you know, again taking into consideration the alternative (unintelligible) and things like that.

And, you know, and for instance we have at least two instances where we have people that are interested in using the eDNA to actually determine if sturgeon are in the system or not, because sampling information or historical information are there. But they can't find them and they want to restock them, but they don't want to restock them if they're already there.

So those are the things that we're doing with the eDNA. We think it's an exciting, really potentially powerful tool for doing things. You know, they're not simple, because again there's all these environmental context and understanding, you know, the nature and the water.

But it really I think will provide capability that nothing else will provide. You know, when you have species that are just so difficult to otherwise detect or monitor or really, really costly to detect and monitor. You know, this is another way to get at it.

And so that's pretty much what we have on eDNA today. And I'd be happy to take any questions.

Courtney Chambers: Great. Thank you very much, Rick.

Rick: I have some on the chat board.

Courtney Chambers: If you don't mind let's go back to that main screen if you could click return for us. And then we can have both of those to look at. Thanks. Okay, yeah, so the question is, (David Ware) how can we develop detection density and distribution thresholds, the number of positive hits over a given area that would realistically represent breeding populations?

Rick: Let's see, yeah, I mean, I think you have to have some caveats, right. So, you know, we have to make some assumptions. So if you say that all the fish in the system are healthy and alive. If you know how much DNA they put out and just sort of what that DNA does once it hits the water, you know, just how far it floats, you know, how quickly it degrades. You know, you could probably do that, you know, based on the number of hits over a given area over a certain timeframe. It's just kind of redundant.

But again you have to realize too that like for instance that 100 fish passing through a body of water might leave behind just as much DNA as 10 fish that live in that same volume of water.

Injured fish that's bleeding and stuff in the water might produce as much eDNA as, you know, 100 healthy fish. So again it can't be just a onetime data point. It has to be something that you see over a period of time, probably over a range, over a certain volume of water and understanding again the system.

Rick: Yeah. So there was a question, what is the cost of the DNA extraction kit? We used the power water kit, but I haven't ordered one for a while. The technicians have been doing that. So that's probably in the range of $200 - $300 or maybe $100 for 300 extractions.

Yeah, so those kits are onetime use. So basically it comes in a box. It's got a bunch of solutions in it that you use to wash the DNA to clean out proteins and things that you don't want in your solution.

And so, yeah, so basically each kit will allow you to do anywhere from 50 to 250 extractions. And usually it's a - once you get to about 250 extractions it's usually $200 per kit.

Courtney Chambers: Great. Thank you. Would anybody else like to ask a question? If you want to verbally ask it please remember to take your phone off of mute first.

(Angie Sowers): Hi. This is Angie Sowers). I have a question about if you're screening an area do you have protocol or recommendations of the number of samples that you would take? Does it depend on I guess the size of the system?

Rick: Right. There's a quality assurance project plan that's been developed for the Asian carp that's a really good guideline for how to handle samples and take samples. The number of samples you take though, yeah, you're right, it probably depends on your system. If it's a closed system, you know, how big your system was. So you really just sort of have to, you know, I guess it would be on a per case basis the number of samples you would need to take. And right now, you know, there's been some debate as to whether you should just take sort of random or grid sample or target certain areas where you think the DNA may accumulate, which we think is probably the best way to do it if you're actually looking for the fish. You want to go to where you think they'd be.

(Angie Sowers): Okay. And then if you get a positive hit are you confident the DNA is recent or how long does the DNA persist, you know, under natural conditions?

Rick: There's been some recent studies that, you know, that they've been able to detect DNA in natural water, environmental water, up to I think two or three weeks after the fish were removed. And some antidotal evidence that it gets into sediment, you know, you can detect on that sediment for quite a while longer. So some of the things that we're going to look at is, you know, can we develop different markers that have different life spans, you know, give a better idea of when that sample may have entered the system. And also some

idea of what effects the life span so that we can have a better idea of, you know, again, how long has that DNA sample been in the water.

(Angie Sowers): Right. Okay, thanks.

Courtney Chambers: Rick, we have another one (Amy) sent to me and I reposted it. It says can you utilize next generation sequencing techniques with more general primers to screen community compositions.

Rick: We have not - since we've been focused just on the carp we have not engaged in next generation sequencing, because, you know, that generates so much data that - and it costs - it's fairly cost prohibitive. If you don't need it, it costs a lot. So it would give us much more data than we could utilize for the carp. But that is - you know, in fact there was just a whole bunch of papers and markers about using this in terrestrial environments at least. They talk a lot about soil. And we actually have a proposal in now to a DOD funding program to do that. Yeah, so that's part of the future, but it's already being done to some degree. I think (Tim King)'s lab.

So basically next generation sequencing allows you to go out and not just focus on detecting one species, but detecting a whole range of potential species in the system. So, yeah, so that's definitely an important application that we're probably going to see a lot of in the future. But it's a bit overkill right now for the carp, because we're just looking for these two species.

Courtney Chambers: Great. Thank you. Feel free to ask any further questions that you might have.

Tim Walker: Hi. This is Tim Walker.

Tim Walker: Hi. (Unintelligible) sturgeon and (Unintelligible) sturgeon hybridized. When you're developing these testing protocols or markers how are you going to handle the hybrid sturgeon?

Rick: Right, so we're facing that same question right now with silver and Asian carp, because they also hybridize. So basically right now with the marker system setup as it is right now we detect a (unintelligible) type. And whether it's a silver carp, (unintelligible), and that's all we know. We don't know if it's a (unintelligible) carp that just so happens to have a silver carp mother somewhere in its lineage.

And so it has other species, (unintelligible) DNA type in it. So right now the current protocols don't discriminate. But, of course, there are genetic ways to do that. And you could potentially - there are some scenarios that identify whether or not you got hybrids in your sample. But you'd have to develop the markets for it. And it would require probably more than just one marker like we typically use now. And use more than one marker here after when we're done with the (ECALS). But the current protocol just incorporates one marker per species.

Courtney Chambers: Any other questions?

(Dave Curran): Yeah. (Dave Curran) here from headquarters. When you start multiplexing that by doing multiple markers what does that do to your cost for analysis? Does it really jump it up there or are we talking...

Rick: No, no, it shouldn't, because if we run them together - really our biggest costs are man hours, just like anything. And some of the genetic supplies, you know, are fairly expensive. But if we multiplex them and run them in the same reactions it shouldn't really increase the cost that much.

(Dave Curran): Yeah, because I'm just concerned about how we can get costs down.

Rick: Yeah, yeah.

(Dave Curran): What is the typical timeframe to run one of these analysis? Can you talk man hours?

Rick: Right now I have a team of four people and not including myself, so five of us. More people to do the bulk of the lab work. And, you know, for the Asian carp monitoring we're sending in at one point 120 samples a week. And so we were able to turn those around in nine working days. But that was because we working on multiple batches at the same time. I think if we were just working on one batch it probably would take us a week.

(Dave Curran): I know they were outfitting the lab up in La Crosse to do a lot more throughput. Is it just multiple individuals or - I mean, we're really talking duplicating equipment?

Rick: So the lab that they're setting up in La Crosse it's basically say an addition to the fish help center in La Crosse. And they're (unintelligible) like an eDNA wing I think.

They basically will have the same sort of lab setup we do and the same amount of equipment personnel. And I think their throughput will be about what ours is.

(Dave Curran): Okay.

Courtney Chambers: Are there any other questions out there? All right, (Rick), it looks like you gave them information they needed. Do you have your contact information anywhere on here, (Rick)?

Rick: No.

Courtney Chambers: Okay, well would you mind maybe in the chat feature maybe typing your email address and making that available?

Rick: Sure. And I just can't thank everyone enough for taking the time to listen in today. I really appreciate it.

Courtney Chambers: All right, great. Thank you very much, (Rick), for sharing today. And thanks for joining us.